ASPIC
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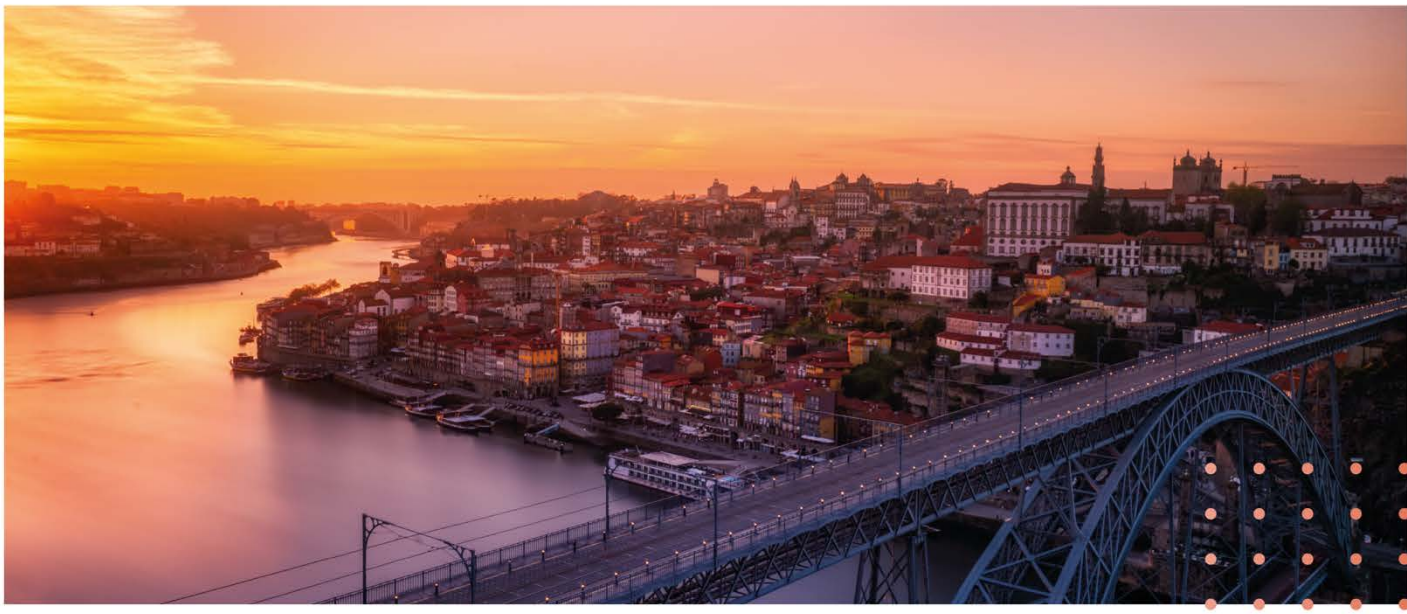
5th ASPIC International Congress

IPO-Porto • 30 June-1 July, 2022

PROCEEDINGS BOOK



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Letter of welcome

Dear Colleagues and Friends,

As Presidents of the 5th ASPIC International Congress, and on behalf of the ASPIC Direction and the Organizing and Scientific Committees, it is with great enthusiasm that we invite you to attend this congress, which will be held on June 30th and July 1st, at the IPO-Porto Auditorium, Porto, Portugal.

The ASPIC Congress is coming back to IPO-Porto, after a big success in 2018. In that meeting, we were thrilled to have 425 participants that created a warm environment to discuss the latest discoveries in cancer research. In 2022, we expect to hold another rewarding meeting, which will also represent an excellent opportunity to physically meet each other again and to learn, face-to-face, about new developments in cancer research, share results and discoveries through oral presentations and through a big and participative Poster Session.

You are invited to take an active part in this conference, which we believe will be an outstanding scientific event. We hope that you will take the opportunity to benefit from the exciting scientific programme and from new contacts and collaborations. During the meeting, there will be great opportunities for young scientists to meet the experts in the field, and we aim to create an open and engaging environment for discussion.

We look forward to welcoming you at the fantastic city of Porto.

Luís Costa and Rui Henrique
Presidents of the 5th ASPIC International Congress

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Congress committees

Congress Coordination

Luís Costa
Rui Henrique

Scientific & Organizing Committee

Joana Paredes
Júlio Oliveira
Jorge Lima
Bruno Costa
Cláudia Faria
Ana Preto
Carmen Jerónimo
Luisa Melo

Scientific Evaluation Committee

Chair: Bruno Costa-Silva
Ana Luísa Correia
Ana Sofia Ribeiro
Célia Gomes
Cláudia Faria
Fátima Baltazar
Peter Jordan
Raquel Almeida

Congress programme

Thursday, 30th June

9:30 Opening Session

Joana Paredes (ASPIC/i3S), **Luís Costa** (CHULN/IMM) and **Rui Henrique** (IPO-Porto/ICBAS)

10:00 ASPIC Lecture

BRCA2-P study: National characterization of the Portuguese founder mutation of the BRCA2 gene

Fátima Vaz (IPO-Lisbon)

11:00 Coffee Break

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Cancer Genomics and Epigenomics

Chair: **Bruno Costa** (ICVS/ U.Minho) and **Cláudia Faria** (CHULN/IMM)

11:30 **Epigenetic contributions to Leukemia**

Christoph Plass (DKFZ German Cancer Research Center)

12:00 Selected Oral Communication

T-cell receptor and Ccr7 chemokine receptor involvement in leukemic T cell dissemination

Ivette Pacheco-Leyva (i3S)

12:15 **The histone methyltransferase DOT1L in cytotoxic T cells: targeting epigenetic regulation at the core**

Fred van Leeuwen (NKI – Netherlands Cancer Institute)

12:45 Selected Oral Communication

SKOR1 mediates FER kinase-dependent invasive growth of breast cancer cells

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14:00 Poster Session

16:00 Coffee Break

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Porto.Comprehensive Cancer Centre

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Irene Braña (Vall d'Hebron University Hospital)

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3D heterotypic culture systems to study immune modulation and antibody response in HER2+ breast cancer microenvironment

Sofia Batalha (iBET/ITQB NOVA)

17:15 **Turning Sweet on Immuno-Oncology: glycans as key immune-checkpoints in cancer with clinical implications**

Salomé Pinho (i3S/FMUP/ICBAS)

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Patient-derived organoids recapitulate the gastric cancer glycosylation profile: a new model to identify novel cancer target therapies

Filipe Pinto (i3S)

18:00 ASPIC General Assembly and Elections

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The potential of liquid biopsy in the management of cancer patients

Evi Lianidou (University of Athens)

10:15 ASEICA Lecture

Breast cancer metastasis cell fate mapping

Roger Gomis (IRB Barcelona)

11:00 Coffee Break

Symposium III
Tumour Microenvironment

Chair : **Joana Paredes** (ASPIC/i3S) and **Ana Preto** (CBMA, U.Minho)

11:30 **The microbiota-immunity axis in gastrointestinal cancers**

Amedeo Amedei (University of Florence)

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Deciphering the impact of cancer cell´s secretome and its derived-peptides on breast cancer brain metastasis

Rita Carvalho (i3S/ICBAS)

12:15 **Neutrophil extracellular traps in the tumour microenvironment: exploring a possible mechanism of acquired immune escape**

Carlos Eduardo de Andrea (Universidade de Navarra) – Sponsored by ILC

12:45 Selected Oral Communication

Cadherin-3 is a novel oncogenic biomarker with prognostic value in glioblastoma

Eduarda P. Martins (ICVS/3B's)

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Chair: **Jorge Lima** (i3S/Ipatimup) and **Júlio Oliveira** (IPO-Porto)

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Marcel Kool (Hopp Children's Cancer Center Heidelberg & Princess Maxima, Center Pediatric Oncology Utrecht)

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Marília Silva (IBB/IST)

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Fernando López-Ríos (12 de Octubre University Hospital Spain) – Sponsored by Thermo Fisher Scientific

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Joana Paredes (ASPIC/i3S)

Abstracts

ASPIC Lecture

BRCA2-P study: National characterization of the Portuguese founder mutation of the BRCA2 gen

Authors and Affiliations

Fátima Vaz

Instituto Português de Oncologia de Francisco Gentil

Abstract

*BRCA2*_P is a multicenter study recruiting patients with a diagnosis of invasive Breast Cancer (BC) associated with the *BRCA2* variant c.156_157insAlu, a founder variant of Portuguese origin. Main objectives of this study are the analysis of the overall and cancer specific survival of *BRCA2* c.156_157insAlu (*BRCA2*_P)-associated breast cancer when compared to non-*BRCA* mutated breast cancer or unknown *BRCA*-status breast cancer, either for non-metastatic or metastatic breast cancer. Other objectives include the descriptive analysis of the demographic, clinical and pathological characteristics of *BRCA2*_P associated breast cancer, as well as the study of the prognostic impact of the *BRCA2*-P mutational status in terms of relapse-free survival and treatment response.

Protocol amendment history as well as results for the primary descriptive case analysis will be presented. Primary analysis included 201 pts from 222 patients' cases identified in 15 study centers across Continental Portugal and Madeira. These cases were all confirmed, as per protocol inclusion criteria, to be associated with the *BRCA* c.156_157insAlu between 2000 and 2021.

Curriculum Vitae

Senior Medical Oncologist with extensive clinical and research experience on Breast, Gynecological and Hereditary Cancer. Coordinator of the Breast, Ovarian and Prostate Hereditary Multidisciplinary Program of the Lisbon center of Instituto Português de Oncologia de Francisco Gentil, (IPOLFG,EPE), a member of the European Genturis network.

Invited Assistant Professor, Nova Medical School Regulatory activities: Member of the Portuguese Central Research Ethics Committee (CEIC) and past experience on request evaluation concerning reimbursement of Health Technologies.

Symposium I
Cancer Genomics and Epigenomics

Epigenetic contributions to Leukemia

Author and Affiliation

Christoph Plass

German Cancer Research Center, Heidelberg

Abstract

Loss of chromosome 7 (-7) or deletion of the long arm (del7q) are recurring chromosome abnormalities in myeloid leukemias. These aberrations are highly associated with MDS and AML (~10%). These chromosome aberrations are associated with a high risk of disease progression and inferior survival. The frequent occurrence of del7q suggests that the affected region(s) may harbour critical tumour suppressor gene(s) acting via dominant silencing mechanisms that remain elusive. Recently, *MLL5* has been proposed as a possible tumour suppressor in chromosomal region 7q22. However, there is data indicating that *MLL5* alone does not account for the properties of AML with del(7q). Mouse modelling promoted another candidate, MLL3, as a haploinsufficient tumor suppressor on 7q36, cooperating with other events (such as hyperactive RAS pathway, or p53 alterations) occurring in monosomy 7 or del(7q). Another candidate tumor suppressor gene represents EZH2 mutated in low frequencies in AML and MDS. EZH2 is a member of the PRC2 complex involved in establishing repressive the H3K27me3 mark. Here, I will describe a novel mechanism where deletions of chromosome 7q or other chromosomal rearrangements result in oncogene activation by juxtaposing hematopoetic enhancers into the vicinity of *Homo sapiens motor neuron and pancreas homeobox 1 (MNX1)* leading to aberrant activation of this homeobox transcription factor by changing gene activation through promoter-enhancer interaction, which is regulated within structural domains of megabase scale, so-called "topologically associating domains" (TADs).

Curriculum Vitae

Prof. Dr. Christoph Plass

Academic education/degrees

1988-1993	PhD study at Institut für Biologie der Medizinischen Universität, Lübeck
1982-1987	Study of Biology at the Freie Universität Berlin

Scientific degrees

1993	Dr rer. nat. (PhD) Universität Lübeck (Prof. Dr. Heinz Winking)
1987	Diploma in Biology, Freie Universität Berlin

Professional career

2007 German Cancer Research Center (DKFZ), Heidelberg, Department of Epigenomics and Cancer Risk Factors, Professor

2005-2007 The Ohio State University, Columbus, USA, Department of Medical Microbiology and Immunology, Division of Human Cancer Genetics, Professor

2002-2005 The Ohio State University, Columbus, USA Department of Medical Microbiology and Immunology, Division of Human Cancer Genetics, Associate Professor

1997-2002 The Ohio State University, Columbus, USA, Department of Medical Microbiology and Immunology, Division of Human Cancer Genetics, Assistant Professor

1996-1997 Roswell Park Cancer Institute, Buffalo, NY, Molecular and Cellular Biology Department, Cancer Research Scientist II

1993-1996 Roswell Park Cancer Institute, Buffalo, NY, Laboratory of Dr Verne Chapman, Molecular and Cellular Biology Department, Postdoc

Honors and other activities

2016 Taiwan Tsungming Tu Award

2007 Stohlman Scholar, Leukemia Lymphoma Society of America

2006 Barbara J. Bonner Chair in Lung Cancer

2005 Elected Fellow, American Association for the Advancement of Science (AAAS)

2003 Honorary Faculty of the Mirrors Honors Society

2002-2007 Leukemia Lymphoma Society of America Scholar

2002-2005 V-Foundation Translational Award

Editor in Chief

International Journal of Cancer

Selected oral communication

T-cell receptor and Ccr7 chemokine receptor involvement in leukemic T cell dissemination

Authors and Affiliations

Ivette Pacheco-Leyva[1,2], Marina Baessa[1,2], Telma Costa[1,2], Telmo Catarino[1,2], Nuno R. dos Santos[1,2]

1. i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto

2. IPATIMUP – Instituto de Patologia e Imunologia Molecular da Universidade do Porto

Presenting Author

Ivette Pacheco-Leyva

Abstract

T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL) involves the bone marrow, blood, and lymphoid organs, but can disseminate to other organs, aggravating disease. It was previously reported that absence of T-cell receptor (TCR) expression in T-ALL/LBL mouse models led to reduced dissemination of leukemic cells to peripheral lymphoid organs, most notably lymph nodes. We aimed to understand which molecules could be involved in this TCR-dependent property.

Gene mRNA expression, flow cytometry immunofluorescence analysis were performed from leukemic cells from the ETV6-JAK2 fusion transgenic mice (EJ-Tg). EJ-Tg mice were bred with Rag2, Ccr7 and Nfkb2 knockout (KO) mice; and with TCR-HY transgenic mice. Leukemic cells were infused intravenously in recipient mice.

Infused EJ-Tg mouse leukemic T cells from mice lacking TCR expression (i.e., EJ-Tg;Rag2-/-) colonized much less efficiently the lymph nodes and spleens of recipient mice than EJ-Tg leukemic cells expressing endogenous or transgenic TCR. Interestingly EJ-Tg;Rag2-/-leukemic cells expressed reduced levels of the Ccr7 chemokine receptor, a T-cell migration mediator involved in both thymic egress and peripheral lymphoid organ homing. By stimulating human T-ALL cell lines with CD3 antibody or the PMA phorbol esters, we confirmed CCR7 expression was induced by TCR signaling. To study the role of CCR7 in vivo, we bred EJ-Tg mice with Ccr7 KO mice. Similarly to EJ-Tg mice lacking TCR (i.e. Tcra-/- or Rag2-/-), EJ-Tg;Ccr7-/- mice presented significantly larger thymic lymphomas and reduced splenic and lymph nodal involvement than Ccr7-sufficient littermates. By breeding EJ-Tg mice with TCR-HY transgene and Ccr7 KO, we observed that although the TCR transgene favored disease dissemination from the thymus to spleen and lymph nodes, the absence of CCR7 had a negative impact. To verify whether Ccr7 was involved in homing to lymphoid organs, Ccr7-expressing EJ-Tg leukemic cells were infused in mice KO for the Nfkb2 gene, which express reduced levels of Ccl19 and Ccl21 Ccr7 ligands in the lymph nodes, or control littermates. Infused leukemic cells colonized less efficiently the lymph nodes of Nfkb2-deficient mice, with no differences in the spleen and liver.

We conclude that TCR signaling is associated with expression of proteins associated with leukemic dissemination to specific niches and that CCR7 is a potential mediator of that property.

The histone methyltransferase DOT1L in cytotoxic T cells: targeting epigenetic regulation at the core

Authors and Affiliations

Muddassir Malik[1,*], Eliza-Mari Kwesi-Maliepaard[1,*], Muhammad Aslam[2], Willem-Jan de Leeuw[1], Teun van den Brand[1], Elzo de Wit[1], Heinz Jacobs[2,#], and **Fred van Leeuwen[1,#]**

1. Netherlands Cancer Institute, Division of Gene Regulation, Amsterdam.

2. Netherlands Cancer Institute, Division of Tumor Biology and Immunology, Amsterdam.

Abstract

T cell differentiation is determined by key transcription factors and guided by epigenetic mechanisms. The toolbox for manipulating epigenetic regulators is rapidly expanding, offering new opportunities to influence T-cell fate and function in the context of cancer immunotherapy. However, the role of epigenetic mechanisms in T cell differentiation is still poorly understood. We recently uncovered a central role for the histone methyltransferase DOT1L in CD8+ (cytotoxic) T cells (CTLs) and thymic lymphoma. DOT1L methylates or 'writes' histone H3K79 (H3K79me) in gene bodies of actively transcribed genes. In a conditional mouse model, loss of DOT1L in the T cell lineage resulted in accelerated, antigen-independent differentiation towards memory-phenotype T cells. This suggest that H3K79 methylation by DOT1L imposes a key epigenetic barrier towards CD8 T cell differentiation, essential in maintaining T cell naivety. At a mechanistic level, our studies in T and B cells indicate that DOT1L indirectly supports the repression of targets of EZH2-containing PRC2 repressor complexes, leading to extensive indirect epigenetic changes that extended beyond H3K79 methylation. Furthermore, we observed a role for DOT1L in T-cell receptor (TCR) signaling: T cells lacking DOT1L displayed altered expression of many genes involved in TCR signaling and showed reduced expression of TCR components and the CD8 co-receptor. Genes controlling TCR surface expression and signaling appeared as direct targets of DOT1L, indicating that DOT1L regulates T cell signaling at multiple levels. We will discuss our recent studies on the role of DOT1L in the execution of cytotoxic T cell responses.

Selected oral communication

SKOR1 mediates FER kinase-dependent invasive growth of breast cancer cells

Authors and Affiliations

Lilian Sluimer[1], Esme Bullock[2], Max Rätze[1], Lotte Enserink[1], Marten Hornsveld[3], Valerie G. Brunton[2], Patrick W.B. Derksen[1,#], Sandra Tavares[1,4,5#]

1. Department of Pathology, University Medical Center Utrecht, Utrecht, The Netherlands.
2. Edinburgh Cancer Research UK Centre, University of Edinburgh, Edinburgh, Scotland, UK
3. Department of Molecular Cell Biology, Cancer Genomics Centre Netherlands and Centre for Biomedical Genetics, Leiden University Medical Center, Leiden, The Netherlands.
4. IPATIMUP - Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Porto Portugal
5. i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal.

Presenting Author

Sandra Tavares

Abstract

Introduction: To this day, targeted treatment options for patients with triple negative metastatic breast cancer (TNBC) are still virtually absent. Previously, we showed that High expression of the tyrosine kinase FER is an independent prognostic factor that correlates with poor survival in high-grade and basal/TNBC patients.

Methods: To investigate whether the kinase activity is essential for FER oncogenic properties, we developed an ATP analogue-sensitive knock-in allele (FERASKI). Using the FERASKI system, we identified a direct FER kinase substrate.

Finally, we studied the role of FER and of one of the candidates in malignant transformation, using imaging, biochemical approaches and functional assays.

Results: Here, we show that specific FER kinase inhibition in MDA-MB-231 cells reduces migration, invasion, and metastasis in a mouse model of breast cancer. Using the FERASKI system, we identified SKI family transcriptional corepressor 1 (SKOR1) as a direct FER kinase substrate. SKOR1 loss phenocopies FER inhibition, leading to impaired proliferation, migration and invasion, and inhibition of breast cancer growth and metastasis formation in mice. We show that the candidate FER phosphorylation residue, SKOR1-Y234, is essential for FER-dependent tumor progression features. Finally, our work suggests that the SKOR1-Y234 residue promotes Smad2/3 signaling through SKOR1 binding to Smad3 attenuation.

Conclusions: Our study thus identifies SKOR1 as a mediator of FER-dependent breast cancer progression, advocating FER kinase inhibition as a candidate strategy to treat high-grade breast cancers.

Symposium II
Porto.Comprehensive Cancer Centre

Epigenetic modulators in solid tumors: drug development guided by clinical and translational research

Author and Affiliation

Irene Brana

Vall d'Hebron Institute of Oncology

Abstract

The new generation of histone modifying agents were initially developed on specific tumor types based on the initial preclinical work with these compounds. The integration new knowledge has identified potential opportunities for personalized medicine, or for synthetic lethality. We will review the development of EZH2 inhibitors in tumors with alterations in theSWF/SNF complex and the development of BET inhibitors in NUT midline carcinoma, salivary gland tumors and central nervous system tumors. The talk will also include the translational research opportunities that these clinical trials and compounds are bringing to the research arena.

Curriculum Vitae

Irene Brana, MD PhD, is a Clinical Investigator at the Phase I Unit at Vall d'Hebron Institute of Oncology and leads the Clinical Research in the Ear-Nose-Throat (ENT) Unit in the Department of Medical Oncology at the same Institution.

She graduates in the University of Oviedo Medical School (Spain) in 2005. In 2010, she completed a four-year specialization in Medical Oncology at Vall d'Hebron University Hospital, a leading Medical Oncology Department in Spain. She performed a one year-fellowship in Drug Development in Vall d'Hebron University Hospital and a three-year fellowship in Drug Development and ENT malignancies at the Princess Margaret Cancer Centre from 2011 to 2014.

She obtained a Master Degree in Molecular Oncology at the Spanish National Cancer Research Center (CNIO) in Madrid in 2012. In 2017, obtained her PhD with honors with her work on anticancer agent combination.

Since November 2015, she works as at the Vall d'Hebron Institute of Oncology at the Vall d'Hebron University Hospital. She is a Clinical Investigator in the Phase I Unit and in the ENT Unit. She has actively worked in the early development of new drugs in solid tumors, and in the early and late development of novel therapeutics in Head and Neck Malignancies. As part of her work in the phase I Units, she has an expertise on epigenetic drugs, novel targeted therapies and novel clinical trial designs including platform, basket and umbrella studies.

Selected oral communication

3D heterotypic culture systems to study immune modulation and antibody response in HER2+ breast cancer microenvironment

Authors and Affiliations

Sofia Batalha[1,2], Giacomo Domenici[1,2], Nuno F. Lopes[1,2], Catarina Brito[1,2]

1. iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal

2. Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal.

Presenting Author

Sofia Batalha

Abstract

Introduction: The poorer prognosis typical of HER2-overexpressing (HER2+) subtype of breast cancer (BC) is known to be influenced by the immune infiltrate, namely monocytic cells (promoters of pro-tumoral immunosuppression) and NK cells, whose basal cytotoxic function may be boosted with therapeutic antibodies. The anti-HER2 antibody trastuzumab is the standard-of-care, however most patients eventually relapse due to therapy resistance. A dual blockade approach with trastuzumab and pertuzumab brought some clinical improvement, but further therapy refinement is still hampered by the fact that the immune mechanism of action of this antibody-based dual HER2 blockade remains largely understudied.

Materials and Methods: To explore how the dual antibody challenge influences the phenotype and function of immune cells infiltrating HER2+ BC, we developed a 3D in vitro heterotypic cellular model. This comprised HER2+ BC cell lines aggregates and primary immune cells from healthy donors, encapsulated in an inert alginate hydrogel and maintained in an agitation-based culture system for up to 7 days.

Results: The model retained original BC molecular features and, after optimization of culture time and cytokine supplementation, it was possible to preserve the NK cell compartment. Challenge with trastuzumab and pertuzumab resulted in enhanced immune cytotoxicity compared with trastuzumab alone and led to induction of immune effector state, including reducing the proportion of the myeloid compartment and expression of immune marker CD16, increase of pro-inflammatory cytokines and downregulation of PD-L1.

Conclusions: This work presents a unique 3D in vitro model for the study of immune effects of anti-HER2 biologicals, which can be used to test novel therapy regimens and improve anti-tumor immune function.

Acknowledgements: FCT/MCTES for funding – iNOVA4Health (UIDB/04462/2020, UIDP/04462/2020), S.B. individual fellowships (PD/BD/135550/2018, COVID/BD/152532/2022); IPST for buffy coat samples.

Turning Sweet on Immuno-Oncology: glycans as key immune-checkpoints in cancer with clinical implications.

Author and Affiliation

Salomé S. Pinho

i3S, University of Porto, Portugal

Abstract

Cancer development is guided by the selective pressure of the immune system on cancer cells (cancer immunoediting) that leads to a progressive disease. These immune escape strategies include the expression of immune inhibitory checkpoints that associated with T cells dysfunction lead to malignant transformation. The contribution of the glycome (biological repertoire of glycans expressed in tumour cells) to cancer immunosurveillance and immunoediting is far from being clarified. Glycans are polysaccharides made by all organisms that covalently conjugate to other biomolecules, such as proteins or lipids. Glycans cover the cell surface of all human cells, being fundamental to define the identity of a cell/organism thereby contributing to discriminating self from non-self (*Alves and Fernandes et al. FEBS 2022*) Tumour growth is accompanied by dramatic changes in the cellular glycome that includes the aberrant expression of complex branched *N*-glycans structures, known to impose pro-malignant and pro-metastatic features to cancer cells, being also associated with poor prognosis and poor survival rates of cancer patients (*Pinho and Reis, Nature Reviews Cancer 2015*). Changes in the cellular glycome of cancer cells can be sensed and recognised by a variety of glycan-binding proteins (GBP) expressed in immune cells, instructing either a pro-inflammatory or anti-inflammatory immune response. We demonstrated that this pro-tumoral branched *N*-glycan structure has a fundamental role in cancer immunoediting and particularly in immune evasion. We revealed that the exposure of branched *N*-glycans is used by colorectal cancer (CRC) cells to escape immune recognition by specific GBP, leading to the creation of immunosuppressive networks through inhibition of IFNγ production. We further demonstrated that the removal of this "glycan-mask" expose immunogenic glycan epitopes that potentiates immune recognition by specific C-type lectin receptors expressed in immune cells resulting in an effective anti-tumour immune response (*Fernandes et al, Cancer Immunology Research 2020*). In summary, we have revealed a novel glycoimmune-checkpoint in cancer, highlighting the therapeutic efficacy of its deglycosylation as a way of potentiating immune recognition and improving cancer immunotherapy.

In fact, the effects of branched *N*-glycans in cancer development can be a "two-sword fencing". Besides their role in defining aggressiveness and metastatic features of epithelial cancer cells, we also have evidences showing that glycans regulate T cells function and activity (*Dias et al, PNAS 2018; Fernandes et al, Immunology 2022*). In fact, in cancer, although T cells recognize transformed and cancer cells, they are often rendered dysfunctional leading to an immunosuppressive microenvironment. Our results on colon-infiltrating T cells from colitis-associated CRC mouse model (AOM-DSS) and from cancer lesions from human Lynch syndrome showed an increased expression of branched *N*-glycans on inflammatory infiltrate associated with immunosuppressive features of infiltrating T cells.

Taken together, we showed that glycans encode fundamental roles in cancer immunosurveillance with potential clinical applications in improving the effectiveness of cancer immunotherapy.

Curriculum Vitae

Salomé Pinho received her D.V.M. from the University of Porto in 2004 and developed her PhD research in cancer glycobiology at the Institute of Molecular Pathology and Immunology of Univ. Porto (IPATIMUP) and at Boston Medical School, MA, USA from 2006 to 2009. She then performed her postdoctoral work at IPATIMUP exploring the glyco-Immunology field in the context of chronic gastrointestinal inflammatory disorders. At present, she is Principal Investigator at i3S-University of Porto and Professor at Faculty of Medicine at University of Porto. She coordinates a research group "*Immunology, Cancer & GlycoMedicine*" at i3S and her research activity is focused on the understanding of the role of glycans and glycosylation in the regulation of key proteins´functions involved in cancer, specifically in the scope of immune-oncology and in chronic inflammatory conditions, envisioning potential clinical applications. She is author of several publications in international peer-reviewed journals (as first and senior author) including Nature Reviews Cancer, Oncogene, PNAS, Gastroenterology, Cancer Immunology Research, among others. She supervises a research team composed by Post-Docs, PhD students, Master students and clinical investigators. She is currently the Principal Investigator of several national/international grants in the field of cancer glycobiology and glyco-immunology. She received many awards such as the "Young Investigator Award" from the European Association for Cancer Research (2012), the 2020 Glycobiology Significant Achievement Award by the American Society for Glycobiology (SfG) and recently the Pfizer Award for Clinical Research in 2021.

Selected oral communication

Patient-derived organoids recapitulate the gastric cancer glycosylation profile: a new model to identify novel cancer target therapies

Authors and Affiliations

Liliana Santos-Ferreira[a,b], Rita Barros[a,c,d], Fabiana Sousa[c,e], Frederica Casanova-Gonçalves[c,e], José Barbosa[c,e], Fátima Carneiro[a,c,d,e], Hugo Santos-Sousa[a,c,e], Celso A Reis[a,b,c,d], Filipe Pinto[a,b,d]

a. I3S-Institute for Research and Innovation in Health, University of Porto, Porto, Portugal;

b. School of Medicine and Biomedical Sciences (ICBAS), University of Porto, Porto, Portugal;

c. Faculty of Medicine of the University of Porto, Porto, Portugal;

d. PATIMUP-Institute of Molecular Pathology and Immunology, University of Porto, Porto, Portugal;

e. Centro Hospitalar Universitário de São João (CHUSJ), Porto, Portugal

Presenting Author

Filipe Pinto

Abstract

Patient-derived cancer organoids (PDO) are a promising tool for gastric cancer (GC) management. These 3D cultures recapitulate the architecture, genetic and phenotypic features of their tissue of origin. Although glycosylation is a well-established component associated with tumor aggressiveness, poor patient survival and therapy resistance, nothing is known about this post-translational process in PDOs. Therefore, we aim to validate organoids as avatars of the glycosylation profile of in vivo tissues and study their potential as predictors of patient drug response.

Healthy, normal adjacent and tumor gastric tissues from CHUSJ-Porto were used to establish PDOs. The glycophenotype of PDOs and respective in vivo tissues were assessed using a robust panel of lectins and antibodies by IHC. Drug response to chemotherapy (cisplatin and 5-FU) was evaluated using CellTiter-Glo® 3D cell viability assay. The predictive value of the glycogene signature associated with therapy resistance was validated in a cohort of stomach cancer patients from TCGA database.

Our results demonstrate that organoids recapitulate the glycosylation profile and heterogeneity of the matched tissues, validating PDOs as avatars of the in vivo glycosylation profile. Importantly, we found sialylated and fucosylated glycoepitopes specifically expressed in tumor when compared with normal organoids. Using in silico analysis we were able to identify a glycosignature of genes associated with resistance to therapy, which was validated in our organoid models. Moreover, we found that this signature is associated with the disease-free survival of GC patients.

This work points to organoids as a promising preclinical model to identify novel therapeutic strategies for resistant GC patients based on their glycosylation profile.

Plenary Lectures

EACR Lecture

The potential of liquid biopsy in the management of cancer patients

Authors and Affiliations

Evi Lianidou

National and Kapodistrian University of Athens, Greece

Abstract

Over the last decade, liquid biopsy has gained much attention as a powerful tool in personalized medicine, since it enables monitoring cancer evolution and follow-up of cancer patients in real time. Through minimally invasive procedures, liquid biopsy provides important information through the analysis of Circulating Tumor Cells (CTCs), and circulating tumour-derived material like circulating tumour DNA (ctDNA), circulating miRNAs (cfmiRNAs) and extracellular vehicles (EVs). CTCs and ctDNA analysis has already an important impact on the prognosis, detection of minimal residual disease (MRD), treatment selection and monitoring of cancer patients, while recent data show also its potential for early cancer diagnosis (Figure 1). Numerous clinical trials include now a liquid biopsy arm, and functional studies mainly based on CTC derived cell-lines and CTC derived explants (CDx) provide important insight on the metastatic process. The recent findings in the field of liquid biopsy and the benefits and main clinical applications of CTC and ctDNA analysis in solid tumors are summarized in this presentation.

Curriculum Vitae

Dr. Evi Lianidou is Professor of Analytical Chemistry and Clinical Chemistry at the Department of Chemistry, University of Athens, Greece. Dr Lianidou has established a Molecular Diagnostics Laboratory focused on Liquid Biopsy at the Department of Chemistry since 1998 (http://en.actc-lab.chem.uoa.gr/). Her lab is specializing in the Liquid Biopsy Analysis, Analysis of Circulating Tumor Cells (ACTC) and cell-free DNA, and has access to many patient samples through extensive clinical collaborations. Her main research interests are on the development and clinical evaluation of: a) single-plex and multiplex quantitative RT-qPCR assays for the detection and molecular characterization of CTCs, b) multiplex assays for gene expression in CTCs based on the liquid bead array (LUMINEX platform), c) DNA methylation assays in CTCs and ctDNA, d) highly sensitive assays for mutation analysis in CTCs and in ctDNA, and e) evaluation of circulating miRNAs as tumor biomarkers in plasma. Dr. Lianidou has 166 publications (https://www.ncbi.nlm.nih.gov/pubmed/?term=lianidou) and has organized six international meetings on liquid biopsy: a) the 7th International Symposium on Minimal Residual Disease in Athens, (http://ismrc2009.chem.uoa.gr), b) the 1st "Advances in Circulating Tumor Cells: From Basic Research to Clinical Practice" (www.actc2012.org), c) the 2nd ACTC meeting, (October 8th-11th, 2014), in Crete, Greece (www.actc2014.org), d) the 3rd ACTC meeting, (October 4th-7th, 2017), in Rhodes, Greece (www.actc2017.org), the 4th ACTC meeting in Corfu, Greece (https://actc2019.org), and the 5th ACTC meeting, Kalamata, Greece, (www.actc2021.org). Prof Lianidou is PI in the European TRANSCAN group "PROLIPSY" and took part in the EU IMI Network Project "CANCER-ID" (www.cancer-id.eu). Dr Lianidou is an elected Board member of the European Association of Cancer Research (EACR), a member of the European Liquid Biopsy

Society (ELBS), and serves on the Editorial Boards of many international journals. Dr. Lianidou served International Federation of Clinical Chemistry (IFCC) as an elected member and Chair of the Committee for Clinical Molecular Biology Curriculum (2014-2019, (http://www.ifcc.org/ifcc-education-division/emd-committees/c-cmbc/) and since 2020 is an elected member of the IFCC Committee on Task Force on Global Lab Quality (TF-GLQ), (https://www.ifcc.org/executive-board-and-council/eb-task-forces/task-force-on-global-lab-quality/).

ASEICA Lecture

ER+ breast cancer metastasis cell fate mapping

Author and Affiliations

Roger R. Gomis PhD

ICREA Research Professor

IRB Barcelona

Abstract

Breast cancers (BCas) are deadly due to their capacity to adapt and thus relapse or resist therapy. BCa metastasize primarily to bone (clinically manageable), but patients ultimately succumb to multi-organ metastases. Recent genomic analyses suggest that most deadly metastases are seeded from secondary bone lesions (metastasis-to-metastasis) rather than from primary tumors[1–3]. Dynamic adaptation to changing environments during the metastatic process requires more rapid change than supported by purely genetic mechanisms[2]. We hypothesize that BCa cell plasticity, imparted by transcriptional and epigenetic adaptive mechanisms, facilitates primary bone metastasis, secondary metastasis to metastasis, and drug resistance, thus resulting in poor patient survival.

Bone metastasis is driven by the interaction between cancer and stroma cells, mainly osteoblasts and osteoclasts, which collectively generate what is defined as an osteolytic vicious cycle. As a consequence of bone resorption, bone matrix growth factors become available and further stimulate metastasis growth. This led to the development of bone modifying agents to manage skeletal related events (i.e. bisphosphonates that inhibit osteoclast activity). MAF gene amplification (16q23), occurring in 20% of BCa, has been clinically validated in two phase III trials to be associated with higher likelihood to develop metastasis. Further, MAF amplification as a biomarker explained for the first time the association between using bone-modifying agents in the adjuvant setting and improved patient outcome, but also the striking harmful effects of bisphosphonates in MAF-amplified particularly in young patients with high systemic E2. These observations suggest that ER signaling may also co-opt metastatic responses to drive dissemination. Given that MAF-amplification is associated with relapse and poor overall survival we hypothesize that uncharacterized epigenetic mechanisms underlie the interplay between ER predisposing signaling and metastasis pathogenesis. We show that metastatic drivers' modification of signal-dependent transcription factors (TFs) may provide cancer cell-type-specific enhancer landscapes, with important implications for understanding the pathology as well as treatment outcome.

Curriculum Vitae

From my PhD at UB and Postdoctoral at MSKCC, I developed an interest in the molecular, metabolic, and genetic mechanisms of metastasis. Since 2010, my laboratory is part of the Cancer Science Program at IRB Barcelona. It seeks to improve the prognosis, prevention, and treatment of cancer by studying the basic principles underlying the development of this disease. We have focused on identifying and functionally validating genes that enable breast and colon cancers to metastasize to clinically relevant sites. In particular, during the last five years, we strived to unravel the tissue-specific mediators and time-dependent components of metastasis processes (Morales et al. **EMBO Mol Med** 2014; Urosevic et al. **Nature Cell Biol** 2014 and **Cancer Res.** 2020; Cejalvo et al. **Cancer Research** 2017).

Remarkably, our findings have the potential to become an objective approach to selection of breast cancer patients for adjuvant bisphosphonate treatment to prevent metastasis (Coleman et al. Lancet Oncol. 2017 and Paterson et al, JNCIcs 2021). In addition, we have provided insights into how breast cancer metastatic epithelial cells remain latent, by controlling luminal differentiation attributes through epigenetic marks, which limits their initiation and expansion at the metastatic site (Gawrzak et al **Nat. Cell Biol.** 2018). Finally, we have significantly contributed to unravelling the metabolic rewiring process required for tumor and metastasis growth (Slebe et al **Nat. Comms**. 2016)

This strong track record of research excellence has provided the laboratory with: *i)* technical competence, background, and expertise in breast and colon cancers and mechanisms of metastasis. This knowledge has been maintained in the laboratory from one "generation" of scientists to the next through training programs as well as by permanent staff; and *ii*) the ability to attract top PhD students and postdoctoral scientists. All of these criteria are fundamental for achieving this ambitious proposal. The Gomis laboratory also participates in numerous (national and international) collaborations, which has allowed us to expand our studies on metastasis beyond breast and colon cancer (PubMed: Gomis, RR).

Symposium III
Tumour Microenvironment

The microbiota-immunity axis in gastrointestinal cancers

Author and Affiliations

Prof. Amedeo Amedei

Department of Experimental and Clinical Medicine

University of Florence (Italy)

Abstract

The human body, including the gut, skin and other mucosal environments, is colonized by a tremendous number of microorganisms (including bacteria, fungi, viruses, parasites) collectively termed microbiome

The microbiota role is crucial for different vital human process such as intestinal mobility, vitamin synthesis or metabolism of dietary components, but notably the microbiota can i) support an appropriate development of immunity and ii) modulate the immune (innate /adaptative) responses.

But, changes in the composition and function of gut microbiota (dysbiosis) can be responsible for the development of various diseases, locally and systemically, including obesity, autoimmune diseases and cancer.

In detail, commensal and pathogenic microorganisms can affect the tumorigenesis by modulating the "cancer hallmarks", including the tumor promoting inflammation and the immune surveillance that are the focus of my research.

Our experimental approach to define the microbiota-immunity axis consists in: i) cellular and molecular characterization of immunity and ii) comparative evaluation of microbiota composition and correlation with immune response.

The colorectal cancer (CRC) is considered the best example of a chronic inflammation–associated tumor (occurring often in patients with inflammatory bowel diseases) and various studies suggest that the breakdown of the intestinal microbiota structure promotes its carcinogenesis.

In a first study (DOI: 10.3389/fimmu.2017.01900), we have we found that the tumor tissue was infiltrated by a large amount of "not effector" T (neT) cells with a regulatory or an anergic profile, which are unable to kill cancer cells, may be contributing to the CRC promotion. The presence of neT cells was investigated also in the peripheral blood of CRC patients, demonstrating that the peripheral T regulatory cells can inhibit the proliferation of effector T cells, confirming their immunosuppressive properties.

Subsequently (DOI: 10.3389/fmicb.2017.02699), we have compared the human microbiota from three different compartments, i.e., saliva, feces, and cancer tissue (CT), of a selected cohort of Italian patients with colorectal cancer (CRC) vs. healthy controls (saliva and feces). The results highlighted the presence of different bacterial compositions; in particular, the fecal samples of CRC patients seemed to be enriched with Bacteroidetes, whereas in the fecal samples of healthy controls Firmicutes were one of the major phyla detected though these differences were not statistically significant. The CT samples showed the highest alpha diversity values. Finally, we investigated the microbiota-immunity axis in healthy and tumor mucosa of CRC patients, focusing on the correlation between cytokine profile and microbiota signature (DOI: 10.3389/fimmu.2020.573158). CRC samples

displayed higher percentages of Th17, Th2, and Tregs. Moreover, CRC tissues showed significantly higher levels of MIP-1α, IL-1α, IL-1β, IL-2, IP-10, IL-6, IL-8, IL-17A, IFN-γ, TNF-α, MCP-1, P-selectin, and IL-9. Compared to CRC-S, CRC samples also showed significantly higher levels of the following genera: Fusobacteria, Proteobacteria, Fusobacterium, Ruminococcus2, and Ruminococcus. Finally, the abundance of Prevotella spp. in CRC samples negatively correlated with IL-17A and positively with IL-9. On the contrary, Bacteroides spp. presence negatively correlated with IL-9.

In addition, we evaluated the type of gastric T cell response elicited by the secreted peptidyl prolyl cis, trans-isomerase of H. pylori (HP0175) in patients with distal gastric adenocarcinoma (DOI: 10.1007/s11739-012-0867-9). The TILs (tumor-infiltrating lymphocytes) from H. pylori infected patients with distal gastric adenocarcinoma produced IL-17 and IL-21 in response to HP0175. HP0175-specific TILs showed poor cytolytic activity while expressing helper activity for monocyte MMP-2, MMP-9 and VEGF production. These findings suggest that the HP0175, by promoting pro-inflammatory low cytotoxic TIL response, matrix degradation and pro-angiogenic pathways, may provide a link between H. pylori and gastric cancer.

In conclusion, increasing studies support the centrality of the microbiota in human "health" and pathological conditions; but the complex relationships between the microbiota and human beings, requires the need to adopt new concepts and new perspectives in order to be properly analysed and utilized it, especially for their therapeutic implementation (DOI: 10.3390/ijms19123756).

Curriculum Vitae

Prof. Amedei graduated with honours in biology from the University of Florence. In 2003, he undertook his doctoral degree in the field of clinical and experimental medicine, studying the role of the *Helicobacter pylori*-specific immune response in gastric diseases. In 2005, he joined the Department of Experimental and Clinical Medicine (University of Florence), where he was appointed an associate professor in 2015. Recently, he has focused his scientific interests on cancer immunology and the role of the microbiota-immunity axis in inflammatory-correlated diseases. Prof. Amedei has published over 185 peer reviewed articles (h-index: 50; 8,351 citations), 9 book chapters, and one patent. He serves as an editorial board member for 13 international journals, as a reviewer on 43 journals, and as co-editor-in-chief of one journal. He also carries out scientific review activities on international research projects for private and public entities. Since 2016, he has been a member of the scientific council of the non-profit organization Toscana Life Sciences.

Selected oral communication

Deciphering the impact of cancer cell´s secretome and its derived-peptides on breast cancer brain metastasis

Authors and Affiliations

Carvalho R[1,2,7], Santos L[3,4,5], Conde I[1,2,6], Leitão R[3,4,5], Gomes C[3,4,5], Silva A[3,4,5], Schmitt FC[1,2], Paredes J[1,2,6], Ribeiro AS[1,2]

1. i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal.

2. IPATIMUP – Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Portugal.

3. Instituto de Farmacologia e Terapêutica Experimental, Faculdade de Medicina, Universidade de Coimbra, Portugal.

4. Instituto de Investigação Clínica e Biomédica (iCBR), Faculdade de Medicina, Universidade de Coimbra, Portugal.

5. CNC. IBILI Consórcio, Universidade de Coimbra, Portugal.

6. Faculdade de Medicina da Universidade do Porto (FMUP), Portugal.

7. ICBAS- Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto, Portugal.

Presenting Author

Rita Carvalho

Abstract

Introduction: Brain metastases present the poorest survival rates, lack efficient therapies, and remain the major clinical problem in breast cancer surveillance. Thus, we aim to dissect the initial steps of the brain metastatic process by identifying a brain cancer cell secretome signature, since it has been seen as a promising source for the discovery of new biomarkers involved in metastatic progression.

Materials and methods: Breast cancer cells 231, and their brain, bone, and lung organotropic variants were used. Their secretome was collected from collagen embedded 3D-spheroids cultures and high throughput proteomic Label-Free Quantitation analysis was performed in order to identify brain enriched metastatic signature. Endothelial and microglia cells were incubated with the secretomes and/or its derived peptides, and in vitro/in vivo blood-brain-barrier (BBB) integrity and microglia activation were evaluated. VGF mRNA and protein expression was analyzed in the TCGA database and in a series of primary breast tumors and breast cancer metastasis.

Results: We found 25 peptides specifically deregulated in the secretome of brain tropic variants. Importantly, their secretome caused significant disruption of BBB, whereas all organotropic secretomes promoted microglia activation. We identified VGF as a brain-specific peptide, promoting BBB dysfunction similar to the secretome of brain tropic cells. In contrast, only a slight increase in microglia phagocytosis and pStat3 expression was observed upon VGF treatment. Importantly, VGF expression is associated with HER2 overexpressing and basal-like tumors, the molecular subtypes of breast cancer that metastasize more frequently to the brain. Furthermore, we observed that approximately 90% of primary tumors that metastasize to the brain show VGF expression.

Conclusion: In conclusion, our data show a specific breast cancer brain metastatic signature. So far, we identified VGF as a key mediator in this process, being associated with a poor prognosis for breast cancer patients.

Neutrophil extracellular traps in the tumour microenvironment: exploring a possible mechanism of acquired immune escape.

Author and Affiliation

Carlos Eduardo de Andrea

Leiden University Medical Center, The Netherlands

Abstract

Neutrophil extracellular traps (NETs) are webs of extracellular nuclear DNA extruded by dying neutrophils infiltrating tissue. NETs constitute a defence mechanism to entrap and kill fungi and bacteria. Tumours induce the formation of NETs to the advantage of the malignancy via a variety of mechanisms shown in mouse models. Here, we investigated the presence of NETs in a variety of human solid tumours and their association with IL-8 (CXCL8) protein expression and CD8+ T-cell density in the tumour microenvironment. Multiplex immunofluorescence panels were developed to identify NETs in human cancer tissues by co-staining with the granulocyte marker CD15, the neutrophil marker myeloperoxidase, and citrullinated histone H3 (H3Cit), as well as IL-8 protein and CD8+ T cells. Three ELISA methods to detect and quantify circulating NETs in serum were optimized and utilized. Whole tumour sections and tissue microarrays from patients with non-small cell lung cancer (NSCLC) (n=14), bladder cancer (n=14), melanoma (n=11), breast cancer (n=31), colorectal cancer (n=20), and mesothelioma (n=61) were studied. Also, serum samples collected retrospectively from patients with metastatic melanoma (n=12) and NSCLC (n=34) were ELISA-assayed to quantify circulating NETs and IL-8. NETs were detected in six different human cancer types with wide individual variation in terms of tissue density and distribution. At least in NSCLC, bladder cancer, and metastatic melanoma, NET density positively correlated with IL-8 protein expression and inversely correlated with CD8+ T-cell densities. In a series of serum samples from melanoma and NSCLC patients, a positive correlation between circulating NETs and IL-8 was found. In conclusion, NETs are detectable in formalin-fixed human biopsy samples from solid tumours and in the circulation of cancer patients with a considerable degree of individual variation. NETs show a positive association with IL-8, and a trend towards a negative association with CD8+ tumour-infiltrating lymphocytes.

Curriculum Vitae

Carlos E de Andrea is a pathologist with a PhD from Leiden University Medical Center (Leiden, the Netherlands) and a Molecular Genetics fellowship from the Royal National Orthopaedic Hospital and University College of London (London, UK). Since July 2015, Dr de Andrea I have been working at Department of Pathology at University of Navarra (Pamplona, Spain) developing platforms to study molecular biomarkers for prediction of response or resistance to immunotherapy and mechanisms of immune evasion.

Selected oral communication

Cadherin-3 is a novel oncogenic biomarker with prognostic value in glioblastoma

Authors and Affiliations

Eduarda P. Martins[1,2]; Céline S. Gonçalves[1,2]; Marta Pojo[1,2]; Rita Carvalho[3]; Ana Sofia Ribeiro[3]; Vera Miranda-Gonçalves[1,2]; Ricardo Taipa[4]; Fernando Pardal[5]; Afonso A. Pinto[6]; Carlos Custódia[7]; Cláudia C. Faria[7,8]; Fátima Baltazar[1,2]; Nuno Sousa[1,2]; Joana Paredes[3,9]; Bruno M. Costa[1,2]

1. Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal
2. ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal
3. i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal
4. Neuropathology Unit, Department of Neurosciences, Centro Hospitalar do Porto, Porto, Portugal;
5. Department of Pathology, Hospital de Braga, Braga, Portugal;
6. Department of Neurosurgery, Hospital de Braga, Braga, Portugal;
7. Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal;
8. Neurosurgery Department, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte (CHLN), Lisbon, Portugal;
9. Faculty of Medicine of the University of Porto, Portugal

Presenting Author

Eduarda P. Martins

Abstract

Introduction: Glioblastoma (GBM) is the most common primary malignant brain tumor. Despite the growing knowledge of the molecular bases of GBM, this understanding is still not sufficiently reflected in patients' clinical outcome, who still present a median overall survival of approximately 15 months. Cadherin-3, widely known as P-Cadherin, is an adhesion molecule encoded by CDH3 gene, known to impact tumoral behavior in different types of cancer. Nevertheless, its relevance in GBM was unknown.

Materials and Methods: P-Cadherin mRNA and protein levels were assessed in glioma samples. Overexpression and silencing models in one commercially available GBM cell line and in two patient-derived cultures, respectively, were established to functionally characterize the effects of P-Cadherin in vitro and in vivo. Molecular signatures associated with CDH3 were evaluated through enrichment and correlations analyses. The significance of CDH3 expression in GBM patients' survival was determined in independent cohorts, including The Cancer Genome Atlas (TCGA) and our cohort with two Portuguese hospitals.

Results: Functional assays show P-Cadherin impacts critical cancer hallmarks in GBM cells, such as cell viability, invasion, migration, stemness, and cell cycle progression. In vivo, P-cadherin-high cells associate with shorter survival of mice when orthotopically injected in the brain, and with larger tumor growth in subcutaneous models. In the clinical settings, a subset of high-grade gliomas overexpresses P-Cadherin at mRNA and protein levels. Mechanistically, genes correlated with CDH3 are enriched for cancer-related genes in GBM patients' samples and in our CDH3-genetically manipulated cell models. Importantly, CDH3 high expression in GBM is associated with shorter overall survival of patients.

Conclusions: These results show that Cadherin-3 is a new oncogenic molecule in GBM, presenting clinically-relevant prognostic value in patients.

Financial support was provided by FCT and Fundação Calouste Gulbenkian.

Symposium IV
Translational Cancer Research

Pediatric brain tumors: how to translate all the molecular data into successful treatments for patients?

Author and Affiliations

Marcel Kool

Hopp Children's Cancer (KiTZ), Heidelberg, Germany

German Cancer Research Center (DKFZ) and German Cancer Consortium (DKTK), Heidelberg, Germany

Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands

Abstract

Thanks to state-of-the-art molecular profiling techniques we by now have a much better understanding of pediatric cancers and what is driving them. On the other hand, we have also realized that pediatric cancers are much more heterogeneous than previously thought. Many new types and subtypes of pediatric cancers have been identified with distinct molecular and clinical characteristics. Also for brain tumors, molecular studies have shown that many genetically and clinically distinct types exist that would not always have been recognized as distinct by histology alone. An important outcome of these studies is the incorporation of molecular data and tools in the recent 5th edition of WHO classification of CNS tumors. However, for many if not most of these new types and subtypes there is no specific treatment available and the outcome of many brain tumor types is still relatively poor. There is thus an urgent need to develop more effective and more specific treatments that are also less damaging to the normal developing brain of a child. In order to develop specific treatment protocols and to increase survival rates for pediatric cancer patients further, both at diagnosis and relapse/metastasis, we need a large collection of well-characterized preclinical models representing all the different types and subtypes. These models can be used for preclinical drug testing to prioritize the pediatric development of anticancer drugs that would be best targeting pediatric tumor biology. The ITCC-P4 consortium, which is a collaboration between many academic centers across Europe, several companies involved in *in vivo* preclinical testing, and ten pharmaceutical companies, started in 2017 with the overall aim to establish a sustainable platform of >400 molecularly well-characterized PDX models of high-risk pediatric cancers and to use them for *in vivo* testing of novel mechanism-of-action based treatments. Currently, 340 models have been fully established, including 87 brain tumor models and 253 non-brain tumor models, together representing many different tumor types both from primary and relapsed/metastatic disease. Out of these 340 models, 252 have been fully molecularly characterized, most of them together with their matching original tumors, and almost of all these models are currently being subjected to *in vivo* testing using three standard of care drugs and six novel mechanism-of-action based drugs. In this presentation, an update on the current status of the ITCC-P4 platform and the data we collectively have generated thus far will be presented.

Curriculum Vitae

Marcel Kool, Ph.D., is a cancer biologist at the Hopp Children's Cancer Center Heidelberg (KiTZ), the German Cancer Research Center (DKFZ) in Heidelberg, Germany, and the Princess Máxima Center (PMC) for Pediatric Oncology in Utrecht, the Netherlands. He is deputy of the division of Pediatric Neurooncology headed by Prof. Dr. Stefan M. Pfister and group leader of the Preclinical Research Group in this division at the KiTZ / DKFZ. Since April 2011 he is working at the DKFZ in Heidelberg and since September 2019 he started his second research group on pediatric brain tumors at the Máxima in Utrecht.

Since 2020 he is also a member of the research management board at the Máxima. Marcel Kool's expertise is the genomics and epigenomics of pediatric brain tumors. He and his teams in Heidelberg and Utrecht aim to (1) characterize each brain tumor entity in full detail at the genomic and epigenomic level in order to identify clinically relevant subgroups; (2) to find the oncogenic driving events in these tumors and the best therapeutic targets; (3) to find diagnostic, prognostic and/or predictive biomarkers for these tumors and their subgroups for use in clinical settings; (4) to build a large repertoire of molecularly characterized tumor models (PDX and organoid models) representing all the different molecular subtypes of pediatric brain tumors and use them for preclinical studies in order to translate the genomic findings into new therapeutic options. Overall, Marcel Kool has co-authored >300 publications of which >150 only in the last five years. Several of these recent papers are landmark papers in the field of pediatric neurooncology describing the identification of new molecular entities and/or molecular subtypes of known entities with their respective oncogenic drivers and mutational landscapes.

Selected oral communication

Genetic engineering of Mesenchymal Stromal Cells to express anti-cancer proteins

Authors and Affiliations

Silva, M.[1], Monteiro, G.A.[1], Bekman, E.[1,2], Fialho, A.M.[1], da Silva, C.L[1], Bernardes, N.[1]

1. Department of Bioengineering and IBB – Institute of Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal
2. The Discoveries Centre for Regenerative and Precision Medicine, Lisbon Campus, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

Presenting Author

Marília Silva

Abstract

Introduction: Due to the lack of specificity of conventional anti-cancer treatments, the development of new tumor-targeted strategies has been a critical focus of cancer research. Novel approaches focus on selective tumor targeting and recently, customized cell-based therapies have been explored as living tools for the delivery of anti-tumor therapeutics. In this perspective, mesenchymal stromal cells (MSC) hold a promising future, due to their innate tumor-specific migratory potential.

Materials and Methods: Here, nonviral, xeno-free, strategies for the engineering of MSC towards the secretion of the potent anti-tumoral protein azurin (hazu) were developed. In a first line of research, a transient gene delivery method was developed as a proof-of-concept to attest the biomedical potential of hazu-expressing MSC's conditioned media (CM) in anti-cancer treatment. In a second line of research, the establishment of a stable transgene-expressing MSC line was developed, through the CRISPR/Cas9 system.

Results: Engineered hazu-MSC were shown to preserve tumor tropism toward breast and lung cancer cell lines, comparable to non-modified MSC. Moreover, hazu was detected in the CM of engineered MSC and, upon treatment with their CM, a decrease in cancer cell proliferation, migration, and invasion was observed, as well as an increase in cell death for both cancer cell lines. Moreover, the AAVS1 locus, a genomic safe harbor (GSH) in the human MSC genome, was targeted by CRISPR/Cas9 achieving 11.6% transgene knock-in efficiency.

Conclusion: By stably inserting the hazu gene into MSC genome, MSC could be employed as a cellular therapy, taking advantage of their tumor tropism and secretory potential; in addition, it could be disclosed a streamline for the production of a bioactive anti-tumoral CM product, by employing hazu-MSC as a continuous living factory. Using the system established here, other therapeutic transgenes could be targeted, aiming at different biological contexts, envisioning a boost in precise MSC genome editing.

Universal fast testing of patients with infiltrating lung adenocarcinomas: from UTOPIA to reality

Author and Affiliation

Fernando López-Ríos

12 de Octubre University Hospital Spain – Sponsored by Thermo Fisher Scientific

Abstract

In this presentation the speaker will discuss latest developments in NSCLC precision therapy and biomarker landscape, and importance of NGS in NSCLC biomarker testing. A special focus will be in patient-centered approaches, including molecular tumor boards. In light of all the discussed he will argue it is time to implement universal fast NGS as the front-line biomarker testing technology in NSCLC.

Curriculum Vitae

Dr. Fernando López-Rios has recently joined the Department of Pathology at "12 de Octubre" University Hospital (Madrid). He received his medical degree and PhD from the Faculty of Medicine at Autonoma University and Complutense University respectively and completed his residency in Pathology at "12 de Octubre" University Hospital (Madrid). Dr Lopez-Rios has also been the Director of Pathology & Laboratory of Therapeutic Targets at "HM Hospitales" and a visiting researcher at Memorial Sloan-Kettering Cancer Center (New York). His main clinical and research expertise is in cancer biomarker testing, with a special interest in lung cancer and mesothelioma. He is currently a member of the IASLC Pathology Committee.

Selected oral communication

Chitosan/γ-PGA nanoparticles-based immunotherapy as adjuvant to IFN-γ in breast cancer

Authors and Affiliations

Flávia Castro[1], Marta L Pinto[1], Catarina L Pereira[1], Karine Serre[2], Mário A Barbosa[1], Karim Vermaelen[3], Fátima Gartner[1], Raquel M Gonçalves[1], Olivier de Wever[3], Maria J Oliveira[1]

1. i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, PT

2. Instituto de Medicina Molecular João Lobo Antunes, Universidade de Lisboa, PT

3. CRIG - Cancer Research Institute Ghent, Ghent University, BE

Presenting Author

Flávia Castro

Abstract

Interferon-γ (IFN-γ) is a key factor for antitumor immunity and immunotherapy response. Most of the clinical trials reported a limited potential of IFN-γ as single therapy, being under clinical evaluation its combination with immunotherapy. Here, we addressed the synergistic effects of chitosan/poly(γ-glutamic acid) nanoparticles (Ch/PGA-NPs) when combined with IFN-γ using 4T1 breast tumor model. We previously showed that Ch/PGA-NPs are able to reeducate immature/immunosuppressive antigen-presenting cells (APCs) to an immunostimulatory profile, leading to T cell activation and impairing APCs-mediated cancer cell invasion. Recently, we demonstrated Ch/PGA-NPs as adjuvants to radiotherapy in breast cancer. Thus, these immunomodulatory abilities of Ch/PGA-NPs point them as appealing adjuvants to IFN-γ-based therapies.

Briefly, after 1 week of 4T1 tumor cell inoculation, animals were subcutaneously injected, 6 times for 2 weeks with Ch/PGA-NPs, IFN-γ or with both (NPs+IFN-γ). While non-treated animals had progressive tumor growth and developed lung metastasis, NPs- and IFN-γ-treated animals significantly decreased primary tumor burden. Remarkably, when both treatments were combined, breast tumor growth was blocked. This impairment was associated with a reduction in splenomegaly, a decrease in the percentage of splenic myeloid-derived suppressor cells and an increase in antitumoral CD4+IFN-γ+ population. Notably, animals from the combinatorial treatment presented lower lung metastatic burden and lower levels of the systemic pro-tumoral cytokines than other groups. Overall, Ch/PGA-NPs potentiate and synergize with IFN-γ to reduce tumor progression and systemic immunosuppression, opening new perspectives to be used in cancer.

Posters

01. Characterizing the inflammatory microenvironment in K14-HPV16 transgenic mice: mast cell infiltration and microRNAs expression

Authors and Affiliations

Alexandra C. Costa[1,2,7,ǂ] , Joana M.O. Santos[1,2,ǂ] , Beatriz Medeiros-Fonseca[3], Paula A. Oliveira[3], Margarida M.S.M. Bastos[4,5], Haissa O. Brito[6], Rui M. Gil da Costa[1,3,6], Rui Medeiros[1,2,7]

1. Molecular Oncology and Viral Pathology Group, Research Center of IPO Porto (CI-IPOP) / RISE@CI-IPOP (Health Research Network), Portuguese Oncology Institute of Porto (IPO Porto) / Porto Comprehensive Cancer Center (Porto.CCC), 4200-072 Porto, Portugal
2. Faculty of Medicine of the University of Porto (FMUP), 4200-319, Porto, Portugal
3. Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), Inov4Agro, University of Trás-os-Montes e Alto Douro (UTAD), Quinta de Prados, 5000-801 Vila Real, Portugal
4. LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
5. ALiCE - Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
6. Postgraduate Programme in Adult Health (PPGSAD), Department of Morphology, Federal University of Maranhão (UFMA), and UFMA University Hospital (HUUFMA), 65080-805 São Luís, Brazil
7. Research Department of the Portuguese League Against Cancer – Regional Nucleus of the North (Liga Portuguesa Contra o Cancro – Núcleo Regional do Norte), 4200-177 Porto, Portugal

ǂ. These authors contributed equally to this work and should be considered joint first authors.

Presenting Author

Alexandra C. Costa

Abstract

Introduction: High-risk human papillomavirus (HPV) is the etiologic agent of several types of cancer. Mast cells' role as either a driving or opposing force for cancer progression remains controversial. MicroRNAs are dysregulated in several HPV-induced cancers, and can influence mast cell biology. The aim of this study was to evaluate mast cells infiltration and to identify microRNAs potentially regulating this process.

Materials and Methods: Transgenic male mice (K14-HPV16; HPV+) and matched wild-type mice (HPV−) received 7,12-Dimethylbenz[a]anthracene (DMBA) (or vehicle) over 17 weeks. Following euthanasia, chest skin and ear tissue samples were collected. Mast cells infiltration was evaluated by immunohistochemistry. MicroRNAs associated with mast cell infiltration were identified using bioinformatic tools. MicroRNAs and mRNA relative expression was evaluated by RT-qPCR.

Results: Immunohistochemistry showed increased mast cell infiltration in both organs from HPV+ mice ($p<0,001$) compared with HPV− mice. DMBA did not have any statistically significant influence in this distribution. Ear tissue of HPV+ mice showed increased mast cell infiltration ($p<0,01$) when compared with chest skin samples. Analyzing both organs together we observed important differences in mast cell infiltration between

the normal epithelium and the hyperplastic epithelium (p<0,001) as well as between the normal epithelium and the dysplastic epithelium (p<0,001). Additionally, reduced relative expression of miR-125b-5p (p=0,008, 2-ΔΔCt=2,09) and miR-223-3p (p=0,013, 2-ΔΔCt=4,42) seems to be associated with mast cell infiltration and increased expression of target gene Cxcl10.

Conclusions: These results indicate that increased mast cell infiltration is associated with progression of HPV-induced lesions, and that miR-223-3p and miR-125b-5p might be assisting this process via the regulation of mast cell chemotactic proteins.

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02. Intra-Hospital Virtual Communities structure: impact on Patient Well-Being and in the Relationship with Physicians and Institutions

Authors and Affiliations

Ricardo Jorge Mendes da Silva

Prof. Dr. Paulo Alexandre Botelho Rodrigues Pires

Presenting Author

Ricardo Jorge Mendes da Silva

Abstract

The use of social media in healthcare settings has proved to be useful, not only for patients, but also for healthcare institutions.

In the specific case of oncological diseases, virtual communities are the social media with the greatest advantage, due to the reduced scope and depth of the topics addressed, although little is known about the structure that both patients and health professionals find most useful in this online environment.

As cancer is the second leading cause of death in Portugal, understanding how to structure virtual communities to improve patients' emotional well-being and collect real-time information about their status is therefore a pressing issue.

This study was conducted in the Portuguese Institute of Oncology of Porto of Francisco Gentil, and they were collected a total of 72 valid questionnaires from patients and conducted 10 semi-structured interviews to health professionals.

All health professionals interviewed shown willing to participate in this social media, although 50% of respondents reported not having available working hours, stating that collecting personalized real-time information about the patient's condition, their physical activity, emotions and nutrition, would allow them to make better decisions.

Patients also want to have this virtual community, where only cancer patients and health professionals shall have access, despite the hospital they are followed in. Patients want to interact with each other, exchanging experiences, testimonies and various types of information, as well as communicating with health professionals who accompany them, providing them with the data they intend to collect.

Conversations and content should be proper moderated by a dedicated team, to avoid online bullying and spam and to guarantee the truth of the shared information.

With this pioneered study, it was possible to fill a gap in the health marketing bibliography and, thus, to identify the basis to define a model structure an oncological virtual community.

03. THOR is a targetable epigenetic biomarker with clinical implications in breast cancer

Authors and Affiliations

Joana Dias Apolónio[1,2], João S. Dias[3], Mónica Teotónio Fernandes[2,4], Ana Teresa Maia[1,5], Uri Tabori[6], Pedro Castelo-Branco[1,2,7]

1. Faculty of Medicine and Biomedical Sciences (FMCB), University of Algarve, Faro, Portugal

2. Algarve Biomedical Center Research Institute (ABC-RI), Faro, Portugal.

3. University Hospital Center of Algarve, Faro, Portugal.

4. Escola Superior de Saúde, Universidade do Algarve, Faro, Portugal.

5. Center for Research in Health Technologies and Information Systems (CINTESIS-UAlg).

6. Arthur and Sonia Labatt Brain Tumor Research Center, University of Toronto, Toronto, Canada.

7. Champalimaud Research Program, Champalimaud Centre for the Unknown, Lisbon, Portugal.

Presenting Author

Joana Apolónio

Abstract

Introduction: The mortality rates still observed among breast cancer (BC) patients demonstrate the urgent need of novel and more effective diagnostic and therapeutic options.

In most BC cases, the process of limitless self-renewal is achieved by telomerase reactivation through upregulation of the human Telomerase Reverse Transcriptase (hTERT). The hypermethylation of the TERT Hypermethylated Oncological Region (THOR) has been associated with increased hTERT expression in cancer. The aim of this study is to investigate the role of THOR as a biomarker and explore the functional impact of THOR methylation status in hTERT upregulation in BC.

Materials and Methods: THOR methylation and hTERT expression from BC tissue and matched normal tissue were analysed using The Cancer Genome Atlas (TCGA). Then, THOR methylation status in BC was assessed by pyrosequencing on discovery and validation cohorts. Luciferase reporter assays and CRISPR-dCas9 system technology were used to assess the biological role of THOR in hTERT regulation.

Results: We found that THOR is significantly hypermethylated in malignant breast tissue when compared to benign tissue (40.23% vs. 12.81%, $p<0.0001$). Using a reporter assay, the addition of unmethylated THOR significantly reduced luciferase activity by an average 1.8-fold when compared to the hTERT core promoter alone ($p<0.01$). Significant demethylation of THOR in MCF-7 cells was achieved using the CRISPR-dCas9 system. Cells previously demethylated on THOR region did not develop a histologic cancer phenotype in in vivo assays. Additional studies are required to validate these observations and to unravel the causality between THOR hypermethylation and hTERT upregulation in BC.

Conclusions: THOR hypermethylation is a relevant epigenetic mark in breast tumorigenesis, representing a promising biomarker and therapeutic target in BC. We revealed that THOR acts as a repressive regulatory element of hTERT, and that its hypermethylation is a relevant mechanism for hTERT upregulation in BC.

04. P-cadherin as a phenotypic stability factor of the hybrid epithelial/mesenchymal phenotype in breast cancer

Authors and Affiliations

Catarina Esquível[1], Tito Teles Jesus[1,2], Maria Miguel Castro[1,2], Rita Canário[1,2], Ana Sofia Ribeiro[1,2], Bárbara Sousa1,2, Joana Paredes[1,2,3]

1. i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto

IPATIMUP – Instituto de Patologia e Imunologia Molecular da Universidade do Porto

FMUP – Faculdade de Medicina da Universidade do Porto

Presenting Author

Catarina Esquível

Abstract

Introduction: Cancer cells harboring the hybrid epithelial-to-mesenchymal transition (EMT) phenotype (hy-phen) plays a crucial role in metastasis, by promoting cellular plasticity, cancer stemness and collective migration. Our group has demonstrated that the expression of the adhesion molecule CDH3/P-cadherin (P-cad) in breast cancer (BC) induces collective cell invasion and migration, stem-like features, and anoikis-resistance in BC cells. Despite the role of P-cad expression in patient's poor prognosis, little is still known about its relevance in EMT. Thus, the aim of this work was to investigate if P-cad expression can act as a PSF (phenotypic stability factor) of a hy-phen in BC cells.

Materials and Methods: Bioinformatic analysis was performed using CCLE database. CDH3/P-cad expression was appropriately manipulated in BC cell lines, either using RNAi or retrovirally transduction of CDH3. Western Blot was used to evaluate protein expression.

Results: Using bioinformatic analysis, we identified 13 EMT genes significantly correlated with CDH3. Specifically, we observed a positive correlation of CDH3 with Epithelial (E) markers and PSFs, as well as an inverse correlation with EMT transcription factors (TFs) and Mesenchymal (M) markers. Interestingly, when we evaluated the distribution of the EMT markers in the different molecular subtypes of BC, CDH3 was the only transcript differentially overexpressed in Basal A cell lines in comparison to Luminal or Basal B cell lines. Finally, we validate these results by western blot in BC cells, and we observed that P-cad expression promotes the expression of EpCAM, OCLN, ΔNp63 and ZEB2 and leads to a decrease in AMPK, GRHL2 and N-cad expression.

Conclusion: In this work, we demonstrate, for the first time, that P-cad can differentially regulate the expression of EMT markers, by promoting the expression of E markers and TFs, as well as a decrease in the expression of M markers and PSFs. So, we can assume that P-cad has a putative role in the maintenance of the hy-phen in BC cells.

05. Activation of the Actin/MRTF-A/SRF signalling pathway in premalignant mammary epithelial cells by P-cadherin is essential for transformation

Authors and Affiliations

Lídia Faria[1,2], Sara Canato[1,2,3], Tito T. Jesus[1,2], Margarida Gonçalves[1,4], Patrícia S. Guerreiro[1,2,5], Carla S. Lopes[1,4], Isabel Meireles[1,2], Eurico Morais de Sá[1,4], Joana Paredes[1,2,6] and Florence Janody[1,2,7]

1. Instituto de Investigação e Inovação em Saúde (i3S), Universidade do Porto, Porto, Portugal.

Instituto de Patologia e Imunologia Molecular da Universidade do Porto (Ipatimup), Porto, Portugal.

Physiology and Cancer Program, Champalimaud Foundation, Lisboa, Portugal.

Instituto de Biologia Molecular e Celular (IBMC), Universidade do Porto, Porto, Portugal.

Vector B2B - Drug Developing - Associação Para Investigação em Biotecnologia, Lisboa, Portugal.

FMUP, Medical Faculty of University of Porto, Porto, Portugal.

Instituto Gulbenkian de Ciência, Oeiras, Portugal.

Presenting Author

Lídia Maria Pereira Faria

Abstract

Introduction: Alterations in the expression or function of cell adhesion molecules have been implicated in all steps of tumor progression. Among those, P-cadherin expression is highly enriched in Basal-like breast cancer, a molecular subset of Triple-Negative breast carcinomas, playing a central role in inducing cancer cell self-renewal, migration and invasion capacity.

Materials and Methods: To decipher the P-cadherin-dependent signalling network, we generated a humanised P-cadherin fly model, establishing a clinically relevant platform for functional exploration of P-cadherin effectors in vivo. In addition, we validated our findings in a human mammary epithelial cell line with conditional activation of the Src oncogene, which recapitulates molecular events taking place during cellular transformation.

Results: We report that actin nucleators, MRTF and SRF are three main effectors of P-cadherin functional effects. We show that prior to triggering the gain of malignant phenotypes, Src induces a transient increase in P-cadherin expression levels, which correlates with MRTF-A accumulation, its nuclear translocation and the upregulation of SRF target genes. Moreover, knocking down P-cadherin, or preventing F-actin polymerization with Latrunculin A, impairs SRF transcriptional activity. Furthermore, blocking MRTF-A nuclear translocation with CCG-203971 hampers proliferation, self-renewal and invasion.

Conclusions: In addition to sustaining malignant phenotypes, P-cadherin can also play a major role in the very early stages of breast carcinogenesis by promoting a transient boost of MRTF-A/SRF signalling through actin regulation.

06. Identification of Sodium Iodine Symporter (NIS) interactome at the surface of thyroid cancer cells

Authors and Affiliations

M. Faria[1,3], J. Vareda, M.J. Bugalho[1,5], A.L. Silva[1,2,†], P. Matos[4,3,†]

1. Serviço de Endocrinologia, Hospital de Sta Maria-CHULN. EPE, Lisboa, Portugal

ISAMB- Instituto de Saúde Ambiental, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal

BioISI- Biosystems and Integrative Sciences Institute, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal

Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal

Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal

†. Co-senior authors

Presenting Author

Ana Luísa Silva

Abstract

Introduction: Radioiodine (RAI) is used as the first-line therapy for metastatic differentiated thyroid cancer (DTC) and is still the most effective treatment available. However, a significant proportion of patients with advanced DTC fail to respond to RAI therapy (refractory-DTC). For these patients, the therapeutic alternatives are limited and ineffective. Since the uptake of RAI by DTC relies on the functional expression of the sodium iodine symporter (NIS) at the plasma membrane (PM), it is now clear that RAI resensitization implicates the development of therapeutic strategies to render functional NIS proteins at the PM thus improving NIS-mediated iodide uptake.

Methods: An innovative proteomics approach was established to identify the set of proteins selectively interacting with the NIS at the PM, potentially modulating its abundance and retention on the cell surface: the refractory-DTC-derived TPC-1 cell line was engineered to stably express a NIS protein harboring an extracellular triple HA tag (3HA-NIS). This allowed the specific isolation by immunoprecipitation (IP) of 3HA-NIS at the PM in intact cells, together with the capture of NIS-associated protein complexes (NIS interactors) which were then subjected to analysis by mass spectroscopy (nanoLC-MS/MS). Selected NIS interactors were validated by co-IP and Western blot and their contribution NIS PM residency and iodide uptake was addressed by cell surface protein biotinylation and iodine influx assays, respectively.

Results: From MS data analysis we identified 128 high confidence (>95%) candidate proteins, putatively interacting with NIS specifically at the PM. Gene ontology analysis of the candidate list (using DAVID, PANTHER and KEGG webtools) identified a significant enrichment in proteins involved in the regulation of the actin cytoskeleton and cell adhesion dynamics. The validation of NIS interactors for their ability to impact NIS functional expression uncovered a central role for the SRC kinase in this process.

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07. Digital expression profile of immune checkpoints genes across medulloblastoma molecular subgroups

Authors and Affiliations

Rui Pedro Ferreira Marques[1, 2, 3], Luciane da Silva[4], Leticia Ferro Leal[4],Terence Duarte[4], Flávia de Paula[4], Gustavo Teixeira[4], Carlos Almeida Junior[5], Fabiano Saggioro[6], Luciano Neder[6], Bruna Mançano[5], Daniel Moreno[4], Rui Manuel Reis[2, 3, 4]

1. School of Medicine, University of Minho, Braga, Portugal

Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

Molecular Oncology Research Center, Barretos Cancer Hospital, Barretos, Brazil

Barretos Children's Cancer Hospital, Barretos, Brazil

Department of Pathology and Forensic Medicine, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil

Presenting Author

Rui Marques

Abstract

Introduction: Medulloblastoma (MB) accounts for 15-20% of all pediatric brain tumors and is a leading cause of cancer-related death in children. MB is a World Health Organization (WHO) malignant grade 4 tumor and comprises at least four molecular subgroups: WNT-activated, SHH-activated and the non-WNT/non-SHH, named "3" (Group 3) and "4" (Group 4). Nowadays, the treatment of medulloblastoma is surgical resection, followed by craniospinal radiation (in patients older than 3 years), and chemotherapy. Although this way of treatment increased the overall survival of patients, it causes significant side effects, mainly because of the radiation effects on brain cells. Therefore, there is an urgency to develop more effective and less toxic therapies. Over the last years, immunotherapies such as immune checkpoints inhibitors have shown promising effects in many cancer types. Currently, attention is being given to monoclonal antibodies that target immune checkpoints such as PD-1/PD-L1, CTLA-4, B7-H3, and Tim-3/4, with clinical trials ongoing with these agents. However, the expression profile of immune checkpoints in medulloblastoma subgroups is poorly described, which hinders the choice for an appropriate target for immunotherapy in this tumor.

Therefore, this study aims to characterize the expression profile of immune checkpoints in a series of molecularly characterized medulloblastomas.

Materials and Methods: Eighty-eight formalin-fixed paraffin-embedded (FFPE) medulloblastoma previously characterized for the molecular subgroups, had the mRNA expression of 770 key immune-oncology related genes determined by nCounter® using the PanCancer Immune Profiling Panel (Nanostring Technologies). The ROSALIND® platform was used to evaluate immune checkpoints gene expression and associate it with medulloblastoma molecular subgroups.

Quality control parameters such as binding density, detection limit, positive controls and housekeeping counts were measured using the nSolverTM Analysis Software v4.0 (NanoString Technologies) and ROSALIND®. The normalized data was downloaded from ROSALIND® platform and subsequent statistical analysis was performed using IBM SPSS version 27.

mRNA levels of the immune checkpoints PDCD1 (PD-1), CD274 (PD-L1), PDCD1LG2 (PD-L2), CTLA4, CD276 (B7-H3), LAG3, PVR (CD155), CD47, CD80, CD86, BTLA, IDO1, HAVCR2 (TIM-3), CD48, TNFSF14, CD160, CEACAM1, CD244 and TIGIT were evaluated. The comparison of these immune checkpoints by medulloblastoma subgroup was made using the nonparametric Kruskall-Wallis test for independent samples and Bonferroni correction for multiple comparisons. Differentially expressed genes were considered when the adjusted p-value was lower than 0.05.

Results: Eight cases did not pass through the experimental quality metrics, leading to a final number of 80 cases, that we molecularly subdivided in WNT (n=13), SHH (n=39), Group 3 (n=10) and Group 4 (n=18).

We observed a shallow expression in all actionable immune checkpoints, except for PVR, CD276 and CD47 that presented mean counts of 100, 490 and 439, respectively. Importantly the expression of pembrolizumab, nivolumab, and ipilimumab targeted genes, namely PD-1, PD-L1 and CTLA4, have normalized mRNA counts below 20, which is below background threshold, indicating a practically null expression.

The CD155 (PVR - poliovirus receptor) gene was overexpressed in WNT compared to SHH and Group 3 and showed low expression in Group 4 compared to other subgroups (p-value < 0,001).

High mRNA counts of CD276 (B7-H3 - B7 Homolog 3) were found across the four subgroups (mean counts = 400 to 700) and this gene was significantly overexpressed in WNT compared to SHH subgroup (p-value < 0,0001).

The CD47 gene showed a significantly higher expression in WNT and SHH (mean counts of 784,2 and 440,7, respectively) compared to Groups 3 and 4.

Conclusion: Actionable immune checkpoints such as PD-1, PD-L1 and CTLA-4 showed very low mRNA expression in medulloblastoma in accordance with reported literature, and corroborates the disappointing clinical trial results of pembrolizumab, nivolumab, and ipilimumab. Notably, the high expression of CD155, CD276 and CD47 suggests that these immune checkpoints could constitute more suitable targets for immunotherapy in medulloblastomas.

08. Motile and adhesive profiles of variants associated with distinct E-cadherin mediated disorders

Authors and Affiliations

Joana Pereira[1,2,3], Soraia Melo[1,2], André Maia[1,4], Annelies de Klein[5], Parry Guilford[6], Raquel Seruca[1,2,3], Joana Figueiredo[1,2]

1. i3S, institute for research and innovation in health, Porto, Portugal

Ipatimup, Institute of Molecular Pathology and Immunology of Porto University, Porto, Portugal

Medical Faculty, University of Porto, Portugal

IBMC, Institute for Molecular and Cell Biology, University of Porto, Porto, Portugal

Department of Clinical Genetics, Erasmus University MC, Rotterdam, The Netherlands

Cancer Genetics Laboratory, Centre for Translational Cancer Research (Te Aho Matatū), Department of Biochemistry, University of Otago, Dunedin, New Zealand.

Presenting Author

Joana Pereira

Abstract

Introduction: Germline mutations of E-cadherin (CDH1 gene) contribute to hereditary diffuse gastric cancer (HDGC) and congenital malformations such as oral facial clefts (OFC). However, the molecular mechanisms through which E-cadherin loss-of-function leads to distinct clinical outcomes remain unknown. We propose that E-cadherin mediated disorders result from abnormal interactions between mutant cells with the extracellular matrix (ECM), and consequently from aberrant integrin activation, affecting the direction and coordination of epithelial migration.

Materials and Methods: To investigate the migratory behavior promoted by OFC or HDGC variants, we characterize cell motility by exploring gap closure migration assays coupled with time-lapse microscopy and bioimaging analysis. For subsequent identification of molecules that control aberrant cell motile behavior, we used a high-throughput ECM array with 36 combinations of ECM components. The number of cells attached to each ECM spot was quantified as a direct measure of cell-matrix adhesive ability.

Results: We verified that OFC and HDGC variants yield distinct motile profiles. Cells expressing OFC variants migrate faster and in a direct way, as observed by low travelled distances and poor diffusion rates. In contrast, HDGC variants show a high cell displacement and low velocity during wound closure. Regarding ECM adhesion, OFC mutants present greater attachment abilities, when compared with wild-type cells. Remarkably, cells expressing OFC variants adhere to a wider variety of matrices than those expressing HDGC variants. This data further suggests that OFC variants lead to increased cell plasticity and high responsiveness to different microenvironments.

Conclusions: Herein, we demonstrated that E-cadherin variants associated with distinct clinical outcomes induce altered cell migratory phenotypes and ECM adhesion preferences, implicating that aberrant integrin activation may underlie CDH1 pleiotropy.

09. Polo-like kinase 4 (Plk4) potentiates anoikis-resistance of p53KO epithelial cells through P-cadherin upregulation

Authors and Affiliations

Irina Fonseca[1,2,4], Cíntia Horta[1,2,4], Ana Sofia Ribeiro[2,4], Barbara Sousa[2], Gaëlle Marteil[3]

Mónica Bettencourt-Dias[1,4,5], Joana Paredes[2,4,5]

1. Instituto Gulbenkian de Ciência, Oeiras, Portugal

i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal

IMoST UMR 1240 INSERM/UCA, Clermont-Ferrand, France

Cancel Stem, Portuguese Consortium on Cancer Stem Cells

Co-Lead authors

Presenting Author

Irina Fonseca

Abstract

Introduction: Polo-like kinase 4 (Plk4) is the major regulator of centriole biogenesis. It has emerged as a therapeutic target in cancer treatment due to its abnormal expression in human cancers, leading to centrosome number deregulation, mitotic defects, and chromosomal instability, promoting tumor growth and metastasis in mouse models, and associated with poor patient prognosis. However, the underlying mechanism of Plk4's role in cancer progression remains to be elucidated.

Methods: MCF10A, a human breast non-cancerous cell line, was engineered to enable the inducible overexpression of Polo-like Kinase 4 (Plk4), an essential regulator of centrosome duplication. With Doxycycline treatment, PLK4 is transiently overexpressed and consequently inducing CA. To ensure cell survival upon centriole manipulation, the p53 tumor suppressor was successfully knockout (KO) through CRISPR/Cas9. Anoikis resistance capacity was assessed by Mammosphere Forming Efficiency (MFE)

Results: Here we show that overexpression of Plk4 significantly potentiates resistance to cell death by anoikis of non-tumorigenic p53 knock-out (p53KO) mammary epithelial cells. Interestingly, this increase is associated with the induction of a stable hybrid epithelial-mesenchymal phenotype and is partially dependent on P-cadherin upregulation. Furthermore, we found that the conditioned media of Plk4-induced p53KO mammary epithelial cells also increases anoikis resistance of breast cancer cells in a paracrine way.

Conclusion: Our work shows, for the first time, that high levels of Plk4 induce anoikis resistance of both mammary epithelial cells with p53KO background, as well as of breast cancer cells exposed to their secretome, partially through P-cadherin upregulation. These results reinforce the idea that Plk4 acts as an oncogene, impacting the tumor microenvironment to promote malignancy.

10. Cell extrusion: a synergistic interaction between E-cadherin and Filamin A

Authors and Affiliations

Soraia Melo[1,2], Joana Figueiredo[1,2], João M. Sanches[4], Raquel Seruca[1,2,3]

1. i3S – Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal;

IPATIMUP – Institute of Pathology and Molecular Immunology of the University of Porto , Porto, Portugal;

Medical Faculty of the University of Porto, Porto, Portugal;

Institute for Systems and Robotics, Instituto Superior Técnico, Lisboa, Portugal.i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal

Presenting Author

Soraia Melo

Abstract

Introduction: E-cadherin mutations are causative events of Hereditary Diffuse Gastric Cancer (HDGC), a highly invasive cancer syndrome with a lethal outcome. In HDGC, abnormal E-cadherin cells spread along and below wild-type cells, representing the first step of invasion and the result from a switch from apical to basal extrusion. Still, the molecular mechanisms through which E-cadherin dysfunctional cells invade instead of being eliminated remain unknown. We hypothesize that at early stages, E–cadherin mutant cells are sensed by wild--type epithelia suffering changes in cytoskeletal organization, cell shape and intracellular signalling, ultimately defining their ability to extrude.

Materials and methods: Herein, we have stably transfected AGS gastric cells with the wild-type protein and a panel of E-cadherin mutants affecting distinct domains of the protein. In particular, we have established a monolayer system where labelled mutant cells were mixed with non-labelled wild-type counterparts to monitor mutant cell fate within a normal epithelium. Structural organization, protrusion formation and dissemination of cells were also assessed in a 3D system.

Results: We verified that the juxtamembrane mutant R749W lead to an increase in basal extrusion phenotype, when compared with mutants affecting the extracellular and intracellular domains. Moreover, R749W mutant cells form aggregates with higher growth ratios and remarkable protrusive abilities. Notably, we found that the R749W mutant impacts the activity of Filamin A, with consequences at leading edge structures and cell movement throughout the extracellular matrix. The implication of Filamin A in extrusive potential was further confirmed by its specific inhibition in wild-type cells that resulted in increased migration capacity and basal position of nuclei.

Conclusions: Our results indicate a molecular signature associated to basal cell extrusion that involves cytoskeletal remodelling and can be explored for the development of new therapeutic strategies.

11. Nuclear signatures of an invasive cancer program triggered by E-cadherin dysfunction

Authors and Affiliations

Joana Figueiredo[1,2], Joana Pereira[1,2,3], Ana Catarina Nunes[1,2], Rui M. Ferreira[1,2], Margarida Gonçalves[1,4,5], Soraia Melo[1,2], Patrícia Carneiro[1,2], Parry Guilford[6], Eurico Morais-de-Sá[1,4], Raquel Seruca[1,2,3]

1. i3S - Instituto de Investigação e Inovação em Saúde, University of Porto, 4200-135 Porto, Portugal;
Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), 4200-135 Porto, Portugal;
Medical Faculty of the University of Porto, 4200-319 Porto, Portugal;
Institute for Molecular and Cell Biology (IBMC), University of Porto, 4200-135 Porto, Portugal;
Instituto de Ciências Biomédicas de Abel Salazar (ICBAS), Universidade do Porto, 4050-313 Porto, Portugal;
Cancer Genetics Laboratory, Te Aho Matatū, Department of Biochemistry, University of Otago, 9016 Dunedin, New Zealand, Universidade do Porto, Portugal

Presenting Author

Joana Figueiredo

Abstract

Introduction: Loss of the cell-cell adhesion molecule E-cadherin occurs in 70% of carcinomas and is widely described as an initiating event in the invasion process. This milestone phenomenon is accompanied by increased cell plasticity and a remarkable ability of cells to overcome the fibrous structure of the extracellular matrix. Since the nucleus is the largest and stiffest organelle in the cell, we hypothesize that it represents a major determinant of cell reshaping and spreading capacity. Our main aim is thus to dissect the mechanisms through which the nucleus supports invasion of cancer cells with E-cadherin dysfunction.

Material and Methods: Herein, we have used cell lines and Drosophila strains expressing either wild-type E-cadherin or the Y755C variant identified in gastric cancer patients. Nuclear morphological features and migratory rates were assessed by fluorescent markers coupled with confocal microscopy and advanced bioimaging techniques. The molecular profile of nuclei envelope from E-cadherin mutant and wild-type cells was subsequently investigated through high-resolution Mass Spectrometry (LC-MS).

Results: We verified that the Y755C E-cadherin mutation induces evident changes in nuclear morphology, and an increased migration performance of border cells in Drosophila. Detailed quantitative measurements revealed differences in nuclear area, mean intensity, perimeter, solidity and relative position to basal surface. Further, proteomic analysis of enriched nuclear fractions disclosed that the composition of the nuclear envelope from E-cadherin mutant cells is significantly different from that of the wild-type counterparts. Differentially abundant molecules include proteins relevant for spatial and structural integrity of the nucleus.

Conclusion: Overall, this work provide evidence that cells with E-cadherin loss of function activate a specific mechanotransduction program that imposes nuclear remodeling at physical and biochemical levels, endowing cells with a pro-invasive signature.

12. Combining Repurposing Drugs with Paclitaxel Has a Synergic Effect in Chemoresistant High-Grade Serous Carcinoma

Authors and Affiliations

Mariana Nunes[1,2], Diana Duarte[3,4], Nuno Vale[3,5], Sara Ricardo[1,6,7]

1. Differentiation and Cancer Group, Institute for Research and Innovation in Health (i3S) of the University of Porto/Institute of Molecular Pathology and Immunology of the University of Porto (Ipatimup), 4200-135 Porto, Portugal. mnunes@ipatimup.pt; sricardo@ipatimup.pt

Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, 4050-313 Porto, Portugal.

OncoPharma Research Group, Center for Health Technology and Services Research (CINTESIS), 4200-450 Porto, Portugal. dianaduarte29@gmail.com; nunovale@med.up.pt.

Faculty of Pharmacy, University of Porto (FFUP), 4050-313 Porto, Portugal.

Department of Community Medicine, Health Information and Decision (MEDCIDS), Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal.

Toxicology Research Unit (TOXRUN), University Institute of Health Sciences, Polytechnic and University Cooperative (CESPU), CRL, 4585-116 Gandra, Portugal.

Faculty of Medicine, University of Porto (FMUP), 4200-319 Porto, Portugal. Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), 4200-135 Porto, Portugal;

Presenting Author

Mariana Nunes

Abstract

The main challenge in ovarian cancer is to unveil therapeutic approaches to overcome chemoresistance. New pharmacological approaches are expected to improve clinical outcomes, nevertheless, long-term and costly research is required to develop new drugs, and soaring healthcare costs are a worldwide concerning. Drug combinations and repurposing of non-oncological agents to treat neoplasms are two attractive strategies which allow higher efficacy, decreased toxicity, and overcoming chemoresistance. The goal of combining drugs is improve therapeutic responses using lower doses of two more drugs comparing with single treatment. Several non-oncological drugs, e.g., antihypertensives, antidiabetics, anthelmintics, antifungal, antibiotics and antivirals display an effective anti-cancer activity and have been studied to be repurposed in multi-drug resistant cancers. The purpose of this study was to explore whether combining Paclitaxel with repurposing drugs (i.e., Pitavastatin, Metformin, Ivermectin, Itraconazole and Alendronate) led to a therapeutic benefit.

Herein, we evaluated the cytotoxic effects of Paclitaxel alone and in combination with several repurposed drugs (mentioned above) in two chemoresistant models, i.e., OVCAR8 (Carboplatin-resistant) and OVCAR8 PTX R P (Carboplatin and Paclitaxel-resistant) cell lines. Different concentrations in a fixed ratio were added to both cell lines for 48 hours. Cell viability was assessed using Presto Blue assay and synergism was evaluated using the Chou-Talalay method.

Pitavastatin, Ivermectin, Itraconazole and Alendronate demonstrate a significant anti-cancer activity at low doses with IC50 values ranging between 1 and 150 μM for both cell lines. Pitavastatin was the most promising candidate alone for drug repurposing with the lowest IC50 value (0,8 μM). In combination, almost all repurposed drugs demonstrate higher ability than Paclitaxel alone to decrease cell viability for both cell lines. Moreover,

the combination of Ivermectin with Paclitaxel demonstrates the highest cytotoxicity and the strongest synergism (CI<1) among all combination for both cell lines.

Overall, our results demonstrate that almost all the repurposed drugs have enhanced anticancer activity at low IC50 values when used alone and in combination can improve the anti-cancer activity of Paclitaxel in chemoresistant high-grade serous carcinoma.

13. Evaluation of secretome associated proteins in glioblastoma patients' tumor tissues and plasma

Authors and Affiliations

1. Bárbara Alves (i3S- Instituto de Investigação e Inovação em Saúde, Portugal / Cancer Signaling and Metabolism Group, IPATIMUP - Institute of Molecular Pathology and Immunology of the University of Porto, Portugal / School of Health, Polytechnique Institute of Porto, Portugal);
2. Sofia Macedo (i3S- Instituto de Investigação e Inovação em Saúde, Portugal / Cancer Signaling and Metabolism Group, IPATIMUP - Institute of Molecular Pathology and Immunology of the University of Porto, Portugal);
3. Joana Peixoto (i3S- Instituto de Investigação e Inovação em Saúde, Portugal / Cancer Signaling and Metabolism Group, IPATIMUP - Institute of Molecular Pathology and Immunology of the University of Porto, Portugal);
4. Jorge Pinheiro (Department of Pathology, FMUP - Faculty of Medicine of the University of Porto, Portugal);
5. Bruno Carvalho (Department of Neurosurgery, Centro Hospitalar Universitário S. João, Porto, Portugal);
6. Jorge Lima (i3S- Instituto de Investigação e Inovação em Saúde, Portugal / Cancer Signaling and Metabolism Group, IPATIMUP - Institute of Molecular Pathology and Immunology of the University of Porto, Portugal / Department of Pathology, FMUP - Faculty of Medicine of the University of Porto, Portugal);
7. Paula Soares (i3S- Instituto de Investigação e Inovação em Saúde, Portugal / Cancer Signaling and Metabolism Group, IPATIMUP - Institute of Molecular Pathology and Immunology of the University of Porto, Portugal / Department of Pathology, FMUP - Faculty of Medicine of the University of Porto, Portugal);
8. Raquel T. Lima (i3S- Instituto de Investigação e Inovação em Saúde, Portugal / Cancer Signaling and Metabolism Group, IPATIMUP - Institute of Molecular Pathology and Immunology of the University of Porto, Portugal / Department of Pathology, FMUP - Faculty of Medicine of the University of Porto, Portugal);

Presenting Author

Bárbara Alves

Abstract

Glioblastoma (GB) is the most aggressive subtype of glioma, accounting for the majority of all primary malignant tumors of the central nervous system in adults. Despite the increased knowledge on its biology and molecular characterization, patients' survival is still extremely low and recurrence mostly inevitable. Studies have been addressing the role of GB secretome in tumor aggressiveness and therapy resistance. Moreover, the fact that GB secretome proteins can extravasate the brain into the blood increases their relevance as biomarkers for diagnosis, progression or prediction of response to therapy. Still, to date, none has translated into effective clinical biomarkers.

This project aims to evaluate the levels of secretome proteins MMP9, MMP2, VEGFA and YKL40 in GB patient samples and to further address their potential as GB biomarkers. For this, protein expression/localization is being immunohistochemically assessed in paraffin embedded tumor tissue samples from 66 GB patients. In some plasma samples, proteomic profile is being evaluated by liquid chromatography mass spectrometry

(LC/MS). Protein expression will be evaluated regarding patients' clinicopathological data, response to therapy and overall survival (OS).

MMP9 expression was mainly observed in inflammatory cells and in areas of microvascular proliferation, being mostly absent in tumor cells (which seems to associate with increased patient OS). In contrast, MMP2, VEGFA and YKL40 expression was mainly found in tumor cells, and MMP2 strong/moderate staining seemed to associate with patients' increased OS. Regarding GB plasma samples' proteomics, a group of proteins was found differently expressed comparing to healthy controls, results that are being further validated.

This ongoing study will allow to further evaluate the relevance of GB secretome proteins as biomarkers to be applied, either individually or in groups, in clinical practice diagnose, monitor and/or prediction of patients' response.

14. DNA methylation biomarkers to residual disease detection in esophageal cancer upon neoadjuvant chemoradiation

Authors and Affiliations

Catarina Macedo-Silva[*,1]; Vera Constâncio[1,2]; Vera Miranda-Gonçalves[1]; Pedro Leite-Silva[1]; Sofia Salta[1]; João Lobo[1,3]; Rita Guimarães[3]; Carina Maia[1]; Davide Gigliano[3]; Mónica Farinha[3]; Olga Sousa[4]; Rui Henrique[1,3,5]; Carmen Jerónimo[1,5]

1. Cancer Biology and Epigenetics Group, Research Center (CI-IPOP) Portuguese Oncology Institute of Porto (IPO Porto), Rua Dr. António Bernardino de Almeida, 4200-072, Porto, Portugal.
2. Department of Precision Medicine, University of Campania "Luigi Vanvitelli", 80138 Naples, Italy
3. Department of Pathology and Molecular Immunology at School of Medicine & Biomedical Sciences– University of Porto (ICBAS-UP), Porto, Portugal.
4. Medical Physics, Radiobiology and Radiation Protection Group - Research Center, Portuguese Oncology Institute of Porto, Portugal, (CI-IPOP).
5. Department of Medical Physics, Portuguese Oncology Institute of Porto, Portugal, (CI-IPOP).

*. correspondence: anacatarina.macedodasilva@unicampania.it / ana.catarina.macedo.silva@ipoporto.min-saude.pt

Presenting Author

Catarina Macedo-Silva

Abstract

Introduction: Esophageal cancer (ECa) is a malignancy with a considerably high mortality. Unfortunately, most of ECa patients are late diagnosed with locally advance disease and not eligible for surgery. Regardless of histological subtype, neoadjuvant chemoradiation (ChRT) is a common procedure recommended by the guidelines. The lack of reliable biomarkers that clearly detect post-ChRT disease and distinguish those with complete response is a major disadvantage. Remarkably, miRNA promotor methylation is a novel approach as detection biomarkers in several cancer models being potential candidates in tissue and plasma detection. Moreover, little has been explored on this topic for ECa model.

Materials and methods: With this work we sought to unveil novel candidate biomarkers as well as strengthen our previously work to properly be able to detect ECa disease prior and post ChRT. Herein, promoter methylation of miR129-2, miR124-3 and ZNF569 was assessed by quantitative methylation-specific PCR (qMSP) in a series of tissue and plasma of ECa naïve and normal esophagus, as well as in post-ChRT tissue samples.

Results: All genes significantly distinguished ECa from normal esophagus and accurately detect post-ChRT minimal residual disease with best biomarker performance for adenocarcinoma subtype. Remarkably, miR129-2me/ZNF569me methylation panel identified ECa in liquid biopsies with 53% of sensitivity and 87% of specificity. Overall, this study provides strong evidence that miR129-2me, miR124-3me and ZNF569me are good candidate biomarkers for prior and post ChRT ECa detection.

Conclusions: Our findings indicate circulating methylated miRNAs as promising minimal invasive strategy to detect ECa disease.

Funding: This study was financial supported by an award from Fundação D. Manuel II, "Principe da Beira, Ciências Biomédicas 2019", in which CM-S was the candidate. Additionally, this work was partially supported by a grant from ESTIMA-NORTE-01-0145-740 FEDER-000027.

15. Cannabidiol plus Exemestane in hormone-dependent breast tumors: effects on sensitive and resistant cells

Authors and Affiliations

Cristina Ferreira Almeida[1,2], Natércia Teixeira[1,2], Georgina Correia-da-Silva[1,2], Cristina Amaral[1,2]

1. UCIBIO.REQUIMTE, Laboratory of Biochemistry, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal
2. Associate Laboratory i4HB - Institute for Health and Bioeconomy –, Faculty of Pharmacy, University of Porto, Rua Jorge Viterbo Ferreira, n° 228, 4050-313 Porto, Portugal2Department of Precision Medicine, University of Campania "Luigi Vanvitelli", 80138 Naples, Italy

Presenting Author

Cristina Ferreira Almeida

Abstract

Introduction: Breast cancer (BC) is the most diagnosed cancer worldwide, being estrogen receptor-positive (ER+) the most common. Exemestane (Exe) is one of the aromatase inhibitors (AIs) used in clinic as first-line treatment. However, its prolonged use may induce resistance and tumor re-growth. Our group has recently showed that cannabidiol (CBD) exerts important anti-tumor effects in ER+ BC. Thus, in an attempt to improve BC therapy, our aim was to understand the effects of CBD when combined with Exe, in sensitive and resistant cells.

Materials and methods: MCF-7aro and LTEDaro cells, a sensitive and a resistant BC cell line, respectively, were used. In both cell lines it was evaluated cell viability (MTT), caspase-7 activity (luminescent assay) and ERK1/2 activation (Western-Blot). ERα protein levels (Western-Blot) and ERα target genes transcription (qPCR) were only studied in MCF-7aro cells.

Results: In both cell lines, the combination induced a higher decrease on cell viability than the compounds per se and caused an activation of caspase-7, as well as, a pronounced decrease on ERK1/2 activation. In MCF-7aro cells, it inhibited the estrogen-like effect induced by Exe by reducing the transcription of ERα target genes.

Conclusions: Although the combination of CBD with Exe had revealed good results in both MCF-7aro and LTEDaro cells, the results on the latter ones are especially important, since it seems to re-sensitize resistant cells to Exe action. Besides the induction of apoptosis, the beneficial effect on LTEDaro cells may be a result of a reduced ERK1/2 activation, while in MCF-7aro cells a consequence of the inhibition of ERα activation. This study opens up the potential of cannabinoids as combined therapy for ER+ BC, though further studies should be carried out to clarify its clinical benefit.

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16. Studing estrogenic and androgenic influences on effects of anticancer drugs using hepatoma cell line Hep G2

Authors and Affiliations

Mohamed A. Hamzawy[1], Alice Ramos[2,3], Eduardo Rocha[2,3]

1. Department of Pharmacology and Toxicology, Faculty of Pharmacy, Fayoum University, Fayoum City, Egypt
2. ICBAS - Institute of Biomedical Sciences Abel Salazar, Department of Microscopy, University of Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
3. CIIMAR - Interdisciplinary Center for Marine and Environmental Research, PATH – Histomorphology, Physiopathology and Applied Toxicology Group, University of Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal

Presenting Author

Mohamed A. Hamzawy

Abstract

Introduction: Hepatocellular carcinoma (HCC) is the fourth and ultimate stage of liver disease after earlier hepatitis, fibrosis, and cirrhosis; actually, it is estimated that 80% of patients with HCC have underlying cirrhosis. Additionally, HCC is the fifth most commonly diagnosed cancer and the second cause of death among other types of cancer. Previous studies reported that gender disparity might play a crucial role in preference of development of HCC, with males having a higher risk. Earlier studies reported that estrogens might act as hepatoprotective by multiple ways, such as inhibitory effects on inflammatory processes and significant promotion of antioxidant enzymes, beside down-regulation of IL-6, which is critical for hepatic lesions. Some estrogenic compounds have a significant role in reversing doxorubicin resistance in human breast cancer. Considering the gender disparity on HCC development and outcome, our aim is to study the potential modeling effects of estrogenic and androgenic compounds on HCC using hepatoma cell lines.

Materials and Methods: doxorubicin, cisplatin, ethinyl estradiol, testosterone, tributyltin chloride was incubated for 48 hr at every of four tested concentrations, from 0.01 µM to 10 µM using Hep G2 as experimental model. MTT assay, clonogenic survival assay and nuclear condensation assay. Using incubation of 48 h and a range of concentrations of the tested compounds, we start getting the first results.

Results: We noticed that EE2 significantly decreased cell viability (at every of four tested concentrations, from 0.01 µM to 10 µM), but under the assayed conditions the estrogen did not potentiate the cytotoxic effect of Dox. As to TBT, it seemed to have potentiated the cytotoxic effect of both DOX and CISP, in opposition to EE2.

Conclusion: Data are being expanded and refined mechanistically, to unveil insights about the influences of estrogenic and androgenic signaling/effects in the development and therapeutics of HCC.

17. Toward understanding other cell communication mechanism of Sorafenib in HCC in rats; comprehensive

Authors and Affiliations

Mohamed A. Hamzawy[1*], Sayed M. Mizar[1], Laila A. Rashed[2]

1. Pharmacology and Toxicology Department, Faculty of Pharmacy, Fayoum University, Fayoum, Egypt

2. Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Cairo University, Cairo, Egypt

Presenting Author

Mohamed A. Hamzawy

Abstract

Introduction: Hepatocellular carcinoma (HCC) is the sixth most frequently diagnosed cancer and shows the third highest mortality rate in cancer patients worldwide. Affected patients have a poor prognosis due to late diagnosis. A tyrosine kinase inhibitor sorafenib has been used as systemic therapy with a demonstrated survival benefit in HCC. However, there are still several limitations to its use, due to acquired resistance. The current study was conducted to explore the other molecular mechanism of sorafenib in experimentally induced liver cancer in rats.

Materials and Methods: Four groups of Swiss albino rats were being treated for 12 weeks as the following: Normal group, (2): the group was treated with DEN (200mg/kg, i.p) + CCL4 (3ml/kg, S.C) weekly for the first 8 consecutive weeks, (3): Sorafenib (10 mg/kg, P.O) daily to for last 4 weeks, (4) Sorafenib after DEN + CCL4 treatment. Blood, liver samples were collected for biochemical analysis, gene expression, western blotting assays, and histological examinations.

Results: DEN and CCL4 showed severe changes in all biochemical and molecular parameters and histological examinations. Two months of daily treatment with sorafenib markedly decreased Bcl-2, Cyclin D1, NF-kB accompanied by improvement of active caspase 3. Sorafenib succeeded to restore the protein expression of P-AKT and P-ERK in beside refinement of histological patterns in animals that pretreated with DEN and CCL4.

Conclusion: Sorafenib interrupts various cell communication pathways that control cancer progression, angiogenesis, and cell survival. Sorafenib regulates the p-AKT/ P-ERK signaling pathway in HCC. Data from the present work address the importance of other therapeutic targets that may help in combating sorafenib resistance and repurpose it in other neoplastic lesions.

18. NRF-1 and PERK protect castration resistant prostate cancer cells from flutamide-induced toxic aggresome accumulation

Authors and Affiliations

Inês Direito[1], Liliana Monteiro[1], Daniela Figueira[1], Margarida Fardilha[1] and Luisa A. Helguero[1]

1. iBiMED - Institute of Biomedicine, University of Aveiro, Aveiro, Portugal

Presenting Author

Inês Direito

Abstract

Introduction: Pre-clinical studies showed that castration resistant prostate cancer cells (CRPC) activate the unfolded protein response (UPR) and the antioxidative response to protect cells from protein damage and misfolding. These prevents protein aggregation and accumulation in toxic aggresomes. Previously, we showed that in breast cancer, aggresome accumulation can be used as a marker of endocrine therapy response. The aim is to study how CRPC cells protect their proteome in response to flutamide (FLUT) and investigate if there is an association of aggresomes with apoptosis.

Material and Methods: Flutamide (FLUT) sensitive (LNCaP) and resistant (22Rv1) prostate cancer cells were treated for 1h to 24h. Specific markers were assessed by WB, immunocytochemistry and qPCR. Proteostat® was used to study protein aggregation. SUnSET was used to monitor protein translation. CellROXTM was used to measure oxidative stress. Small interfering RNA and GSK2606414 were used for PERK inhibition.

Results: FLUT lead to aggresome accumulation only in apoptotic LNCaP cells. In 22Rv1 cells, FLUT induced a transient antioxidative response through NRF1 (at 3h) to maintain redox homeostasis and selectively activated the PERK/eIF2α pathway of UPR (at 12h) to temporarily reduce protein translation and prevent toxic aggresome formation. PERK inhibition increased aggresome accumulation, re-sensitizing CRPC cells to therapy.

Conclusions: FLUT treatment induces a first wave of antioxidant response followed by transient inhibition of protein translation, sustaining survival when AR signaling is blocked. PERK inhibition re-sensitized CRPC cells to therapy. Aggresome levels could be used as markers of therapy response.

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19. Hyaluronic acid as a potential target ligand for CD44+ Oral Cancer Cells – a preliminary approach

Authors and Affiliations

Cátia Domingues[1,2,3]; Ivana Jarak[1]; Carmen Alvarez-Lorenzo[4]; Francisco Veiga[1,2]; Marília Dourado[3,5]; Ana Figueiras[1,2]

1. Univ Coimbra, Faculty of Pharmacy, Drug Development and Technologies Laboratory, Coimbra, Portugal
2. REQUIMTE/LAQV, Drug Development and Technologies Laboratory, Faculty of Pharmacy, University of Coimbra, Portugal
3. Univ Coimbra, Institute for Clinical and Biomedical Research (iCBR) area of Environment Genetics and Oncobiology (CIMAGO), Faculty of Medicine, Coimbra, Portugal
4. Departamento de Farmacología, Farmacia y Tecnología Farmacéutica, I+D Farma (GI-1645), Facultad de Farmacia, iMATUS, and Health Research, Institute of Santiago de Compostela (IDIS), Universidade de Santiago de Compostela, Santiago de Compostela, Spain
5. Univ Coimbra, Center for Studies and Development of Continuous and Palliative Care (CEDCCP), Faculty of Medicine, Coimbra, Portugal

Presenting Author

Cátia Domingues

Abstract

Introduction: Oral Squamous Cell Carcinoma (OSCC) is a multistep disease with a poor prognosis. CD44 receptor is commonly overexpressed in cancer cells with stemness potential and epithelial-mesenchymal phenotype. Hyaluronic acid (HA) is recognized as a target ligand for CD44, including in OSCC. Hence, this study aimed to assess the role of HA in OSCC and, based on this data, to synthesize a nanosystem using Pluronic L121®-PEI for potential drug/gene co-delivery using HA as a target to CD44+ cells.

Materials and Methods :A two-step chemical approach was performed to covalent link Pluronic L121® with Polyethyleneimine (PEI). After, HA was electrostatically conjugated. Structural analysis of the different formulations was evaluated by FTIR and 1H-NMR spectroscopy. The physicochemical characterization was achieved by Dynamic/Electrophoretic Light Scattering (DLS and ELS, respectively). Immortalized OSCC cell lines were used to assess the expression of CD44 by flow cytometry in the absence/presence of HA. The resazurin assay was used to evaluate cell metabolic activity.

Results: Pluronic L121®-PEI was successfully synthesized, demonstrated by the appearance of characteristic bands in FTIR and NMR spectra. The conjugation with HA was also detected. The different nanosystems presented a hydrodynamic diameter ranging between 100-200 nm with a polydispersity of ca. 0.200. The in vitro studies revealed that the expression of CD44 is cell type-dependent. The presence of HA can modulate the expression of CD44 receptors and can also impact cell metabolic activity.

Conclusions: Overall, these preliminary results indicated that HA could constitute a potential target ligand to CD44+ OSCC cells, and its conjugation with the synthesized nanosystem can benefit the development of new vectorized drug/gene delivery therapies for OSCC.

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20. The role of cell-cell adhesion in the survival of inflammatory breast cancer tumour emboli

Authors and Affiliations

MM Castro[1,2,3], C Esquível[1], TT Jesus[1,4], AS Ribeiro[1,4], B Sousa[1,4], C Jerónimo[3], J Paredes[1,2,4]

1. i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto

2. FMUP – Faculdade de Medicina da Universidade do Porto

3. CI-IPOP – Centro de Investigação do Instituto Português de Oncologia

4. IPATIMUP – Instituto de Patologia e Imunologia Molecular da Universidade do Porto

Presenting Author

Maria Miguel Castro

Abstract

Introduction: The expression of cell-cell adhesion molecules E- and P-cadherin (E-cad and P-cad, respectively), is a recognized biomarker of tumor cell emboli, which is a special feature of the most aggressive forms of advanced breast cancer, the IBC (Inflammatory Breast cancer). The main goal of this project is to understand if cell-cell adhesion and anoikis-resistance, mediated by the co-expression of both cadherins, can be a metastasis-promoter and a candidate to control survival and therapeutic resistance of matrix-detached IBC cells.

Materials and Methods: The silencing of E- and/or P-cadherin expression was performed through RNAi in two breast cancer cell lines with high levels of E- and P-cad, IBC SUM149PT and MDA-MB-468. Cell-cell adhesion and anoikis-resistance were evaluated by slow aggregation and mammosphere forming efficiency assays, respectively.

Results: We observed that the simultaneous silencing of both cadherins induced a statistically significant decrease cell-cell aggregation, in comparison to the control cells in SUM149PT ($p= 0.0173$) and a tendency in MDA-MB-468 cells. We also observed a decrease in MDA-MB-468 cell aggregation upon CDH1 silencing; moreover, no differences were found upon CDH3 silencing in both cell lines. Interestingly, we observed a decrease in anoikis-resistance of SUM149PT and MDA-MB-468 cells when we simultaneously silence the expression of both cadherins ($p=0.0286$); additionally, silencing CDH3 in MDA-MB-468 decreased anoikis-resistance, while no alterations were found in SUM149PT cells. No significant effect was found upon CDH1 silencing in both cell lines, in comparison to control cells.

Conclusion: In conclusion, the simultaneous silencing of both cadherins promotes a decrease on anoikis-resistance and aggregation.

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21. Concerted metabolic and transcriptomic rewiring dictates glioblastoma cells survival and stemness

Authors and Affiliations

Joana Peixoto[1,2,3], Sudha Janaki-Raman[3], Werner Schmitz[3], Susanne Walz[3], Céline S. Gonçalves[4,5], Bruno M. Costa[4,5], Paula Soares[1,2,6], Almut Schulze[3,7], Jorge Lima[1,2,6]

1. i3S – Instituto de Investigação e Inovação em Saúde, Porto, Portugal

2. Ipatimup – Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Porto, Portugal

3. Theodor-Boveri-Institute, Biocenter, Würzburg, Germany

4. ICVS – Life and Health Sciences Research Institute, Braga, Portugal

5. ICVS/3B's-PT Government Associate Laboratory, Braga, Portugal

6. Faculty of Medicine of the University of Porto, Porto, Portugal

7. German Cancer Research Center, Heidelberg, Germany

Presenting Author

Joana Peixoto

Abstract

Altered metabolic processes contribute to carcinogenesis by modulating proliferation, survival and differentiation. Tumours are composed of different cell populations, with cancer stem-like cells being one of the most prominent examples. These cells are thought to be responsible for cancer growth and recurrence and play a particularly relevant role in glioblastoma (GBM), the most lethal form of primary brain tumours.

Using GBM cells under neurosphere (NS) conditions, we have selected cells exhibiting anoikis resistance and self-renewal capacity with an upregulation of a stemness gene signature and downregulation of differentiation signatures, when compared with monolayer (ML) cultures.

Metabolic flux analysis with [U-13C6] glucose revealed increased lactate levels along with lower acetyl-CoA and citrate production in NS, suggesting an impairment of pyruvate dehydrogenase (PDH), a pivotal enzyme that connects glycolysis with the TCA cycle. Indeed, NS presented increased levels of the inactive form of PDH, along with higher glycolysis and lower respiration, compared with ML. Modulation of PDH activity by DCA treatment or by genetic manipulation of PDKs and PDP1, showed a higher sensitivity of NS towards PDH activation. Additionally, metabolomics analysis showed that the TCA cycle intermediate α-KG is reduced in NS. Exogenous α-KG supplementation or modulation of the glutamine pathway, the main fuel for α-KG synthesis, confirmed the deleterious effect of this metabolite on NS. Furthermore, RNAseq data showed specific gene regulation of PSAT1 and GPT1 in DCA-treated NS, linking PDH regulation and α-KG availability. Finally, α-KG levels may be regulating NF-kB pathway, by increasing p65 activation and upregulating inflammation-related signatures in NS, while not affecting ML cells.

Collectively, these results demonstrate that NS and ML differ in their metabolism, further suggesting that specific metabolites can control non-metabolic processes that are essential for cell survival and may regulate stemness.

22. Proteome alterations and chromatin remodeling as mechanisms of resistance to KRAS inhibition

Authors and Affiliations

F Martins[1,2,3]; PD Carvalho[2,3,4]; J Carvalho[2,3]; R Matthiesen[5]; S Velho[2,3]

1. FMUP – Faculdade de Medicina da Universidade do Porto, Doctoral Program in Biomedicine, Porto, Portugal
2. i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal
3. IPATIMUP - Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Porto, Portugal
4. ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Doctoral Program in Pathology and Molecular Genetics, Porto, Portugal
5. CEDOC - Chronic Diseases Research Centre, NOVA Medical School, Faculty of Medical Sciences, NOVA University of Lisbon, Lisboa, Portugal

Presenting Author

Flávia Martins

Abstract

Introduction: Phase I clinical trials to evaluate the therapeutic efficacy of KRAS-targeted inhibition yielded promising, yet far from ideal, responses, revealing that resistance mechanisms rapidly emerge to bypass KRAS loss. Therefore, we aim to understand, predict, and overcome KRAS-targeted therapy resistance.

Materials and Methods: KRAS expression was silenced through siRNAs in colorectal cancer (CRC) cell lines that carry KRAS mutations. Silenced cells or their control were cultured as spheroids. The proteome characterization was obtained by mass spectrometry and the expression of significantly altered proteins was validated by western-blot. Chromatin remodeling was investigated using electron microscopy.

Results: Our results using 3D CRC cell culture models indicate that, upon KRAS inhibition, spheroids size decreases. Proteomics analysis revealed that KRAS-inhibited persister cells upregulated several proteins associated with the extracellular exosome or nuclear compartments. Molecular function and biological process gene ontology terms revealed an up-regulation of proteins mainly associated with binding activities (RNA, protein, nucleosomal, and core promoter binding) as well as gene expression regulation, mRNA splicing and processing, and nucleosome assembly. In addition, proteomic analysis also revealed an upregulation of proteins involved in nucleosome assembly and repositioning such as core and variant histone, and an increase of proteins associated with active chromatin states. Additionally, several mRNA splicing related proteins were also upregulated in KRAS-silenced cells. Moreover, KRAS-silenced persister cells grown in 3D also presented a gain in some histone post-translational modifications and an increase in the percentage of euchromatin, both suggesting a more active state of gene transcription.

Conclusions: Overall, our results suggest a novel mechanism of resistance to KRAS targeted therapies that involve chromatin structural alterations and gene expression deregulation, which we are currently pursuing.

23. Effects of protein fractionation, thermal denaturation and lipids isolation from a human amniotic membrane extract in hepatocellular carcinoma cell lines

Authors and Affiliations

Ricardo Jorge Teixo[1,2,3,4], Mafalda Laranjo[1,2,3,4], Maria João Carvalho[1,2,3,4,5], Beatriz Serambeque[1,2,3,4], Ana Margarida Abrantes[1,2,3,4], Pedro Domingues[6], Maria Filomena Botelho[1,2,3,4]

1. Biophysics Institute, Faculty of Medicine, University of Coimbra, Coimbra, Portugal
2. Institute for Clinical and Biomedical Research (iCBR) area of Environmental Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, Coimbra, Portugal
3. Clinical Academic Center of Coimbra, Coimbra, Portugal
4. Center for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, Coimbra, Portugal
5. Gynecology Service, Coimbra Hospital and University Centre, Coimbra, Portugal
6. Centro de Espetrometria de Massa, Departamento de Química & QOPNA& LAQV-REQUIMTE, University of Aveiro, Aveiro, Portugal

Presenting Author

Beatriz Serambeque

Abstract

Introduction: Hepatocellular carcinoma (HCC) is most common liver cancer and presents poor prognosis. Human amniotic membrane (hAM) presents interesting anti-tumor properties, such as anti-angiogenic and pro-apoptotic activity. Our team previously showed that total human amniotic membrane extract (hAME) leads to decreased viability and increased cell death on HCC cells. We aimed to assess effects of hAME fractions (protein/lipidic) and thermal denaturation on HCC cells.

Materials and Methods: We previously verified that hAME comprises a complex protein mixture. Thus, fractionation was performed considering solubility, through ammonium sulphate (AS) precipitation. Fractionation was performed through ammonium sulphate (AS) precipitation by sequentially adding 10%, 25% and 50% AS to hAME, on ice, 15min, centrifuged at 14000 G, 15 min. Precipitated fractions (10P/25P/50P) were resuspended on PBS; soluble fractions (10S/25S/50S) were submitted to salting out with PBS by centrifugation (4000G, 60 min), on VivaSpin® tubes (30 kDa cutoff). Lipid isolation was performed by Folch method (chloroform:methanol (2:1), two extraction steps and solvent evaporation on vacuum). hAME thermal denaturation was performed at 100°C, 5min. HepG2, Hep3B and HuH7.sil cells were incubated with total hAME, fractions, thermal denaturated hAME and isolated lipids (1µg/µL), for 72h. Metabolic activity was accessed by MTT assay.

Results: Our results demonstrated that hAME induced a decrease on metabolic activity on HCC cells, compared to control (HepG2: 57.07±9.26; Hep3B: 50.66±9.78; HuH7.sil: 58.16±6.05). Incubation with fractions from AS precipitation induced a lower metabolic activity compared to total hAME. Values of metabolic activity varied from 2 to 30%, depending on HCC cell line. Metabolic activity of HCC cells incubated to isolated lipids decreased compared to control and total hAME. Thermal denatured induced a partial inhibition of total hAME effects.

Conclusions: These findings indicate that both proteins and lipids could have an important role on hAME induced anti-tumor activity.

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24. DNA methylation biomarkers to residual disease detection in esophageal cancer upon neoadjuvant chemoradiation

Authors and Affiliations

Catarina Macedo-Silva[*1]; Vera Constâncio[1, 2]; Vera Miranda-Gonçalves[1,5]; Pedro Leite-Silva[1]; Sofia Salta[1]; João Lobo[1,3,5]; Rita Guimarães[1,3]; Carina Carvalho-Maia[1]; Davide Gigliano[1, 3]; Mónica Farinha[1, 3]; Olga Sousa[4]; Rui Henrique[1,3,5]; Carmen Jerónimo[1,5]

1. Cancer Biology & Epigenetics Group, Research Center of IPO Porto (CI-IPOP) / RISE@CI-IPOP (Health Research Network), Portuguese Oncology Institute of Porto (IPO-Porto) / Porto Comprehensive Cancer Centre (PCCC), R. Dr. António Bernardino de Almeida, 4200-072, Porto, Portugal;
2. Doctoral Programme in Biomedical Sciences, School of Medicine & Biomedical Sciences, University of Porto (ICBAS-UP), Rua Jorge Viterbo Ferreira 228, 4050-513, Porto, Portugal
3. Department of Pathology, Portuguese Oncology Institute of Porto, Porto, Portugal;
4. Department of Radiation Oncology, Portuguese Oncology Institute of Porto, Porto, Portugal;
5. Department of Pathology and Molecular Immunology, School of Medicine & Biomedical Sciences, University of Porto (ICBAS-UP), Porto, Portugal

*. correspondence: anacatarina.macedodasilva@unicampania.it / ana.catarina.macedo.silva@ipoporto.min-saude.pt

Presenting Author

Catarina Macedo-Silva

Abstract

Introduction: Esophageal cancer (ECa) is a malignancy with a considerably high mortality. Unfortunately, most of ECa patients are late diagnosed with locally advance disease and not eligible for surgery. Regardless of histological subtype, neoadjuvant chemoradiation (ChRT) is a common procedure recommended by the guidelines. The lack of reliable biomarkers that clearly detect post-ChRT disease and distinguish those with complete response is a major disadvantage. Remarkably, miRNA promotor methylation is a novel approach as detection biomarkers in several cancer models being potential candidates in tissue and plasma detection. Moreover, little has been explored on this topic for ECa model.

Materials and methods: With this work we sought to unveil novel candidate biomarkers as well as strengthen our previously work to properly be able to detect ECa disease prior and post ChRT. Herein, promoter methylation of miR129-2, miR124-3 and ZNF569 was assessed by quantitative methylation-specific PCR (qMSP) in a series of tissue and plasma of ECa naïve and normal esophagus, as well as in post-ChRT tissue samples.

Results: All genes significantly distinguished ECa from normal esophagus and accurately detect post-ChRT minimal residual disease with best biomarker performance for adenocarcinoma subtype. Remarkably, miR129-2me/ZNF569me methylation panel identified ECa in liquid biopsies with 53% of sensitivity and 87% of specificity. Overall, this study provides strong evidence that miR129-2me, miR124-3me and ZNF569me are good candidate biomarkers for prior and post ChRT ECa detection.

Conclusions: Our findings indicate circulating methylated miRNAs as promising minimal invasive strategy to detect ECa disease.

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25. Characterization of a 3D rectal cancer model

Authors and Affiliations

Estêvão D.[1,2]; Ribeiro M.[1,2] ; Sousa O.[3]; De Wever O.[4]; Oliveira MJ.[1,2,5], Cruz T.[1]

1. i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

2. ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal.

3. IPO-Porto – Instituto Português de Oncologia do Porto, Porto, Portugal

4. Laboratory of Experimental Cancer Research, Department of Human Structure and Repair, Ghent University, Ghent, Belgium

5. Departamento de Patologia, Faculdade de Medicina, Universidade do Porto, Porto, Portugal.

Presenting Author

Estêvão D.

Abstract

Most colorectal cancer studies are not only focused exclusively on the colon section, disregarding the rectum, but also on 2D, completely ignoring cell-to-cell interaction, in vivo tissue architecture and microenvironment. To address this void in cancer research, we developed a biomimetic 3D rectal cancer spheroid that can be used as a tool for rectal cancer studies, cultivated in different cell culture media, and generated with different cell-seeding numbers. The 3D rectal cancer spheroid model was characterized for their tumour features, namely 3D dynamics and morphology, metabolic and viability properties, as well as proliferative and necrotic areas. The influence of T lymphocytes and macrophages as well as of ionizing radiation, mimicking the standard one-week treatment of rectal cancer patients, was also used to validate the usefulness of the model in studying radiotherapy and resistance mechanisms. The 3D rectal cancer spheroid model is clearly influenced by the different cell culture media and different cell-seeding numbers. The overall dynamics and morphology as well as the metabolic and proliferative activity change according to the culture conditions. Additionally, the 3D rectal cancer model is characterized by a proliferative region and a well-formed necrotic area, especially in larger spheroids cultivated in a poorer cell culture media. These tumour morphological features were accentuated after ionizing radiation treatment and affected by T lymphocytes and macrophages. This is the first detailed characterization of a 3D rectal cancer model that can not only be used to expand drug screening studies but also metabolic, radioresistance, and therapeutic responses in rectal cancer.

26. Tribbles gene expression in glioblastoma brain tumors

Authors and Affiliations

Ana Luísa De Sousa-Coelho[1,2,3], Wolfgang Link[4], Bibiana I. Ferreira[1,2,5]

1. Algarve Biomedical Center Research Institute (ABC-RI), Universidade do Algarve, Campus de Gambelas, Faro, Portugal.

2. Algarve Biomedical Center (ABC), Universidade do Algarve, Campus de Gambelas, Faro, Portugal.

3. Escola Superior de Saúde (ESS), Universidade do Algarve, Campus de Gambelas, Faro, Portugal.

4. Instituto de Investigaciones Biomédicas "Alberto Sols" (CSIC-UAM), Madrid, Spain

5. Faculdade de Medicina e Ciências Biomédicas (FMCB), Universidade do Algarve, Campus de Gambelas, Faro, Portugal.

Presenting Author

Ana Luísa De Sousa-Coelho

Abstract

Glioblastomas (GBM) are very aggressive brain tumors, which are among the most deadly and incurable types of human cancer. GBM display considerable heterogeneity and are greatly resistant to the standard treatments currently available. GBM that harbor isocitrate dehydrogenase (IDH) mutations, have a better prognosis than IDH wild type GBM. Recurrent H3F3A mutations affecting two critical positions of histone H3.3 (K27, G34) can be found in pediatric GBM, and are mutually exclusive with IDH1 mutation. Tribbles homolog 1, 2 and 3 (TRIB1, TRIB2 and TRIB3, respectively) belong to the conserved mammalian Tribbles family of pseudokinases proteins. Recently, TRIB2 was recognized as one of the genes that was most correlated with pathological classification and therapeutic resistance in GBM, while TRIB3 was identified to facilitate GBM progression via limiting autophagy.

To further dissect the transcriptomic profiles of glioblastoma samples, we evaluated the relative gene expression of TRIB1, TRIB2 and TRIB3, in a subset of 40 samples with a defined status of IDH1 and H3F3A mutations, obtained from GEO profiles (GDS4470/GSE36245). The statistical analysis was performed at GraphPad Prism. Outliers identified by the ROUT method were removed.

From the selected gene data set of GBM brain tumors, we found higher transcript levels of both TRIB2 and TRIB3 in samples with wild type vs mutated IDH1 (1.9-fold, $p=0.011$ and 1.6-fold, $p=0.029$, respectively). As anticipated, GBM with H3F3A mutations were from younger patients. Although Tribbles gene expression levels were not different in H3F3A mutated samples vs H3F3A wild-type samples (all IDH1 wild type), and there were no differences in the age of the patients based on the IDH1 status, the levels of TRIB1, TRIB2 and TRIB3 were higher in patients younger than 18 years old ($p<0.05$).

Tribbles pseudokinases might be involved in the development and aggressiveness of GBM, which should be further investigated.

27. Helicobacter pylori activates Laminin γ2 promoting survival and invasion of gastric epithelial cells with E-cadherin impairment

Authors and Affiliations

RM Ferreira[1,2#], J Figueiredo[1,2#], I Pinto-Ribeiro[1,2], I Gullo[1,2,5], DN Sgouras[3], L Carreto4, P Castro[1,2], MA Santos[4], F Carneiro[1,2,5], R Seruca[1,2,5] and C Figueiredo[1,2,5]

1. i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

2. IPATIMUP – Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Porto, Portugal

3. Laboratory of Medical Microbiology, Hellenic Pasteur Institute, Athens, Greece

4. Department of Biology and CESAM, University of Aveiro, Aveiro, Portugal

5. Department of Pathology, Faculty of Medicine of the University of Porto, Porto, Portugal

#. These authors contributed equally to this work.

Presenting Author

Rui Ferreira

Abstract

Introduction: Helicobacter pylori promotes cell motility and invasion in epithelial cells, which are relevant phenotypes for cancer progression. Herein, we test whether extracellular matrix (ECM) could be involved in deleterious effects mediated by H. pylori.

Materials and Methods: Microarray analysis was used to uncover ECM interactors in cells infected with H. pylori. LAMC2 was selected as a candidate gene and its altered expression was assessed in vitro and in vivo. The role of LAMC2 was investigated by siRNA combined with TUNEL and matrigel assays. Laminin γ2 and E-cadherin were evaluated by immunohistochemistry in gastric cancer cases.

Results: We identified Laminin γ2 as an ECM component significantly overexpressed in H. pylori infected gastric cancer cells. This result was validated in vitro by infection of different gastric cell lines with H. pylori clinical isolates, and in vivo by using human gastric biopsies of infected and non-infected individuals. In addition, we showed that Laminin γ2 overexpression is dependent on the H. pylori type IV secretion system and on the CagA oncoprotein. H. pylori-induced Laminin γ2 promotes cell invasion and resistance to apoptosis through modulation of Src, JNK and AKT activity. Importantly, these effects were impaired in infected cells containing a functional E-cadherin.

Conclusion: H. pylori CagA-positive strains support survival and invasion of cancer cells with E-cadherin impairment in a mechanism implicating Laminin γ2 expression and deposition. Our results highlight the importance of H. pylori eradication in carriers of E-cadherin alterations as a preventive strategy aiming to delay gastric cancer onset and progression.

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28. Assessing METTL3 inhibitor efficacy in Renal Cell Carcinoma

Authors and Affiliations

Catarina Guimarães-Teixeira[1], Daniela Barros-Silva[1], Yaara Ofir-Rosenfeld[2], Carmen Jerónimo[1,3]

1. Cancer Biology and Epigenetics Group, Research Center of IPO Porto (CI-IPOP) / RISE@CI-IPOP (Health Research Network), Portuguese Oncology Institute of Porto (IPO Porto) / Porto Comprehensive Cancer Center (Porto.CCC), Portugal
2. Storm Therapeutics, Cambridge, United Kingdom
3. Department of Pathology and Molecular Immunology, Institute of Biomedical Sciences Abel Salazar, University of Porto (ICBAS-UP), Porto, Portugal

Presenting Author

Catarina Guimarães-Teixeira

Abstract

Introduction: N6-methyladenosine (m6A) is the most prevalent mRNA modification catalyzed predominantly by the methyltransferase complex METTL3/METTL14. STORM therapeutics Lda. recently identified novel first-in-class inhibitors of METTL3 that show pronounced anti-tumor efficacy in animal models of Acute Myeloid Leukemia (AML). Nevertheless, the effectiveness of those compounds in solid tumors is yet poorly explored. Remarkably, transcripts containing differential m6A sites were enriched in kidney cancer-related signaling pathways. Thus, we hypothesized that inhibition of METTL3 in Renal Cell Carcinoma (RCC) with consequent m6A depletion may represent a promising approach for anticancer therapy.

Materials and Methods: METTL3/14 proteins expression were evaluated in a panel of RCC cells (769-P, 786-O, CAKI1, CAKI2 and ACHN) in comparison with a normal kidney cell line (HK2). Then, RCC cells were treated for 6 days with 0.5-100 μM of 3 different METTL3 inhibitors, kindly provided by STORM.

Results: METTL3/14 protein expression was higher in RCC cell lines, specifically in 786-O and ACHN, when compared to normal kidney cells. During the 6 days of treatment, the effect of the m6A level depletion by at least one of the 3 tested METTL3 inhibitors impacted in RCC cells' growth inhibition (IC50 = 2-9 μM). For all tested cells, the reduced viability in presence of the best tested drug is in line with increased cell apoptosis after 6 days of incubation, as evaluated by Annexin-V staining.

Conclusion: Our results suggest that pharmacological METTL3 targeting is a potential therapeutic strategy for RCC and prove that RNA modifying enzymes represents a promising new anti-cancer therapy.

29. A new aromatase inhibitor (AI) as multi-target compound in estrogen receptor-positive (ER+) breast cancer cells

Authors and Affiliations

Cristina Amaral[1,2], Cristina F. Almeida[1,2], Tiago Augusto[1,2], Carla Varela[3,4], Fernanda F.M.F Roleira[3,4], Elisiário Tavares-da-Silva[3,4], Georgina Correia-da-Silva[1,2], Natércia Teixeira[1,2]

1. UCIBIO.REQUIMTE, Laboratory of Biochemistry, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal;
2. Associate Laboratory i4HB - Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Porto, Portugal;
3. Laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal;
4. CIEPQPF Centre for Chemical Processes Engineering and Forest Products, University of Coimbra, Coimbra, Portugal.

Presenting Author

Cristina Amaral

Abstract

Introduction: Around 70-85% of all breast cancer (BC) cases are estrogen receptor-positive (ER+). The third-generation of aromatase inhibitors (AIs) are the first-line treatment option for these tumors. Despite their therapeutic success, they can induce several side effects and resistance, which limits their efficacy. Thus, it is crucial the search for novel and more potent therapeutic molecules. Currently, multi-target drugs are emerging, as they present higher efficacy and lower toxicity in comparison to standard options. In this work, it will be investigated the anti-cancer properties and their multi-target potential of the compound 1α,2α-epoxy-6-methylenandrost-4-ene-3,17-dione (3), a molecule that we previously demonstrated to be a potent AI in ER+ BC cells.

Material and Methods: The ER+ BC cell line that overexpresses aromatase, MCF-7aro cells, was used. Cell viability/proliferation were studied by MTT and 3H-thymidine incorporation assays, respectively and apoptosis by the analysis of caspase-7/-8/-9 activities (luminescent assays). The involvement of ERα and androgen receptor (AR) was explored, at protein and gene level, using qPCR and Western-blot and by evaluating the transcription of ER-responsive genes.

Results: AI 3 reduced cell viability, impaired DNA synthesis and induced apoptosis of MCF-7aro cells. Moreover, it decreased ERα expression, inhibited ERα activation and induced AR overexpression with a pro-death effect.

Conclusion: Our work highlights the discovery of a new and promising multi-target compound, that besides acting as an AI also modulates ERα and AR, decreasing ER+ BC proliferation. This may represent a therapeutic advantage over other molecules used in BC treatment.

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30. Antitumor activity evaluation of new synthetic 1,2-epoxide steroids in lung and liver cancer

Authors and Affiliations

Ana R. Gomes[1,2], Saúl C. Costa[3], Ana M. Abrantes[2,4,5], Carla L. Varela[4,5,6], Maria F. Botelho[2,4,5], Fernanda M.F. Roleira[1], Elisiário J. Tavares-da-Silva[1], Ana S. Pires[2,4,5]

1. Univ Coimbra, CIEPQPF, Faculty of Pharmacy, Laboratory of Pharmaceutical Chemistry, Coimbra, Portugal
2. Univ Coimbra, Coimbra Institute for Clinical and Biomedical Research (iCBR) area of Environment Genetics and Oncobiology (CIMAGO), Biophysics Institute of Faculty of Medicine, Coimbra, Portugal
3. Univ Coimbra, Faculty of Pharmacy, Laboratory of Pharmaceutical Chemistry, Coimbra, Portugal
4. Univ Coimbra, Center for Innovative Biomedicine and Biotechnology (CIBB), Coimbra, Portugal
5. Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal
6. Univ Coimbra, CIEPQPF, Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, Coimbra, Portugal

Presenting Author

Ana R. Gomes

Abstract

Cancer incidence and mortality rates have been promptly increasing worldwide, being that liver and lung cancer are currently amongst the deadliest cancers, which points out the need for new emerging therapies. Steroidal compounds are one of the most diversified therapeutic classes of compounds and they were proven to be efficient against several types of cancer. The epoxide function has been frequently associated with anticancer activity. Moreover, previous studies by our group demonstrated that 1,2-epoxides are very potent against several types of cancer cell lines. Thus, we combine this chemical function with the steroidal backbone, by synthesizing steroidal epoxides and evaluating their potential antitumor activity against liver and lung cancer cells, ultimately to find new antitumor agents with fewer side effects.

The compounds 1α,2α,4β,5β-diepoxyandrostane-3,17-dione (EP2) and 1α,2α-epoxyandrosta-4,6-diene-3,17-dione (EP3) were synthesized and their cytotoxicity evaluated in liver and lung cancer cell lines (HepG2 and H1299, respectively). To assess cell proliferation, SRB assay was performed after treating cancer cells with the synthesized compounds (1-75 μM).

Preliminary results suggest that both compounds successfully decreased H1299 and HepG2 proliferation in a dose-dependent manner. EP2 was the most active compound in both cell lines with an IC50 of 2.8 μM in H1299 and 3.7 μM in HepG2 cells. EP3 was also quite active in both cell lines (IC50≈12.75 and 14 μM in H1299 and HepG2, respectively). On the contrary, the parent compounds failed to decrease proliferation of both cancer cell lines, which shows that the 1,2-epoxide function seems to be important for the cytotoxicity observed.

These preliminary results suggest that both EP2 and EP3 might have an antitumor effect, which encourages further studies.

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31. Microbiota-derived short-chain fatty acids as new potential therapeutic agents against colorectal cancer

Authors and Affiliations

Sara Gomes[1,2,3,4], Fátima Baltazar[3,5], Elisabete Silva[2], Ana Preto[1,4*]

1. Center of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Braga, Portugal

2. Department of Life Sciences, Brunel University (BU), London, United Kingdom

3. Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal

4. IBS - Institute of Science and Innovation for Bio-Sustainability, Braga, Portugal

5. ICVS/3B's-PT Government Associate Laboratory, Braga/Guimarães, Portugal

*. Correspondence to: apreto@bio.uminho.pt

Presenting Author

Sara Gomes

Abstract

Diet is a significant risk factor for colorectal cancer (CRC), being responsible for the majority of cancer-related deaths worldwide. Specific dietary habits are able to modulate the colon microbiota composition which interfere with the production of short-chain fatty acids (SCFA), namely, acetate, butyrate, and propionate. CRC patients have been associated with a gut dysbiosis profile and a decrease in SCFA concentration. Colonocytes are exposed to these three SCFA simultaneously, and, despite all the reported effects of each SCFA individually, to our knowledge, their combined effect is still unknown. Our aim was to study the effects of each SCFA alone or in combination, at physiological molar ratio, to unveil their biological impact on CRC cells phenotype.

We used a mathematical model for the calculation and prediction of the expected SCFA mixture effects and found that SCFA, when in mixture, exhibit a concentration addition behaviour. Our results indicate that all SCFA, alone or in mixture, show an anticancer effect by inhibiting colony formation and cell proliferation, increasing apoptosis, disturbing the energetic metabolism, inducing lysosomal membrane permeabilization and decreasing cytosolic pH.

Here we show for the first time, that SCFA act more specifically on colon cancer cells, showing promising therapeutic effects against CRC cells. Our results suggest that the modulation of the SCFA concentrations in the colon through specific diet-derived microbiota strategies could constitute a new road for the development of alternatives for CRC therapy.

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32. Establishing a veterinary oncology network to improve knowledge of the role of companion animals in comparative oncology. Vet-OncoNet, a pioneering Portuguese approach

Authors and Affiliations

Katia Pinello[1,2,3,*], Paulo Tiago Carvalho[1], Ana Filipa Castro[1], and João Niza-Ribeiro[1,2,3]

1. Vet-OncoNet, Departamento de Estudo de Populações, ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal

2. EPIUnit—Instituto de Saúde Pública, Universidade do Porto, Porto, Portugal

3. Laboratório para a Investigação Integrativa e Translacional em Saúde Populacional (ITR), Porto, Portugal

Presenting Author

João Niza-Ribeiro

Abstract

Study of cancer burden of animals enhances the translational value of domestic animals in comparative oncology. Only with accurate cancer surveillance in animals is possible to produce relevant scientific evidence disclosing the translational role of companion animals in comparative studies. In this context, Vet-OncoNet network was launched in December 2019 by ICBAS, University of Porto. Inspired by the One Health vision, Vet-OncoNet uses business intelligence tools to optimize the process of collecting, treating, and reporting animal cancer data in Portugal through three interfaces: ACR (Animal Cancer Registry, pathology-based), COR (Clinical Oncology Registry, veterinary practice-based) and RFR (Risk Factor Registry, owner-based).

ACR includes animal cancer diagnostic submitted by 6 (in eight) national veterinary laboratories between January 2019 and April 2022. Each dataset is classified using the Vet-ICD-O-canine-1 classification system, the canine counterpart of the human ICD-O-3.2 classification and topographies are grouped after the Regional Cancer Registry (ROR), to ensure comparability with the human registry. The dataset includes benign and malignant tumors. Data from the National Companion Animal Registration System (SIAC) are used as the denominator for incidence estimation.

ACR has reached the milestone of 20,000 national cancer records: 80.1% are dogs and 19% cats; 54.6% are females and 44.8% live in Lisbon: Cats are 6 times more likely to have malignant tumors than dogs. In the Porto district, the estimated annual cumulative incidence risk (Ire), calculated as "tumors cases /100,000 animals" is 373 for dogs (females 444; males 262) and 198 for cats (females 240; males 152). The IRe for mammary gland tumors is 163 for female dogs and 134 for female cats. Women's crude incidence rate in Porto region is 139 (RON, 2018).

This study represents the continuous animal cancer registration system in Portugal, which the authors expect to contribute positively to research on comparative oncology.

33. The dual role of tumour-associated macrophages in anaplastic thyroid cancer

Authors and Affiliations

Ricardo Rodrigues[1], Ana Pinto[1,2], Marta Pojo[1], Diana Pacheco Sousa[1], Ana Sofia Carvalho[3], Hans C. Beck[4], Rune Matthiesen[3], Carolina Pires[1], Joana Simões Pereira[1,5], Valeriano Leite[1,5], Branca Maria Cavaco[1]

4. Unidade de Investigação em Patobiologia Molecular (UIPM), Instituto Português de Oncologia de Lisboa Francisco Gentil (IPOLFG), Lisboa, Portugal
5. Instituto de Biomedicina (iBiMED), Universidade de Aveiro, Aveiro, Portugal
6. Centro de Estudo de Doenças Crónicas, Faculdade de Ciências Médicas da Universidade NOVA de Lisboa
7. Centre for Clinical Proteomics, Department of Clinical Biochemistry and Pharmacology, Odense University Hospital, DK-5000 Odense, Denmark
8. Serviço de Endocrinologia, IPOLFG, Lisboa, Portugal.

Presenting Author

Ricardo Rodrigues

Abstract

Introduction: Anaplastic thyroid cancer (ATC) is a rapidly growing, highly dedifferentiated and invasive tumour, with high metastatic potential, usually unresponsive to conventional treatments, being the most aggressive thyroid cancer subtype. ATCs are characterized by intense tumour-associated macrophage (TAM) infiltration. However, the role of these immune cells in ATC aggressiveness remains poorly defined.

Aim: To investigate the role of TAMs in ATC's aggressiveness and associated molecular mechanisms.

Methodology: Transwell co-cultures between macrophages (THP-1) and 4 ATC cell lines (T235, T238, C3948, C643) were established. ATC cells' migration, invasion, actin cytoskeleton, proteome profile, as well as macrophage markers expression were assessed. After proteomic data validation by Western blot, siRNA assays were performed. Co-cultures were compared with the respective monocultures.

Results: Invasion and migration of T235 and C643 cells increased in co-culture with macrophages compared with monoculture, contrasting with a decrease in C3948, which was corroborated by actin cytoskeleton alterations. Flow cytometry showed CD80 and CD163 upregulation in THP-1 co-cultured with T235 cells and downregulation when co-cultured with C3948. Western blot validated that SPRY4 (sprouty RTK signaling antagonist 4), an inhibitor of MAPK (mitogen-activated protein kinase) signaling pathway, was downregulated in T235 co-cultures and upregulated in C3948 co-cultures, confirming proteomic data. SPRY4 silencing increased C3948 and T235 invasion in mono- and co-cultures, which was confirmed by cytoskeletal alterations.

Conclusions: TAMs appear to have a crucial role in ATC cells migration and invasion. Reciprocally, ATC altered TAM markers' expression. SPRY4 was identified as a possible modulator of ATC-TAM crosstalk, with a tumour suppressor role.

34. In silico approach to identify Brachyury target genes in prostate cancer

Authors and Affiliations

Mendes, A.[a,b,c], González, R. D.[a,b,d,e], Fontão, P.[a,b], Martinho, O.[a,b], Reis, R. M.[a,b,f]

a. Instituto de Investigação em Ciências da Vida e Saúde. Escola de Medicina da Universidade do Minho. Braga, Portugal;

b. Laboratório Associado ICVS/3B's. Universidade do Minho, Braga, Portugal;

c. Stemmatters, Biotecnologia e Medicina Regenerativa S.A, Guimarães, Portugal;

d. Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Portugal;

e. Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal;

f. Centro de Pesquisa em Oncologia Molecular, Hospital do Cancro de Barretos, Barretos. SP, Brasil.

Presenting Author

Mendes, A.

Abstract

Introduction: Prostate cancer(PCa) is the second most frequently diagnosed neoplasia and the sixth cause of cancer mortality. Androgen deprivation therapy has been the gold standard of care for advanced PCa. Initially, this treatment benefits, but eventually, the tumor recurs as castration-resistant prostate cancer, which has restricted treatment options and poor survival benefits. In previous studies, our group reported the role of Brachyury (TBXT), a T-box transcription factor, as a new independent and important biomarker of poor prognosis and metastization. We also found that Brachyury is a molecular driver of the major mechanisms of prostate tumor therapy resistance, inducing resistance to chemotherapy in vitro. Thus, a better understanding of these driving resistance mechanisms is crucial to developing more effective therapeutics and potentially the discovery of prognostic and therapeutic biomarkers. Therefore, we aim to unveil Brachyury target genes in prostate cancer using an in silico approach, with the prospect to identify novel biomarkers for potentially therapeutic prediction in PCa patients using liquid biopsies.

Materials and methods: Cancer data available in user-friendly portals and online databases were used to study Brachyury expression association in prostate cancer. Most of the tools were built based on The Cancer Genome Atlas (TCGA) dataset.

Results: Brachyury was found significantly overexpressed in carcinomas and metastasis in different CBioPortal datasets. Regarding STRING analysis, Brachyury directly interacts with FOXA1/2, NKX2-5, and WNT3A genes, but not with PDE10A and NKX2-6. Additionally, it was observed that the probability of Brachyury and AR proteins interacting is 59,8 %, which supports the previous findings reported by the group. Structural analysis of both proteins also corroborates this observation.

Conclusion: Identifying potential target genes of Brachyury contributes to a step forward in establishing new prostate cancer biomarkers.

35. Can anesthetics influence the therapeutic response of breast and urological cancers? An in vitro study

Authors and Affiliations

Ana Catarina Matos[1,2,3], Inês Alexandra Marques[1,2,3], Ana Salomé Pires[1,3,4], Ana Valentim[4,5], Ana Margarida Abrantes[1,3,4] and Maria Filomena Botelho[1,3,4]

1. Coimbra Institute for Clinical and Biomedical Research (iCBR), Area of Environment, Genetics and Oncobiology (CIMAGO), Institute of Biophysics, Faculty of Medicine, University of Coimbra, 3000-548 Coimbra, Portugal;
2. Faculty of Pharmacy, University of Coimbra, 3000-548 Coimbra, Portugal;
3. Center for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, 3000-548 Coimbra, Portugal;
4. Clinical Academic Center of Coimbra (CACC), 3004-561 Coimbra, Portugal;
5. Anaesthesiology Service, Centro Hospitalar e Universitário de Coimbra (CHUC), 3004-561 Coimbra, Portugal.

Presenting Author

Inês Alexandra Marques

Abstract

Introduction: Local anesthetics (LAs) such as lidocaine (Lid), ropivacaine (Rop) and levobupivacaine (Lev) are used during cancer treatment approaches and as adjuvants in pain control. Studies report that these drugs present anticancer effects or can act in synergy with chemotherapy. Thus, this study aims to evaluate the potential anticancer effect of these LAs alone or in combination with chemotherapy.

Materials and Methods: Cell metabolic activity of breast (BC, MCF-7), bladder (bCa, TCCSUP and HT1376) and prostate (PCa, LNCaP and PC3) cancer cells and in non-tumor breast, prostate, and fibroblasts cell lines (MCF12A, RWPE-1 and HaCaT) was determined by MTT assay after 48h incubation with Lid (1-10mM), Rop (0.01-1mM), Lev (0.01-1mM) or Docetaxel (DTX, 0.01-250nM). These results allow to assess cellular cytotoxicity by tracing dose-response curves to obtain the respective IC50 values (half-maximal inhibitory concentration). In combination studies, PC3 cells were treated with the IC50 value of each LA, and concomitantly with the same dose range of DTX used previously, in order to draw a new dose-response curve.

Results: In general, LAs alone led to a decrease in cell metabolic activity of BC, bCa and PCa cells compared to normal cell lines, suggesting the presence of a selective cytotoxic effect. Higher evidence was shown for Lev in MCF-7 cells (IC50≈0.18 mM) and lower evidence was shown for Rop in LNCaP cells (IC50<1 mM). All the combinations of LA+DTX48h in PC3 cells resulted in lower cell metabolic activities compared to the cells treated only with DTX.

Conclusions: Overall, results showed a selective cytotoxic effect of LAs against BC, bCa and PCa cells, which could influence its application in specific clinical settings. Albeit preliminary, Lev alone appears to be the most promising LA and drug combination results suggest the potential use of LAs to promote synergistic therapeutic effects in PCa during DTX treatment. The combinatory effects, possible synergy and combination index will be further determined for other tumor types.

36. Differential SETD7 expression correlates with survival, immune score and response to therapy in specific molecular subtypes

Authors and Affiliations

Fátima Liliana Monteiro[1], Lina Stepanauskaite[2], Cecilia Williams[2,#] and Luisa A. Helguero[1,#]

1. Department of Medical Sciences, Institute of Biomedicine—iBiMED, University of Aveiro, Aveiro, Portugal;
2. SciLifeLab, Department of Protein Science, KTH Royal Institute of Technology, Stockholm, Sweden

#. Co-senior authors

Presenting Author

Fátima Liliana Monteiro

Abstract

Introduction: SETD7 is a lysine N-methyltransferase which methylates several proteins important in breast cancer (BC). However, the role and clinical significance of SETD7 in BC is still unclear. The aim was to explore SETD7 expression in human samples using public datasets to assess its association with BC outcomes, clinical factors and cancer-related bioprocesses.

Materials and methods: KM-Plotter, GEO and cBioportal were used to retrieve and analyze all gene chip, RNA-seq and proteomics datasets. Additionally, the TCGA-BRCA data was analyzed to identify the significantly overrepresented biological processes and pathways associated with SETD7 differential expression. The analysis divided two groups based on SETD7 mRNA upper and lower quartile (SETD7-H and SETD7-L, respectively).

Results: SETD7 mRNA was highest in HER2+ and Luminal A and lowest in Basal subtype. When grouped by subtypes, SETD7-H correlated with worse RFS for Basal subtype ($p< 0.009$). The SETD7-H group had significantly higher ERBB2 mutation (20% event frequency vs 5% in SETD7-L). In all BC subtypes pooled and in Luminal A, significantly higher stromal scores and lower immune scores in SETD7-H (based on mRNA and protein) were related with higher number of cancer-associated fibroblasts but lower B and T cell signatures. The genes up in SETD7-H and SETD7-L were overrepresented in several immune responses processes. All immune genes in SETD7-H were unique to Basal and Luminal B subtypes, and in SETD7-L were found in Luminal A and HER2 subtypes. Interestingly, the SETD7-H in HER2+ subtype did not respond well to anti-HER2 therapy.

Conclusions: SETD7 clinical potential must be evaluated in the molecular subtype context and its expression appears strongly associated with the tumor stromal and immune signatures. High SETD7 may be predictive of bad prognosis in basal subtype and may have a predictive value for HER2+ subtype since its expression is associated with ERBB2 mutation and less response to anti-HER2 therapy.

37. The interplay between KRAS/Galectin-3/ Tumor-associated macrophages in colorectal carcinogenesis

Authors and Affiliations

F Pereira[1,2,3,4], T Cruz[3,4], CA Reis[3,5,6], MJ Oliveira[3,4,5*], A Preto[1,2*]

1. Centre of Molecular and Environmental Biology (CBMA), Braga, Portugal;
2. Institute of Science and Innovation for Bio-Sustainability (IB-S), Braga, Portugal;
3. Institute for Research and Innovation in Health (i3S), Porto, Portugal;
4. Institute of Biomedical Engineering (INEB), Porto, Portugal;
5. Institute of Biomedical Sciences Abel Salazar (ICBAS), Porto, Portugal;
6. Institute of Molecular Pathology and Immunology from University of Porto (IPATIMUP), Porto, Portugal.

*. These senior authors contributed equally to this work

Presenting Author

F Pereira

Abstract

KRAS mutations (KRASmut) are present in 30%-50% of colorectal cancer (CRC) cases, contributing to CRC cell survival, increased tumor aggressiveness and resistance to anti-EGFR therapies. Galectin-3 (Gal-3) is a multifunctional protein also important in CRC and correlated with cancer cell invasion, metastasis and angiogenesis. Gal-3 seems to interact and affect KRAS expression levels. Furthermore, Gal-3 secreted by cancer cells is also capable of influencing tumor-associated macrophages (TAMs), which are important players at the tumor microenvironment (TME). On its turn, our group has demonstrated that TAMs stimulate CRC cell motility and invasion.

To the best of our knowledge, the role of KRAS on Gal-3 expression and function in CRC is not fully understood. Since KRAS seems to interfere with Gal-3, which is known to regulate TAMs, here, we aim to explore the interplay KRASmut/Gal-3/TAMs and uncover their impact in colorectal carcinogenesis.

For this purpose, co-immunoprecipitation and co-localization studies were performed and demonstrated that KRAS/Gal-3 physically interact and form a complex in CRC cells. Moreover, KRAS/Gal-3 exhibits a feedback loop regulation, being Gal-3 able to interfere with KRAS expression, as also KRAS affects Gal-3 expression levels. Additionally, we also found that the disruption of the KRAS/Gal-3 complex significantly decreases CRC cell migration and invasion. Further, we established co-cultures of CRC cells with human macrophages isolated from healthy blood donor buffy coats aiming to evaluate the impact of co-cultures on macrophage polarization and on CRC cell immunophenotype.

In summary, our results appoint KRAS and Gal-3 as relevant players in colorectal carcinogenesis. Additionally, we expect that understanding the KRASmut/Gal-3/TAMs interaction and its impact in CRC phenotypic alterations will bring valuable insights to unveil the role of TME in resistant CRC harboring KRAS mutations and to the development of novel therapeutic approaches.

38. Chemical hypoxia originates gastric cancer stem-like cells via upregulation of SOX2 and c-MYC expression

Authors and Affiliations

Diana Pádua[1,2,3], Mariana Pinto[1,2,4], Paula Figueira[1,2], Carlos Filipe Pereira[5,6,7], Raquel Almeida[1,2,8], Patrícia Mesquita[1,2]

1. i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal
2. IPATIMUP – Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Portugal
3. ICBAS - Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto, Portugal
4. Universidade Católica Portuguesa, Porto, Portugal
5. CNC – Centro de Neurociência e Biologia Celular, Universidade de Coimbra, Portugal
6. Lund Stem Cell Center, Universidade de Lund, Suécia
7. Wallenberg Center for Molecular Medicine, Universidade de Lund, Suécia
8. FCUP – Departamento de Biologia da Faculdade de Ciências da Universidade do Porto, Portugal

Presenting Author

Diana Pádua

Abstract

Introduction: Hypoxia is an important feature of the solid tumor microenvironment. Its master regulators – hypoxia-inducible factors (HIFs) – participate in the maintenance of a cancer stem-like state. Moreover, HIF1α is known to be differentially expressed between cancer stem cells (CSCs) and non-CSCs. Using the SORE6-GFP reporter system, which relies on SOX2 and/or OCT4 activities to drive GFP expression, we isolated and characterized a gastric CSC subpopulation (SORE6+). Moreover, SORE6+ express HIF1α, contrarily to what happens to SORE6-. Our aim was to investigate if either the overexpression of HIF1α or the treatment with the hypoxia mimetic $CoCl_2$ are able to reprogram SORE6- cells into CSCs.

Materials and Methods: HIF1α was overexpressed in SORE6- cells using the DOX-inducible lentiviral FUW-tetO-HIF1α vector. Cells were treated with $CoCl_2$ (0, 100, 200 and 300 uM) for 24h and 48h. GFP levels were analyzed by flow cytometry, as a readout of cellular reprogramming, and SOX2 and c-MYC expression was evaluated by western blot. Clonogenic assays were used to evaluate if HIF1α have an impact on the formation of colonies in SORE6- cells. PrestoBlue was used to assess cell viability.

Results: In SORE6- cells with HIF1α overexpression, despite the obvious increase in colony formation and in the levels of SOX2 and C-MYC, no activation of GFP occurred. After 24h of treatment with 200 and 300 uM of $CoCl_2$, we observed the activation of the reporter system in 17% and 15% of the cells, respectively. Cell viability increased in all tested conditions except for the treatment with $CoCl_2$ for 48h.

Conclusions: Further studies are needed to elucidate the mechanism of gastric CSCs reprogramming by hypoxia, but we concluded that hypoxia is able to induce the activation of stemness-related genes like SOX2 and c-MYC.

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39. SPINT2 downregulation increases melanoma aggressiveness features

Authors and Affiliations

Sónia Pires Celeiro[1,2], Fátima Baltazar[1,2], Rui M. Reis[1,2,3], Marta Viana-Pereira[1,2]

1. Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus Gualtar, 4710-057 Braga, Portugal
2. ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal
3. Molecular Oncology Research Center, Barretos Cancer Hospital, Pio XII Foundation, Barretos, S.Paulo, Brazil

Presenting Author

Sónia Pires Celeiro

Abstract

Introduction: Melanoma is the most fatal and aggressive skin cancer type, and its incidence has been raising in the last years, with 324 635 new cases in 2020. Several signaling pathways have been related to melanoma development and therapeutic resistance. Hepatocyte growth factor (HGF)/c-MET signaling leads to melanoma cell proliferation, survival and migration. HGF is the only ligand known for c-MET receptor and its secreted levels seem to be related to BRAF inhibitors' resistance.

Serine protease inhibitor Kunitz type 2 (SPINT2) inactivates serine proteases responsible for pro-HGF conversion into its active form (HGF). SPINT2 is downregulated through gene promoter hypermethylation and identified as a tumor suppressor in various solid tumors. However, little is known about SPINT2 in melanoma. Therefore, our work aim is to understand the role and influence of SPINT2 in melanoma.

Materials and Methods: Melanoma clinical cohort and in silico analysis were used to evaluate SPINT2 expression and methylation status and perform clinicopathological correlations. Stable transfectants with SPINT2 overexpression in melanoma cell lines (A375; WM9) were obtained. Two and three-dimensional melanoma cell culture models were used to understand SPINT2 functional role. SPINT2 influence on ex vivo tumor growth and angiogenesis was evaluated by Chick Chorioallantoic Membrane (CAM) assay.

Results: SPINT2 downregulation and promoter methylation seem to be highly frequent in melanoma. Decreased cell viability, migration, and proliferation were verified in SPINT2 overexpressed cell lines in two and three-dimensional models. CAM assay confirmed this SPINT2 suppressive role. Thus, SPINT2 seems to reduce melanoma aggressiveness showing potential as a biomarker for melanoma patients.

Conclusions: The results suggest that SPINT2 exhibits a tumor suppressor activity in melanoma being downregulated by gene promoter hypermethylation.

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40. Pro-Inflammatory Cytokines induced changes in expression of Tumour-Related Splice Variant RAC1B in Polarized Colorectal Cells

Authors and Affiliations

Joana F. S. Pereira, Cláudia Bessa, Vânia Goncalves Paulo Matos and Peter Jordan

- Department of Human Genetics, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal
- BioISI—Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, Portugal

Presenting Author

Joana F. S. Pereira

Abstract

An inflammatory microenvironment is a tumour-promoting condition that provides sur- vival signals to which cancer cells respond with gene expression changes. One example is the alternative splicing variant Rat Sarcoma Viral Oncogene Homolog (Ras)-Related C3 Botulinum Toxin Substrate 1 (RAC1)B, which we previously identified in a subset of V-Raf Murine Sarcoma Viral Oncogene Homolog B (BRAF)-mutated colorectal tumours. RAC1B was also increased in samples from inflammatory bowel disease patients or in an acute colitis mouse model. Here, we used an epithelial-like layer of polarized Caco-2 or T84 colorectal cancer (CRC) cells in co-culture with fi- broblasts, monocytes or macrophages and analysed the effect on RAC1B expression in the CRC cells by RT-PCR, Western blot and confocal fluorescence microscopy. We found that the presence of cancer-associated fibroblasts and M1 macrophages induced the most significant increase in RAC1B levels in the polarized CRC cells, accompanied by a progressive loss of epithelial organization. Under these conditions, we identified interleukin (IL)-6 as the main trigger for the increase in RAC1B levels, associated with Signal Transducer and Activator of Transcription (STAT)3 activation. IL-6 neutraliza- tion by a specific antibody abrogated both RAC1B overexpression and STAT3 phosphorylation in polarized CRC cells. Our data identify that pro-inflammatory extracellular signals from stromal cells can trigger the overexpression of tumour-related RAC1B in polarized CRC cells. The results will help to understand the tumour-promoting effect of inflammation and identify novel therapeutic strategies.

41. The disease spectrum of CTNNA1 germline variants goes beyond Hereditary Diffuse Gastric Cancer

Authors and Affiliations

Silvana Lobo[1,2,3], Patrick R Benusiglio[4,5], Florence Coulet[6], Lise Boussemart[7,8], Lisa Golmard[9], Isabel Spier[10,11], Robert Hüneburg[11,12], Stefan Aretz[10,11], Liselotte P Van Hest[13], Judith Balmaña[14], Sigrid Tinschert[15,16], Bryson Katona[17], Melyssa Aronson[18], Chrystelle Colas[9], Paulo Pereira[1,19], Carla Oliveira[1,2,20]

1. i3S – Instituto de Investigação e Inovação em Saúde, Porto, Portugal
2. IPATIMUP – Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal
3. International Doctoral Programme in Molecular and Cellular Biotechnology Applied to Health Sciences (BiotechHealth) from Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Porto, Portugal
4. Unité fonctionnelle d'Oncogénétique clinique, Département de Génétique, Groupe Hospitalier Pitié-Salpêtrière, AP-HP. Sorbonne Université, Paris, France
5. Chirurgie générale et digestive, Hôpital Saint-Antoine, AP-HP. Sorbonne Université, Paris, France
6. Unité fonctionnelle d'Onco-angiogénétique et génomique des tumeurs solides, Département de Génétique médicale, Hôpital Pitié-Salpêtrière, AP-HP. Sorbonne Université, Paris, France
7. Université Nantes, Centre de Recherche en Cancérologie et Immunologie Nantes Angers, Institut National de la Santé et de la Recherche Médicale, Nantes, France
8. Department of Dermatology, Nantes University Hospital, France
9. Department of Genetics, Institut Curie, University Paris Sciences Lettres, Paris, France
10. Institute of Human Genetics, Medical Faculty, University of Bonn, Bonn, Germany
11. National Center for Hereditary Tumor Syndromes, University Hospital Bonn, Bonn, Germany
12. Department of Internal Medicine I, University Hospital Bonn, Bonn, Germany
13. Amsterdam UMC, Vrije Universiteit Amsterdam, Department of clinical genetics, Amsterdam, Netherlands
14. Hereditary Cancer Group, Medical Oncology Department Hospital Vall d'Hebron, and Vall d'Hebron Institute of Oncology, Barcelona, Spain
15. Division of Human Genetics, Medical University Innsbruck, Innsbruck, Austria
16. IFLb Laboratoriumsmedizin Berlin GmbH, Windscheidstraße, Berlin-Charlottenburg, Germany
17. Division of Gastroenterology and Hepatology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA
18. Zane Cohen Centre, Sinai Health System, Toronto, Canada
19. IBMC – Instituto de Biologia Molecular e Celular, Porto, Portugal
20. FMUP – Faculty of Medicine of the University of Porto, Porto, Portugal

Presenting Author

Silvana Lobo

Abstract

Introduction: CTNNA1/αE-catenin germline variants cause Hereditary Diffuse Gastric Cancer (HDGC) in 2% of families meeting clinical criteria. CTNNA1-associated tumor spectrum, variant-type causality and genotype-phenotype associations are yet to be defined.

Methods: We gathered CTNNA1 germline variant carriers' clinical data from ERN-GENTURIS collaborating Institutes, others worldwide, and the literature. We classified variants for variant type and actionability; evaluated ascertainment according to HDGC-criteria; and performed genotype-phenotype associations. Furthermore, we developed a Drosophila melanogaster model to study tissue-specific αE-catenin impairment.

Results: We collected 66 CTNNA1 carrier families, bearing 32% Pathogenic (PV), 32% Likely Pathogenic (LPV) and 32% Variants of Unknown Significance (VUS). From 21 VUS, 13 are missense. PV carriers mainly presented Diffuse Gastric Cancer and Lobular Breast Cancer, but also non-classical-HDGC phenotypes, as Colorectal, Prostate and Thyroid Cancer. LPV disease spectrum includes Breast Cancer Unknown Histotype, Melanoma and Multinodular Goiter. Most PV families met HDGC criteria (81%), while all LPV families didn't. A strong association was found for missense VUS and the heritable eye disorder Macular Dystrophy Patterned 2. Knockdown of Dα-cat caused complete lack of eye development and severe lethality, phenotypes that could be rescued by switching Dα-cat for its human version in Drosophila's' eye primordial tissue.

Conclusions: Disease spectra associated with CTNNA1 PV, LPV and missense VUS present differences between one another. Moreover, CTNNA1 LPV and missense VUS disease spectrum diverge from the one associated with CDH1. This claims for phenotype-driven CTNNA1-specific variant classification rules supported by robust in vivo testing models. The humanized Drosophila model that we developed will enable the study of CTNNA1 specific variant types in a tissue-specific manner to better define CTNNA1 disease spectrum.

42. Antibody blockade of the PSGL-1 checkpoint regulator promotes human T cell responses against lymphoma cells

Authors and Affiliations

JL. Pereira[1,2,3], RF. Santos[1], AM. Carmo[1], NR. dos Santos[1,2]

1. i3S-Instituto de Investigação e Inovação em Saúde-Universidade do Porto, Porto, Portugal;
2. IPATIMUP-Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal;
3. Faculty of Medicine of the University of Porto, Porto, Portugal.

Presenting Author

JL. Pereira

Abstract

P-selectin glycoprotein ligand-1 (PSGL-1) is a membrane-bound glycoprotein expressed in immune cells and involved in T cell interactions. Recently, mouse PSGL-1 was found to be an immune checkpoint protein negatively regulating T cell responses, promoting T cell exhaustion and inducing immune tolerance. However, an immune regulatory function in human cells has so far remained to be demonstrated. We assessed PSGL-1 surface expression in healthy donor CD4 and CD8 T cells and found that it decreased upon T cell activation. By performing immunofluorescence staining of T cell immunological synapses with Raji antigen-presenting cells, we observed that PSGL-1 expression polarized to the opposite pole of the synapse in contrast to expression throughout the entire surface in non-engaging T cells. To determine whether PSGL-1 expression restrains human T cell responses against tumor cells, we cocultured these cells with irradiated Raji lymphoma cells with or without PSGL-1 blocking antibody and evaluated CD25 and CD69 surface expression. When T cells were primed with a first round of irradiated Raji cells, they showed a small percentage of CD69+ and/or CD25+ populations. When cocultured with a fresh batch of irradiated Raji cells, the percentage of activated cells increased. We tested the effect of anti-PSGL-1 on CD3/CD28 pre-activated T cells upon coculture with irradiated Raji cells. Pre-activated T cells upregulated CD69 and CD25, and anti-PSGL-1 antibody blockade promoted and sustained T cell activation throughout time. To assess whether anti-PSGL-1 could enhance the response of exhausted T cells against lymphoma cells, we repeatedly activated donor T cells by CD3/CD28 stimulation, resulting in higher expression levels of PD-1, TIM-3, and LAG-3. These in vitro exhausted T cells were cocultured with irradiated Raji cells and blocking PSGL-1 increased the percentage of CD4+CD69+ T cells. We conclude that PSGL-1 blockade is sustaining human T cell responses against lymphoma cells.

43. Ancestry-guided drug response in triple-negative breast cancer cell lines: unravelling new putative targets in the African scope

Authors and Affiliations

Ricardo J. Pinto[1,2,3], Cristina P. R. Xavier[1,2], Ana C. Magalhães[1,2,3], Noémia Afonso[4], M. Helena Vasconcelos[1,2,5], Lúcio Lara Santos[3,6,7], Luísa Pereira[1,2]

1. i3S-Instituto de Investigação e Inovação em Saúde-Universidade do Porto, Porto, Portugal;
2. IPATIMUP-Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal;
3. Faculty of Medicine of the University of Porto, Porto, Portugal.

Presenting Author

Ricardo J. Pinto

Abstract

Introduction: African people have a disproportionally high burden of triple-negative breast cancer (TNBC), due to increased incidence, younger age at presentation and high metastatic potential. The absence of known druggable molecular targets leaves chemotherapy as the favoured therapeutic option, although the aggressive pattern of TNBC often drives treatment evasion. Thus, understanding the impact of the ancestry on the TNBC cellular response to standard therapy regimens is vital. Here, we present the rationale and preliminary data from a drug response study in African and European TNBC cell lines, which will guide the selection of the most promising drugs for transcriptomic analyses to identify deregulated pathways and new therapeutic options.

Materials and Methods: Ancestry data from over 1000 cell lines was mined from Dutil et al. 2019 and filtered to retain only TNBC cell lines. Eleven antitumor drugs used in the clinical practice were selected for studies of drug response. The cytotoxicity of these drugs on TNBC cell lines is currently being assessed, using the sulforhodamine B assay to determine cell growth.

Results: Five European TNBC cell lines were selected to compare with five African ones. The effect of eleven drugs on the growth of these cell lines is being tested, totaling over 100 drug-cell-line interactions. Preliminary results show a trend for increased sensitivity of African cells to gemcitabine. More conclusive data will be included on the poster presentation.

Conclusions: The choice of the appropriate therapeutic regimen for TNBC patients assumes greater relevance in Africa since the limited access to effective treatment options is a major drawback for the successful management of this disease. We expect that this ancestry-stratified study of drug response in TNBC cell lines will identify new deregulated pathways in the cells from African origin, revealing novel therapeutic options for a pathology associated with poor prognosis.

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44. Clinical therapeutic targets in endometrial cancer stem cells: a proteomic overview

Authors and Affiliations

Catarina Mestre[1], Beatriz Serambeque[1,2], Ana Sofia Carvalho[3], Hans C. Beck[4], Maria João Carvalho[1,5,6], Maria Filomena Botelho[1,2,5], Rune Matthiesen[3], Mafalda Laranjo[1,2,5]

1. Coimbra Institute for Clinical and Biomedical Research (iCBR), area of Environment Genetics and Oncobiology (CIMAGO), Institute of Biophysics, Faculty of Medicine, University of Coimbra, Coimbra, Portugal
2. Center for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, Coimbra, Portugal
3. Computational and Experimental Biology Group, Chronic Diseases Research Centre, NOVA Medical School, Faculty of Medical Sciences, NOVA University of Lisbon, Lisbon, Portugal
4. Centre for Clinical Proteomics, Department of Clinical Biochemistry and Pharmacology, Odense University Hospital, DK-5000 Odense, Denmark
5. Clinical and Academic Centre of Coimbra (CACC), Coimbra, Portugal
6. Department of Gynaecology, Coimbra Hospital and University Centre, and University Clinic of Gynaecology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Presenting Author

Catarina Mestre

Abstract

Endometrial cancer is the most frequent gynaecological malignancy of the female genital tract in developed countries. Tumours include a subpopulation of undifferentiated cells, Cancer Stem Cells (CSC), with self-renewal, high differentiation, and proliferation potential. These are pointed out as responsible for resistance to chemo and radiotherapy. Therefore, the aim of this study is to identify potential therapeutic targets with the endpoint of applicability in the clinic. ECC-1, a human endometrioid carcinoma type I cell line was propagated in adherent conditions. The sphere forming protocol allowed to obtain the CSC population. Cells were collected, lyzed, reduced and alkylated with dithiothreitol and iodoacetamide, respectively, and finally digested with trypsin. Afterwards, each condition was analysed through liquid chromatography with tandem mass spectrometry. Finally, data was analysed using R package "limma" for system biology. R was used for functional enrichment analysis the annotations in the R package "org.Hs.eg.db". The proteomic data was obtained by comparison of duplicate analysis of 4 samples from ECC-1 cell line and first generation of spheres. The framework analysis was done using the following criteria: comparison of first generation of spheres versus ECC-1 cell line, proteins significantly up-regulated by a 40-fold-change and cellular localization (plasmatic membrane, mitochondria membrane and cytosolic). Thus, getting from over five thousand identified proteins to a total of 18 proteins significantly up-regulated, from which 7 proteins were associated with the plasmatic membrane, 2 associated with the mitochondrial membrane and 8 associated with the cytosol. From the mitochondrial (ARG2) and plasmatic membranes (GPX1, FTL, CDA and PEF1) one and four proteins were identified as a potential option for targeted therapy, related to cellular metabolism. Additional proteins were identified that may contribute to a very promising discovery path.

45. Fishing for new immunotherapy compounds to boost innate-tumor rejection

Authors and Affiliations

Ana B. Machado[1], Luís Leite[1], Domenico Calafato[1], Cátia Almeida[1], Bruna Costa[1], Rita Fior[1]

1. Champalimaud Foundation, Champalimaud Research, Lisbon, Portugal

Presenting Author

Ana Machado

Abstract

Despite significant advances in cancer immunotherapy, tackling immunosuppression remains critical for more effective responses. While developing zebrafish xenograft, we found that some tumors engraft very efficiently (progressors), while others get cleared (regressors), indicating that some cancer cells are recognized and eliminated, while others evade/suppress innate immunity. This opens an opportunity to perform an in vivo phenotypic drug screen to find compounds that induce this clearance by boosting innate tumor rejection.

Zebrafish xenografts of human colorectal (CRC) and breast cancer (BC) cell lines that show a high engraftment/low clearance phenotype (progressors) were used to screen a 774-compound FDA-approved library. Tumor cells are injected into 2 days post-fertilization embryos and subjected to compound testing. At 3-5 days post-treatment, clearance rates are quantified, and hits are defined as compounds that significantly increase clearance.

To scrutinize the effects of the hits on innate immunity, we perform rescue experiments using zebrafish mutants lacking myeloid cells (macrophages and neutrophils) and generate xenografts on transgenic reporters that reveal the myeloid inflammatory state. Live microscopy allows the study of immune cell behavior and interaction with tumor cells.

We have already screened ~400 drugs and obtained a total of 8 confirmed hits, 3 for CRC and 5 for BC. Interestingly, one of the hits is common for both cancer types. So far, hits belong to the following pharmacological groups: non-steroidal anti-inflammatory drugs (NSAID), vasoactive agents, anti-malarial, anti-fungal, bronchodilators, and anti-parkinsonian. We are in the process of confirming more hits and characterizing how these compounds modulate the tumor microenvironment (TME).

These compounds that boost innate-tumor clearance may help overcome the suppressive TME and can hopefully be used to prime the response to immune checkpoint blockers, engaging both the innate and the adaptive immune arms to fight cancer.

46. Hypoxia-driven adenosinergic signaling promotes the aggressiveness of bladder cancer

Authors and Affiliations

Margarida Pereira[1,2], Frederico Furriel[3], Célia Gomes[1,2]

1. Coimbra Institute for Clinical and Biomedical Research, Faculty of Medicine, University of Coimbra, Coimbra, Portugal (iCBR);
2. Center for Innovation in Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal (CIBB);
3. Urology and Renal Transplantation Department, Coimbra University Hospital Centre, Coimbra, Portugal (CHUC)

Presenting Author

Margarida Pereira

Abstract

Introduction: Hypoxia is a critical hallmark of malignant disease often linked to a poor prognosis. The HIF-1α is a master regulator of the transcriptional response of cancer to hypoxia that upregulates the expression of the ecto-nucleotidases CD39 and CD73 involved in the generation of extracellular adenosine (ADO). Besides generating an immunosuppressive microenvironment, ADO exerts direct effects on tumor cells through P1 receptors. Herein, we explored the role of the adenosinergic pathway in the adaptation of bladder cancer (BC) to hypoxia for identify potential targets for therapeutic intervention.

Materials and Methods: Two human BC cell lines (UM-UC3 and HT-1376) were exposed to hypoxia with and without inhibitors of ectonucleotidases. The levels of HIF-1α, CD73, CD39 and ADO receptors A2A and A2B were analyzed by Western blot. ADO levels in supernatants were measured using a fluorometric assay kit. Cells' proliferation and chemosensivity to cisplatin were evaluated using a WST-1 assay. Cell migration was analyzed by a wound-healing assay. Epithelial-mesenchymal transition markers were analyzed by RT-PCR.

Results: BC cells activate the adenosinergic pathway under hypoxic conditions and upregulate A2BR expression. Hypoxia cells exhibited a higher proliferation rate, enhanced resistance to cisplatin and acquired a migratory mesenchymal phenotype. The pharmacological inhibition of ectonucleotidases prevents the generation of ADO and reverses the chemoresistance to cisplatin. Blockade of A2BR attenuated ADO-induced proliferation

Conclusion: Hypoxia induces the activation of adenosinergic pathway and exacerbates the malignant features of BC. Therapeutics targeting this pathway might be beneficial in controlling BC growth and response to therapy.

47. Expression of PD-L1 in pituitary adenomas: review of three large original series

Authors and Affiliations

R. Joaquim[1,2], M.J. Bugalho[1,3], A.L. Silva[1,2], P. Marques[1]

1. Serviço de Endocrinologia, Hospital de Sta Maria-CHULN. EPE, Lisboa, Portugal;
2. ISAMB- Instituto de Saúde Ambiental, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal,
3. Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal

Presenting Author

Rita de Faria Joaquim

Abstract

Pituitary adenomas (PAs) are amongst the most common intracranial tumours. PAs can be clinically classified as non-functioning PAs (NFPAs) or as functioning PAs, which usually secrete prolactin (PRL), growth hormone (GH) or adrenocorticotropic hormone (ACTH). The first line treatment is, in most cases, surgery. Often, further treatments such as medical therapy or radiotherapy or even both are needed. Recently immunotherapy, particularly immune checkpoint blockade (ICB) therapy, raised interest and may be useful to treat advanced or resistant PAs. Programme death protein 1 (PD-1) interacts with PD-L1 establishing an "immune checkpoint" that undermines the normal function of lymphocytes enabling tumours to suppress the immune system. Such blockade of this interaction facilitates T-cell response. ICB has shown good results in lung cancer, renal cell carcinoma and even melanoma. We aimed to review the studies that assessed the expression of PD-L1 in PAs in order to better understand the potential relevance for such immunotherapy in PAs.

We identified 3 main studies analysing the expression of PD-L1 in PAs, comprising a total of 394 patients with PAs, 201 of which were NFPAs and the remaining functioning PAs. Among functioning PAs, 70 were GH secreting PAs, 66 were PRL secreting PAs and 57 were ACTH secreting PAs. PD-L1 was expressed in 110 out of the 394 PAs (28%). Generally, functioning PAs express more PD-L1 than NFPAs, with GH-producing adenomas being those showing higher expression levels of PD-L1.

Data presented herein, deriving from a revision of the literature, suggest that up to a third of PAs express PD-L1. Such noteworthy proportion of PD-L1 positive PAs underlies some rationale for attempting testing and using ICB in patients with PAs, particularly those that are refractory to conventional therapies. Further studies must be performed to better understand the mechanisms underlying PD-L1 expression in PAs and whether PD-1/PD-L1 targeted immunotherapy may indeed be extended to PAs alone or combined with other therapies.

48. T cell glycosylation at the interface of inflammatory bowel disease and colorectal cancer

Authors and Affiliations

Guilherme Faria (Institute for Research and Innovation in Health, Porto, Portugal; Faculty of Medicine, University of Coimbra, Coimbra, Portugal); Mariana Silva (Institute for Research and Innovation in Health, Porto, Portugal; Instituto de Ciências Biomédicas Abel Salazar, Porto, Portugal) Eduarda Gomes (Institute for Research and Innovation in Health, Porto, Portugal; Instituto de Ciências Biomédicas Abel Salazar, Porto, Portugal); Catarina Azevedo (Institute for Research and Innovation in Health, Porto, Portugal; Instituto de Ciências Biomédicas Abel Salazar, Porto, Portugal); Ângela Fernandes (Instituto de Ciências Biomédicas Abel Salazar, Porto, Portugal); Salomé S. Pinho (Institute for Research and Innovation in Health, Porto, Portugal; Instituto de Ciências Biomédicas Abel Salazar, Porto, Portugal; Faculty of Medicine, University of Porto, Porto, Portugal)

Presenting Author

Guilherme Faria

Abstract

Inflammatory bowel disease (IBD), encompassing ulcerative colitis and Chron's disease, is an idiopathic condition defined by chronic inflammation of the gastrointestinal tract. Long-standing IBD is associated with increased risk for colorectal cancer (CRC) development and current standard of care lacks preventive measures to intercept malignant transformation.

Glycosylation consists of the synthesis of glycans – macromolecular structures formed by linkages between sugar monomers – and their addition to proteins and lipids. In the context of IBD, our group has shown that diminished levels of branched N-glycans in the T cell receptor result in enhanced T cell activation, driving disease-associated hyperactive phenotypes (Dias et al., PNAS, 2018). Opposingly, in CRC, expression of branched N-glycans in tumor cells favors immune evasion, thereby facilitating tumor development (Silva and Fernandes et al., Cancer Immunol Res, 2021). However, the influence of glycosylation in immune reprogramming in inflammation-associated carcinogenesis remains unexplored.

To address the contribution of T cell glycosylation in this setting, this work combines analysis of human- and mice-derived samples, organoid models and adoptive T cell transfer in mice.

Glycan characterization of paraffin-embedded clinical samples has shown a progressive increase in branched N-glycan levels throughout the inflammation-dysplasia-carcinoma sequence, suggesting the implication of these glycan structures in this progression. Preliminary data from adoptive cell transfer experiments have revealed notable differences in T cell glycophenotype along the transition from homeostasis to acute inflammation. In addition, mice with branched N-glycan deficiency in T cells appear to develop more severe inflammation. Ongoing experiments will further assist in understanding whether T cell N-glycan branching can influence disease progression, with potential applications in CRC prevention and treatment.

49. CITED4 hypomethylation in the 3'UTR is associated with an adverse prognosis in glioblastoma

Authors and Affiliations

Mónica Teotónio Fernandes[1,2,3], Joana Dias Apolónio[2,3,4], Pedro Castelo-Branco[2,3,4], José Bragança[2,3,4]

1. School of Health, University of Algarve, Faro, Portugal
2. Algarve Biomedical Center Research Institute (ABC-RI), Faro, Portugal.
3. Algarve Biomedical Center, Faro, Portugal.
4. Faculty of Medicine and Biomedical Sciences (FMCB), University of Algarve, Faro, Portugal

Presenting Author

Mónica Teotónio Fernandes

Abstract

Introduction: Malignant gliomas are the most common and deadly brain cancers, resulting from the transformation of glial cells like oligodendrocytes and astrocytes. The median survival of patients with glioma has improved over the past years but is still relatively low and new biomarkers and therapeutic targets are sorely needed. CITED4 (CREB-binding protein/p300-interacting transactivator with E/D-rich tail 4) has been reported as a potential tumor suppressor gene in gliomas since, mainly in oligodendrogliomas, it is downregulated by the common 1p/19q deletions and promoter methylation, leading to a favorable prognosis. Nevertheless, these genetic and epigenetic alterations are not generally detected in glioblastoma (grade IV glioma). Our aim was therefore to explore the potential of CITED4 methylation as a biomarker in glioblastoma.

Materials and Methods: We analyzed data from the publicly available databases Genotype-Tissue Expression (GTEx) Data Set and The Cancer Genome Atlas (TCGA), including the lower grade glioma (LGG) and glioblastoma (GBM) cohorts. The collected data included RNA expression by RNA sequencing (Illumina HiSeq), copy number variation, DNA methylation (450K array), and clinical characteristics.

Results: In gliomas from 659 patients, we confirmed that deletions in the CITED4 gene and promoter methylation are more common in oligodendrogliomas and oligoastrocytomas and associated with lower CITED4 expression and a better prognosis, and rare in astrocytomas and glioblastomas. Moreover, we found hypomethylation in a CpG site located in the 3'UTR (cg26834479), which was associated with higher CITED4 expression in glioblastomas and an adverse prognosis. Interestingly, CITED4 was hypomethylated at the same site in patients with no IDH1 mutations and those classified as non G-CIMP, which present a worse overall survival.

Conclusions: CITED4 methylation in the 3'UTR (cg26834479) is a novel potential prognosis biomarker that deserves being validated to be used in the clinical practice.

50. Trastuzumab-resistant HER2+ Brain Tropic Breast Cancer Cells Disrupt the Blood-Brain Barrier: a Potential Route for Brain Drug Delivery

Authors and Affiliations

Liliana Santos[1], Francesca Tomatis[2,3], Hugo Ferreira[4,5], José Sereno[1], Sara Almeida[1], Lino Ferreira[2,3], Ana Paula Silva[4,5], João Nuno Moreira[2,5], Antero Abrunhosa[1], Célia Gomes[4,5]

1. CIBIT/ICNAS - Institute for Nuclear Sciences Applied to Health, University of Coimbra
2. CNC - Center for Neuroscience and Cell Biology, University of Coimbra
3. IIIUC - Institute of Interdisciplinary Research, University of Coimbra
4. iCBR - Institute for Clinical and Biomedical Research, Faculty of Medicine, University of Coimbra
5. CIBB - Center for Innovative Biomedicine and Biotechnology Consortium, University of Coimbra

Presenting Author

Liliana Santos

Abstract

Introduction: Up to 50% of HER2+ breast cancer patients eventually develop brain metastasis (BM), with a median survival of less than one year after diagnosis. Systemic anti-HER2 therapies are highly effective for extracranial metastasis but ineffective on brain metastases due to inadequate drug penetration through the blood-brain barrier (BBB) and acquired resistance mechanisms. Herein, we evaluated the molecular mechanisms underlying brain metastasis formation in HER2+ breast cancer (BC).

Materials and methods: HER2+ BC cell lines and brain-tropic derivatives were characterized regarding cell surface HER2 density, chemosensitivity to trastuzumab, invasion and migratory ability. The BBB integrity was evaluated in vitro and in vivo in mouse models carrying brain metastasis or a primary orthotopic tumor by near-infrared fluorescence imaging.

Results: Brain tropic HER2+ cells are more resistant to trastuzumab, despite not differing significantly in HER2 expression levels, and display a more invasive phenotype than their corresponding parental cells. The BBB is selectively disrupted by brain-tropic cells indirectly through secreted factors. Animals with brain metastases showed structural and functional alterations in the BBB integrity (perceived by a decrease of collagen IV and an increase of albumin immunoreactivity) that allowed the passage of a 20kDa dextran. Similar changes were observed in the presence of a localized primary tumor.

Conclusions: Our results suggest that brain tropic HER2+ breast cancer cells are less susceptible to trastuzumab while maintaining HER2 expression. Brain metastasis causes dynamic changes in the BBB permeability that start before brain colonization, which in a therapeutic perspective could be beneficial to novel anticancer drugs in alternative to trastuzumab.

51. Understanding the mechanism of ARL1 and ARL15 in the aggressiveness of cutaneous melanoma

Authors and Affiliations

Inês Saraiva[1], Pedro Chaves[1], Cheila Brito[1], Ana Tomás[1], José Maximino[1], Andreia Ferreira[2], Victor Farricha[3], Hugo Nunes[4], Guadalupe Cabral[2], Duarte Barral[2], Marta Pojo[1]

1. Unidade de Investigação em Patobiologia Molecular, Instituto Português de Oncologia de Lisboa Francisco Gentil, Lisboa, Portugal
2. Centro de Estudos de Doenças Crónicas (CEDOC), NOVA Medical School, Universidade NOVA de Lisboa, Lisboa, Portugal
3. Serviço de Cirurgia, Instituto Português de Oncologia de Lisboa Francisco Gentil, Lisboa, Portugal
4. Serviço de Oncologia Médica, Instituto Português de Oncologia de Lisboa Francisco Gentil, Lisboa, Portugal

Presenting Author

Inês Saraiva

Abstract

Introduction: Cutaneous melanoma (CM) is the skin cancer with the worst prognosis due to its high metastatic rate. Although targeted and immunotherapies have improved the overall survival of patients, resistance to therapy is still the main hurdle in CM treatment.

ARLs are small GTPases responsible for controlling cell signaling, membrane traffic and cytoskeletal organization. In a previous study carried out by our group high ARL1 expression was correlated with prolonged overall survival, whereas high ARL15 expression was shown to be linked to poor prognosis. A role for these ARLs in immune modulation was also suggested.

Here we aimed to investigate the mechanism by which ARL1 and ARL15 are associated with CM prognosis.

Methods: ARL1 and ARL15 expression was evaluated by RT-qPCR in 17 CM surgical fragments from Instituto Português de Oncologia de Lisboa Francisco Gentil, and flow cytometry was performed for immune cell characterization. ARL1 or ARL15 silencing was performed in A375 and WM115 CM cell lines and cell viability, migration and invasion were assessed.

Results: ARL15 is downregulated in CM patients, with a significant decrease in stage IV CM. No differences were found in ARL1 expression in any of CM stages. Nevertheless, we observed a positive correlation between ARL1 expression and tumor infiltration of helper and cytotoxic T cells, as well as regulatory T cells expressing CCR4. In vitro assays showed that ARL1 and ARL15 expression modulation has no impact on cell viability. However, ARL1 silencing decreases cell migration in the A375 CM cell line.

Conclusion: ARL1 potentiates the recruitment of immune cells with antitumor and immunosuppressive activity, suggesting a role of ARL1 in CM immune modulation. ARL1 or ARL15 silencing has no effect on cell viability, but ARL1 potentiates cell migration. More samples will be added to our patient cohort in order to corroborate this data, and we will continue to perform in vitro assays with different genetic backgrounds to further demonstrate the role of these ARLs in CM pathogenesis.

52. A pilot study of the clinicopathological potential of BiP/GRP-78 and RTCB immunohistochemistry in breast cancer

Authors and Affiliations

Daniela Gomes[1], Inês Direito[1], Liliana Monteiro[1], João Lobo[2,3,4], Rui Henrique[2,3,4], Carmen Jerónimo[2,3,4], and Luisa A. Helguero[1]

1. iBiMED - Institute of Biomedicine, University of Aveiro, Agra do Crasto 30, Aveiro, Portugal
2. Department of Pathology, Portuguese Oncology Institute of Porto (IPOP), R. Dr. António Bernardino de Almeida, 4200-072, Porto, Portugal
3. Cancer Biology and Epigenetics Group, IPO Porto Research Center (GEBC CI-IPOP), Portuguese Oncology Institute of Porto (IPO Porto) & Porto Comprehensive Cancer Center (P.CCC), R. Dr. António Bernardino de Almeida, 4200-072, Porto, Portugal
4. Department of Pathology and Molecular Immunology, Institute of Biomedical Sciences Abel Salazar, University of Porto (ICBAS-UP), Rua Jorge Viterbo Ferreira 228, 4050-513, Porto, Portugal

Presenting Author

Daniela Gomes

Abstract

Introduction: BiP/GRP78 is chaperone that prevents toxic protein aggregation, RTCB acts downstream of BiP to restore protein homeostasis. Overexpression of BiP has been correlated with poor breast cancer (BC) prognosis. We showed that luminal A cells resistant to antiestrogens enhance BiP expression and that RTCB sustains tamoxifen resistance, while sensitive cells accumulate RTCB into aggresomes in apoptotic cells. There is no systematic evaluation of the prognostic value of BiP, RTCB or Aggresomes as immunohistochemical (IHC) markers for BC.

Material and Methods: 1) Metanalysis: Eligible studies of BiP IHC were identified (PubMed, May 2022). Analysis was done with R (meta and dmetar), random-effect model, significant at the level of 5% ($p<0.05$). 2) Public datasets: TCGA PanCancer Atlas and CPTAC datasets were stratified by higher and lower quartile (BiP-H and BiP-L). 3) IHC: FFPE tissues from 14 BC patients that received neoadjuvant hormonal therapy were processed for RTCB and Aggresome staining. Pre and post-treatment differences studied by Wilcoxon test.

Results: BiP+ cells=78% (0.62-0.89; 10 studies). Tumor stage, lymph node metastasis and HER2 expression influenced %BiP. RFS was inversely correlated with %BiP+ cells [RR=3.05 (1.70; 5.47)]. HER2+ and TNBC correlated with BiP-H and Luminal with BiP-L. Antiestrogen treatment reduced Ki67 (15% to 7%, $p<0.0001$) and increased RTCB+ cells colocalizing with aggresomes (15 to 26%; $p<0.05$).

Conclusion: These pilot study showed that BiP correlates with aggressive BC subtypes and poor prognosis factors. In ER-positive tumors RTCB and aggresomes may be indicators of response to therapy. These results deserve more in-depth studies

53. CCR4high regulatory T cells and polarized monocytes as potential circulating biomarkers of cutaneous melanoma prognosis

Authors and Affiliations

Ana Tomás[1a], José Maximino[1a], Hugo Nunes[2], Rute Salvador[3], Rafael Luís[1], Cheila Brito[1], Diana Saraiva[3], Emanuel Gouveia[2], Carolina Pereira[2], Victor Farricha[4], Cecília Moura[5], Elisabete Lopez Carvalho[6], Maria José Passos[2], Guadalupe Cabral[3], Sofia Cristóvão Ferreira[2], Marta Pojo[1]

1. Unidade de Investigação em Patobiologia Molecular,
2. Serviço de Oncologia Médica do Instituto Português de Oncologia de Lisboa Francisco Gentil (IPOLFG) E.P.E, Lisbon, Portugal;
3. Chronic Diseases Research Center (CEDOC), NOVA Medical School, Universidade Nova de Lisboa, Lisbon, Portugal;
4. Serviço de Cirurgia, 5Serviço de Dermatologia, 6Unidade de Investigação Clínica do IPOLFG E.P.E, Lisbon, Portugal

a. Ana Tomás and José Maximino contributed equally as first authors.

Presenting Author

Ana Tomás

Abstract

Introduction: Cutaneous melanoma (CM) is a deadly type of skin cancer that is becoming increasingly prevalent. Even though immunotherapy helps manage this disease, half of CM patients will not respond to treatment. Macrophage and regulatory T cell (Treg) recruitment to the tumor may impair antitumoral immune responses, with CCR4 as one of the main Treg receptors associated with immunosuppression. Thus, we aim to clarify the potential role of CCR4high Tregs and monocytes as potential circulating prognostic biomarkers in CM.

Methods: Blood and tumor samples were obtained from 66 CM patients undergoing resection surgery or immunotherapy, at Instituto Português de Oncologia de Lisboa. Flow cytometry was performed for immune cell characterization. THP-1 monocyte model was cultured in CM conditioned media from A375 and WM3211 cell lines, and changes in gene expression were assessed by RT-qPCR.

Results: Preliminary results show a more immunosuppressive systemic immune profile in stage IV CM patients, with a higher level of CCR4 expression in Tregs associated with worse overall survival. CCR4 expression in Tregs tends to also decrease throughout immunotherapy in responders. M1/M2-like monocytes appear to be elevated in CM patients compared to healthy controls, and to increase along with staging, indicating a possible association with worse prognosis. In vitro, CM derived soluble factors resulted in increased expression of genes related to recruitment, matrix degradation, angiogenesis, inflammation, and differentiation in a monocyte model, some of which are positively correlated with monocyte attractant CCL2 expression in CM samples.

Conclusions: Systemic immune profiling of advanced CM patients seems to be a promising prognostic tool, highlighting CCR4high Tregs and M1/M2 monocytes as subpopulations of interest. Currently, we are increasing sample size and further characterizing the potential clinical impact of these populations. Furthermore, we intend to explore the putative role of CCR4 as a therapeutic target.

54. Photodynamic therapy based on novel ring-fused chlorins: the future targeted therapeutic approach for endometrial cancer?

Authors and Affiliations

Beatriz Serambeque[1,2], Gabriela Correia-Barros[1], Maria João Carvalho[1,3,4], Nelson AM Pereira[5], Marta Pineiro[5], Teresa MVD Pinho e Melo[5], Maria Filomena Botelho[1,2,3], Mafalda Laranjo[1,2,3]

1. Coimbra Institute for Clinical and Biomedical Research (iCBR), area of Environment Genetics and Oncobiology (CIMAGO), Institute of Biophysics, Faculty of Medicine, University of Coimbra, Coimbra, Portugal
2. Center for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, Coimbra, Portugal
3. Clinical and Academic Centre of Coimbra (CACC), Coimbra, Portugal
4. Department of Gynaecology, Coimbra Hospital and University Centre, and University Clinic of Gynaecology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal
5. Coimbra Chemistry Centre (CQC) and Department of Chemistry - Institute of Molecular Sciences, University of Coimbra, Coimbra, Portugal

Presenting Author

Beatriz Serambeque

Abstract

The management of endometrial cancer (EC) became a challenge for clinicians, particularly due to the diagnosed postmenopausal women, usually with comorbidities and a high surgical risk, but also due to young women whose fertility may be compromised. Thus, to preserve fertility and to provide a new therapy to ineligible patients for surgery, conservative and minimally invasive therapeutic approaches must be explored. Photodynamic therapy based on 5-tetrahydropyrazolo[1,5-a]pyridine-fused chlorins has demonstrated effective outcomes as a promising anticancer therapy. Therefore, this work aims to evaluate the EC cells uptake and the internalization of a chlorin of this group named Px1. Endometrioid EC cell lines, ECC-1 and RL95-2, were incubated with 500 nM of Px1 during four, eight and twenty-four hours. Afterwards, to determine the uptake, cells suspensions were prepared and the fluorescence intensity were measured using an excitation wavelength of 410 nm by spectroscopy. Confocal microscopy was used to evaluate the colocalization of Px1 in the nucleus, mitochondria and plasma membrane, of EC cells. Thus, ECC-1 and RL95-2 cells were incubated with 500 nM of Px1 for 24 hours. Images were acquired with a 40x oil objective on a laser-scanning confocal system. The preliminary results of cell uptake showed internalizations of less than 50 nM for both cell lines, considering the incubation times of 4, 8, and 24 hours. Confocal studies corroborated the Px1 internalization by endometrioid EC cells. Moreover, the colocalization analysis demonstrated that Px1 seems to accumulate into cytoplasm and organelles, mitochondria and plasma membrane, with an absence of colocalization in the nucleus. Cell uptake can contribute to define the best drug-light interval. Px1 subcellular localization may allow the development of a novel therapy targeting subcellular molecular targets for EC.

55. The anticancer potential of a novel benzo[a]phenoxazines derivative for colorectal cancer therapy

Authors and Affiliations

João Carlos Canossa Ferreira[1,2,3*], Maria Sameiro Torres Gonçalves[3], Ana Preto[1,2#], Maria João Sousa[1,2#]

1. Centre of Molecular and Environmental Biology, University of Minho, Braga, Portugal
2. Institute of Science and Innovation for Bio-Sustainability, University of Minho, Braga, Portugal
3. Centre of Chemistry, University of Minho, Braga, Portugal

#. Co-senior authorship

*** .** E-mail: canossa100@hotmail.com

Presenting Author

João Carlos Canossa Ferreira

Abstract

Cancer is expected to rank as the leading cause of death and the most important barrier to increase life expectancy in the 21st century. Colorectal cancer (CRC) has been positioned as one of the most incident cancer types and one of the most mortal.

Overall, the number of effective anti-cancer agents approved for use in humans is still very limited. Moreover, tumor resistance and secondary effects stemming from classical chemotherapy remain a major clinical problem, reinforcing the need for the development of novel drugs.

In the recent years, benzo[a]phenoxazines derivatives have shown to possess anticancer activity, which has created interest in exploring the potential of these compounds as anticancer drugs.

In this context, we have synthetized and evaluated the anticancer activity of different benzo[a]phenoxazine derivatives in CRC- derived cell lines.

Our results revealed that one particular compound, BaP1, displayed promising anticancer activity against CRC cells. We found that BaP1 is selective for CRC cells, reduces cell proliferation, cell survival and cell migration. Furthermore, we observed that the compound is associated with ROS generation, accumulates on the lysosome and leads to lysosomal membrane permeabilization, cytosolic acidification and apoptotic cell death. In vivo results using CAM assay showed that BaP1 inhibit tumor growth and proliferation. These observations highlight BaP1 as a very interesting agent to disturb and counteract the important roles of lysosome in cancer and suggest BaP1 as a promising candidate to be exploited as new anticancer targeted agent, using LMP as a therapeutic approach in CRC.

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56. Unravelling a Cancer Stem Cell Signature Associated with Breast Cancer Metastatic Organotropism

Authors and Affiliations

I Conde[1,2,3], R Carvalho[1,2], MT Pinto[1,2], N Mendes[1,2], A Ribeiro[1,2], J Paredes[1,2,3]

1. i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto
2. IPATIMUP – Instituto de Patologia e Imunologia Molecular da Universidade do Porto
3. FMUP – Faculdade de Medicina da Universidade do Porto

Presenting Author

Inês Conde

Abstract

Introduction: Breast cancer (BC) is a major cause of cancer death, mainly due to distant metastases. It has been described that there is a small subpopulation of cancer cells within the tumour, known as cancer stem cells (CSCs), that have stem-like properties and are involved in tumorigenesis and metastasis formation. Therefore, the search for new CSC biomarkers and improvements in CSC-targeted therapies are crucial to tackle metastatic progression. Thus, our major goal is to unravel a CSC signature associated with BC metastatic organotropism.

Materials and Methods: To achieve our aim, we used a cell model composed by the MDA-MB-231 parental cell line and its organotropic variants to bone, brain and lung. First, we characterized their CSC properties by performing the mammosphere formation assay, as well as by characterizing the breast CSC phenotype ($CD44^+/CD24^{low}$). Then, we evaluated the tumorigenic potential and stem cell frequency through the limiting dilution assay in the chick chorioallantoic membrane (CAM) and in mice models. We also performed proteomic analysis of membrane proteins to establish a CSC signature associated with different metastatic sites.

Results: We observed that all organotropic variants showed an increased mammosphere-forming efficiency, as well as an enriched $CD44^+/CD24^-$ phenotype, when compared with the parental cell line. Moreover, they displayed a significantly increased tumorigenic capacity and stem cell frequency in both in vivo models. Regarding the proteomic analysis, we validated a list of significantly deregulated peptides, that have been already described as CSC biomarkers from the CSC database.

Conclusion: Our data shows that organotropic cells have increased CSC activity and present a distinct CSC profile associated with organotropism. Importantly, we validated potential biomarkers of CSCs associated with breast cancer metastatic organotropism, opening new therapeutic strategies to overcome metastatic disease.

57. Oncogenic KRAS mutations modulate the glycolytic metabolism and the activity of the pro-apoptotic protein BAX

Authors and Affiliations

Anabela Ferreira, Manuela Côrte-Real, Ana Preto*, Maria João Sousa*

- CBMA-Centre of Molecular and Environmental Biology, University of Minho, Braga, Portugal
- IBS-Institute of Science and Innovation for Bio-Sustainability, University of Minho, Braga, Portugal

* co-senior authorship

Presenting Author

Anabela Ferreira

Abstract

KRAS belongs to the GTPase RAS superfamily which regulates several cell-signaling pathways involved in the control of important cellular functions, including apoptosis and metabolism. Oncogenic mutations in KRAS are considered the most common gain-of-function mutations and affect 30-50% of the colorectal cancer (CRC) patients.

RAS proteins usually have an anti-apoptotic role, but little is known about the role of KRAS mutations in apoptosis regulation. The activation of anti-apoptotic pathways is also a consequence of the metabolic adaptation in cancer cells. In fact, CRC cells harboring KRAS mutations can adapt their cellular metabolism, in order to favor tumor progression.

Here, we aimed to uncover the role of mutated human KRAS in the regulation of the glycolytic metabolism and in the regulation of apoptosis and its interaction with mitochondria, specifically with the pro-apoptotic protein BAX, using the yeast Saccharomyces cerevisiae as a model.

To achieve our aims, KRAS-humanized yeasts expressing KRASWT or KRAS bearing the hot spot mutations found in CRC, KRASG12D, KRASG12V or KRASG13D, were used, and, to assess the influence of the different KRAS isoforms on BAX activity, these cells were co-transformed with the human BAX.

Regarding the role of mutated KRAS in the metabolic adaptation, our results showed that, comparing with KRASWT, KRAS mutations lead to a decrease in biomass yield in agreement with a decrease in respiration in the presence of glucose, but, in its absence, KRAS mutations present a higher respiration rate. Furthermore, in medium containing lactic acid, cells expressing KRAS mutations present higher specific growth rates and values of cell survival.

Concerning the regulation of apoptosis by KRAS, our results support an interaction between BAX and KRAS mutants and showed that these mutations confer resistance to BAX-induced cell death. Remarkably, we also observed that KRASG13D, but not KRAS codon 12 mutations, physically interacts with BAX.

Altogether these findings sustain that oncogenic KRAS mutations are sufficient to confer an advantageous ability for metabolic adaptation in tumor microenvironment containing increased lactate and also to overcome BAX-induced cell death.

58. Multifunctional nano-therapy to overcome pancreatic cancer immune evasion

Authors and Affiliations

Mariana Bento[1], Liane Moura[1], Rita Acúrcio[1], Nicole Mendes[1], Erik Wegener[2], Rainer Jordan[2], Ronit Satchi-Fainaro[3*], Helena F. Florindo[1*]

1. Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal
2. Department Chemie, Technische Universität Dresden, Dresden, Germany
3. Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

*. Co-corresponding authors

Presenting Author

Mariana Bento

Abstract

Pancreatic Ductal Adeno Carcinoma (PDAC) is one of the deadliest cancers and no effective treatment is available yet. Polymeric nanoparticles (NP) are potential tools to regulate host effector immunity in vivo against cancer by enabling the delivery of regulators of dendritic cell (DC)-T lymphocyte crosstalk. The main goal of this work is to develop a polyoxazolines (POx)-coated polycaprolactone (PCL)-based nanoplatform to deliver antigens (KRAS G12D) and other bioactive molecules to DC.

The double emulsion solvent evaporation method was used to produce three biodegradable NP composed by PCL and Poly(2-butyl-2-oxazoline-block-2-methyl-2-oxazoline) (POx#1) or Poly(2-nonyl-2-oxazoline-block-2-methyl-2-oxazoline) (POx#2). The type of POx did not significantly impact NP surface charge (ZP≈-1.5 mV), polydispersity index (PdI≤0.1), nor mean average size (Z-Ave): 186 ± 3.0, 175 ± 4.0 and 182 ± 4.0 nm for NP composed by PCL, PCL-POx#1 and PCL-POx#2, respectively. Stability studies show that NP physicochemical properties remain unaltered at 4, 25 or 37□C at least for 60 days. The POx changed the ability of NP to incorporate short KRASG12D peptide: PCL-POx#1 NP and PCL-POx#2 NP presented an entrapment efficiency (EE%) of 67% for and 48%, respectively. However, no difference was observed in the EE% for longer KRAS G12D (EE%≈70% for all formulations). We obtained higher loading capacity (LC) for POx#1 when compared to POx#2 using short KRASG12D, while no differences were obtained for the LC of longer KRASG12D. Ongoing studies are being performed to analyze the impact of NP in immature-DC and to evaluate the internalization profile of these NP, as well as their impact on the maturation of DC.

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59. Targeting KRAS mutated colorectal cancers with new ruthenium-based drugs

Authors and Affiliations

Ana Rita Brás[1,2,3*], Pedro Fernandes[1,2], Tiago Moreira, Andreia Valente[3#], Ana Preto[1,2#]

1. Centre of Molecular and Environmental Biology, University of Minho, Braga, Portugal
2. Institute of Science and Innovation for Bio-Sustainability, University of Minho, Braga, Portugal
3. Centro de Química Estrutural, Institute of Molecular Sciences and Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal

#. Co-senior authorship

***.** E-mail: pg31015@alunos.uminho.pt

Presenting Author

Ana Rita Brás

Abstract

Colorectal cancer (CRC) is an important cause of global morbidity and mortality. CRC harboring KRAS mutations, present in 40% of all CRCs, are resistant to available EGFR inhibitors. Moreover, specific targeting of KRAS hotspot mutations is very difficult to achieve, highlighting the need of developing new specific target drugs. Ruthenium (Ru) drugs emerged as one of the most promising metallodrugs with features that increase their selectivity to cancer cells.

In this work, a new family of Ru-cyclopentadienyl agents was designed, using different approaches to increase the targeting to CRC cells.

Here, we aimed to assess the anticancer effects and mechanism of action of these new Ru agents in CRC cell lines harboring KRAS mutation, by studying in vitro cell viability, proliferation, cell death mechanism, migration, actin cytoskeleton alterations and KRAS signaling pathways molecules expression.

Our results revealed that the new Ru agents present promising anticancer activity being selective to CRC cells, inducing apoptosis and decreasing proliferation and migration. The lead agent PMC79 affects the actin cytoskeleton and specifically decreases the expression levels of KRAS, ERK and AKT proteins only in CRC-derived cells with KRAS mutation, to a higher extent when compared with KRAS siRNA.

PMC79 has a noticeable effect inhibiting KRAS on CRC cells harboring KRAS mutation and not on CRC cells with KRAS wild type, suggesting to be a specific inhibitor of KRAS mutations and a potential "magic bullet" for CRCs harboring mutated KRAS (Submitted patent nr 20221000000928).

Summing up, the new Ru agents are promising new drugs for CRC therapy, which could bring new exciting therapeutic avenues, especially in CRC harboring KRAS mutations, which treatment constitutes a clinically relevant problem that needs to be overcome.

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60. Bladder stiffness after cystectomy in bladder cancer patients: a pilot study

Authors and Affiliations

Sara Monteiro-Reis[1,2#], João P. Ferreira[2,3#], Ricardo Pires[4], João Lobo[1,5], Renato Natal Jorge[2,3], Carmen Jerónimo[1,6]

1. Cancer Biology and Epigenetics Group, Research Center of IPO Porto (CI-IPOP) / RISE@CI-IPOP (Health Research Network), Portuguese Oncology Institute of Porto (IPO Porto) / Porto Comprehensive Cancer Centre (Porto.CCC), Porto, Portugal.
2. Institute of Science and Innovation in Mechanical and Industrial Engineering (INEGI), Porto, Portugal.
3. Department of Mechanical Engineering, Faculty of Engineering-University of Porto (FEUP), Porto, Portugal.
4. 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Guimarães, Portugal.
5. Department of Pathology, Portuguese Oncology Institute of Porto (IPO Porto), Porto, Portugal.
6. Department of Pathology and Molecular Immunology, School of Medicine and Biomedical Sciences Abel Salazar-University of Porto (ICBAS-UP), Porto, Portugal.

#. Equally contributing authors.

Presenting Author

Sara Monteiro-Reis

Abstract

Introduction: Bladder cancer (BlCa) is the ninth most common cancer worldwide, associated with significant morbidity and mortality. Most bladder tumors derive from the urothelial lining – the urothelial carcinomas. Distinguishing the specific microenvironment which cancer cells experience between mucosa and muscularis layers can help elucidate how these cells acquire invasive capacities. Thus, the current work aims to measure the mechanical properties of both mucosa and muscularis layers of the bladder wall.

Materials & Methods: Measures of the micromechanical properties of bladder tissue from BlCa patients were performed using Atomic Force Microscopy (AFM). For that, fresh tissue samples from patients diagnosed with BlCa and treated with cystectomy were collected. Specifically, two cross-sections (transversing all layers) of both the macroscopically normal urinary bladder wall (away from the tumor area) and from the bladder wall adjacent to the tumor were collected and immediately frozen, prior to AFM samples preparation. The respective twin formalin-fixed paraffin-embedded specimens were processed and later evaluated by a uropathologist for routine histopathological examination.

Results: The average Young's modulus (representing cells stiffness) in tumor-adjacent specimens was significantly higher in the muscularis when compared the mucosa. Similarly, tumor-free specimens presented significantly higher Young's moduli in the muscularis than the mucosa samples. Moreover, medium Young's moduli values were higher in all layers of tumor-adjacent tissues, when compared with respective tumor-free samples.

Conclusions: AFM measurements clearly distinguish the stiffness of both mucosa and muscularis layers, being the muscularis the stiffest for all conditions. The quantitative assessment of the bladder wall stiffness range presents essential data for future research on BlCa cells culture substrates, and for understanding how cancer cells invade through the different bladder layers.

61. Role of the ubiquitin hydrolase ATXN3 in glioblastoma: a new tumor suppressor gene?

Authors and Affiliations

Margarida Machado-Sousa[1,2,*]; Céline S. Gonçalves[1,2,*]; Andreia Carvalho-Neves[1,2]; Eduarda P. Martins[1,2]; Liliana Costa[1,2]; Sara Duarte-Silva[1,2]; Patrícia Maciel[1,2,*]; Bruno M. Costa[1,2,*]

1. Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus Gualtar, 4710-057 Braga, Portugal;
2. ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal;

*. These authors contributed equally to this work

Presenting Author

Céline S. Gonçalves

Abstract

Introduction: Glioblastoma (GBM) is the most malignant and common primary brain tumor in adults, with an average median survival of 15 months. To improve the clinical management of GBM patients it is crucial to identify new biomarkers to contribute to a better stratification of these patients in molecular subgroups. Here, we explored the role of the ubiquitin hydrolase ATXN3 in glioblastoma.

Material and Methods: ATXN3 expression was evaluated in publicly available datasets of glioma patients at the mRNA level, and in GBM patient-derived and commercially available cell models, at the mRNA and protein level. ATXN3 was overexpressed in a GBM cell line and its effects on cell viability, proliferation, invasion, migration, and cell death after treatment with temozolomide were assessed. Survival analyses were performed with Cox regression and Log-rank tests. Meta-analyses were used to calculate the estimated pooled effect.

Results: We show that ATXN3 expression decreases significantly along glioma grade, being less expressed in GBM when compared to lower-grade gliomas, and that it is associated with IDH mutation and 1p/19q codeletion. In vitro, ATXN3 was expressed in all GBM cells tested, both at the mRNA and protein level. Functionally, ATXN3 overexpression was associated with a significant decrease in the cell viability and invasion of GBM cells. No effect was observed regarding cell proliferation and migration. This data suggests that ATXN3 may have tumor suppressive functions in GBM, decreasing its aggressiveness in vitro. In GBM patients, we found that ATXN3 has clinical prognostic value, being associated with longer overall survival, independently of other potential prognostic factors.

Conclusions: In summary, this work shed light on the role of ATXN3 in GBM by identifying it as a tumor suppressor gene, and as a new prognostic biomarker of favorable outcome, bringing new knowledge about the molecular mechanisms underlying this deadly disease.

62. Lactate – Sirtuin 6 axis mediating a metabolic reprogramming in renal cell carcinoma

Authors and Affiliations

Vera Miranda-Gonçalves[1,2], Ana Lameirinhas[1,2], Catarina Macedo-Silva[1], João Lobo[1,2,3], Paula C. Dias[1,3], Verónica Ferreira[1,3], Rui Henrique[1,2,3], Carmen Jerónimo[1,2]

1. Cancer Biology and Epigenetics Group, Research Center of IPO Porto (CI-IPOP)/RISE@CI-IPOP (Health Research Network), Portuguese Oncology Institute of Porto (IPO Porto)/Porto Comprehensive Cancer Center (Porto.CCC), Portugal.
2. Department of Pathology and Molecular Immunology, School of Medicine & Biomedical Sciences-University of Porto (ICBAS-UP), Portugal.
3. Department of Pathology, Portuguese Oncology Institute of Porto (IPOP), Portugal.

Presenting Author

Vera Miranda-Gonçalves

Abstract

Introduction: Renal cell carcinomas (RCCs) are the most lethal of the common urological cancers, being metastasis the foremost cause of cancer-related mortality. Despite improvements in adjuvant therapies, the impact on survival of patients with advanced and metastatic cancer has been feeble. Recently, it has been acknowledged the relevance of metabolic-epigenetic interplay in RCC, where metabolic fluctuations dictate cancer cells' epigenetic plasticity. Moreover, RCC display a characteristic Warburg effect, producing high levels of lactate oncometabolite. The effect of extracellular lactate on the epigenetic landscape, particularly in sirtuins (SIRTs) has been explored in some tumour types, however it remains uncharted territory in RCC.

Materials and Methods: The effects of lactate, nicotinamide (NAM) and alpha-cyano-4-hydroxycinnamate (CHC) on SIRT6 and histone acetylation levels were evaluated in normal kidney and RCC cell lines. Additionally, lactate derived SIRT6 effect on metabolic enzymes was assessed by qRT-PCR, western blot and Immunofluorescence. Finally, SIRT6 immunoexpression was tested in human RCC and normal renal tissues.

Results: Lactate inhibited SIRT6 expression in RCC cells, increasing histone acetylation levels. This effect was paralleled by NAM treatment and reverted by lactate transporter inhibitor (CHC). Cells exposed to lactate exhibited increased PKM2, LDHA, MCT1 and MCT4 expression, as well as of lipid synthesis (FASN and ACAC1) related genes. Moreover, lactate increased the HIF-1α and HIF-2α transcript levels along with SREBP1 transcription factor in RCC cell lines. In patients, RCC tissues presented lower SIRT6 expression than normal kidney samples.

Conclusions: Lactate oncometabolite seems to regulate SIRT6 expression promoting a metabolic reprogramming in renal cell carcinoma cells.

63. Redesigned case-control study using allelic expression as a quantitative phenotype identifies new risk loci for breast cancer

Authors and Affiliations

Ghezzo, MN[1], Magno, R[1], Xavier, JM[1], Maia, AT[1,2]

1. Center for Research in Health Technologies and Information Systems (CINTESIS), Universidade do Algarve, Faro, Portugal
2. Faculty of Medicine and Biomedical Sciences, Universidade do Algarve, Faro, Portugal

Presenting Author

Ghezzo, MN

Abstract

Despite the recent identification of hundreds of risk loci by genome-wide association studies (GWAS), half of the genetic burden of breast cancer (BC) is still unaccounted for. Considering that most of the identified risk loci lie outside of genes and that the characterised ones cis-regulate the expression of target genes near and far, we hypothesise that the missing heritability of BC is mostly attributable to unidentified cis-regulatory variants. Hence, we have redesigned case-control studies to focus genetic risk identification on the variants with greater cis-regulatory potential.

Using RNA-seq data from breast normal-tissue samples from BC patients (TCGA, n = 97) and healthy individuals (GTEx, n = 72), we performed a case-control genome-wide association study using allelic expression (AE) ratios as a quantifiable trait. Reference genome mapping of RNA-seq data was done using STAR, and variant calling was performed with GATK and samtools. Obtained read counts (for both reference and alternative alleles) were used to calculate AE ratios at variants identified as heterozygous at the DNA level. Of the 14887 SNPs tested, over 600 (annotated to 500+ genes) were significantly associated with BC risk (FDR 1%). These variants were located both at novel and previously reported BC loci. These associations now warrant further validation in an independent dataset, as well as mapping of the candidate causal variants and functional characterisation at the top loci.

Here we propose and show the power of using a functional approach to redesign case-control studies – using AE ratios as a quantitative phenotype - to identify risk for a polygenic complex disease.

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64. Assessment of radiation sensitivity in BRCA mutations carriers compared to non-carriers from HBOC families

Authors and Affiliations

Diana Gonçalves[1,3], Inês Marques[1,2,4], Ana Salomé Pires[1,2,5], Patrícia Paiva[5,6,7], Ana Jardim[5,6,7], Joana Santos[8], Graciano Paulo[8], Ilda Ribeiro[5,6,7], Isabel Carreira[5,6,7], Gabriela Sousa[9], Maria Filomena Botelho[1,2,5], Ana Margarida Abrantes[1,2,10]

1. University of Coimbra, Coimbra Institute for Clinical and Biomedical Research (iCBR) Area of Environment Genetics and Oncobiology (CIMAGO), Institute of Biophysics of Faculty of Medicine, Coimbra, Portugal;
2. University of Coimbra, Center for Innovative Biomedicine and Biotechnology (CIBB), Coimbra, Portugal;
3. University of Coimbra, Faculty of Science and Technology, Coimbra, Portugal;
4. University of Coimbra, Faculty of Pharmacy, Coimbra, Portugal;
5. Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal;
6. University of Coimbra, Cytogenetics and Genomics Laboratory, Institute of Cellular and Molecular Biology, Faculty of Medicine, Coimbra, Portugal;
7. University of Coimbra, Coimbra Institute for Clinical and Biomedical Research (iCBR) Area of Environment Genetics and Oncobiology (CIMAGO), Faculty of Medicine, Coimbra, Portugal;
8. Medical Imaging and Radiotherapy Department, Polytechnic Institute of Coimbra, ESTESC-Coimbra Health School, Coimbra, Portugal;
9. Portuguese Oncology Institute of Coimbra Francisco Gentil, Coimbra, Portugal;
10. ESTESC-Coimbra Health School, Coimbra, Portugal

Presenting Author

Inês Alexandra Marques

Abstract

Introduction: Hereditary Breast and Ovarian Cancer Syndrome (HBOC) is a genetic disorder mostly caused by mutations in the BRCA1 DNA associated gene (BRCA1) and BRCA2 DNA associated gene (BRCA2) tumour suppressor genes. Individuals with HBOC have a greater susceptibility to develop different types of cancer compared to the general population. Thus, they have a regular follow-up for cancer detection based mainly on imaging procedures with ionizing radiation (IR). However, it is hypothesized that HBOC individuals may have increased radiosensitivity. Aim: Evaluate the radiosensitivity of BRCA2 mutation carriers (BRCAmC) comparatively to non-carriers and the possible biological effects induced by the exposure to IR during diagnostic imaging procedures.

Materials and Methods: Genetic characterized individuals with BRCA2 mutation or non-carries from HBOC families were included in this study. Blood samples from these individuals were directed cultured or used to obtain lymphoblastoid cell lines (LCLs). Then, both cultures were irradiated using single (0,19 – 18,23 mSv) and cumulative (23,84 mSv in each irradiation) IR diagnostic doses. Radiobiological assays were performed for the analysis of reactive oxygen species formation (ROS), cell viability (XTT and Alamar blue assays) and genotoxicity (micronucleus and dicentric assays).

Results: Till now, 14 blood samples of BRCAmC and non-carriers of BRCA2 mutations were irradiated with single doses. No alterations were observed related to viability, ROS or micronucleus and dicentric chromosome formation. Moreover, 14 LCLs were obtained, and cumulative IR dose protocol is being performed.

Conclusions: The study is still ongoing, but the results obtained till now showed that single exposure to low doses of IR (up to 18,23 mSv) do not induced biological damage for BRCAmC. However, more experiments are being conducted to clarify the effects related to cumulative doses of IR, which will indicate the effects of performing various imaging procedures throughout life.

65. Comparison of Radium-223 biological effects in 2D and 3D cell cultures of metastatic prostate cancer

Authors and Affiliations

Carolina Fernandes[1,3*], Inês Alexandra Marques[1,2,4*], Afonso Pedrosa[1,4], Ana Salomé Pires[1,2,5], Edgar Tavares-da-Silva[1,5,6], Gracinda Costa[7], Francisco Caramelo[1,2,5], Ana Margarida Abrantes[1,2,5] Arnaldo Figueiredo[5,6], Maria Filomena Botelho[1,2,5]

1. University of Coimbra, Coimbra Institute for Clinical and Biomedical Research (iCBR) area of CIMAGO, Institute of Biophysics of Faculty of Medicine, Coimbra, Portugal;
2. University of Coimbra, Center for Innovative Biomedicine and Biotechnology (CIBB), Coimbra, Portugal;
3. University of Coimbra, Faculty of Science and Technology, Coimbra, Portugal;
4. University of Coimbra, Faculty of Pharmacy, Coimbra, Portugal;
5. Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal;
6. Department of Urology and Renal Transplantation, Centro Hospital e Universitário de Coimbra (CHUC), Coimbra, Portugal;
7. Department of Nuclear Medicine, Centro Hospitalar e Universitário de Coimbra (CHUC), Coimbra, Portugal.

*. Both authors contributed equally for this work

Presenting Author

Inês Alexandra Marques

Abstract

Introduction: Metastatic castration-resistant prostate cancer (mCRPC) is an incurable disease with only palliative treatments available. Radium-223 (Ra-223) was the first alpha-particle emitting radioisotope approved for the treatment of patients with mCRPC and bone metastases. The clinical outcomes do not meet the expectations, mostly justified by the doubts about Ra-223 localization and how it interacts within the metastatic niche. Using 3D cell cultures allows to mimic in vivo tumour microenvironment. Aim: Analyse the radiobiological effects of radium-223 in 2D and 3D models of metastatic prostate cancer (mPCa) to better understand its mode of action and the molecular pathways involved in the biological response.

Materials and Methods: mPCa cells (PC3) in 2D culture were exposed to increasing activities of Ra-223 (55 - 7040 kBq/kg) during 4, 24 or 48 h. The effects on cellular protein content were evaluated by SRB assay 5 and 7 days post-irradiation. PC3 spheroids (5000 cells) were created and irradiated with the same activities used for 2D cultures. The spheroid's size and disintegration were followed during 8 days by optical microscopy and images analysed in ImageJ. Cell viability was measured by CellTiter-Glo® 3D Assay.

Results: In 2D, it was observed a decrease in protein content with increasing activities of Ra-223, mainly for exposures to 3520, 5280 and 7040 kBq/kg. This decrease is evident for all times of exposure, with no significant differences between 24 and 48 h of irradiation. A decrease in spheroid size, integrity and proliferation was also observed, especially for exposures to 5280 and 7040 kBq/kg.

Conclusions: Higher activities of radium-223 have a greater cytotoxic effect, even in 3D structures. More studies regarding cell death, genotoxicity and DNA damage are under development to disclosure the radiobiological effects of Ra-223. We aim to develop future experiments in 3D heterotypic cultures to consider the role of angiogenesis, osteoblastic cycle, and immune system in Ra-223 therapeutic effects.

66. Generation of tumor-immune spheroids to tackle triple-negative breast cancer radioresistance and immune escape

Authors and Affiliations

Ana Canha-Borges[1,2,3*], Filipa Leal[1,2,3*], Catarina Moreira-Barbosa[1,2,3], Flávia Castro[1,2], Joana Paredes[1,4], Maria José Oliveira[1,2]

1. i3S – Institute of Investigation and Innovation in Health, University of Porto, Portugal;
2. INEB – Institute of Biomedical Engineer, University of Porto, Portugal;
3. ICBAS – Institute of Biomedical Sciences Abel Salazar, University of Porto, Portugal;
4. IPATIMUP – Institute of Pathology and Molecular Immunology, University of Porto, Portugal

*. equally contributing first authors.

Presenting Author

Ana Canha-Borges

Abstract

Triple-negative breast cancer (TNBC), the most aggressive subtype, lacks expression of therapeutic targets. Being more radioresistant than others, TNBC associates with higher risk of recurrence following radiotherapy (RT). This points the need to unveil mechanisms underlying TNBC radioresistance, disclosing novel targets for radiosensitive therapies that improve disease management. To overcome radioresistance, attention must be paid to other elements of the tumor microenvironment, as macrophages.

This study aimed to develop a 3D-model that closely mimics TNBC microenvironment to address the role of macrophages in radioresponse.

For this, 3D tumor-immune spheroids gathering MDA-MB-231 TNBC cells and human macrophages were established. After 3 days, spheroids were submitted, or not, to two RT schemes commonly used in clinic: 2.67Gy and 5.2Gy, for one or five cumulative fractions, representing one day or week of patients treatment. Then, spheroids were dissociated to evaluate macrophage inflammatory profile and cancer cell immunogenicity by flow cytometry. Results show that cancer cells promoted macrophages to express high levels of CD40 and CD206. In return, macrophages modulated cancer cells to increase CD47 and PD-1 immune checkpoints expression. After RT, macrophages acquired a more pro-inflammatory phenotype, with increased CD86 and CD40 and decreased CD163 and CD206 expression. Interestingly, and despite this phenotype, macrophages protected tumor cells from death caused by RT and promoted an increase of cancer cells PD-L1 expression and IL-6 secretion, which may favor immune escape.

Overall, this study highlights the importance of combining RT with immunotherapy in TNBC patients. This model can be used to study therapy-resistance mechanisms and to unveil novel pharmacological targets to improve TNBC patients treatment.

67. Challenges related with germline variants of uncertain significance in BRCA1 and BRCA2 and other promising targets of PARPi

Authors and Affiliations

Pedro Rodrigues[1,2]; Patrícia Theisen[1]; Catarina Silva[1,2]; Joana Mendonça[1], Dina Carpinteiro[1]; Luís Vieira[1,2]; João Gonçalves[1,2]

1. Human Genetics Department, National Institute of Health Dr Ricardo Jorge, Lisbon, Portugal
2. Center for Toxicogenomics and Human Health, Nova Medical School, Lisbon, Portugal

Presenting Author

Pedro Rodrigues

Abstract

Introduction: The growing use of PARP inhibitors (PARPi) in the treatment of patients with homologous recombination-deficient (HRD) tumors contributes to an increasing number of patients being screened for BRCA variants (Vs), even when family history of cancer is absent, resulting in a growing number of Vs that need to be classified at the pathological level. In the absence of BRCA germline pathogenic (P)/Likely pathogenic (LP) Vs, screening of other genes related with Homologous Recombination Repair (HRR) pathway, might benefit patients candidates for PARPi treatment. The goals of this study, were to identify P and LP Vs in BRCA1, BRCA2, ATM, CHEK2 and PALB2, to analyze uncertain significance Vs (VUS) and bring to discussion their uncertainties and impact on patients and relatives.

Materials & Methods: We analyzed 146 patients with personal history of breast cancer (124), ovarian cancer (12) and breast plus other type of cancer (10) and mainly with family history of cancer (137). NGS data were obtained using TruSight® Cancer and MiSeq. Annotation was performed with Variant Interpreter, VEP, HSF, IGV, HGMD, Alamut and Varsome. Vs were analyzed considering allele frequency (AF) in population databases and classified according to ACMG-AMP guidelines.

Results: We identified 128 Vs with AF< 1% (BRCA1: 13, BRCA2: 34, ATM: 58, CHEK2: 8 and PALB2: 15). Among the Vs, 17 were classified as P (BRCA1: 3, BRCA2: 8, ATM: 3, CHEK2: 1 and PALB2: 2), 2 as LP (BRCA2: 1 and ATM: 1) and 31 as VUS (BRCA1: 6, BRCA2: 7, ATM: 12, CHEK2: 2 and PALB2: 4), We highlight that, VUS were more frequent than P and LP Vs.

Conclusion: Patients without BRCAs germline P/LP Vs but that are carriers of P/LP Vs in other genes related with HRR-pathway, might benefit from PARPi. VUS pose difficulties related to the clinical management of patients and relatives, for that reason functional studies are of major importance to assess their biopathologic impact.

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68. Searching for biomarkers: impact of early molecular changes in centrosome function and Barrett's esophagus progression

Authors and Affiliations

Miguel A. Pereira[1,2], Leonor Nunes[3], Inês S. Gomes[1], Mónica Bettencourt-Dias[3], Carla A.M. Lopes[1,3]

1. Instituto Português de Oncologia de Lisboa Francisco Gentil, Lisboa, Portugal
2. Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal
3. Instituto Gulbenkian de Ciência, Oeiras, Portugal

Presenting Author

Miguel A. Pereira

Abstract

Introduction: Barrett's Esophagus (BE) is a pre-malignant condition and the only known precursor of esophageal adenocarcinoma. Its malignant transformation is a multistep process from metaplasia (premalignant condition) to dysplasia, adenocarcinoma and metastasis. Despite the low progression rate of BE, the current lack of progression biomarkers results in the inclusion of all BE patients into surveillance programs to detect early malignant progression. Recent studies from our group suggest the possibility of using centrosome aberrations (CA) as biomarkers of progression: while CA were never detected in metaplasia from patients that did not progress, CA were detected in metaplasia of patients who progressed to neoplasia and may contribute to malignant transformation. We therefore hypothesize that molecular changes occurring early in BE leading to CA may be potential progression biomarkers

Materials and Methods: Analysis of transcriptome datasets of metaplasia clinical samples from patients that progressed versus patients that did not progress revealed deregulated expression of several centrosome components. Taking advantage of a validated panel of cell lines that represent all stages of disease found in vivo, we validated a candidate protein and tested its impact in centrosome function and BE progression.

Results: We confirmed that the candidate protein is downregulated (qPCR, WB, IF) in BE progression. Notably, its down-regulation (by siRNA) in a normal esophageal cell line led not only to centrosome number abnormalities, but also to centrosome structural changes that may also impact its microtubule nucleation capacity and therefore potentially contribute to the acquisition of malignant features such as invasiveness capacity.

Conclusions: By revealing the role of molecular changes occurring early in BE in its malignant transformation, these results may be used to develop new tools that explore CA as biomarkers of progression, and thus contribute to the improvement of surveillance programs.

69. A variant of unknown significance in the BRCA2 gene alters pre-mRNA splicing and protein expression

Authors and Affiliations

Pais, ACC[1]; Lima, B[1]; Custódio, N[1]; Dias, P[2]; Dupont, J[2]; Sousa, AB[2]; Carmo-Fonseca, M[1]; Carvalho, C[1]

1. Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal
2. Serviço de Genética, Departamento de Pediatria, Hospital de Santa Maria, Centro Hospitalar Universitário Lisboa Norte, Lisboa, Portugal

Presenting Author

Célia Carvalho

Abstract

Introduction: Germinal mutations that cause dysfunction of BRCA1 and BRCA2 proteins are a hallmark of the Hereditary Breast and Ovarian Cancer syndrome (HBOC). Somatic mutations in the BRCA1/2 genes are also detected in a sub-set of non-familiar cancers. Both familial and sporadic BRCA-deficient cancers are exquisitely sensitive to drugs such as platinum salts and poly (ADP-ribose) polymerase (PARP) inhibitors. For these reasons, BRCA genetic testing is extensively used in the clinic. However, a considerable number of tests are inconclusive because no known BRCA pathogenic mutation is detected. Here, we investigated how intronic alterations in BRCA1/2 genes impact on RNA splicing and protein expression.

Materials and methods: Mononucleated blood cells were isolated from germline mutation carriers. An immortalized cell line was generated. Splicing isoforms were analyzed by RT-PCR, sequenced, and quantified by droplet digital PCR (ddRT-PCR); protein expression by immunoblot.

Results: We observed that the pathogenic variant BRCA1: c.212+1G>A promotes the usage of a natural, underused, alternative splice donor; the resulting mRNA contains a premature termination codon (PTC). Analysis of BRCA2: c.7618-10T>G, classified as variant of uncertain significance (VUS), revealed activation of a cryptic splice and PTC creation.

The lymphoblastoid cell line carrying a BRCA1: c.212+1G>A showed decreased levels of BRCA1 protein. Next, we investigated the impact of BRCA mutations on DNA repair: RAD51 foci formation after etoposide treatment was comparable with normal cells and treatment with mitomycin C did not induce chromosomal breakage in these cells.

Conclusions: We show that a BRCA2 variant currently classified as VUS alters mRNA splicing and creates a PTC. However, if the cellular function of BRCA2 is affected remains unclear. We used standard assays to measure DNA repair and found no defects in cells carrying a monoallelic BRCA1 pathogenic variant. Although further studies are needed, our results suggest that heterozygous BRCA mutations are not drivers of genomic instability.

70. Impact of BRCA1/2 variants on ovarian cancer survival

Authors and Affiliations

Sofia Fragoso[1], Teresa Duarte1, Pedro Meireles[2], Inês Oliveira[2], Paula Rodrigues[3], Sidónia Santos[1] Ana Opinião[2,3], Ana Carla Luís[2,3], Isália Miguel[2,3], Carolina Pereira[2], Beatriz Mira[2,3] and Fátima Vaz[2,3]

1. Molecular Pathobiology Research Unit, Portuguese Institute of Oncology of Lisbon Francisco Gentil;
2. Medical Oncology Department, Portuguese Institute of Oncology of Lisbon Francisco Gentil;
3. Family Risk Clinic, Portuguese Institute of Oncology of Lisbon Francisco Gentil

Presenting Author

Sofia Fragoso

Abstract

BRCA1/2 are the major tumor suppressor genes associated with ovarian cancer (OC). In contrast to most previously described data, our cohort of BRCA1/2 OC pts includes more BRCA2 pts. A trend to better survival for BRCA2 compared with BRCA1 pts was previously observed for OC as the first or subsequent diagnosis. We hypothesized that c.156_157insAlu variant in BRCA2 (BRCA2 Alu) and copy
number variations in BRCA1 (BRCA1 CNV) might play a more influential role in OC due to their higher frequency in our cohort. In this study our objective is to study the impact of the BRCA2 Alu and BRCA1 CNV on pts survival outcomes.

Methods: Survival analysis of BRCA1 and BRCA2 OC pts; subgroup analysis of BRCA2 Alu and BRCA1 CNV OC pts.

Results: The median age of diagnosis was 64 yrs for BRCA2 Alu and 58 yrs for non-Alu pts. Both groups had pts treated with olaparib (31% BRCA2 Alu and 21% non-Alu). For a median follow up was 57 months, the median OS for OC as 1st diagnosis was 73 months for BRCA2-Alu pts while median OS has not yet been reached in non Alu subgroup.

For BRCA1 the median age of diagnosis was 53 yrs in the BRCA1-CNV and 52 yrs for non-CNV pts. Both groups had pts treated with olaparib (20% BRCA1-CNV and 17% non-CNV). For a median follow up was 57 months, median OS was 44 months for BRCA1-CNV pts and 77 months for all others BRCA1 pts.

Conclusion: In our cohort, BRCA1 OC pts have worse median OS than BRCA2 pts ($p<0.05$). Regarding subgroup analysis, a trend to better OS was observed for BRCA2 non-Alu pts while BRCA1 CNV pts have a worse OS than all other BRCA1 pts.

71. Impact of early centrosome deregulation in malignant transformation

Authors and Affiliations

Inês S. Gomes[1], Carla A.M. Lopes[1,2]

1. Instituto Português de Oncologia de Lisboa Francisco Gentil, Lisboa, Portugal
2. Instituto Gulbenkian de Ciência, Oeiras, Portugal

Presenting Author

Inês S. Gomes

Abstract

Introduction: Centrosome amplification (CA) is a hallmark on human cancer and is therefore an appealing feature for prognosis and therapy. As the major microtubule-organizing center in animal cells, the centrosome has key roles in cell division, signaling and migration. However, the poor understanding of its origin and impact in cancer has limited its use in the clinic. We used Barrett's esophagus (BE) tumorigenesis as a cancer model to test if CA contributes to tumorigenesis and progression. BE's neoplastic pathway of progression has well characterized steps: from metaplasia (premalignant condition) to dysplasia, adenocarcinoma and metastasis. Given that CA arises in metaplasia and that its incidence significantly increases in dysplasia, we hypothesized that CA may play an important role in the acquisition of malignant properties. If this is true, then an increase of CA in metaplasia and/or a decrease of CA in dysplasia would be sufficient to respectively promote or reduce malignant properties.

Materials and Methods: Here, we took advantage of a validated panel of cell lines that represent all stages of disease found in vivo, particularly three distinct dysplasia cell lines derived from different patients. To reduce CA levels in dysplasia cells we downregulated key molecules of the centrosome duplication cycle by either siRNA or chemical inhibition. We then assessed their invasive potential using invasion assays and 3D cell cultures

Results: Both strategies were effective in reducing CA and 3D cultures showed that reduction of CA levels decreased the invasiveness capacity of all dysplasia cell lines tested.

Conclusions: By showing that CA contributes to the invasiveness potential in dysplasia, our results reveal the importance of CA in the acquisition of malignant properties in BE progression. These findings may contribute to new clinical tools, namely using CA as a biomarker of progression. Given widespread occurrence of CA in human tumors, our results may be extended to other cancers where CA is prevalent.

72. Recapitulating the Tumor Microenvironment of Hodgkin Lymphoma disease: adipocytes friends or foe?

Authors and Affiliations

Andreia Matos[1,2,3,4], Joana Marinho-Dias[4,5], Dulcineia Pereira[6], Ana Miranda[7], Helena Brízido[7], Manuel Bicho[2,3], Yan Cheng[8], Pingzhao Hu[8], Alan G Ramsay[9], Maria J. Oliveira[1], Ricardo Ribeiro[1,10]

1. Tumour & Microenvironment Interactions Group i3S -Instituto de Investigação e Inovação em Saúde, University of Porto, Rua Alfredo Allen, 208, 4200-135 Porto, Portugal
2. Genetics Laboratory and Environmental Health Institute, Faculdade de Medicina, Universidade de Lisboa, Av. Prof. Egas Moniz, 1649-028 Lisboa, Portugal
3. Instituto de Investigação Científica Bento da Rocha Cabral, 1250-047, Lisboa
4. Abel Salazar Institute for the Biomedical Sciences (ICBAS), University of Porto, Rua de Jorge Viterbo Ferreira No. 228, 4050-313 Porto, Portugal
5. Molecular Oncology Group-CI, Portuguese Institute of Oncology, Edificio Laboratorios-Piso 4, Rua Dr António Bernardino Almeida, 4200-072 Porto, Portugal
6. Onco-hematology Department, Portuguese Institute of Oncology Porto Centre, Rua Dr António Bernardino Almeida, 4200-072 Porto, Portugal
7. Immunotherapy Unit and Clinical Pathology Department of Sta Maria Hospital, Lisbon, Portugal
8. Department of Biochemistry and Medical Genetics & Department of Electrical and Computer Engineering, University of Manitoba, Winnipeg, Canada
9. School of Cancer and Pharmaceutical Sciences, Faculty of Life Sciences & Medicine, King's College London, London, UK
10. Centro Hospitalar Universitário do Porto EPE, Portugal

Presenting Author

Andreia Matos

Abstract

Introduction: Accumulating evidence indicates that the tumor microenvironment (TM) plays a key role in the pathogenesis of Hodgkin Lymphoma (HL), both at lymph nodes (LN) and in infiltrated bone marrow (BM). Evidence suggests that not only T cells, but also monocytes contribute to the TM in HL disease. Adipocytes are now starting to be implicated as regulators at BM and we envisage an intricate role surrounding LN.

Material and Method: From our cohort, we analyzed interstitial marrow fluid (IMF) from BM aspirate of 16 HL patients at diagnosis and 11 controls. Human Obesity Array C1 (RayBiotech®) was used to determine protein expression in IMF of 8 HL (4 with increased adiposity, IA, and 4 with normal adiposity, NA) and 8 age, gender- and body mass index-adjusted controls (4 IA and 4 NA). Adiposity was measured through abdominal circumference (AC) measurement and Body mass index. We validate those findings measuring some adipokine-related molecules determined by ELISA. Gene expression analysis was conducted through RT-qPCR. Further, L428 cells were cultured with human macrophages and T cells (isolated from healthy blood donor buffy coats). Hypertrophied adipocytes were isolated from obese patients submitted to bariatric surgery and directly cultured with L428, Mac and T cells. The interaction between these distinct cells were investigated by flow cytometry and soluble factors quantify by ELISAs.

Results and discussion: From the BM niche, we found a downregulation of interleukins (IL-1a/b, IL-6sR, IL-12), chemokines (CCL2, CCL3, CCL16), IGF-axis mediators (IGFBP-1, IGFBP-2, IGFBP-3, IGF-1sR), sTNFRII, TGFβ1, leptin, Osteoprotegerin (OPG), and Fas ($P<0.1$) at IMF of HL comparing to controls. Even when analyzed

according with adiposity status (HL vs controls), for some of these, we continued to observe a downregulated profile. Interestingly, HL overweight/obese subjects presented up-regulation of OPG and lymphotactin (P<0.1). From the IMF milieu, the IGFBP-3 levels continue to be decreased, independently of adiposity status (P=0.008), and OPG levels were increased in HL disease (P=0.047). The transcripts from fractionated BM-adipocytes (BMA) and stromal cells (SC) demonstrated that LEPR (2.6-fold, P<0.001), TGFβ1 (4.57-fold, P=0.038), and IGFBP3 (22.21-fold, P=0.003) were significantly overexpressed in HL compared to controls in BMA, while the IFG2R was upregulated in SC (P=0.047). Peripherally, we observed a decreased levels of serum IL-8 and increase levels of serum HGF and MMP-9 in HL compared to controls (P<0.05). From the in vitro perspective, co-culture L428 with macrophages, resulted in M2-like phenotype, as seen through the upregulation of CD40, and CD206. The conditioned media of L428 supported M2-like differentiation through upregulation of CD40, CD163, CD206, and PD-L1. Moreover, the CD14+PD-L1 cells may modulate the expression of CD3+PD1+ cells through direct contact with PD-L1 macrophages and HRS cells. These cells induce an immunosuppressive microenvironment and thereby escape antitumor immunity. Now, we are dissecting the role of hypertrophied adipocytes in this set-up, considering a direct contact system between adipocytes, macrophages, T and L428 cells.

Conclusions: We have performed extensive in vitro studies using bi-, tri- and tetra-cultures including malignant HRS cells, T-cells, macrophages, and adipocytes, determining its functional role in the regulation of immunobiology of HL.

73. P-cadherin overexpression is associated with shorter overall survival in high grade serous ovarian cancer

Authors and Affiliations

R. Canario[1,2,3], I. Morgado[1,2,4], N. Mendes[1], P. Lopes[2], M. R. Teixeira[2,3], A.S. Ribeiro[1], C. Bartosch[2,3], J. Paredes[1,5]

1. i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto
2. Instituto Português de Oncologia do Porto Francisco Gentil
3. Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto
4. Faculdade de Medicina, Universidade de Coimbra
5. Faculdade de Medicina, Universidade do Porto

Presenting Author

Rita Canário

Abstract

Introduction: Epithelial-mesenchymal transition (EMT) is a developmental program often reactivated during cancer progression, where classic cadherins are known to be deregulated. Growing evidence associates hybrid EMT phenotypes with increased metastatic potential. Due to the unique anatomical position of the Fallopian tube, these phenotypes co-expressing epithelial and mesenchymal markers may be involved in the peritoneal dissemination of high grade serous ovarian carcinoma (HGSC).

Material and Methods: Expression of E-cad (CDH1), N-cad (CDH2) and P-cadherins (CDH3) was analyzed in tumours (immunohistochemistry of 130 Fallopian tube epithelium (FTE) and 135 HGSC) and in cell lines, both in silico (CCLE database) and in vitro (BG1 and OVCAR4 cell lines).

Results: HGSC had significant upregulation of P-cad and downregulation of N-cad, when compared with the FTE. All tumors co-expressed the 3 cadherins; however, only 28% had high co-expression scores. Tumours with high cadherin co-expression and high P-cad expression were significantly associated with shorter overall survival (OS). CDH3 expression was positively correlated with CDH1 but not with CDH2. Interestingly, the 2 cell lines used for in vitro characterization co-expressed high levels of P- and E-cad, but lack N-cad expression. Correlation with other EMT-related genes/proteins was observed, both in silico and in vitro, namely a positive correlation with EpCAM (epithelial marker) and GRHL2 (hybrid marker) and an inverse correlation with ZEB1, ZEB2 (EMT transcription factors) and VIM (mesenchymal marker). Currently, we are testing the functional impact of P-cad silencing in vitro in terms of cell migration, anoikis resistance, resistance to carboplatin and invasion.

Conclusions: HGSC with high P-cad expression have significantly shorter OS. P-cad de novo expression is correlated with co-expression of epithelial and hybrid EMT markers, supporting our hypothesis that this transient state may have a role in HGSC carcinogenesis.

74. Unravelling the target-specific anticancer action of THEDES

Authors and Affiliations

Filipe Oliveira, from LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, Caparica, Portugal; Eduardo Silva, from 3B's Research Group, University of Minho, Avepark 4805-017 Barco, Guimarães, Portugal and ICVS/3B's PT Government Associated Laboratory, Braga/Guimarães; Joana M. Silva, from 3B's Research Group, University of Minho, Avepark 4805-017 Barco, Guimarães, Portugal and ICVS/3B's PT Government Associated Laboratory, Braga/Guimarães; Rui L. Reis, from 3B's Research Group, University of Minho, Avepark 4805-017 Barco, Guimarães, Portugal and ICVS/3B's PT Government Associated Laboratory, Braga/Guimarães; Ana Rita C. Duarte, from LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, Caparica, Portugal.

Presenting Author

Filipe Oliveira

Abstract

The remarkable bioactive properties of DES have now been widely described. Eutecticity has proven to be a promising tool in the development of new and effective therapeutic agents, contributing to modern medicine most challenging battles, such as antibacterial and antibiofilm, antifungal, and anticancer. Additionally, the tailor-made versatility and compliance with the green chemistry metrics, have pushed forward the possible therapeutic applications of eutectic formulations. In this work, we aim to unravel what we consider to be the next step in the comprehension and consolidation of such therapeutic systems – target-specificity. By allying the anticancer and anti-inflammatory properties of terpenes with nonsteroidal anti-inflammatory drugs (NSAID), we have already observed and described THEDES selective action towards cancer cells (Colorectal cancer cells), without compromising normal intestinal cells viability. So, what are the specific cellular targets and consequent responses from the eutectic exposure? Using a multidisciplinary approach, from the design, preparation and characterization of THEDES, to the study of their bioavailability and bioactivity; to the evaluation of THEDES specific cell target, we expect to untangle THEDES cytotoxic mechanisms and establish this technology as an innovative approach in the cancer challenge.

75. Overcome colorectal cancer using therapeutic deep eutectic systems

Authors and Affiliations

Joana Pereira, Maria Castro, Filipe Oliveira e Ana Rita Duarte

- LAQV-REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal

Presenting Author

Joana Pereira

Abstract

Cancer remains a major health problem worldwide, with colorectal cancer (CRC) being the third most incident. Inflammation has been highly associated with cancer development and maintenance, therefore, the reduction of the inflammatory microenvironment represents a promising therapeutic strategy. The use of nonsteroidal anti-inflammatory drugs (NSAIDs) to reduce CRC risk has already been described.

Deep eutectic systems (DES) are based on the combination of different components which together present a deep decrease in their melting point. These systems are most widely in compliance with the green chemistry metrics and have low production costs, thus presenting an alternative to conventional solvents. When an active pharmaceutical ingredient is part of a DES it is designated by therapeutic deep eutectic system (THEDES). To evaluate THEDES potential therapeutic activity, three THEDES based on natural occurring molecules with anticancer properties combined with NSAIDs were prepared: safranal (Saf):ibuprofen (IBU) (3:1), Saf:IBU (4:1) and Menthol (Me):IBU (3:1). The evaluation of these THEDES physico-chemical properties alongside with the assessment of its bioavailability and bioactivity, were explored in this work as an integrative approach to determine their anti-CRC activity. Our results show that these THEDES present promising therapeutic activity towards CRC cells due to a selective cytotoxic action towards cancer cells. These antiproliferative activity seems to occur through the induction of apoptosis via caspase-3 and in the case of Me:IBU (3:1) also via LDH release. Additionally, Me:IBU (3:1) seems to have an anti-inflammatory activity by reducing ROS production. In conclusion, THEDES present high value enhanced therapeutic properties, namely increased bioavailability and anticancer specific activity, suggesting lower side effects.

76. Peritumoural adipose tissue at the crossroad of clear cell renal cell carcinoma - friend or foe?

Authors and Affiliations

Mendes-Ferreira, M (Instituto de Investigação e Inovação em Saúde, i3S); Oliveira, MJ (Instituto de Investigação e Inovação em Saúde, i3S); Ribeiro, R (Instituto de Investigação e Inovação em Saúde, i3S)

Presenting Author

Mendes-Ferreira, M

Abstract

Excess adipose tissue is a well-established factor for cancer incidence. Notably, clear cell renal cell carcinoma (ccRCC) is among the cancers for which this effect is more prominent. Nevertheless, the molecular features behind this paradox remain elusive.

Here, we take advantage of ex vivo perirenal (PrAT) and subcutaneous (ScAT) adipose explants from ccRCC subjects to assess the influence of these depots on tumour progression. PrAT and ScAT were phenotypically characterized and the conditioned media (CM) from these depots were collected and used to evaluate the impact of adipose secretome on ccRCC cells functional and transcriptomic traits.

PrAT displayed a decreased adipocyte cross sectional area compared to ScAT and higher amounts of brown- and beige-selective genes, hinting a thermogenic-laden phenotype. Intriguingly, PrAT also displayed increased amounts of white-selective genes in comparison to ScAT, revealing an heterogeneous profile. Adipose secretome decreased the number of EdU+ events and increased the number of apoptotic cells. Overall, these results suggest an influence of the adipose secretome to impair tumour expansion. As adipose tissue is a source of fatty acids (FA), we looked into the FA metabolic profile of ccRCC cells following treatment with adipose CM. Our findings suggest an increase in lipolytic-related genes when cells are cultured under the influence of ScAT secretome. We substantiated these results resorting to the KIRC-TCGA dataset.

Site-specific differences of distinct adipose depots seem to contribute differently to ccRCC progression. Whereas both adipose depots seem to impair cancer cell proliferation, ScAT increased apoptosis and the lipolytic machinery within the remaining cells, adding yet another factor to the intricate relationship between adipose tissue and ccRCC. Our results open an avenue to assess the influence of these depots on the tumour immune compartment as well as towards the influence of distant adipose depots on cancer biology.

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