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ESE Basic Endocrinology Course on Endocrine and Neuroendocrine Cancer 2016

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Invited Speakers' Biographical Notes and Lectures

L1 – Biography

Pilar Santisteban is a Full Professor at the Biomedical Research Institute of the Spanish Council of Research, Madrid, Spain. Among other responsibilities, she has been a Vice President of the Spanish Endocrine Society and a member of the Executive Committee of the European Thyroid Association (ETA). Currently, she is the manager of the Spanish National Grants Program, the Spanish Member of the Joint Research Center of the European Union, and since October 2015, she is the president-elected of the ETA.

She is a specialist in molecular biology of the thyroid gland, focused on deciphering the role of thyroid transcription factors and signals that control differentiation and proliferation in development and cancer. Her work was pioneer describing the mechanism by which BRAF represses iodide uptake and promotes metastases. Recently, her laboratory, using the next-generation sequencing, has described genes that confer thyroid cancer susceptibility and miRNAs that target genes involved in Iodide metabolism and invasiveness. Her work tries to combine basic research and its clinical application.

L1

Advances in molecular pathogenesis of thyroid cancer

Pilar Santisteban
Instituto de Investigaciones Biomédicas. CSIC-UAM, Madrid, Spain.

The presence of differentiated follicular thyroid cells in thyroid cancer is critical for the antitumor response to radioactive iodide treatment, and loss of the differentiated phenotype is a key hallmark of iodide-refractory metastatic disease. Thus, one of the objective of our laboratory is to study the signals and molecular mechanisms involved in thyroid differentiation in cancer. Among genes responsible of thyroid differentiated phenotype are the transcription factor PAX8 and its target gene SLC5A5, also named as NIS (Na/I Symporter), which function is to uptake iodide. It is well accepted that the RET-RAS-BRAF-ERK signaling pathway leads to thyroid cellular transformation being the BRAF mutation the most frequent genetic event that confers aggressive biological behavior to papillary thyroid cancer (PTC). We have shown that BRAF impairs NIS function by a mechanism involving the TGFβ/Smad signaling and accordingly causes radioiodide-resistant metastasis. In addition TGFβ has a strong cooperative effect with ERK signal in BRAF-induced epithelial mesenchymal transition (EMT), migration, and invasion. We have generated an orthotopic mouse model of thyroid cancer to study primary tumor formation and its metastases and how the inhibition of the TGFbeta pathway could abolish metastases and recover Iodide uptake.

By Next Generation Sequencing we have identified the miRNome and characterized the miRNAs (miRs) regulatory network of normal thyroid and PTC samples unveiling that the most abundant deregulated miRs in PTC regulates genes essential for thyroid differentiation. Among these miRs are the mature products of miR-146b (miR-146b-5p and -3p) that targets genes involved in iodide metabolism such as PAX, NIS and IYD (DEHAL1), the iodotyrosine deiodinase that controls the recycling of iodide for thyroid hormone synthesis. In addition, miR-146b-5p targets DIO2, the iodothyronine deiodinase that converts T4 into T3. We have performed functional studies found that miR-146b-3p binds to the 3′UTR of PAX8 and NIS leading to impaired protein translation and a subsequent reduction in iodide uptake. Interestingly miR-146b and PAX8 regulate each other and share common target genes, thus highlighting a novel regulatory circuit that governs the differentiated phenotype of PTC.

In summary, all these data describe molecular determinants that may be exploited therapeutically to modulate thyroid cell differentiation and iodide uptake for improved treatment of advanced thyroid cancer.

DOI: 10.1530/endoabs.40.L1

L3 – Biography

Paula Soares, BSc, MSc, PhD, is an Assistant Professor of Biopathology at the Medical Faculty of the University of Porto and coordinates the Group of Cancer Biology at the Institute of Pathology and Immunology of the University of Porto (Ipatimup) – Instituto de Investigação e Inovação em Sáude (i3S), Portugal. She received her degrees in Biology, Master in Oncobiology, and PhD in Human Biology at the University of Porto. Her main interests include oncobiology of thyroid and neuroendocrine tumours mainly addressing thyroid cancer genetic alterations and signal transduction molecules involved in the MAPK and mTOR pathways. Paula Soares has a particular interest in the clinical translation of the pathologic meaning of genetic alterations. The metabolic deregulation in cancer is also an interest in her research. Paula Soares team has provided important insights into the genetics of thyroid tumors concerning the discovery of BRAF mutations and of TERT promoter mutations in thyroid cancer. She is also a member of numerous scientific committees and evaluation boards and serves on grant review committees and in journal editorial and review boards.

L3

Telomerase promoter mutations in cancer: beyond immortalization?

Paula Soares & Cancer Biology Group
I3S/IPATIMUP and Faculdade de Medicina da Universidade do Porto, Porto, Portugal.

Cell immortalization has been considered for a long time as a classic hallmark of cancer cells. Besides telomere maintenance due to the 'alternative mechanism of telomere lengthening' it was advanced that such immortalization could be due to telomerase reactivation, but the mechanisms underlying such reactivation remained elusive.

Mutations in the coding region of telomerase gene are very rare in the cancer setting, despite being associated with some degenerative diseases. Recently, mutations in telomerase (*TERT*) gene promoter were found in sporadic and familial melanoma and subsequently in several cancer models, notably in gliomas, thyroid cancer and bladder cancer. In thyroid cancer, the relevance of these findings has been reinforced by the association of *TERT* mutations with tumour aggressiveness, presence of distant metastases and poor patient survival. Specifically, in differentiated thyroid cancers TERT promoter mutations are an indicator of clinically aggressive tumours, being correlated with worse outcome and higher disease-specific mortality. TERT promoter mutations have an independent prognostic value in differentiated thyroid cancer and, notably, in papillary thyroid carcinoma, the most common type of endocrine neoplasia. We will address the role of telomerase genetic alterations in the metastatic profile of thyroid cancer and discuss the value of telomerase as a new biomarker with impact on the prognosis and survival of the patients and as a putative therapeutic target.

DOI: 10.1530/endoabs.40.L3

L4 – Biography

Valeriano Alberto Pais Horta Leite, MD, PhD was born in Lisbon in 1958.
He completed his MD from the Faculty of Medicine of Lisbon in 1982 and Fellowship in Endocrinology 1991, Portuguese Institute of Oncology, Lisbon. In 1996, he was awarded the PhD degree by the Faculty of Medical Sciences, Lisbon.
He is currently the Head of the Department Endocrinology, Portuguese Institute of Oncology, Lisbon; Professor of Endocrinology, Faculty of Medical Sciences, Lisbon; Investigator of iNOVA4Health, Lisbon.
He has published more than 65 papers in refereed journals such as Journal of Clinical Endocrinology and Metabolism, Thyroid, Clinical Endocrinology, European Journal of Endocrinology, Endocrine Pathology, British Journal of Cancer, Endocrine Related Cancer, Journal of Endocrinology, Neuroendocrinology, Biology of Reproduction, Molecular and Cellular Neurosciences.
He is also a referee of several peer-reviewed journals and is the recipient of 21 prizes and awards.

L4

Molecular targeted therapy in medullary and well-differentiated thyroid carcinoma

Valeriano Leite
Department of Endocrinology, Instituto Português Oncologia, Lisboa, Portugal.

Differentiated thyroid cancer (DTC) of papillary (PTC) and follicular (FTC) types with invasive loco-regional or metastatic disease, poorly differentiated (PDTC) and anaplastic thyroid cancers (ATC), as well as the C-cell derived medullary thyroid cancers (MTC), represent the major cause of mortality amongst thyroid cancer patients. Because such patients are resistant to standard therapy, they may be excellent candidates for innovative adjuvant approaches such as molecular targeted therapies. Thus, a better understanding of the molecular mechanisms underlying these advanced forms of thyroid cancer is essential for the design of newer and more efficient forms of treatment. Our knowledge of the molecular pathogenesis of thyroid carcinomas has improved dramatically in the past few years. Recently, the whole-exome sequencing of ~500 PTCs confirmed the dominant role of *MAPK* and *PI3K* pathways in PTC and also revealed other new driver mutations.[1] Mutational analysis of PDTC and ATC showed that most mutations were present in *TP53, RAS, TERT, PIK3CA*, *PTEN* and CDKIs[2,3] and mutually exclusive *RET* and *RAS* mutations represent the major mutational events in MTC.[4]

Knowledge of key oncogenic mutations in differentiated thyroid cancer rose interest on tyrosine kinase inhibitors and several phase III studies with these drugs have been performed in both follicular-derived and C-cell derived carcinomas with promising results. Unfortunately, studies on target-therapy of specific oncogenic mutations and immune-modulating approaches are yet to be published.

Next-generation sequencing, which enables analysis of multiple genes in DNA extracted from tumour biopsies or in tumour circulating DNA, combined with specific inhibition of target gene(s), will represent a powerful tool to manage advanced thyroid cancer analogously to other tumour models, such as the lung, in which this approach has revolutionized the treatment of the disease.

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DOI: 10.1530/endoabs.40.L4

L5 – Biography

Professor Barbara Jarzab MD, PhD, endocrinologist and nuclear medicine specialist, head of Department of Nuclear Medicine and Endocrine Oncology at Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Gliwice, Poland; a comprehensive cancer center and research institute that takes care for Silesian patients, an industrial part of Poland with 4.6 million population. Areas of research involvement: functional genomics of endocrine tumors with focus on gene expression profiling of thyroid cancer and genetic predisposition to endocrine disease (thyroid cancer, pheochromocytomas, Graves' disease). Clinical activity: radioiodine therapy of differentiated thyroid cancer and benign thyroid disease, radiopeptide imaging and therapy of neuroendocrine tumors, new methods of diagnosis and therapy of progressive endocrine gland cancer, including multikinase inhibitors clinical trials in thyroid cancer. Coordinator of multidisciplinary consortium which introduced first in Poland DNA microarray technologies implemented in analysis of cancer transcriptome. Correspondent Member of Polish Academy of Arts and Sciences and correspondent Member of Polish Academy of Sciences, member of the Committee for Human Genetics and Molecular Pathology of the Polish Academy of Sciences, President of Polish Group of Endocrine Tumors of Polish Society of Endocrinology. Previous positions: Secretary of European Society of Endocrinology, Secretary of European Thyroid Association – Cancer Research Network, member of European Thyroid Association Executive Committee.

L5

Endocrine cancer: in the light of recent molecular studies

Barbara Jarząb & Jolanta Krajewska
Department of Nuclear Medicine and Endocrine Oncology, MSC Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Gliwice, Poland.

Endocrine cancer constitutes a term and simultaneously a definition of a type of cancer, that is common in American literature whereas so far absent in European nomenclature. Detailed analysis of scientific programs of European endocrinological and oncological congresses proves that also these societies do not mention this type of cancer. To change this unfavorable situation a new clinical platform called European Task Group for Endocrine Cancer (ETEC), working under the auspices of the ESE Clinical Committee, has been created.
The lecture will discuss the features characteristic for endocrine cancer and why it is worthy to look for them. Molecular features that join malignant tumors in a one separate group named endocrine cancer will be analyzed. We also attempt to answer the question how the molecular characteristic of endocrine cancer may influence our clinical practice, both its diagnostic and therapeutic aspects.

DOI: 10.1530/endoabs.40.L5

L6 – Biography

Prof. Scarpa is Chair of the Department of Pathology and Diagnostics and Director of the ARC-Net Research Center for Applied Research on Cancer. He leads the Cancer Biobank Network Programme in the Veneto Region (Italy) (population 4 600 000). Chief of the Diagnostic Molecular Pathology Laboratory officially recognized by Veneto Government as one of two referral centres for cancer related molecular diagnostics in the region. Prof. Scarpa's focus: translation into clinical practice of molecular subclassifications of cancers with prognostic-diagnostic-therapeutic relevance. Leader of the Italian effort in the International Cancer Genome Consortium (www.icgc.org) funded by Italian Ministry of Research and Ministry of Health. PI of Italian National Consortium for early diagnostics in cancer funded by Italian Association for Cancer Research (AIRC). He has participated in EU funded consortia such as MOLDIAG-PACA (www.moldiagpaca.eu), EPC-TM-NET (www.EPC-TM-NET.eu), CAM-PAC (www.cam-pac.eu). Over 360 publications in peer reviewed journals, 82 publications in the last 4 years.

L6

The genomic landscape of pancreatic neuroendocrine tumors

Aldo Scarpa
ARC-Net Research, University of Verona, Verona, Italy.

Pancreatic neuroendocrine tumours (PanNETs) are clinically challenging diseases as they display variable outcomes. We performed genome sequencing on 100 sporadic, primary pancreatic neuroendocrine tumours (PanNETs) and defined the underlying mutagenic mechanisms and mutations promoting this rare malignancy. Genome-wide analysis showed PanNETs to have a lower mutation burden and greater genomic stability than pancreatic exocrine cancers. The most common mutational signatures were Deamination, APOBEC, and the unknown aetiology signature five of Alexandrov *et al*. (Nature, 2013). In addition, 6/100 patients had a novel mutational signature. A single PanNET had a BRCA-deficient mutational signature due to a BRCA2 homozygous deletion. Chromothripsis occurs rarely and involves chromosome 11q inactivating *MEN1* gene. Alternative Lengthening of Telomere (ALT) was detected in 60% of cases and these were heavily enriched for *DAXX* and *ATRX* mutations. Driver gene analysis confirmed pathogenic mutations in *MEN1* (42% mutated, 3% homozygously deleted, 5% damaged by SV), ALT promoting mutations in *DAXX* (24%) and *ATRX* (11%), recurrent mutations mTOR signalling components (20%). CNV analysis found three major molecular tumour classes confirming previous published suggestions. Integrated mRNA and miRNA profiling of human PanNETs identifies three distinct subtypes classified into insulin-like (IT), metastasis-like (MLP), and MEN1-like. MLP tumors express pancreas development genes, whereas IT tumors express mature islet cell genes.

DOI: 10.1530/endoabs.40.L6

L8 – Biography

Dr Oriol Casanovas is an internationally renowned laboratory researcher with ample knowledge and expertise in basic and translational research of mouse models of cancer and tumor angiogenesis. His research at ICO-IDIBELL in Barcelona has always been focused on determining the consequences of adaptation and resistance to anti-angiogenic therapies against cancer. His scientific achievements have been published in highly prestigious journals specialised in the field of cancer research, and for their particular impact, his papers have frequently been published with 'preview' articles and associated to several highlights or commentary articles in the most prominent scientific journals. Accumulated IF: 1805 (Scopus), H-Index Score: 13 (Scopus).

L8

Anti-tumor effects of Semaphorin 4D blockade unravel a novel pro-invasive mechanism of vascular targeting agents in pNETs

Marta Pàez-Ribes, Laura Martín, Patricia Carrasco & Oriol Casanovas
Tumor Angiogenesis Group, Translational Research Laboratory, Catalan Institute of Oncology – IDIBELL, Barcelona, Spain.

One of the main consequences of vessel pruning and inhibition of neovessel growth produced by angiogenesis inhibitors is increased intra-tumor hypoxia. Nevertheless, growing evidence indicates that tumor cells escape from this hypoxic environment to better nourished locations, presenting hypoxia as a positive stimulus for invasion. Indeed, we and others have described a pro-invasive and pro-metastatic adaptation to VEGFR-targeted antiangiogenic therapy that implicates hypoxia as a triggering event.

Using the spontaneous mouse model of pancreatic neuroendocrine cancer (RIP1-Tag2) we describe here the vascular targeting and antitumor effects of Sema4D pro-angiogenic molecule blockade that consequently impairs tumor growth and prolongs survival. Mechanistically, vessel structure is altered at the pericyte coverage level, while vessel density is maintained. Consistently, there is no vascular trimming or induction of intra-tumor hypoxia by anti-Sema4D therapy, but surprisingly there is a significant increase in local invasion and distant metastases, comparable with VEGFR inhibition. When dissecting the mechanisms of these hypoxia-independent pro-invasive and pro-metastatic effects, we discovered a novel Sema4D-positive population of macrophages that are actively recruited by Sema4D blockade to the tumor parenchyma and tumor invasive fronts. Furthermore, *in vitro* studies demonstrate that Sema4D blockade in macrophages alters their secretome to promote tumor cell invasion and dissemination.

Overall, this study demonstrates beneficial anti-tumor and pro-survival effects of Sema4D blocking antibody but also unravels a novel mechanism of tumor aggressivity that implicates Sema4D+ macrophages that mediate local tumor cell invasion and distant metastasis.

Publication List (related to the topic of the talk)

Moserle, Jiménez-Valerio & Casanovas *Cancer Discovery* 2014
Moserle & Casanovas *J. Internal Medicine* 2013
Casanovas O. *Nature* 2012
Moserle & Casanovas *EMBO-MM* 2012
Casanovas O. *J. Clin. Invest.* 2011
Pàez-Ribes *et al. Cancer Cell* 2009
Casanovas *et al. Cancer Cell* 2005
Casanovas *et al. Oncogene* 2005

DOI: 10.1530/endoabs.40.L8

L9 – Biography

Corinne Basquet is currently Research Director (DR2), INSERM U1037 – Cancer Research Center of Toulouse (CRCT), Toulouse France and is co-Leader of the Team 'Protein synthesis & secretion in carcinogenesis'.
In 1999, she had obtained her PhD from the Cedars-Sinai Medical Center, Los Angeles, USA and also completed her post-docorate from there. In 2001, she was recruited as Research Scientist (Chargée de Recherches) at INSERM. In 2012, she was promoted to the rank of Research Director at INSERM.
Her fields of expertise are Somatostatin and somatostatin receptors, G Protein-coupled and Cytokine Receptor Signalings, Pancreatic cancer biology, Microenvironment, Neuroendocrine and Pituitary tumors, PI3K-mTOR signalling and Transgenic models of pancreatic cancer.
Her current research projects are to i) explore the role of the tumor microenvironment in the initiation/progression phases of pancreatic cancer using transgenic models, and primary cultures of human cancer-associated fibroblasts; ii) identify stroma-derived proteins by implementing challenging proteomic approaches; iii) investigate the tumor suppressive action of somatostatin and its receptors during pancreatic carcinogenesis, more specifically at the interface between pancreatic epithelial and stromal cells.

L9

Deciphering the antitumoral potential of stromal somatostatin receptor signal

Corinne Bousquet
INSERM U1037, Cancer Research Center of Toulouse, France.

Pancreatic ductal adenocarcinoma (PDA) remains a highly lethal malignancy. Therapeutic strategies aimed at targeting pancreatic cancer cells have failed. We hypothesized that such pitfall is due to the functional heterogeneity of this tumor which comprises both cancer and stromal cells. It is now recognized that cancer-induced but host-derived stromal events support tumor growth and chemoresistance. In order to reach efficacy with chemotherapies, we believe that stroma has to be co-targeted.

Cancer-associated fibroblasts (CAFs) are the most abundant cells present in PDA stroma. We showed that human activated (αSMA-expressing) pancreatic CAFs (primary cultures isolated from human tumor resections) express the somatostatin receptor subtype sst1, but not the other receptor subtypes (sst2-sst5), by opposition to non-activated (αSMA-negative) pancreatic fibroblasts (PaSC, pancreatic stellate cells isolated from normal pancreas) which do not express any sst. This was confirmed by immunohistochemistry on human PDA.

Somatostatin receptors are known to be highly expressed in neuroendocrine tumor cells where they are used in clinic as therapeutic targets for their antisecretory and antitumoral role. However, these receptors are not expressed in pancreatic cancer cells (PDA). Data regarding the expression of somatostatin receptors in the tumor stroma are quasi-inexistent.

Interestingly, we observed a hyperactivation of the PI3K-mTORC1 pathway in CAFs as compared to PaSC, which was blunted by the somatostatin analog SOM230 (pasireotide) through activation of sst1, but not by octreotide. The observed elevated protein synthesis rates in CAFs were also dramatically decreased by SOM230 treatment, resulting in the inhibition of soluble and extracellular matrix protein secretion. Chemoprotection provided by CAF secretome on cancer cells was blunted by SOM230 treatment, involving interleukin-6 the expression of which was decreased by SOM230 specifically at the protein, but not mRNA, level. *In vivo,* athymic mice orthotopically co-xenografted with the human pancreatic cancer cells and CAFs developed tumors, the growth of which was dramatically reduced upon mouse treatment with the combination SOM230 + gemcitabine (chemotherapy of reference for PDA), but not by each single drug. Interestingly also, we showed that 60% of untreated, and 100% of gemcitabine-treated mice, developed metastases (lungs and liver), whereas none of the SOM230-treated or SOM230 + gemcitabine-treated mice showed any metastases. This is consistent with the ability of CAF conditioned media to induce epithelial-to-mesenchymal transition in pancreatic cancer cells (decrease or increase of E-cadherin or N-cadherin expression, respectively, and increase of expression of the EMT Zeb1, Snail & Slug transcription factors), which is reverted with conditioned media from SOM230-treated CAFs.

Our results highlight a novel promising anti-tumor activity for SOM230 indirectly targeting pancreatic cancer cell chemoresistance, invasion and metastasis through pharmacological inhibition of stromal CAFs.

DOI: 10.1530/endoabs.40.L9

L10 – Biography

Professor Aurel Perren is Director of the Institute of Pathology at the University Bern since 2009. He did his pathology training in Zürich with a special focus on endocrine tumors. 2007 he was appointed Professor of Pathology in the Technische Universität München. His research focus lies on the histopathological, molecular and genetic analysis of familial and sporadic NEN with a special interest in pancreatic NEN. He was involved in the WHO classification of endocrine tumors 2004 and since the first Frascati meeting in 2005 Aurel Perren is regularly involved in European Neuroendocrine Tumor Society (ENETS) activities. His research group in Bern as a focus on the progression of pancreatic NEN and thyroid carcinomas. The group integrates molecular *in-vitro* and *in-vivo* results with clinical and *ex-vivo* results gained on human tumor tissues.

L10

ALTered telomeres in pancreatic neuroendocrine tumors

Aurel Perren & Ilaria Marinoni
Institute of Pathology, University Bern, Switzerland.

Neuroendocrine tumors do not share many classical pathways of tumorigenesis with their non-endocrine counterparts. In recent years, the molecular understanding of of pancreatic neuroendocrine tumors (PanNET) tumorigenesis has made major advances. A whole exome NGS analysis described frequent mutations of two new genes *DAXX* and *ATRX*. 40% of unselected panNET show mutations in one of these genes. Mutations in these genes are associated with loss of the respective proteins.[1,2] Interestingly, DAXX and ATRX negative panNET are characterized by a telomerase independent mechanism of telomere length maintenance termed ALT.[3] The mechanisms leading to ALT activation upon DAXX/ATRX loss are still unknown. DAXX or ATRX knock down is not sufficient to induce ALT activation in telomerase positive PanNET cell lines[4,5] (and Marinoni unpublished data). Increasing evidence indicates that DAXX and ATRX are involved in histone 3 deposition at telomeric sequences thereby participating in the maintenance of the epigenetic status of telomeres. Additionally, ATRX is part of the ADD domain (ATRX-DNMT3-DNMT3L) of DNMT3A, responsible of *de novo* DNA methylation while DAXX binds and mediates DNMT1 recruitment at specific genes promoters. We have shown that ALT as well as loss of DAXX/ATRX is associated with chromosomal instability in PanNET and with the adverse outcome associated with this.[2] DAXX/ATRX loss seems to be a late event in PanNET tumorigenesis leading to a potential advantage of clonal heterogeneity via chromosomal instability and telomerase independent survival mechanisms.

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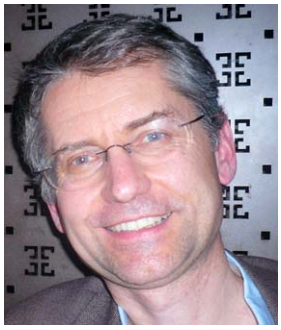
L11 – Biography



Jérôme Bertherat is professor of Endocrinology at Paris Descartes University, Chief of the Endocrinology Department of Cochin Hospital, head of the National Center for Rare Adrenal Diseases and of the research team 'Genomics and Signaling of Endocrine Tumors' in the Cochin Institute (INSERM U1016 & CNRS UMR8104), Paris, France. He serves as the coordinator of the French National Network for Rare Endocrine Disorders (FIRENDO) and as chair of the Clinical Committee of the European Society of Endocrinology (ESE). He has served as deputy Chief Editor of the European Journal of Endocrinology, and in the nomination Committee of the European Society of Endocrinology. He is currently member of the executive committees of the ESE, of the ENSAT network dedicated to adrenal tumors and of the French Endocrine Society. His main research specialties include Cushing's syndrome, the genetics of familial adrenal tumors and the molecular genetics of endocrine tumors.

L11

New insights on adrenocortical tumors OMICs

Jérôme Bertherat
Center for Rare Adrenal Diseases, Hôpital Cochin & INSERMU1016, CNRS 8104, Université Paris-Descartes, Paris, France.

There is a variety of adrenocortical tumors that can be responsible for different cortisol excess levels. Genomics allowed recently many progress in this field. Exome sequencing allowed to identify somatic activating mutations of the catalytic subunit of PKA (*PRKACA*) in cortisol secreting adenomas responsible for overt-Cushing. Combining pangenomic snp analysis and whole genome sequencing led to the identification of inactivating germline mutations of a new tumor suppressor gene, ARMC5, in primary bilateral macronodular adrenal hyperplasia. The use of genomics in adrenocortical cancer give now a clear description of the mRNA and miRNA expression profile (transcriptome), as well as chromosomal and methylation alterations. Exome sequencing combined with pangenomic snp analysis revealed alterations of key driver genes in adrenocortical cancer, like *CTNNB1*, *TP53*, *CDKN2A*, *DAXX*, *TERT*, *MEN1*, *RB1*… It also revealed that the most frequently altered gene is *ZNRF3*, a new tumor suppressor gene identified by this approach, and likely to control the Wnt/β-catenin pathway. Interestingly, genomics study of adrenocortical cancer also revealed subtype of malignant tumors with different pattern of molecular alterations associated with different outcome.

Clearly genomics studies led to a new vision of adrenocortical tumors classification based on molecular analysis. This open new perspectives not only to unravel the adrenal tumorigenesis but also for the development of various molecular tools to classify adrenocortical tumors and guide patient management for a precision medicine.

DOI: 10.1530/endoabs.40.L11

L12 – Biography

Alberto Cascon obtained his BSc (1993) and PhD in Biology (2000) from the University of León (Spain). In 2001 he was awarded a Postdoctoral Fellowship by the Madrid City Council and took up position with the Hereditary Endocrine Cancer Group at the Spanish National Cancer Research Centre (CNIO) in Madrid. In 2004, he was awarded a Fellowship by the Spanish Department of Health, and became a Staff Scientist in the same group at the CNIO, led by Dra. Mercedes Robledo. Since 2001 he has been investigating the genetics of pheochromoctymas and paragangliomas, a subject on which he has focused 40 of his more than 70 published manuscripts. During the last years, he has conducted various exome sequencing projects which have led to the discovery of two new pheochromocytoma/paraganglioma susceptibility genes: *MAX* and *MDH2*. In 2012, his paper reporting the discovery of MAX was awarded publication of the year by the *Fundación Mutua Madrileña*.

L12

Identification of new familial pheochromocytoma/paraganglioma genes using next generation sequencing (NGS)

Alberto Cascon
Hereditary Endocrine Cancer Group, Spanish National Cancer Research Centre (CNIO), Madrid, Spain.

The high percentage of germline mutations found in patients with pheochromocytoma (PCC) and/or paraganglioma (PGL) in recent years has made this rare disease the most heritable of all tumors. Whole-exome sequencing (WES) has played a substantial role in deciphering the genetic causes of many of these hereditary cases. Thus, *MAX*, *FH* and *MDH2* have been identified as PCC/PGL susceptibility genes by sequencing the whole exome of patients selected because of the presence of a particular phenotype or distinctive molecular signature. Hereditary PCC/PGL is a good example of how WES has led to breakthroughs in understanding the genetic basis of an exceptionally rare syndrome.

DOI: 10.1530/endoabs.40.L12

L13 – Biography

Prof. Massimo Mannelli is Full Professor of Endocrinology in the Department of Experimental and Clinical Biomedical Sciences 'Mario Serio', University of Florence. He is Senior Lecturer in Endocrinology at the Postgraduate School of Endocrinology, University of Florence. He is Director of the Post-graduate School of Endocrinology and Metabolism.

He is member of the Italian Society of Endocrinology, the European Society of Endocrinology, the Endocrine Society, the European NeuroEndocrine Association. In the period 2002–2005 he was the coordinator of the Scientific Committee of the Italian Society of Endocrinology. He is reviewer of many international journals in the field of Endocrinology and Hypertension.

Prof. Mannelli has a wide experience in clinical endocrinology in view of his responsibility in the In-patient Clinic and Day Hospital Service of the Endocrinology Unit of the University of Florence. His main research field encompasses the pathophysiology of the sympathetic-adrenal system as well as of the adrenal cortex and the endocrine hypertension.

Prof. Mannelli has been founding member of the ENS@T (European Network for the Study of Adrenal Tumors).

He published more than 50 book chapters (mostly on physiology and pathology of the adrenal gland) and more than 200 original papers in peer-reviewed international journals (among which *Lancet*, *JAMA*, *Nature Genetics*, *NEJM* etc) for a total impact factor greater than 1000.

L13

Cellular and animal models in pheochromocytoma/paragangliomas research: role of microenvironment

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Pheochromocytomas (PHEOs) and paragangliomas (PGLs) are rare neuroendocrine tumors. About 30-40% of these tumors are due to e germ-line mutation in one of the 13 main susceptibility genes which include: the tumor-suppressor gene *VHL*; the proto-oncogene *RET*; the tumor-suppressor gene *Nf1*; the genes encoding the four subunits of the succinate dehydrogenase (SDH); the *SDHAF2/SDH5* gene that is responsible for the flavination of the SDHA subunit; *TMEM127*; *MAX*; HIF2α; the gene encoding the fumarate-hydratase (FH) and, the gene encoding the TCA cycle enzyme malate-dehydrogenase type 2 (*MDH2*).

PHEO/PGL are mostly benign and in such cases they are cured by surgical removal. On the contrary, in PHEO/PGL due to *SDHB* mutations up to 80% of affected patients develop metastatic disease and no successful cure is at present available.

Tumor tissue, obtained at surgery, does not permit dynamic studies that can be performed only *in vitro* on living cells or *in vivo* in animal models. Unfortunately, the research on malignant PHEO/PGL is made difficult by the limited research models so far available.

In fact, at present no SDH knockdown animal models nor suitable human neural crest-derived cell lines are at disposal. The cell lines most widely utilized for the study of PHEO/PGL tumorigenesis are: PC12, derived from an irradiated rat PHEO; MPC, derived from an irradiated heterozygous *Nf1* knockout mouse; MTT, a MPC-derived more aggressive cell line. The only human PHEO cell line (hPheo1), obtained by immortalizing sporadic PHEO primary cultures by hTERT, apart from not being commercially available, appears to be minimally or not at all functional. A surrogate model is offered by human neuroblastoma cell lines, such as SK-N-AS, that share with PHEO/PGL the same embryologic origin.

An additional factor to be considered when studying tumorigenesis is the role of microenvironment. Solid tumors are in fact very complex tissues comprising not only cancer cells, but also non-malignant stromal cells such as endothelial cells, fibroblasts, immune cells and extracellular matrix. It is becoming more and more evident that a continue interplay between cancer and stromal cells generates a positive loop that leads cancer cells to survive the hostile environment, to grow and spread metastases to healthy tissues.

At this aim, co-cultures of stromal cells (fibroblasts) along with cancer cells (SK-N-AS, MPC or MTT control or *SDHB* silenced) have been performed in an attempt to recreate an *in vitro* cellular microenvironment as close as possible to the *in vivo* tissue conditions.

Either SDHB silencing or the fibroblasts were able to modify the metabolism and the functional characteristics of tumor cells. Silencing and fibroblasts caused additional effects.

The comprehension of the mechanisms by which *SDHB* mutations cause mitochondrial impairment, cell metabolic reprogramming and tumor development as well as the comprehension of the mechanisms driving the cross-talk between tumor cells and microenvironment might possibly represent the first step towards the development of potential novel pharmacological approaches aimed at limiting the proliferative effect and the tumor invasive/metastasizing potential of malignant PHEO/PGL.

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L15 – Biography

Born in Madrid, Mercedes Robledo studied at the Universidad Autónoma de Madrid where she graduated in Biology in 1987. She then joined the Genetic Department of the same University supervised by J Fernández-Piqueras, working on the cytogenetic and molecular features of heterochromatin. In 1989, under the supervision of J Benitez, she studied the characterization of chromosomal and molecular alterations of lymphomas for which she was awarded her PhD.

In 1995, she was appointed as staff member of the Genetics Service of the Fundación Jiménez Díaz (Madrid). Robledo moved to the CNIO in 2000 as the head of the Hereditary Endocrine Cancer Group. Her group has made substantial contribution to understanding the genetic bases of endocrine tumours, identifying two new genes involved in hereditary pheochromocytomas, and genes responsible for part of the hidden heritability of follicular cell derived thyroid carcinomas. Her research is supported by the FP7-HEALTH program of the European Commission, Spanish Ministry of Health, Autonomous Community of Madrid, Spanish Fundación Mutua Madrileña, and GETNE (Grupo Español de Tumores Neuroendocrinos).

She has authored more than 120 articles on genetic characterization of hereditary diseases as well as seven books chapters on molecular diagnosis in medical genetics. She has supervised 12 doctoral theses.

L15

Meet-the-expert: from genetics to the bedside

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Pheochromocytomas (PCC) and paragangliomas (PGL) are rare neuroendocrine tumours, located in the adrenal medulla (PCC) and in the intra-abdominal, thoracic or head and neck paraganglia (PGL). They have the highest heritability of all human neoplasms being a good example of diseases with underlying genetic heterogeneity. In this regard, at least 40% of PCC/PGL (PPGL) patients carry a germline mutation in one of the 22 genes described so far as related to the disease. In addition to the complexity of the genetics of PCC/PGL (from now on, PPGL), we need to consider the role of somatic mutations, which to date, have been identified at least in 20–30% of tumours. The latter have been observed to occur not only in the same genes involved in heritable susceptibility, but also in new ones, which have thus recently emerged as key players in the sporadic presentation of these diseases.

Other remarkable feature is the clinical heterogeneity among patients diagnosed with PPGL. Part of them develops additional tumours or disorders that serve as clues for deciding the most appropriated genetic test to perform. However, the main proportion of patients develops non-syndromic PPGLs, being necessary to use other information (such as biochemical secretion, tumour location, immunostaining data, etc.) that helps to address the genetic screening. In this regard, several algorithms have been proposed, but they usually exclude sporadic-PPGLs and none include somatic testing. Our group aimed to genetically characterize S-PPGL cases and propose an evidence-based algorithm for genetic testing, prioritizing also the DNA source.

Nowadays, new approaches based on targeted next generation sequencing (NGS) are allowing to simultaneously analyze all genes related to a disease, but interpretation of variants found is a new challenge to deal with, which must catch our attention in order to offer genetic counseling.

In addition, despite of the increasing proportion of patients already explained by germline or somatic genetic defects, there are still patients with clinical indicators of hereditary disease (i.e. familial antecedents, multiple tumors and/or young age) without a molecular diagnosis. In this regard, the genomic characterization has demonstrated to be an efficient tool for identifying not only diagnostic and prognostic markers, but also for discovering new genes related to the disease, after applying whole-exome sequencing to candidate patients clustered according to their genomic features.

The talk will review algorithms to apply in the genetic screening of patients diagnosed with PPGLs.

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L16 – Biography

Prof. Márta Korbonits, MD, PhD, DSc, FRCP, is a clinical academic endocrinologist. She graduated in medicine at Semmelweis Medical School in Budapest and works in the Department of Endocrinology at Barts and the London School of Medicine, where she is Co-Centre Head. She shares her time between clinical patient care, clinical research and laboratory-based research as well as teaching at undergraduate and postgraduate level. Her current interests include endocrine tumorigenesis, especially the genetic origin of pituitary adenomas and she works on both the clinical characterisation as well as molecular aspects of this disease. She leads a large international consortium to study this issue. In addition, she focuses on translational research on the hormonal regulation of the metabolic enzyme AMP-activated protein kinase. She was a recipient of a Medical Research Council Clinician Scientist Fellowship, the Society for Endocrinology Medal, and the Endocrine Society Delbert Fischer award. She published over 200 papers and has an H index of 47 on Scopus. She is currently the Head of the Science Committee of the European Society of Endocrinology. She served on numerous Program Organising Committees for ECE, ENEA and SfE and was member of the executive board of the Society for Endocrinology, Pituitary Society and ENEA and European Society of Clinical Investigation. She is on the editorial board of *Journal of Clinical Endocrinology and Metabolism* and *Pituitary* and the endocrine editor of *Scientific Reports* and *Annals of Human Genetics*.

L16

AIP and the somatostatin signalling in pituitary tumours

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Germline mutations in the *AIP* gene predispose to the development of pituitary adenomas, most often GH secreting tumours. These patients often poorly respond to medical therapy with somatostatin analogues.
There are two mechanisms suggested to be involved in this poor response.
One suggests that the somatostatin-induced upregulation of the tumour suppressor gene *Zac1* involves AIP. SSTR2 agonist treatment leads to AIP upregulation *in vivo* and *in vitro* and AIP overexpression or downregulation leads to increase or drop of ZAC1 levels.
The other mechanism suggests the involvement of the Gαi-2 protein, one of the G proteins known to be activated by SSTRs, as this was found to be downregulated in AIP insufficient cells.
These mechanisms could be operational not just in patients with germline AIP mutation, but also in patients with low AIP expression due to other factors.
It is currently unknown what are the exact molecular mechanisms involved in these effects. Future data on multireceptor ligand pasireotide in patients with AIP mutation or low AIP levels will help to clarify the mechanism.
Further deciphering these molecular pathways will provide a better understanding of the mechanism of action of this class of drugs and will have important therapeutic implications.

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L17 – Biography

Raul M Luque is an Associate Professor of Cell Biology at the University of Cordoba (UCO), Spain. Dr. Luque graduated (1997) and obtained his PhD (2003) in Biological Sciences from the UCO and completed his postdoctoral training at the Department of Medicine, University of Illinois, Chicago (USA). He currently serves as Co-Head of the Hormones and Cancer Research Group of the Department of Cell Biology, Physiology and Immunology of the UCO and the 'Maimonides' Biomedical Research Institute. His research over the last 15 years has been focused on the regulation of pituitary cell types by somatostatin, cortistatin, ghrelin, insulin and IGF-I and on the molecular biology of these peptides, their receptors and signaling pathways in health and disease. Dr. Luque has published over 100 scientific articles and 25 book chapters and has contributed over 200 congress communications. He has supervised 13 doctoral theses. He is a member of the Editorial Board and has served as ad hoc reviewer of several journals including *Journal of Clinical Endocrinology & Metabolism*, *Journal of Endocrinology*.

L17

Truncated sst5 receptor variants in pituitary tumors and cancer

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Endocrine-related cancers comprise a complex group of heterogeneous pathologies whose development and progression are profoundly conditioned by endocrine–metabolic deregulations. The well-known capacity of somatostatin (SST) to inhibit hormone secretion and cell proliferation in a wide variety of cell types, coupled to the ubiquitous expression of SST receptors (ssts) in normal and tumoral tissues, has led SST analogs (SSAs) to be extensively used in clinical practice for the treatment of endocrine-related cancers, such as pituitary and neuroendocrine tumors (NETs). However, despite ssts being present in their tumor cells, a significant proportion of patients are (or become) resistant to SSA therapy, and effectiveness of SSAs is thus limited to certain groups of patients. Moreover, clinical studies exploring the utility of SSAs in other ssts-positive, endocrine-related tumors, such as breast or prostate cancers, are lacking or also unsatisfactory. Lack of responsiveness to SSA therapy has been suggested to be associated to the intrinsic nature of ssts. In an attempt to further characterize the sst family, our group has identified new functional truncated variants of the sst5, with less than 7TMD, in various mammalian species (human, pig, mouse and rat). These truncated receptors are originated by the elimination of a cryptic intron in the sst5 sequence during the mRNA maturation through a non-canonical splicing event. Remarkably, these truncated variants have unique, ligand-selective signaling properties, distinct distribution in normal tissues, and different subcellular localization to that shown by the originally identified, long sst5 variant. Interestingly, human sst5 truncated receptors, and specially the truncated receptor with 4TMDs (sst5TMD4), are barely expressed in normal tissues, but have been found to be highly expressed in a subset of pituitary tumors, NETs, thyroid or breast cancers, wherein its expression has been associated to poorer prognosis. Indeed, the expression of sst5TMD4 has been correlated with impaired response to SSA treatment in pituitary adenomas and, likely, in thyroid carcinoma. Our data suggest that sst5TMD4 could act, at least partially, through the blockade of the normal activity of full-length canonical receptors, particularly sst2, thus acting as a dominant-negative receptor. Finally, sst5TMD4 has been linked to increased malignant phenotype in *in vitro* models of NETs, thyroid and breast cancer through increased proliferation, migration and invasion abilities, which altogether indicate the pivotal role of sst5TMD4 in the malignancy of these tumors, and pave the way to the identification of new molecular targets for the diagnosis, prognosis and therapy in endocrine-related tumors.

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L18 – Biography

Clara V Alvarez MD, PhD, graduated in Medicine at the University of Santiago de Compostela (USC). In parallel she initiated studies in hypothalamus-pituitary regulation of somatotroph and follicular thyroid cell function and proliferation. Upon completion of her Ph.D. she extended her training with a first postdoc at the Institut für Tumorforshung (IMT) in Marburg (Germany) and later on in the DIBIT, San Raffaele Hospital (Milan, Italy) where she developed functional analysis of RAS and EGFR mutations respectively. For the last 20 years she has been attached to the University of Santiago, where she is now Professor Accredited in Physiology in the School of Medicine and leader of the group, Neoplasia and Endocrine Differentiation at the Centre for Research in Molecular Medicine and Chronic Diseases (CIMUS).

Her major focus is in furthering our understanding of the basic mechanisms involved in pituitary cell renewal and cellular homeostasis, and the alterations in thyroid cell proliferation/differentiation leading to cancer. Her group has helped in the elucidation of fundamental physiological and molecular mechanisms in these two glands. In addition they have illuminated pathophysiologic mechanisms, improved diagnosis and treatment in patients with pituitary or thyroid disease. She has published articles in top peer reviewed journals and is frequently lecturing in International Congress and Symposia.

Dr CV Alvarez has a pro-European view of Endocrinology. She has served in the Executive Committee of the European Society of Endocrinology (ESE) under both Prof. Steve Lamberts and Prof. Ebo Nieschlag as presidents.

L18

Stem cells in pituitary… and in pituitary tumours?

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The demonstration of postnatal stem cells in the adenopituitary (PSC) a few years ago has made us to redraw many of the cell biology concepts that we previously had for this gland. PSC organize in a three-dimensional structure, the niche, where they combine with other populations. How many populations cohabit in the niche, and which markers should we use to define cells associated to PSC we still do know not. However, we have advanced in the definition of a PSC as a cell co-expressing markers of different families of proteins: stem cell markers (e.g. Sox2, Sox9, Klf4), pituitary development factors (e.g. Prop1), epithelial markers (cytokeratins, β-catenin) and survival factors (Ret and Gfr α co-receptors and ligands). In PSC, a careful balance of expression among all these markers exists to ensure that stemness is maintained. In fact, overexpression of one marker over the others from the same family appears to indicate recruitment from the niche and loss of the stemness state. Little is known about the processes of commitment and differentiation once a stem cell has left the niche. In the other hand, once accepted that the pituitary is renewed by stem cells, we should take up in parallel the existence of physiological pathways of apoptosis for old or unfitting secretory cells. The study of how the pituitary maintains a balanced 'cellular plasia' will help us to better understand human disease due to insufficient (hypopituitarism) or excess (pituitary tumor) cell numbers.

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L19 – Biography

Prof. Adrian Daly MB BCh, PhD, was born in Boston, Massachusetts and raised in the United States and Ireland. He received an Honors Bachelor of Science degree in Physiology from University College Dublin, Ireland in 1991 and his medical degree (MB BCh BAO) from the Royal College of Surgeons in Ireland Medical School in 1996. After undertaking postgraduate medical training, he received an MSc in Molecular Medicine (Trinity College Dublin, 2002). In 2003 Dr Daly joined Prof. Albert Beckers at the Department of Endocrinology at the CHU de Liege, University of Liege in Belgium, where he received his doctorate in 2008 for the study of the epidemiology and genetics of pituitary adenomas. At the Beckers group, Dr Daly leads international research projects that have characterized the genetics and clinical features of the new diseases, familial isolated pituitary adenomas (FIPA) and X-linked acrogigantism (X-LAG syndrome), in addition to studies on aggressive pituitary adenomas, inherited disorders of calcium metabolism and gonadotrope signalling and the immunotherapy of parathyroid carcinoma.

L19

Gigantism, acromegaly and GPR101

Adrian Daly
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Pituitary gigantism occurs due to hypersecretion of GH and insulin-like growth factor-1 (IGF-1) in children and adolescents before the closure of epiphyseal growth plates. This hormonal disorder is usually due to an adenoma or hyperplasia of the anterior pituitary gland and like other tumoral conditions in children often has a genetic background. Recently we demonstrated that a genetic predisposition is present in just under half of cases of pituitary gigantism. The best recognized causes are *aryl hydrocarbon receptor interacting protein* (AIP) gene mutations occurring sporadically or as familial isolated pituitary adenomas (FIPA), McCune Albright syndrome, and Carney Complex. In addition, we recently discovered and characterized a novel cause of pituitary gigantism, termed X-linked acrogigantism (X-LAG) syndrome. This condition differs from other forms of pituitary gigantism as it occurs at a significantly younger age and generally presents in the first year of life. It is associated with marked overgrowth due to GH, IGF-1 and often prolactin hypersecretion from a pituitary adenoma and or hyperplasia. Recent evidence suggests that X-LAG syndrome may originate from a central (e.g. hypothalamic) source of GHRH dysregulation. Patients with X-LAG syndrome have been shown to have a microduplication on chromosome Xq26.3 that includes the gene *GPR101*.

This gene encodes for an orphan GPCR that is highly expressed in the hypothalamus and in various areas of the brain. Adenomas/hyperplasia from patients with X-LAG syndrome over-express GPR101 both at the RNA and protein level, indicating that this receptor represents a novel pathway for regulation of growth signals. X-LAG syndrome is very challenging to treat as it gives rise to very marked hormonal excess and large pituitary adenomas in young children. Control is challenging but necessary as if left uncontrolled, chronic GH hypersecretion can lead to extreme gigantism. Standard somatostatin analog therapy is usually not effective and a combination of radical surgery plus pegivisomant may be required to halt overgrowth. Even small residual tumor remnants appear capable of inducing acromegaly in X-LAG syndrome patients, 40 to 50 years after initial disease onset. Diagnosis of X-LAG syndrome has important clinical genetic implications as the microduplication can be transmitted from affected parent to child in the setting of rare FIPA families.

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L20 – Biography

Maria João Mascarenhas Saraiva received a BSc in Biology from the University of Porto, Portugal, in 1976, and an MSc in Biochemistry from the University of London, in 1978. Between 1980 and 1984, she did a PhD in biochemistry at the University of Porto, and qualified as Professor of Biochemistry in the University of Porto in 1991. She worked for different periods as a Visiting Scientist at the College of Physicians and Surgeons at Columbia University, New York. She is Director of the Molecular Neurobiology Group at Instituto de Biologia Molecular e Celular (IBMC).

Maria J Saraiva was awarded the Seiva Prize for Services to Science by the City of Porto, in 1996, and the Gulbenkian Prize in Science, in 2009. She has published over 220 articles in peer reviewed journals, several reviews on the subject of molecular biology of misfolding diseases of the central and peripheral nervous system.

L20

The bright and dark side of transthyretin, a thyroxine plasma transporter

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Transthyretin (TTR) is a plasma and cerebrospinal fluid (CSF)-circulating protein. Besides the primordially attributed systemic role as transporter molecule of thyroxine (T_4) and retinol (through the binding to retinol-binding protein (RBP)), TTR has been recognised as a protein with important functions in several aspects of the nervous system physiology. TTR has been shown to play an important role in behaviour, cognition, amidated neuropeptide processing, and nerve regeneration. Further, it has been proposed that TTR is neuroprotective in Alzheimer's disease and cerebral ischemia. Mutations in TTR are a well-known cause of familial amyloidotic polyneuropathy (FAP), an autosomal dominant neurodegenerative disorder characterised by systemic deposition of TTR amyloid fibrils, particularly in the peripheral nervous system. The purpose of this review is to highlight the roles of TTR in the nervous system, beyond its systemic role as transporter molecule of T_4 and RBP-retinol.

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Oral Communications

OC1

A new chemotherapy combination (U0126+SN50) potentiates apoptosis in thyroid cancer but induced survival in normal thyroid

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Papillary thyroid carcinoma (PTC), the most frequent thyroid cancer, has low proliferation but no apoptosis, presenting frequent lymph-node metastasis (Ordonez *et al*., 2004).

SMAD3 has two opposite phosphorylation sites: the TGF-βRI–induced C-terminal Serine SMAD3 phosphorylation and the phosphorylation induced by CDK2 (T8, T179 and S213) and ERK (T179, S204, S208) (Wang *et al*. 2005, Matsuura *et al*. 2005).

In our previous studies, we have demonstrated that in normal thyroid, TGF-β/SMAD represses *p27/CDKN1B* gene, activating CDK2-dependent T179-SMAD3 phosphorylation to induce p50 NFκB-dependent BAX upregulation and apoptosis.

In thyroid cancer, oncogene activation prevents TGF-β/SMAD-dependent *p27* repression, and CDK2/SMAD3 phosphorylation, leading to p65 NFκB upregulation which represses *BAX*, induces *cyclin D1* and prompts TGF-β-dependent growth.

The *p27/CDKN1B* gene is downregulated by SMAD3 in normal thyroid but not in thyroid cancer. This could be used because both PTC groups (those originated in oncogenic BRAF and RAS or similar mutations) have a high activation of the MEK/ERK pathway (Agrawal *et al*. 2014). A high MEK/ERK activity phosphorylates SMADs at the linker region preventing p27 downregulation by TGF-β in thyroid cancer cells, blocking the apoptotic pathway.

The aim of this work is to study if combined therapies based on the p27/SMAD/NFkB pathway could be use in future clinical trials.

MEK inhibitor U0126 was used alone or combined with NFkB inhibitor (SN50) in thyroid cancer cells and in normal thyroid cells cultured in human homeostatic conditions (h7H) (Bravo *et al*. 2013).

Combined ERK and p65 NFkB inhibitors reduce p27 and potentiate apoptosis in thyroid cancer while survival in normal thyroid.

Our study suggests that a combination of chemotherapy (U0126+SN50) could kill cancer cells but prevent death in normal thyroid cells. This could open a new avenue to treat thyroid cancer patients after surgery preventing recurrences and shifts to malignancy.

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OC2

mTOR pathway activation in papillary thyroid carcinomas: associations and correlations

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Background

Mammalian target of rapamycin (mTOR) is a downstream effector of the PI3K/AKT pathway. It can be activated by diverse stimuli, such as growth factors, nutrients, energy, stress signals and signaling pathways such as PI3K, MAPK and AMPK, in order to control cell growth, proliferation and survival. The active form of mTOR (pmTOR) may form two distinct complexes: mTORC1 that activates S6K1 and 4EBP1 which are involved in mRNA translation; and mTORC2 that activates PKC-α and AKT, regulating actin cytoskeleton organization, cell migration and survival[1]. mTOR deregulation is observed in multiple cancers[2]. In papillary thyroid carcinoma (PTC), mTOR pathway was reported to be overactivated in PTC[3], particularly in those with BRAF mutation compared to BRAF wt[4]. Sodium iodide symporter (NIS) is a transmembrane glycoprotein that transports iodine from bloodstream to the interior of follicular cells to participate in the normal process of thyroid hormone synthesis[5]. Thyroid cancer therapy is based on surgery followed by ^{131}I to treat possible tumor remnants and metastases. NIS is a vital protein in this process because it transports the radioactive iodine to the interior of cancer cells, resulting in their death. Approximately 20% of well differentiated thyroid tumors lose NIS expression becoming refractory to therapy[6]. The mechanisms of loss of NIS expression remain poorly understood, but it has been described that mTOR pathway activation decreases iodine uptake by thyrocytes[5] and that inhibition of the pathway in thyroid tumor cell lines increases differentiation resulting in higher iodine uptake[6].

Aim and methods

In order to study mTOR pathway status and possible associations with clinicopathological (age, gender, tumor capsule, tumor capsule invasion, extrathyroidal invasion, multifocality, lymphocytic infiltration, lymph node metastases, vascular invasion, status of tumor margins, staging, distant metastases, persistence of disease) and molecular features (BRAF, NRAS, TERT promoter mutations, RET/PTC rearrangements) as well as with NIS expression, we studied by immunohistochemistry pmTOR and pS6 in a series of 192 PTCs with detailed clinicopathological informations, addressing NIS expression in 44 of them by real time PCR.

Results

pmTOR expression was significantly higher in PTCs presenting absence of capsule, distant metastases and persistence of disease. Furthermore, pmTOR expression was significantly correlated with higher number of ^{131}I therapies, higher cumulative dosis and with lower NIS expression. Additionally, pmTOR revealed to be an independent risk factor for distant metastases. The expression of pS6 was significantly associated with presence of capsule and absence of extrathyroid extension and of lymphocytic infiltration, and with BRAF wt status. There was no correlation between pmTOR and pS6 expression.

Conclusions

Despite pS6 being a downstream effector of pMTOR in the mTOR pathway, we observed, in this work, a different behavior of both markers. pmTOR expression is associated with aggressiveness and worse prognosis, while pS6 associates with less aggressive clinicopathological features. The discordant results obtained with both markers and the lack of correlation of their expression, lead us to hypothesize that mTOR activation preferentially induces the formation of mTORC2 complex, activating other downstream effectors than pS6.

Further studies are needed to confirm this hypothesis, namely regarding the dissection of which mTOR complex (1 or 2) is being preferentially activated in PTCs, so that we can use the most appropriate pharmacological strategy to block the pathway. Such blockage may be very important to overcome tumor progression and refractoriness to radioactive iodine therapy.

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OC3

Mesoporous silica nanoparticles for somatostatin targeted Notch activation in animal model of pancreatic neuroendocrine cancer

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Pancreatic neuroendocrine tumors (PNETs) form a distinct entity of malignant lesions with a dreadful prognosis. More than 50% of the patients already have distant metastasis by the time of diagnosis, which underpins the urgent need for more effective treatment modalities. Notch pathway, evolutionary old and highly cellular context dependent signalling mechanism, was shown to regulate growth and development of normal neuroendocrine cells and PNETs, with experimental data implying its function as a tumor suppressor gene in these malignancies. Development of nanoparticulate system for delivery of Notch activators directly to pNETs may solve the long-standing problem of unwanted systemic effects of such compounds. Our group & collaborators have engineered, synthesized and successfully tested mesoporous silica nanoparticles (MSNPs) in different cell lines and in a breast cancer animal model for Notch inhibitor delivery. PNETs, as unique tumors with repressed Notch pathway represent an appealing testing platform for nanocarrier-mediated Notch activator delivery. MSNPs, decorated with tumor-inhibiting somatostatin analogues and loaded with Notch activators, may make an elegant vehicle, selectively steering this 'tumoricidal cocktail' to PNETs with inherently high levels of somatostatin receptors.

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OC4

TERT promoter mutations in pancreatic endocrine tumours are frequent in tumours from patients with hereditary syndromes

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Objective
One of the hallmarks of cancer is its unlimited replicative potential. Recently, telomerase promoter (TERTp) mutations were presented as novel mechanism for telomerase re-activation/expression. Due to this fact, it was our objective to study pancreatic endocrine tumours (PET) and related cell lines, to determine if TERTp mutations were present.
Design
We performed characterization of the TERTp in a series of 55 PETs and three common PET cell lines. TERTp mutations were evaluated if they could represent an alternative mechanism in PETs. Additionally, we tested functionally the mutations *in vitro*, and performed chromatin immunoprecipitation to determine what were the fundamental transcription factors acting on the novel binding sites generated by the mutations.
Results
TERTp mutations were detected in 7% of the cases studied and were mainly associated to patients harbouring hereditary syndromes. In vitro, these mutations confer a two to fourfold increase in telomerase transcription activity. They are able to recruit ETS transcription factor members, in particular GABP-α and ETV1. The findings obtained in tumours are also recapitulated in the cell lines, where TERTp mutated cell line does not rely on alternative lengthening of telomeres mechanism.
Conclusions
We report for the first time TERTp mutations in PETs and derived cell lines. In our series, the mutations were noticeably prevalence in cases with a hereditary component. The data we present point these mutations as an alternative mechanism and in an exclusive manner. *In vitro*, these alterations are functional and perform in the same manner as presented in other cancer models.

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OC5

The anti-proliferative effect of metformin in a model of adrenocortical carcinoma

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Adrenocortical carcinoma (ACC) is a rare, heterogeneous malignancy with aggressive behavior and poor prognosis, particularly when metastatic at diagnosis. To date, radical surgery, possibly associated to mitotane adjuvant therapy, is considered the best option for ACC treatment. However, the mean 5-year survival rate diminishes dramatically in metastatic ACC and chemo-resistance often develops. Thus, more specific and effective drugs for ACC treatment are urgently required. The use of metformin, a well-established and effective agent for the management of type 2 diabetes mellitus, has been associated with decreased cancer incidence and mortality in several human malignancies, leading to increasing interest in its potential use as an anticancer agent.
In our study, we evaluate the potential *in vitro* anti-cancer effect of metformin on the adrenocortical cancer cell line H295R, looking for an alternative therapeutic approach to ACC treatment.
We observed that increasing doses of metformin (0.5–250 mM), administered to H295R cells for 1, 2, 3 and 7 days, inhibit cell viability and proliferation in a dose- and time-dependent manner, as demonstrated by MTS, cell counting and [^{3}H]thymidine incorporation assay. This anti-proliferative effect seems to be mediated by metformin-induced apoptosis, as investigated by cytofluorimetric assay. Western blot analysis of cell lysates after 6- and 24-h metformin treatment (20, 50 and 100 mM) was used to identify the molecular pathways involved in mediating the drug effect: it revealed a dose-dependent increase in phosphorylated AMPK, which associates with a decreased mTOR phosphorylation, and a reduction in IGF1R expression and ERK1/2 phosphorylation, suggesting that metformin may exert its effect through these signaling pathways.
In conclusion, our data demonstrate that metformin is particularly effective in inhibiting proliferation in H295R adrenocortical cancer cells in a manner that depends on the drug concentration and treatment duration.
Further studies are necessary to validate these findings *in vivo,* to assess direct and indirect anti-cancer effects of metformin and to better elucidate the intracellular mechanisms involved in metformin action.

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OC6

Norepinephrine transporter as a predictive marker of response to PI3K/mTOR inhibition in pheochromocytoma

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Phaeochromocytomas (PCs) are neuroendocrine tumors derived from neural crest-derived chromaffin cells of the adrenal medulla and sympathetic ganglia (the latter referred to as paragangliomas), which produce significant amounts of catecholamines. Although PCs are mostly benign, 10% of all PCs can be malignant evidenced by the presence of metastases, predominantly in bone, lungs, liver and lymph. Currently, there is no effective therapy for malignant PCs.
In many neuroendocrine tumors, including PCs, the PI3K/AKT/mTOR survival pathway is hyperactivated, thereby mediating signals of tumor cell proliferation and survival. Therefore, the inhibition of this signaling cascade may exert an antitumor effect by inhibiting tumor angiogenesis, tumor cell growth and proliferation.
In our study, we evaluated the efficacy of BEZ235, a dual PI3K/mTOR inhibitor, against PC *in vivo*. We used a dual inhibitor to avoid the re-activation of upstream

AKT signaling following mTOR inhibition via a well-documented negative feedback loop. We took advantage of a unique *in vivo* model of endogenous PCs: MENX-effected rats. We found that BEZ235 exerts antitumor effects on PCs cells. Gene expression analyses of tumors from rats treated with placebo or with BEZ235 identified the *Slc6a2* gene as being down-regulated following drug treatment. *Slc6a2* encodes the norepinephrine transporter (NET), which is responsible for the intracellular re-uptake of norepinephrine in chromaffin cells. The fact that NET is expressed by PC cells has been extensively exploited for the functional imaging of these tumors using radiolabeled norepinephrine analogues. We observed a dose-dependent reduction of both *Slc6a2* (by TaqMan) and NET (by immunohistochemistry) expression in rat PCs following BEZ235 administration, which associated with decreased uptake of the radiolabeled norepinephrine analogue 18F-LMI1195 *in vivo*.

To assess the relationship between NET levels and response to BEZ235, we generated a drug-resistant derivative of the MPC (mouse PC) cell line. While incubation with BEZ235 reduced NET expression in MPC cells, no reduction was observed in the resistant derivative cells. This suggests that decreased NET expression associates with the ability of PC cells to respond to PI3K/mTOR inhibition.

Altogether, our data demonstrate that targeting PI3K/mTOR signalling is effective against PCs and suggest that NET levels may represent a surrogate marker of therapy response to PI3K/mTOR inhibitors, which can be monitored by functional PET imaging.

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OC7

Bifocal intracranial germinoma presenting as adipsic diabetes insipidus

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Introduction

Intracranial germ-cell tumors (GCT) are rare, occurring in less than 4% of pediatric patients. Histologically, they are divided into several types, among which germinomatous GCTs represent over 50% of cases. Depending on their location, GCTs usually present with symptoms of intracranial hypertension for pineal lesions, whereas suprasellar ones lead to visual disturbances or pituitary hormonal defects.

We present the case of a bifocal intracranial germinoma with an unusual clinical presentation of adipsic diabetes insipidus, a condition with significant morbidity and mortality.

Case report

A 19-year-old female patient was addressed to the emergency department with fatigue, dizziness and unsteady gait. She reported a rapid decline of visual function over the previous 15 days, although certain complaints (blurred vision of the right eye, considered to be due to myopia) had already been investigated 2 years earlier. Clinically, the patient was dehydrated, with normal blood pressure, but tachycardic. Homonymous left lateral hemianopsia was objectified as well as severely impaired vision (1/10 – right eye, 2/10 – left eye).

Biochemistry found severe hypernatremia – 177 mmol/l with hyperosmolality – 379 mOsm/kg and acute prerenal renal failure. Urines were normally diluted and thirst sensation was lost. Cerebral MRI revealed two lesions in the suprasellar and pineal regions with intense contrast-enhancement, compatible with GCTs. The suprasellar lesion extended up to the lateral ventricular floor.

Further hormonal evaluation revealed pan-hypopituitarism and hyperprolactinemia most likely due to stalk compression. Tumor markers (AFP and bhCG) were negative in serum, while bhCG was slightly elevated in CSF.

As the lesion was bifocal, with slightly elevated tumor markers, it was considered a germinoma. Treatment with chemotherapy and adjuvant radiotherapy was decided. The favorable response to treatment confirmed the diagnosis.

Conclusion

We report the case of a bifocal germinoma presenting as adipsic diabetes insipidus. To our knowledge, this is the case of intracranial CGT with this presentation. In our patient's history, a period of polydipsia and polyuria was noted several months prior to diagnosis, but medical attention was not sought at the time. As the lesion expanded towards the hypothalamus, thirst sensation was lost, therefore leading to adipsic diabetes insipidus. Our case also underlines the importance of a thorough investigation of apparently benign symptoms, such as visual complaints or polyuria/polydipsia, especially when occurring simultaneously.

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OC8

Small cell lung carcinoma presenting as ophthalmoplegia due to pituitary metastases: case report

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We are presenting a case report of 82-year-old patient admitted at Cork University Hospital on 3 July 2015 for investigation of complete ptosis and ophthalmoplegia of left eye which had occurred in couple of weeks time. Initially patient was referred to ophthalmology department by his GP and ophthalmologist found left ophthalmopathy with complete ptosis and referred patient to medical for stroke work up. CT Brain showed abnormal mass in pituitary fossa. MRI Pituitary showed multiple small lesion involving pituitary and impression of metastatic disease was given.

Chest X-ray showed left hilar mass and subsequent CT showed left lilar mass encasing main pulmonary artery with metastatic to pleura and liver with widespread mediastinal lymph adenopathy.

Transbronchial Biopsy showed small cell carcinoma of lung with stage IV disease. After multidisciplinary meeting concluded palliative treatment. He was given cranial and thorax radiotherapy. He did not tolerated chest radiotherapy. After 4 cycles of cranial radiotherapy, there was 25% improvement in his ptosis. His hospital course was complicated by aspiration pneumonia which settled after broadspectrum antibiotics. He was then transferred to Marymount University Hospital and Hospice, Cork. He developed fracture at the junction of the superior pubic ramus and acetabulum and right inferior pubic ramus. He was seen by orthopedic team and advised conservative treatment. He passed away on 27 August 2015.

Keywords: pituitary metastases, small cell carcinoma, palliative radiotherapy
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Poster Presentations

P1

Genetic heterogeneity of medullary thyroid carcinoma

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Genetic intratumor heterogeneity has been recently demonstrated in some solid human cancers and a few years ago RET mutated and not mutated cells were described in medullary thyroid carcinoma (MTC). Nobody reported the presence of two different RET mutations.
Aim of our study was to investigate the RET somatic mutation profile in primary MTC (pMTC) and in the corresponding metastatic tissues (mets).
We studied pMTC and mets of 22 MTC sporadic cases. Altogether 86 samples were screened for the presence of a RET somatic mutations in exons 10, 11, 13-16.
In 18 cases (81.8%), 57 different tissues, a correspondent mutation profile was found in the pMTC and in their mets. In 4 cases (18.2%), 29 different tissues, a different RET mutation profile was observed in pMTC and in their mets. In particular in one case a M918T was found in the pMTC but only in 3/5 lymphnode mets; in another case, a 3 bp in frame deletion in exon 15 was found in 8 lymphnode mets but not in the primary tumor and in 4 additional lymphnode mets. Interestingly we found one patient with a S891A somatic mutation in the primary tumor that was absent in a kidney distant metastases that was indeed characterized by the presence of a M918T mutation. A complex genetic heterogeneity was finally demonstrated in one MTC patient with a very severe disease. The primary tumor displayed a heterozygous 6 bp in frame deletion in exon 11 that was found also in 4/5 lymphnode metastases and in 1/2 liver metastatisis. In 1/5 lymphnode and in 1/2 liver metastasis the deletion was homozygous. The analysis of several RET SNPs demonstrated that in this case 1 RET allele was missing. In addition both the primary tissue and 4 lymph node metastases harbored a V804M mutation.
In conclusion our study shows that i) 81% of cases had a correspondent RET mutation profile, although in these cases we cannot exclude the simultaneous presence of RET positive and RET negative cells; ii) 19% of cases are clearly heterogeneous and among them 2/4 have different RET mutations in different tissues. This information should be taken into consideration in the planning of personalized target therapies and raise the question of whether RET mutations play a real driving role in the development of MTC.

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P2

Methimazole induced agranulocytosis side effect-not always a bad effect: case report

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Background
Malignancy and hyperthyroidism used to be considered mutually exclusive, but increased association was observed. These two conditions incidence is variable, ranging between 0 and 9%, Graves' disease is especially linked to differentiated thyroid carcinoma type.
Case presentation
We report the case of a 33 year old, female, smoker, diagnosed with Basedow–Graves disease in may 2015, on methimazole 20 mg/day. She was admitted to our department with palpitations, ocular pains, bilateral exophthalmos and severe neutropenia (150/mm^3). The physical exam revealed normal skin temperature, sinus tachycardia, bilateral exophthalmos with no diplopia, diffuse painless goiter. Laboratory tests showed TSH <0.004 mUI/ml, free thyroxine (FT_4) 1.76 ng/dl, total triiodothyronine (TT_3) 246.3 ng/dl, anti TSH receptor antibody (TRAb) 2.27IU/l. Thyroid ultrasound revealed diffusely enlarged thyroid gland with heterogeneous echotexture with increased color Doppler flow and no pathological cervical nodes. The administration of methimazole was stopped and treatment with lithium carbonate and potassium iodide was started. For the neutropenia she was given dexamethasone, injections of granulocyte colony stimulating factor. When controlled levels of thyroid hormones (T_3 and T_4) and neutrophils were ensured, the patient underwent total thyroidectomy. The histopathology revealed a 5 mm foci of papillary microcarcinoma of follicular type, with no invasion of the thyroid capsule.
The postoperative follow-up showed no remnant thyroid on ultrasound of the neck, Tireoglobulin (Tg), levels on LT_4 <1 ng/ml, Tg antibodies in normal range. Tg antibodies are periodically measured and TSH serum level is ranged between 0.1 and 0.5 mUI/l. The patient had no more ocular complaints.
Conclusion
Thyroid cancer is an unusual finding in a patient with Basedow–Graves disease, the most commonly type of cancer is papillary and follicular. The particularities of the case were the coexistence of these two conditions and the fact that severe neutropenia lead to total thyroidectomy and permitted a fast cure.

Keywords: Basedow-Graves disease, differentiated thyroid cancer, thyroidectomy
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P3

Prevalence of BRAF V600E mutation in Romanian thyroid tumors patients

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Introduction
BRAF V600E mutation is reported to occur in 28–83% of papillary thyroid cancer, being associated with increased tumour aggressiveness.
Objective
To determine the prevalence of BRAF V600E mutation in Romanian patients with thyroid nodules referred to surgery in a reference endocrinology centre.
Materials and methods
140 patients were included in the study: 70 patients with papillary thyroid carcinoma (PTC), 42 patients with follicular adenoma, 22 patients with hyperplastic thyroid nodule and six patients with autoimmune thyroiditis. DNA was isolated from thyroid tissue using PureLink Genomic DNA Kits (Invitrogen, Life Technologies). BRAF V600E mutation was determined by PCR-RFLP using TspRI as restriction enzyme and confirmed by sequencing on Beckman Coulter CEQ8000 genetic analyser. Patients were enrolled after they gave their informed consent.
Results
Patients with PTC were divided into following histological subtypes: Classical PTC – 27 patients, PTC 'follicular variant' – 35 patients, aggressive forms – eight patients. BRAF V600E analysis was done in all enrolled patients. We did not find this mutation in patients with follicular adenoma, hyperplastic thyroid nodule or thyroiditis. In PTC group we found 10/70 mutations (14.28%): 8/27 (29.63%) in classical PTC and 2/8 (25%) in the histological aggressive forms. There were no mutations in PTC follicular variant.
Conclusion
BRAF V600E prevalence in Romanian patients varies depending on the histological type of the tumour. Overall BRAF V600E prevalence in our classic PTC group (including aggressive phenotype) was 28.57%.

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P4

Expression of osteopontin isoforms is related with thyroid cancer growth and invasion

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Osteopontin (OPN) is a matricellular protein highly expressed in cancer cells, which is able to modulate tumorigenesis and metastasis in several malignancies, including follicular cell-derived thyroid cancers (TC). OPN is one of the gene products aberrantly expressed in TC, but the contribution of each OPN isoform (OPNi), named as OPNa, OPNb and OPNc, is currently unknown. This study aims

to analyze the expression profile of OPNi in TC tissue samples, correlate its expression with molecular and clinicopathologic features, and evaluate the role of OPNi in TC cell lines. The expression profiles of OPNi in TC cell lines and in thyroid tissue samples were evaluated by q-RT-PCR and immunohistochemistry. In order to address the putative roles of OPNi in TC, we overexpressed OPNi in TC cell lines. c643 and 8505c cells were transfected with vectors containing OPNi as well as empty vector, and stable overexpressing clones were selected with geneticin. Functional assays (proliferation, migration, motility, gelatin zymography and invasion) were also performed. We found that the OPNa and the OPNb isoforms are expressed in higher levels in classic papillary thyroid carcinoma (cPTC) samples than in non-tumoral thyroid, adenomas and follicular thyroid carcinoma tissues. Conversely, OPNc isoform transcript levels are similar among samples from the aforementioned pathologies. In cPTC samples, high OPNa and OPNb expression levels were significantly associated with higher tumor size, presence of vascular invasion and $BRAF^{V600E}$ mutation. In eight distinct TC cell lines, we observed differential expression of the three OPNi, of which cell line c643 expressed the lower levels of OPNi. Higher proliferation, migration and motility were associated with c643 and 8505c cells overexpressing OPNa. In both cell lines overexpressing OPNa, we observed an increase of matrix metalloproteinase 2 in the extracellular medium. Further, in *in vivo* CAM assay we found that cells overexpressing OPNa are significantly more invasive when compared to the control cells. Taken together, our data indicate that both OPNa and OPNb are overexpressed in cPTC samples and OPNa is the isoform that is significantly associated to promotion of cell growth, migration, motility and invasion in TC cells.

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P5

Mouse model of *BRAF*V600E-induced papillary thyroid carcinoma – summary of our results

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Introduction

*BRAF*V600E mutation is the most frequent alteration in papillary thyroid carcinoma (PTC). Although its relation to factors of poor prognosis was demonstrated in many studies the use of *BRAF* as a predictive marker in clinical treatment of PTC patients is controversial. The aim of our study was to analyze molecular consequences of *BRAF*V600E mutation in a transgenic mouse model performed for this purpose and to refer the obtained results to human PTCs.

Material and methods

Gene expression profiling was performed for the selected mouse samples obtained from the transgenic mouse model of *BRAF*V600E-induced PTC with the affymetrix platform and the data were referred to human thyroid dataset with particular emphasis on early steps of PTC carcinogenesis.

Results and conclusions

Most *BRAF*(+) mice developed malignant lesions (83%), of which 15% presented metastases to lungs or extrathyroidal invasion to muscles. These results confirm the initiating potential of the V600E mutation and its association with the aggressive PTC phenotype. Nevertheless 16% of *BRAF*(+) mice displayed non-malignant benign hyperplastic lesions or healthy thyroids. Gene signature obtained from the comparison of mouse non-malignant *BRAF*(+) to *BRAF*(−) thyroids enabled selection of 862 significantly deregulated genes, of which 532 were identified on the human HG-U133A microarray. We believe that the list of 532 genes represents the human signature of an early stage of *BRAF*V600E-derived PTC. On comparison of *BRAF*(+) PTCs to *RET*(+), *RAS*(+), or PTCs without *BRAF* and *RET* alterations and to healthy thyroids, 18 of the 532 genes displayed significantly deregulated expression in all comparisons. Seven out of 18 genes have not been previously reported to be related to *BRAF* mutation or thyroid carcinoma. Obtained results may be useful in development of new therapeutic strategies that would be able to overcome resistance to *BRAF*-targeted therapies.

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P6

Toxicity of tyrosine kinase inhibitors in the treatment of thyroid cancer – a 10-year experience resume

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Targeted therapy based on tyrosine kinase inhibitors (TKIs) constitutes a new treatment modality in thyroid cancer (TC). Their efficacy in prolongation of progression free survival in comparison to placebo has been documented in phase III studies. However, a problem of their tolerability has recently risen as numerous side effects influencing the quality of life may potentially limit their clinical use. Therefore, we decided to carry out a retrospective analysis of the frequency and severity of adverse effects (AEs) related to TKI administration (VEGFR inhibitors only) in patients treated due to advanced TC within different prospective phase II and III clinical trials. The comparison of the efficacy between particular drugs was not aimed.

Material

In total 55 courses of TKI therapy were evaluated. 19 subjects were given vandetanib, 15 – lenvatinib, 14 – sorafenib, 4 – motesanib and 3 – axitinib. Median treatment duration was 21.3 months (range: 0.7–100.8). All AEs were assessed on the basis of Common Terminology Criteria for Adverse Events (CTCAE) version 3.0.

Results

The drug was discontinued due to poor treatment tolerability in eight subjects (14.5%) whereas 29 (52.7%) of patients required dose reduction. Among the most common TKI-related AEs were arterial hypertension (73.0%), skin reactions (70.3%), diarrhea (54.1%), weight loss (54.1%) and stomatitis (43.2%). Most of AEs fulfilled G1 and G2 criteria except of hypertension mainly classified as G3 (at least two antihypertensive drugs required). However, dose reduction was mostly related to weight loss, diarrhea and skin toxicity, while among AEs leading to treatment withdrawal were: weight loss (2), myocardial infarct (2) lymphopenia (1), QTC prolongation (1), tracheo-esophageal fistula (1) and purulent meningitis (1).

Conclusion

TKIs constitute a safe and well-tolerated treatment method in TC patients. Frequent, mostly mild or moderate side effects may be alleviated by additional pharmacological therapy or by dose modification.

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P7

The genetic screening of RET proto-oncogene in Polish population during the past two decades

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Introduction

Gain of function mutations of *RET* protooncogene are associated with hereditary medullary thyrpoid cancer. There are mainly specific hot-spot *RET* gene mutations however they may differ between population.

Aim of the study

In this study we report the prevalence of *RET* mutations in Polish population based on 20 years of experience of referral polish centers.

Material and methods

RET genetic screening was performed in 2405 patients of Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology (1975 from Gliwice Branch and 430 patients from Warsaw). There were 1712 probands and 693 family members.

Results
We have found 268 RET positive families (16% of all probands) and 259 *RET* gene carriers. In total we identified 527 patients with *RET* mutations (22% of all analyzed MTC patients). Codon 634 was the most frequent *RET* alteration among all *RET* mutations (80/296; 41%) in MEN2A/FMTC patients and only codon 918 (27/27; 100%) was observed in MEN2B patients. Those results are similar to the other European countries (average was 39% of all *RET* mutation, based on data from Germany, Italy, France, Greece and Czech Republic). Characteristic for Polish population is relatively high frequency of aminoacid substitution in codon 791 (48/296; 25%) and mutation in codon 649 (12/296; 6,1%) which is not observed in other European populations. Routinely we did not analyze mutation in codon 533 (exon 8) of *RET* gene which is characteristic for Greek population, however we performed such screening in 104 MTC patients who were negative in standard hot-spot analysis. We did not find any mutation in codon 533.
Conclusion
The most frequent alteration of *RET* gene in Polish population is mutation in codon 634 of *RET* protooncogene which is characteristic for all European populations. However variation related to different ethnic origin is also reflected in Polish population and is related to two *RET* gene SNP changes: codon 649 and codon 791.

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P8

Medullary thyroid cancer in a *RET*-negative patient with a germline *SDHB* mutation

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Introduction
Medullary thyroid cancer (MTC), in its familial forms, is usually associated with pheochromocytoma and primary hyperparathyroidism, related to an underlying germline *RET* mutation. *SDHx* germline mutations associated with MTC have not yet been reported.
Case report
We report the case of a 60-years-old woman, who was submitted, elsewhere, in November/2013, to a total thyroidectomy + right lymph-node dissection due to a nodule suspicious of MTC on fine-needle biopsy. Histology confirmed a MTC with blood vessel invasion (pT3N1bMx). Serum calcitonin was 264 pg/ml before surgery and <2 pg/ml 3 months after surgery (NR <2). Surgery induced a bilateral vocal cord paralysis and hipoparathyroidism. A pre-operatory CT scan revealed a 17 mm left adrenal nodule. She was normotensive and urinary metanephrines were normal. Her daughter had been previously diagnosed with a carotid body paraganglioma and multinodular goiter. Gene testing revealed a deletion on *SDHB* gene (p.Ser198AlafsX22 (c.591delC), exon 6) in both the patient and daughter; no germline mutations were found on *RET, VHL, TMEM127* or other *SDH* genes.
Nine months after surgery, the patient was diagnosed with a biochemical (calcitonin 6.2 pg/ml (N <2.0)) and neck recurrence and she was re-operated. There were metastases in 3/29 lymph-nodes resected. Serum calcitonin increased after surgery to 27.9 pg/ml and a PET-CT showed neck and bone disease (skull, mandible, dorsal vertebrae, ribs and hip). She was submitted to radiotherapy of the mandible and dorsal spine in September/2015.
Bone disease has been progressing and the pain difficult to control. Her last PET-CT revealed new neck lesions and extensive bone disease: skull, mandible, scapula, sternum, ribs, cervico-dorso-lombar column, hip, humerus and femur. Serum CT and CEA levels were 362 pg/ml and 53.1 ng/ml, respectively. Recently, she developed high blood pressure, slight face rounding and hypokalemia. ACTH level of 58 pg/ml (NR <46) confirmed ectopic Cushing syndrome. Given the progressive and symptomatic disease she started sunitib on December 17th 2015.
Conclusion
To our knowledge, no germline mutations have been found on *SDHx* genes in MTC patients, turning our case the first demonstrating this association. An increased frequency of some *SDHx* polymorphisms in patients with sporadic and familial MTC has been reported.
Other interesting point is that our patient exhibits an extensive plurimetastatic disease with a relatively modest serum calcitonin elevation. Some authors believe that, in contrast with *RET* and *NF-1* mutations, pheochromocytomas/paragangliomas with SDHB/D and VHL mutations are characterized by low tissue levels of epinephrine with corresponding usually normal levels of plasmatic and urinary metanephrines. Our hypothesis is that the modest elevation of serum calcitonin may be related to a poorly differentiated phenotype in patients with *SDHB* mutations.

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P9

Case report of ACTH-secreting tumour of the liver

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Introduction
We report the clinical presentation, immunohistochemistry, imaging, histopathology, treatment and outcome of a patient with ACTH-secreting tumour of liver.
Case report
Cushing's Syndrome due to ectopic ACTH production is uncommon and due to neuroendocrine tumour of liver is extremely rare. We discuss the case of a 27-year-old female who initially presented with vague, non-specific symptoms, such as general and muscle weakness, weight gain, hirsutism, increase in fasting blood glucose, in which an ACTH-secreting tumor found to be the cause of her clinical presentation. At admission: Height 166 cm, weight 70 kg. normosthenic constitution, diffusely hyperpigmented skin, darkened skin around elbows, striae on the stomach. Laboratory showed AM cortisol of 1750 mmol/l, PM cortisol more than 1750 mmol/l, 24-h urinary free cortisol more than 6700 nmol/day, AM ACTH level of 211.2 mg/ml, PM ACTH level of 148.0 mg/ml and non-suppression of cortisol with overnight dexamethasone suppression test (1 and 8 mg). Brain MRI showed no pathological changes. CT scan showed tumor of the right lobe of the liver ($7.5\times6.8\times5.8$ cm, density 40H). Selective sampling of the lower sinuses showed no gradient. Because of severity of the condition for health reasons she had bilateral adrenalectomy clinical and laboratory signs of hypercortisolism disappeared after surgery, but ACTH level was very high. In 2 months she had right-sided hemihepatectomy. ACTH level next day after surgery was 1 mg/ml. Immunohistochemistry showed primary neuroendocrine tumour, Grade 2. We still observe her 1 year and 8 months and during this period she felt fine and she has a laboratory and clinical remission.
Conclusion
Despite numerous guidelines in diagnosis and treatment of hypercortisolism, there are still diagnosis and treatment mistakes due to rarity and complexity of clinical presentation in ACTH-ectopic syndrome. So, we need to improve the guidelines for diagnosis and treatment of ACTH-ectopic tumors.

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P10

A case of ectopic adrenocorticotropic hormone syndrome in bronchial carcinoid

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Introduction
Despite advances in analytic and imaging techniques, the syndrome of ectopic ACTH secretion from a tumour resulting in Cushing's syndrome continues to pose difficult diagnostic and therapeutic challenges. We report a patient with ectopic ACTH from a bronchial carcinoid tumour highlighting the unusual presentation and difficulties in management.
Case description
A 23-year-old man with a past medical history of Cushing disease presented with typical Cushingoid appearance which had become apparent for about a year prior to presentation. He had hypokalemia, metabolic alkalosis, hypertension, osteoporosis and weight gain. Following thoracotomy and resection of his lung lesion, the plasma ACTH decreased significantly. When reviewed in clinic 12 months after surgery, he was symptomatically well, with good lung function.
Conclusion
Ectopic ACTH-secreting tumours present some of the most challenging differential diagnoses in endocrinology and require careful clinical, biochemical, radiological, and pathological investigation.

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P11

Acromegaly caused by hepatic metastasis of a pulmonary neuroendocrine tumor

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Introduction
Acromegaly is usually caused by a pituitary adenoma; ectopic or eutopic secretion of GHRH is a rare condition, responsible for $<1\%$ of the cases. Ectopic secretion of GH itself is even rarer.
Case report
A 34-year-old woman was referred to the Endocrine Clinic with a multinodular goiter. However, on physical examination there were some physical signs of acromegaly: coarsening of facial features, protusion of the lower jaw and thick, oily skin. In her past medical history there was a right lung lower lobectomy with a histologic diagnosis of a typical carcinoid. Laboratory investigation showed a seric concentration of IGF1 of 826 ng/ml (136–449), and a fine-needle aspiration of a thyroid nodule revelead a cytologic diagnosis suspicious of papillary carcinoma. A random GH level was 7.7 ng/dl, and after a 75 g oral glucose load the GH was 9.4 ng/dl. The pituitary MRI, however, did not show a tumor, but its dimensions were increased, which raised the possibility of ectopic acromegaly. Since our patient had an history of a pulmonary carcinoid, it was asked a chest and abdominal CT, which showed 'eight nodules in the liver, highly suspicious of metastatic involvement by the primary neuroendocrine tumor'. She had a discrete elevation of chromogranin A and no elevation of 5-hydroxyindoleacetic acid. A Ga^{68} – PET – DOTANOC revealed an increased captation in the hepatic lesions, and a biopsy was performed, confirming the neuroendocrine origin of the metastasis; immunohistochemical staining was positive for GH and negative for GHRH (Cohen antibody, 1:2000, automated method). The patient was initiated on monthly intramuscular octreotide (20 mg), and was submitted to radioembolization of the hepatic lesions, with a notable reduction in the size of the metastasis. A total thyroidectomy confirmed the diagnosis of papillary thyroid carcinoma, but with no indication for radioiodine ablation. Posteriorly it was asked a plasmatic determination of GHRH, which was negative (<60 ng/dl). At the moment the IGF1 levels are in the normal range and the GH level is <1 ng/dl, and the size of the hepatic metastasis is stable.
Clinical lessions
The diagnosis of ectopic secretion causing acromegaly may be suspected in any case of clinically suspected and biochemically proven acromegaly with no pituitary adenoma. However, this case has many peculiarities: in spite of the hyperplasia of the pituitary (which is suspicious of GHRH hypersecretion), the immunohistochemical staining was negative for this hormone and positive for GH; it is important to note that the biopsy sample was very small and the expression of GHRH is usually focal. So, to clarify this situation it was asked a determination of plasmatic GHRH, which is the gold-standard for the diagnosis: it was negative (even when the patient is under somatostatin analogues this value is positive in cases of ectopic secretion of GHRH). Therefore, it remans the doubt: is this clinical picture caused by ectopic secretion of GH? Or it may be caused by ectopic secretion of a truncated form of GHRH not detectable by radioimmunoassay but with biological activity?

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P12

Ectopic cushing syndrome by an aggressive gastroenteropancreatic neuroendocrine carcinoma

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Introduction
Gastroenteropancreatic neuroendocrine tumors have a highly variable presentation, which can range from benign neoplastic growths to rapidly aggressive malignancies. Neuroendocrine carcinomas of the digestive system are relatively rare. The incidence of GEP NEC in Spain is estimated to be 2.5 – five cases per 100 000 inhabitants.
Case
We describe the case of a 67-year-old man, previously diagnosed with hypertension and dyslipidemia. He was admitted due to hyperglycemia. The clinical examination was not remarkable and the first blood sample showed elevated transaminases (with progressive worsening) and hypokalemic metabolic alkalosis. The CT scan showed hypodense liver lesions with peripheral enhancement, suggestive of metastases, and lymph nodes located in pancreatoduodenal space, that were associated with a thickening of the third duodenal portion wall. The result of the biopsy sample was a neuroendocrine carcinoma with Ki- 67 of 80% and a rate of mitosis of 10/10 HPF representing WHO Grade 3. Because there was contraindication of other alternative treatment due to the hepatic involvement, and the positive uptake in the octreoscan, symptomatic treatment with somatostatin analogues was considered. Also, the patient received treatment with cisplatin and etoposide. Despite the treatment, patient's condition progressively deteriorated and he died 1 month after the diagnosis of NEC.
Conclusion
Neuroendocrine carcinomas are high-grade malignancy tumors that can be metastatic at diagnosis and have a high index of cell proliferation, with poor prognosis for all stages of disease, with a median survival of 5 months for metastatic disease from the time of diagnosis.

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P13

Multiple endocrine neoplasia type 1 – retrospective analysis of five families

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Introduction
Multiple endocrine neoplasia type 1 (MEN 1) is a rare syndrome with autosomal dominant inheritance. It mainly involves the parathyroid glands (90%), the pancreas (60%) and the pituitary (40%). More than 300 different MEN1 germline mutations were already described.
Objective
To characterize families with MEN1 followed at the Department of Endocrinology of the University and Hospital Center of Coimbra, Portugal, from 1990 until 2015.
Methods
Data collection from clinical records of patients admitted to the Department of Endocrinology with a confirmed diagnosis of *MEN1*.
Results
A total of 14 patients with *MEN1* mutation were identified, grouped into five families. Of the patients studied, 50% were women, with a mean age at diagnosis of 35.4 ± 14.3 years. Patients were followed for 11.1 ± 8.8 years after diagnosis and the survival rate was 93%. On average, each patient had 2.7 ± 1.4 tumors. Primary hyperparathyroidism was present in 64% of patients and was the first manifestation in 43%. On the other hand, 43% of patients had pituitary adenoma and this was the initial manifestation in 22% of the cases. Neuroendocrine tumors of the pancreas were found in 43% of the cases (83% nonfunctioning) and were the first manifestation in 7% of the patients. Adenomas of the adrenal glands were found in 50% of the patients, and were the first manifestation in 7% of the cases. Five different mutations were found: c.1546delC, c.1357C$>$T, 735del14, c.1A$>$T and c.637delG. The most frequently comorbidities observed were urolithiasis (43%), osteoporosis/osteopenia (36%) and hypertension (21%). Half of the patients studied had evidence of pulmonary nodules and in 43% of these the diagnosis of bronchial carcinoid was confirmed. Metastatic disease occurred in 14% of patients.
Conclusions
MEN1 is a rare entity in which an insightful clinical suspicion is essential to enable timely diagnosis of the syndrome and of its various components, since the prognosis improves with early detection of its characteristic neoplasias.

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P14

A novel germline mutation of the MEN 1 gene associated with multiple endocrine neoplasia type 1 (MEN1 syndrome) followed over three generations of a family

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Background
Germline heterozygous mutations in the MEN1 gene located on chromosome 11q13, predisposes to the development of tumors in multiple endocrine tissues. The MEN1 gene encodes a protein of 610 amino acid residues, known as menin which is involved in genome stabilization as well as in several steps of cellular division, conferring tumor suppression activity in MEN1-associated target tissues. The three

main endocrine tissues affected by tumors in MEN1 are parathyroid (95%), enteropancreatic neuroendocrine (50%) and pituitary (40%). Primary hyperparathyroidism from parathyroid hormone-secreting adenomas is usually the first clinical manifestation presenting typically between 20 and 25 years of age.
Objective
The present work is aimed at searching for mutations in the MEN1 gene in members of a family with multiple endocrine neoplasias across three generations.
Patients
The index-case, a 46-year-old woman, presented symptomatic hypercalcemia and markedly raised serum PTH. Neck exploration revealed a parathyroid adenoma which was successfully removed. She also reported a history of recurrent peptic ulcers including two duodenal perforations at the age 25 and 30 years suggesting a Zollinger–Ellison syndrome. Gastrin levels were found to be elevated and a hepatic gastrinoma was identified in the Octreoscan. Excision of the tumor led to remission of gastro-intestinal complaints. The father of the index-case presented a long standing symptomatic hyperparathyroidism and had a history of gastric perforation at 40-yr-old due to a carcinoid tumor. The index-case sister, complained of recurrent renal stones since her twenties and suffered of an acute episode of renal failure due to obstructive nephropathy. A parathyroid adenoma was identified and successfully removed. Two of hers three sons presented symptomatic hypercalcemia during their twenties and were submitted to parathyroidectomy. The other son was asymptomatic as well as the two children of the index-case.
Methods
Written informed consent was obtained from each available member of the family. DNA was extracted through conventional methods. Sequencing of the coding exons and splicing regions of the MEN1 gene were carried out after DNA amplification with PCR.
Results
In five members of the family herein reported, it was identified a novel missense germinal mutation involving the exon 2 of the MEN1 gene (c.124G>C) which leads to substitution of glycina by arginina at position 42 (Gly42Arg). The mutation was not found in non-affected members of the family.
Conclusion
Identification of a MEN1 gene mutation is an important tool in the follow-up of asymptomatic carriers avoiding unnecessary tests in noncarriers relatives.

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P15

Post-pancreatectomy persistent adult nesidioblastosis: follow-up of 24 years

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Introduction
Adult nesidioblastosis is a rare entity that has motivated the publication of several case reports, but long-term outcomes are rarely described. Distal or sub-total pancreatectomy is indicated in cases of severe symptoms or lack of response to medical treatment. We report the case of a patient with persistent nesidioblastosis after pancreatectomy keeping under medical treatment for 24 years.
Clinical case
Woman currently with 81-years old, diagnosed with diffuse nesidioblastosis at the age 57 (1991), after distal pancreatectomy performed on suspicion of insulinoma. Because of lack of response to medical treatment the patient underwent re-pancreatectomy 2 years after the first surgery (1993), but again showed no cure criteria. Since diagnosis to the current time, the patient still need medical treatment with two daily injections of octreotide. Diabetes mellitus was diagnosed 5 years after the near-total pancreatectomy (1998), with blood tests showing persistent hypoglycemia. In the last years, the patient maintains unstable glycemic control with hypoglycemia and hyperglycemia, with HbA1c between 6.3 and 9.3%; the attempts to reduce octreotide dose increase the frequency and severity of hypoglycemia. Today, with 81 years old, the patient is treated with octreotide 70 μg/day, keeping glycemic instability, but being clinically well, with no evidence of chronic complications of diabetes.
Discussion and conclusion
The definitive treatment of diffuse nesidioblastosis is difficult and imposes a real challenge to the endocrinologist. The prognosis depends on surgical treatment: if resection is not wide enough, the disease can persist; on the other hand, if it is too large there is a high risk of endocrine and exocrine pancreatic insufficiency. The case we report, despite the persistence of the disease and association with diabetes mellitus, has shown a good performance without major complications throughout these 24 years of follow-up.

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P16

Insulinomas at São João Hospital between 1980 and 2015

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Introduction
Insulinomas are pancreatic endocrine tumors originating in the beta cells, characterized by hypoglycemia resulting from insulin hypersecretion.
Objectives
To characterize the demography, the clinical features, imagiological findings and pathological evaluation of the cases of insulinomas identified in the previous 35 years at São João Hospital.
Methods
We retrospectively analyzed the cases diagnosed as insulinomas at São João Hospital in the period between January 1980 and December 2015.
Results
We identified 19 patients, 68% women, with a median age at onset of symptoms of 49 years. All patients presented neuroglycopenic symptoms and 79% also presented neurogenic symptoms. Whipple triad was present in all cases at the diagnosis and no case was associated with MEN1 syndrome. 17 patients (89%) had an abdominal CT performed, contributing to the location of the tumor in 12 patients. Abdominal magnetic resonance was performed and localized the tumor in five patients. Octreoscan was performed in three patients, detecting the tumor in only one case. Of the eight cases in whom abdominal ultrasound was performed, the tumor was localized in only two of them. Regarding the treatment, five patients were treated with diazoxide before surgery, and all patients were submitted to surgery. Enucleation of the tumor was performed in four cases and the remaining 15 were submitted to partial pancreatectomy. In one patient, the surgery did not remove the tumor and was unable to correct the fasting hypoglycemia, and so total pancreatectomy was performed. All tumors were solitary, with a median diameter of 1.8 cm. Ten tumors were located at the head of the pancreas, four at the body, four at the tail and one at the uncinate process. One tumor was associated with endogenous hyperinsulinism but negative insulin imunohistochemical evidence. There were no cases of lymphatic or vascular invasion. Surgical complications (fistula and infection) were observed in three patients. No patient presented evidence of recurring disease.
Conclusion
Our series of insulinomas, in agreement with the previous literature, has a predominance of female patients, with a peak age of onset between the third and fifth decade of life, and a predominance of neuroglycopenic symptoms.

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P17

Aggressive multifocal angiomyxoma – a surgical challenge

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Introduction
Aggressive angiomyxoma is a rare, benigne, misdiagnosed tumor that occurs preferentially in women during the reproductive age.
Case report
We present a 43-year-old Caucasin woman diagnosed with aggressive multifocal angomyxoma developing for the last 8 years. The patient underwent five surgical procedures during this period of time in order to debulk the pelvis and subperitoneal space of a large invasive tumor. The last surgical approach revealed a gigantic tumor of elastic consistency that presented perirectal, periuterin and perivaginal extension. Also, the tumor expanded in the right iliac fossa and further to the femoral ring. In addition to hysterectomy with bilateral salpingo-oophorectomy due to its increased size and the invasive character, a massive resection of the tumor was performed. The histopathological and immunohisto-chemical staining confirmed the aggressive angiomyxoma with intense positive reaction for estrogen and progesterone receptors and a proliferation index (ki67%) of 3%. The 3 months postoperative evaluation consisting in abdominal and pelvic IRM revealed no tumor reccurence.
Conclusion
Despite its benign features, the aggressive angiomyxoma should be regarded as a tumor with a high tendency to reccurence. The lack of specificity of its clinical presentation can lead to a delay in diagnosis. The angiomyxoma should be considered as a possible diagnosis in incidental finding of a mass in the pelvic or genital area in women of reproductive age.

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P18

Massive bilateral pheocromocitomas: a rare case

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Introduction
Pheochromocytoma is a rare catecholamine-secreting tumor that arises from the chromaffin tissue of the adrenal medulla. Of the reported cases, only 10% consist in bilateral lesions and the probability of multiple endocrine neoplasia should always be investigated.
Clinical case
Female patient, 19 years old, presented with a clinical history with 2 years of evolution, characterized by episodes of palpitations, headache, nausea and abdominal discomfort.
Because of persistent abdominal pain, it was performed ultrasound study that showed 'bilateral cystic perirenal lesions' and after that, abdominal CT confirmed 'cystic adrenal formations, with multiple septations, the right with 11.3 cm and the left with 7.8 cm of larger diameter, without infiltrative aspects.'
The complementary diagnostic exams revealed: serum metanephrines 7386.4 pg/ml (<60), calcitonin 55 pg/ml (<10) and PTH 41 pg/ml (9–72). The 123I-MIBG scintigraphy showed 'massive bilateral pheochromocytomas and abnormal fixation on the topography of the left thyroid lobe' and cervical plus thoracic CT indicated the presence of a thyroid nodule on the left lobe, with larger diameter 1.1 cm, without evidence of other lesions'. Thyroid fine-needle aspiration biopsy was performed, with pathological results compatible with medullary carcinoma.
Patient underwent bilateral laparoscopic adrenalectomy and after that, total thyroidectomy with central lymph node dissection, both without complications. Anatomopathological study revealed bilateral benign pheochromocytoma (Ki67 2%) and medullary carcinoma of the thyroid T1bN0M0;R0.
Genetic analysis confirmed mutation c.2080 T> C in exon 11 of the RET gene, consistent with a diagnosis of MEN2A. The genetic study of relatives in the first degree was negative.
Currently, the patient is clinically and analytically stable and presented: calcitonin <2.0 pg/ml (<10), PTH 32 pg/ml (9–72), calcium 9.5 mg/dl (8.8–10.6) and serum metanephrines 47.2 pg/ml (<60).
Conclusions
This report illustrates an uncommon case of massive bilateral cystic pheocromocitomas in a young patient. In the presence of bilateral adrenal tumors and young age, multiple endocrine neoplasia probability is higher, and should be carried out biochemical, imaging and genetic investigation. If confirmed, genetic evaluation of first-degree relatives should be performed. Furthermore, because of the high possibility of recurrence, these patients should maintain close and long-term monitoring.

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P19

Recurrent adrenal pheochromocytoma – a benign condition?

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Introduction
Pheochromocytomas are adrenomedullary catecholamine-secreting tumors. These account for <0.1% of all causes of hypertension and may be related to potentially fatal hypertensive crises. Can appear as sporadic tumors or associated with familial syndromes.
Malignancy occurs in 15–20% of the cases and is characterized by local invasion or distant metastasis rather than capsular invasion. Tumor recurrence has been reported with a frequency between 6 and 23% wherein the highest rate of recurrence is usually associated with familial syndromes.
Case report
A 48-year-old woman was investigated at General Surgery Department in 2011 referring severe headaches refractory to analgesic therapy, episodic postural hypertension and palpitations. Initial investigation identified a nodular lesion of left adrenal gland with 3.5 cm major axis and catecholamine hypersecretion so left adrenalectomy was performed. First histopathological study was compatible with pheochromocytoma probably with benign behavior.
At April 2015, the patient started to complain again of palpitations and was referred to the Endocrinology Department. Abdominal CT was performed showing remaining glandular tissue in surgical loci. Elevated catecholamines on urinary samples (metanephrines 1572.76 μg/24 h (normal range (NR) 0–350); normetanephrines 1681.68 μg/24 h (NR 0–600); Vanillylmandelic acid 13.60 mg/24 h (NR 1–13.6)) were also demonstrated. 123I-MIBG scan showed and abnormal uptake in the left adrenal loci and the patient went surgical reintervention 1 month later. Second histopathological study reaffirmed the presence of pheochromocytoma – score 10 PASS classification by Thompson; pTNM: rT3; R1. The tumor demonstrated positive immunoreactivity for chromogranin and synaptophysin and the Ki-67 index was <2%.
CT and 123I-MIBG scan performed 2 months later confirmed a persistent lesion on the left adrenal loci and secondary lesions in left subdiaphragmatic topography; plasma and 24-hour free catecholamine remain elevated.
According to these findings, the patient was proposed for second surgical re-intervention with block excision of tumor mass, spleen, perirenal fat, parietal peritoneum and left diaphragmatic crus in August 2015. Third histopathological study showed none metastatic invasion in four lymphnodes and no tumoral infiltration on parietal peritoneum; R0 margin was confirmed.
Last laboratory findings showed near normal plasma Metanephrines (61.4 pg/ml; NR <60) and plasma Normetanephrines (124.4 pg/ml; NR <120); normal Vanillylmandelic acid (3.99 mg/24 h; NR <7), normal urinary Normetanephrines 484 μg/24 h (NR 50–650) and lower urinary Metanephrines (557.65 μg/24 h; NR 30–350).
Post second surgical re-intervention 123-I MIBG showed no abnormal uptake on left adrenal loci and low uptake area in left sub diaphragmatic which did not justify additional therapy.
Since the patient remains asymptomatic with favorable laboratorial evolution and no further therapy was recommended in multidisciplinary decision, it was decided to maintain clinical, analytical and imagiological surveillance.
Genetic analysis did not confirm any clinically relevant mutation in SDHAF2, SDHB, SDHC, SDHD, MAX, TMEM127 and VHL genes.
Conclusions
Pheochromocytoma is a rare tumor and patients with localized disease can develop a recurrence in 6–23% of the cases, usually 5 to 15 years after initial surgery.
Near 50% of patients with recurrent disease experience distant metastasis, so, long-term follow-up is essential for all patients with pheochromocytoma even when initial pathology is not suspicious of malignancy.

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P20

The importance of fully investigating adrenal incidentaloma: two pheochromocytoma cases

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Introduction
In most cases, adrenal incidentalomas are non-functioning adrenocortical adenomas, but may also represent conditions in which therapeutic intervention is essential, such as pheochromocytomas, even with low index of suspicion.
Case reports
Case report 1. Fifty-three-year-old male with history of arterial hypertension (HT), type 2 diabetes *Mellitus* and myocardial infarction, with a right adrenal incidentaloma found in abdominal ultrasound in February/2011. Abdominal CT revealed a 36 mm nodule in the right adrenal gland with solid homogeneous density. The preoperative analytical study was normal, including values of urinary fractionated metanephrines. In 2012 the control abdominal CT continued to show a nodule with the same characteristics, 'suggestive of adrenal adenoma', 40 mm. The patient underwent laparoscopic right adrenalectomy on 22 October 2013. Pathology report diagnosed 'pheochromocytoma'. Although he did not undergo preoperative adrenergic receptor blockade, he maintained hemodynamic stability throughout the surgical intervention and the postoperative period. The analytical study after the adrenalectomy was normal, with normal metanephrine levels and no adrenal insuffiency. Genetic testing did not find any clinically relevant mutations.
Case report 2. Fifty-year-old female with no relevant medical history. Right adrenal incidentaloma found in abdominal ultrasound in January 2015. Abdominal CT showed a well defined 35 mm nodule, with characteristics suggestive of adrenal adenoma. Preoperative investigation revealed significantly

elevated plasma free and urinary fractionated metanephrines. She underwent laparoscopic right adrenalectomy on 28 October 2015; the pathology report confirmed a pheochromocytoma, with no criteria for suspicion of malignancy (PAS Score <4). Postoperative study showed normalization of metanephrine values and absence of adrenal insufficiency. Genetic testing result is pending.
Conclusion
Both the cases concern patients with an incidentally found adrenal mass, who did not have the typical signs and symptoms of pheochromocytoma. In case 1, we describe a man with HT but no other symptoms and no biochemical evidence of catecholamine hypersecretion, and the patient in case 2 had a completely silent tumor. Both had abdominal CT sugestive of adenoma. As these case reports illustrate, the exhaustive evaluation of all adrenal incidentalomas is leading to a rising prevalence of diagnosed pheochromocytomas, allowing for safer surgical procedures and reducing immediate and long lasting morbidity and mortality.

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P21

Pituicytoma: a rare tumor

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Introduction
Pituicytoma is a low-grade glioma of the suprasellar and sellar regions that is rarely described (about 60 cases described in the literature). The clinical, laboratory and neuroradiological findings are not pathognomonic, and therefore definitive diagnosis is only possible after surgery and histopathological study. Total resection is the treatment of choice, since subtotal removal can often lead to recurrence or progression.
Case report
We report the case of a Caucasian male with no significant medical history who develops isolated frontal headache at the age of 48. The use of magnetic resonance imaging (MRI) revealed an expansive sellar and suprasellar lesion, with 14 mm, well-defined limits, bright homogeneous enhancement with gadolinium, associated with a slight deviation of the optic chiasm. Hormonal study was normal. The patient continued to be followed in Neurosurgery consultation and after 4 years presented complaints of gradually worsening visual disturbances, progressively decreased libido and erectile dysfunction. On visual field testing, a bitemporal hemianopia was noted. MRI revealed millimetrical increase of the tumor. He underwent transnasal/transsphenoidal surgery, which resulted in a partial removal due to difficulty in controlling bleeding. The histopathological examination showed a tumor composed of bipolar cells, with immunohistochemical positivity for vimentin and S100 protein, Ki-67 <2% – pituicytoma. The postoperative hormone study revealed panhypopituitarism. The control MRI detected significant residual tumor, so it was decided to propose the patient for radiosurgery treatment.
Conclusions
This pituicytoma case illustrates a diagnosis that, although rare, should not be excluded from the possibilities available before a suprasellar or sellar lesion that presents with certain cardinal radiological features. The persistence of significant residual tumor was due to the bleeding tendency of the lesion during surgery, which is frequent in pituicytomas. Awareness of the possibility of pituicytoma before surgery would be ideal for appropriate treatment planning, with possible preoperative embolization of the tumor.

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P22

Hypothyroidism and thyroid autoimmunity in metastatic renal cell carcinoma patients treated with sunitinib: 2 years follow-up of long survivors from a sigle centre experience

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Background
The development of thyroid dysfunction is a frequent side effect associated to sunitinib therapy but scanty data are available on thyroid function on long-term sunitinib-treated patients.
Objective
Prospective evaluation of 28 patients suffering from mRCC treated with sunitinib, enrolled between September 2013 and October 2015. 28 patients (24 men and 3 women (median age 57.7, range 51–77)) with comparable tumor staging, normal thyroid function and no evidence of thyroid autoimmunity (TA), were studied before and every 4 weeks after beginning Sunitinib (Sutent). In all patients, serum levels of TSH, FT3, FT4, thyroid antibodies (TgAb and TPOAb) and morphological evaluation were measured up to 24 months (median 17.6 months). We analyzed cumulative thyroid dysfunction and TA in surviving patients.
Results
6 (21%) patients died with no evidence of thyroid dysfunction (after 2–5 months of treatment) and TA. 22 (78%) developed primary hypothyroidism and 11 (39%) developed detectable TPOAb (TPOAb+). TPOAb+ patient had higher degree of hypothyroidism (median TSH 14.1 mIU/l vs 8.8 mIU/l in TPOAb-negative hypothyroid patients). Cumulative prevalence of hypothyroidism and TPOAb-positivity was 33% after 6 months, 46% after 12 months, 57% after 18 months to 78% at 24 months. Cumulative prevalence of TPOAb was 21% at 4 months, 32% at 9 months and 39% at 24 months. A remarkable decrease of thyroid volume (12.5 ± 11.6 ml to 2.2 ± 0.5 ml ($P < 0.0001$)) was observed in almost all patients, during the first 9 months of treatment, associated with the complete disappearance of thyroid nodules in four patients with thyroid nodular disease before sunitinib therapy. Presently, the survival of patients developing more severe hypothyroidism (TSH > 15 mUI/l) was significantly longer (17.8 ± 6.2 months) when compared to the other patients (13.5 ± 5.3 months $P < 0.05$).
Conclusions
Prolonged sunitinib treatment is associated with progressive increase of hypothyroidism and TA, affecting almost all patients after 24 month of therapy. Severity of thyroid dysfunction is associated with longer survival and may represent a biomarker of response in sunitinib treated mRCC patients.

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P23

A rare synchronous presentation of aldosterone producing adenoma and multifocal papillary thyroid microcarcinoma

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Introduction
In this report, we describe a rare coexistence of a multifocal papillary thyroid (micro)carcinoma and aldosterone-producing adenoma.
Case presentation
A 60-year-old man attended our clinic with a mass in the right adrenal gland was identified during the work-up in 2007. The patient presented complaints on anxiety, tachycardia, and arterial hypertension. He had several episodes of severe hypertension (220/120 mmHg) while sleeping. In 2014, an ultrasound guided fine needle aspiration of the thyroid nodule was performed; cytology showed Hurthle cell neoplasm suspicious for malignancy. The diagnosis was confirmed by laboratory findings and a magnetic resonance imaging which revealed a mass in the right adrenal gland. The tumorous mass in the right adrenal gland was removed surgically and a total thyroidectomy was performed. Pathological findings revealed a multifocal papillary (micro)carcinoma and adrenocortical adenoma.
Conclusion
Most aldosterone-producing adenomas and papillary thyroid carcinomas are sporadic; and the association between the tumors was ruled to be most likely accidental because of no family history or genetic testing. However, while no hereditary cancer syndromes were identified in this case, this does not preclude the possibility of an untested or unknown disorder.

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P24

Functional role of somatostatin receptor subtype 1 (sst1) in prostate cancer: an *in vitro* approach

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Prostate cancer (PC), the most commonly diagnosed malignancy among men, is a complex and heterogeneous disease that is highly influenced by the endocrine environment, which makes difficult the identification of novel therapeutic biomarkers to treat this pathology. Somatostatin (SST) is a pleiotropic neuropeptide that exerts its multiple biological functions, including tumor cell regulation, through a family of receptors (named sst1-5). Particularly, in this study we have found that sst1 is overexpressed in human PC samples compared with normal prostate (NP); however, the functional relevance of these alterations in PC is still unknown. Therefore, we aim to determine the potential functional role and mechanism of action of sst1 in human PC cells (using 22Rv1 and C42B cell lines). Functional parameters (i.e. proliferation and/or signaling pathways) were analyzed in response to treatments with two sst1-agonists or by silencing sst1 expression (using small interfering RNA). Moreover, we analyzed potential microRNAs (miRNAs) that could regulate sst1 expression in PC (using *in silico* approaches) and then, some selected miRNAs were used to perform functional assays. Our results showed that treatment with sst1-agonist decreased, while silencing of sst1 expression increased, cell proliferation in 22Rv1 cells. The effects of sst1-agonist in these PC cells were probably mediated through the regulation of AKT and PTEN but not ERK or calcium mobilization. *In silico* analyses revealed four putative miRNAs (miR-24/27/383/488) that could interact with sst1 at the 3′UTR. Interestingly, these miRNAs were negatively correlated with sst1 in 414 tumors (using The Cancer Genome Atlas (TCGA) data portal). Moreover, overexpression of miR-24 decreased sst1 expression, cell proliferation and migration, but increased cell death, in 22Rv1 and/or C42B cells. Finally, an inverse correlation between sst1 and miR-24 expression was found using the MSKCC dataset which include the combined analysis of 29 NP, 131 primary PC and 19 metastatic PC samples (i.e. expression of miR-24 gradually decreased, while sst1 expression increased, in these samples). In conclusion, our results indicate that sst1 is over-expressed in PC, where it can exert a relevant pathophysiological role by enhancing cell proliferation through AKT and PTEN signaling pathways. The observation that miR-24 can regulate sst1 expression and aggressiveness features in PC cells supports the idea that the combination of sst1 and miR-24 expression might be used as a novel tool to explore therapeutic targets in PC.

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P25

Metformin suppressed the proliferation of prostate cancer cells *in vitro* and reduced prostate tumor growth *in vivo* under low-fat and, especially, under high-fat fed conditions

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Obesity (Ob) is a chronic endocrine-metabolic disease and one of the most serious and complex threats for the human health, which is associated with an increased incidence of some types of cancers such as prostate cancer (PC), the second most common cancer in men worldwide. Interestingly, metformin (Met), an antidiabetic drug, might represents a very promising opportunity to treat Ob and PC as some retrospective clinical studies have shown that the incidence of PC is lower in patients treated with Met. However, the endocrine-metabolic, cellular and molecular mechanisms underlying the association between Ob and higher incidence/aggressiveness of PC and the putative pharmacological effectiveness of Met in PC are still unknown. We used primary normal prostate (NP) cell cultures from mice and human PC cell lines (PC3, 22Rv1 and LNCaP) as well as, immuno-suppressed mice inoculated with PC3 cells, fed a high-fat diet (HFD) or low-fat diet (LFD), as models to test the beneficial effect of Met (doses used: *in vitro* (10 μM–10 mM) or, *in vivo* (250 mg/Kg per day)). Our results indicate that Met modulates key metabolic, endocrine and pathologic components (e.g. expression of different components of insulin/IGF-I/somatostatin/ghrelin systems) in NP cell cultures. Interestingly, Met had no evident effect on 22Rv1 cells proliferation but significantly reduced PC3 and LNCaP cells proliferation and/or migration. Remarkably, we found that Met also have a significant beneficial *in vivo* effect as it reduced tumor volume and weight in mice fed a LFD and, specially, on those fed a HFD compared to their vehicle-treated control mice. In line with this, Met regulated the expression of key markers involved in cellular proliferation (i.e. PTTG and p53) and glucose/insulin homeostasis (i.e. IRS and AKT isoforms, IGFIBP3 and receptors for insulin, IGFI and GH) in tumors of mice fed a LFD and/or a HFD. In addition, Met significantly decreased the percentage of tumor cells mitosis and tumor necrosis under HFD, but not LFD, conditions and tends to activate ERK signaling (increased p-ERK/total-ERK; $P = 0.09$), but not AKT signaling, only in tumors of mice fed a HFD. Altogether, our data suggest that Met modulates NP cell function and exerts beneficial effects in the inhibition of PC cells growth *in vitro* and *in vivo*, specially, under HF-conditions.

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