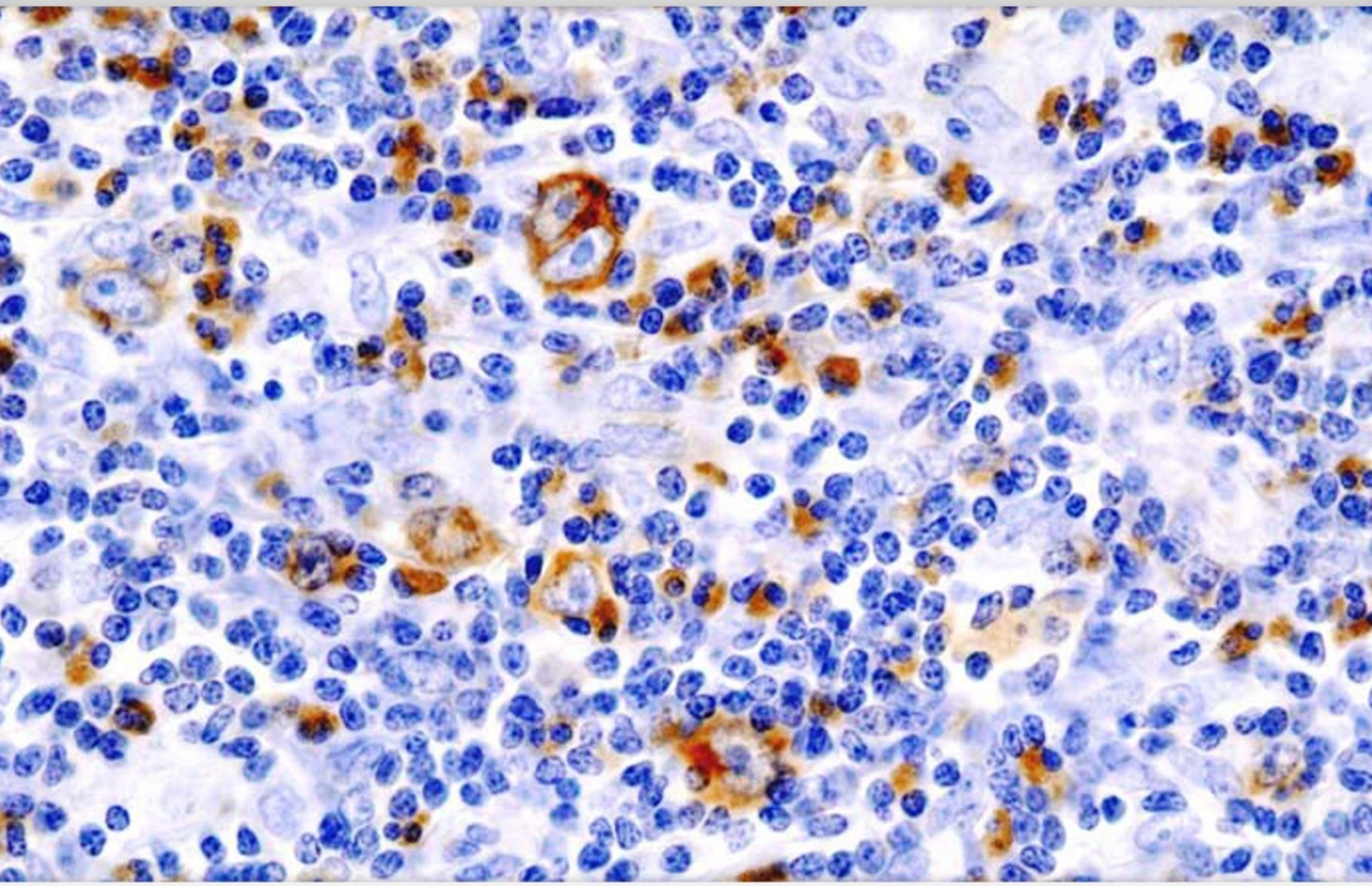
THE 2014 PATHOLOGY CONGRESS

ABSTRACTS



2nd - 4th December
London, UK

EuroSciCon

This meeting draws together international experts to discuss current techniques and research involved in cellular and molecular pathology. This year focuses on three specific areas

- Progress in Molecular and Cellular Pathology
- Developments in immunohistochemistry for diagnostic cellular pathology
- Biomarkers
- Histopathology and Cytopathology 2014: Advances in research and techniques

With plenty of opportunity for networking and debate, this informal international meeting will bring you up to date with current research and thinking regarding pathology. This meeting gathers together workings from clinical, academic and pharmaceutical organisations.

This event has CPD accreditation

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Day 1: Progress in Molecular and Cellular Pathology

Invited Speakers Abstracts

MicroRNAs and the contribution of cellular pathology to understanding their role in health and disease.

Tony Warford, Senior Lecturer in Cellular Pathology, University of Westminster, London

Nearly 2000 human microRNAs sequences currently populate miRBase. Is this the final total?; almost certainly not. MicroRNAs provide regulatory control of mRNA translation. Quantitative analysis of cell and tissue homogenates has shown that their expression is often altered in disease. This global information can be supplemented by the use of *in situ* hybridisation (ISH) to give precise cellular localisation of microRNAs. Whilst microRNAs are have very short sequence lengths of around 20 nucleotides they are often expressed in high copy number and are stable in formalin fixed and paraffin embedded tissue. Accordingly, they are amenable to ISH demonstration and in this presentation the contribution of cellular pathology to understanding the role of microRNA in health and disease will be discussed.

Update in the molecular pathology of uveal melanoma

Professor Sarah Coupland, Professor and Honorary Consultant in Pathology, University of Liverpool, UK

Uveal Melanoma (UM), the most common primary intraocular cancer in adults, is fatal in 50% of patients, because of metastatic spread involving the liver. Chemotherapy of metastases has limited success and disseminated disease occurs in most patients <2 years of diagnosis. Clinical, histopathological and genetic risk factors for UM metastasis are documented. UM is characterised by frequent non-random gross chromosomal changes, the most common being monosomy 3, gain of 8q, loss of 1p, gain of 6p and loss of 6q. The first two chromosomal abnormalities in particular are the strongest predictors for metastasis development. The purposes of this presentations are to review: a) described genetic abnormalities of UM, and relate these to hypotheses regarding tumour development and spread; b) current methods used in UM prognostication.

From Eustachius to Schuknecht: What have human temporal bone pathology studies taught us?

Panos Dimitriadis, Luton and Dunstable Hospital, UK

The first human temporal bone (TB) histopathology studies were conducted as early as in the Middle Age when Vesalius described the malleus and incus. There are 10 TB collections in Europe, two of which are in London. The amount of time and effort expended in maintaining a TB lab is staggering. To be cost effective it must lead to improved health care. We assess the clinical relevance of pathologic findings of human TB studies in diseases like: Meniere's disease, presbycusis, otosclerosis, sudden deafness, and vertigo. We include recommendations regarding the setup of a cost-effective TB lab of today.

Procurement Time of Human Tissue Biospecimens – Significant increases reported using AQIX® RS-I fluid technology

Dr. Douglas Rees PhD MRSNZ, Founder, Director & CSO, AQIX Ltd, London BioScience Innovation Centre, UK

Introduction: The quality of RNA subunits and morphology is directly impacted by the quality of human tissue samples procured. It was hypothesised that if fresh, human tissue biopsies could be immediately immersed in a solution that closely resembles the interstitial fluid which lies juxtposed to every human cell, then these cells would maintain homeostatic balance during storage and transportation. An essential requisite was that there be optimal preservation of tissue viability with no aberrant changes in RNA integrity or gene expression profiling.

Results: Normal human colon tissue samples stored and transported in AQIX® RS-I solution showed excellent preservation of both morphology and RNA integrity (RIN) in comparison to RPMI over 30 hours. Equally, cancerous human breast tissue samples stored and transported in AQIX® RS-I solution over 24 - 46 hours again indicated that both the morphology and RNA integrity was better preserved than that previously reported.

Conclusion: The results to date indicate that AQIX® fluid technology will significantly advance the ability to store and transport human tissue samples over geographic distances in preserving their morphology and RNA integrity to improve diagnostic and prognostic outcomes.

Whole Slide Imaging using Hamamatsu NanoZoomer Slide Scanner

Dr Matthew Burke, Hamamatsu Photonics, UK

Digital Pathology is pushing new boundaries all over the world and its use is increasing day by day. More and more people are looking to use whole slide imaging to improve efficiency in clinical environments, open up new cutting edge research opportunities and using it as a tool to teach the next generation of pathologists.

Hamamatsu have been developing and providing the NanoZoomer slide scanner range for a decade and have always been at the cutting edge for this technology using our advance camera imaging systems and high level engineering capabilities.

This presentation will give an overview of the current benefits and issues with traditional imaging techniques and then providing solutions through use digital pathology, the NanoZoomer slide scanner and associated software. Some real world examples from customers in the UK will be provided where the system is being used in clinical, research and teaching applications to confirm the benefits of whole slide imaging in a range of different pathology applications.

What can NGS offer to the cancer pathologist?

Dr Sterghios Moschos, Redear in Industrial Biotechnology and Biochemistry; Director, Westminster Genomic Services, Department of Biomedical Sciences, University of Westminster, UK

The talk will overview the requirements and landscape of diagnostics development for the next generation sequencing arena, and contextualise these in the recent developments in cancer diagnosis. Key research outcomes and examples of studies will be overviewed with a focus on clinically actionable output delivery and the impact to the patient.

Quantifying digital pathology in a large human brain cohort

Dr Atticus H Hainsworth, Stroke & Dementia Research Centre, St George's University of London, UK

Small vessel disease is the most prevalent cause of lacunar stroke, vascular cognitive impairment and white matter lesions in older people. The pathogenesis is poorly understood. we have explored vascular histological markers in large cohorts (OPTIMA and MRC-CFAS).

Oral Presentation Abstracts

THE TUMOUR-STROMA RATIO (TSR) ADDITIONAL TO THE TNM CLASSIFICATION?

W.E. Mesker[1], G.W. van Pelt[1], V.T.H.B.M Smit[2], J. Morreau[2], J.H.J.M. van Krieken[3], R.A.E.M. Tollenaar[1].
Departments of Surgery (1) and Pathology (2), LUMC, Leiden and Department of Pathology, UMCN, Nijmegen (3), The Netherlands.

Dr. W.E.Mesker, Associate professor, Department of Surgery, Leiden University Medical Center, Albinusdreef 2, 2300 RC Leiden, The Netherlands telnr +31715262987 / 4005; w.e.mesker@lumc.nl

Objective

The tumour-stroma ratio (TSR) can be applied for better selection of colon cancer patients for adjuvant therapy. The TSR is an independent prognostic marker and was discussed by the TNM Evaluation Committee (UICC) and the CAP (College American Pathologists). They stated that our observations are important and novel and may potentially be included in the TNM staging algorithm. The TSR-parameter can be determined at routine diagnostics and has been validated in national and international research studies resulting in a high inter-observer agreement K>0.80.

Study method

Tissue samples were analysed for their tumour-stroma percentage, consisting of 5µm Haematoxylin and Eosin (H&E) stained sections from the most invasive part of the primary tumor. Stroma-high (>50% stroma) and stroma-low (≤50% stroma) groups were evaluated with respect to survival times.

Results

In different studies significant differences in survival were observed between stroma-high and stroma-low patients showing poor survival for stroma-high patients ($p<0.0001$, HZ 2.5). Results have been validated in various studies (stage II, III) (OS $p<0.0001$, HR=2.0) with clear five year differences for both stroma groups. When adding the stroma-parameter to the ASCO criteria the number of correctly classified patients increased with an additional 14%.

Conclusion

We propose the tumour-stroma parameter as an additional marker to select patients at high-risk of recurrence of disease. This parameter is to be expected to be used in clinical practice for better risk-

classification, is simple to determine and reproducible and should therefore be considered for implementation in standard pathology reports in addition to the current TNM classification.

EXPERIENCES WITH THE LATEST GENERATION *in situ* HYBRIDISATION METHODOLOGIES

Paul Murdock.
Stemgent-Asterand, Orchard Road, Royston Hertfordshire, SG8 5HD

The visualisation of specific mRNA sequences in tissue sections using *in situ* hybridisation has been widely used over several decades, but with limited levels of success. The latest generation of *in situ* hybridisation technology offers unparalleled image quality, multiplexing, specificity and sensitivity. This presentation will provide our independent experiences using branched nucleic acid-based *in situ* hybridisation technology for visualising mRNA in human tissue sections, and provide examples of how this technology can be applied to aid your research. The presentation will be of particular interest to researchers seeking an independent view of how the latest *in situ* hybridisation methodology has come of age, as well as to scientists working with this platform.

Poster Presentation Abstracts

PANCREATIC METASTASIS IN LARYNX

Rosa Maria Hernandez-Cancela MD,
Anatomia patológica, Complejo Hospitalario Universitario A coruñaLa coruña, 15006 Spain

Introduction: Metastases of the larynx from distant primary malignancies are uncommon, accounting for fewer tan 0, 5% of all laryngeal tumors. Metastases originating from pancreas are extremely rare with research of the literature revealing only two documented cases. Laryngeal metastases may occasionally simulate primary tumors and represent a diagnostic dilemma. Fine needle aspiration can provide an accurate diagnosis. This is the first description of laryngeal metastases from pancreatic carcinoma diagnosed by FNA cytology and immunocytochemistry. **Case history:** A 53 year old man with a 2 month history of dysphagia and dysphonia was referred to us for FNA on a rapidly enlarging cervical mass. Four years earlier de patient had undergone Pancreaticoduodenectomy because of pancreatic cancer. On the patients' examination a 2cm firm and painless mass was identified, FNA was performed and a diagnosis of metastases of primary pancreatic carcinoma was given. The adverse clinical course caused the performance of a total larynguectomy and left hemithyroidectomy and histopathology confirmed the cytological diagnosis and no lymph nodes were affected. Postoperative radiotherapy was administered but finally the patient succumbed to the disease 6 months later. **Conclusion:** Laryngeal metastases are thought to arise as a result of lymphatic dissemination or from passage through the bloodstream. The rare occurrence of metastases to the larynx may therefore be related to the terminal position of the organ in the lymphatic and vascular circulation. Laryngeal metastases are infrequent. We have only two reports of laryngeal metastases originating from pancreatic neoplasms. In rare occasions the metastases is the only evidence of an otherwise occult primary tumor, but when a primary tumor has been discovered a histological and immunohistochemical comparison of both tumors should be considered. FNA and immunocytochemical profile of the laryngeal tumor is of great value to rule out the possibility of metastasizing process. CDX2 Being a useful marker to determine the pancreatic origin of the metastasis in our case.

NEUROPROTECTIVE PROPERTIES OF PICEATANNOL AND XANTHOHUMOL ON NEURONAL HIPPOCAMPAL CELLS UNDER OXIDATIVE STRESS CONDITIONS

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Over the past few years, the numerous studies have been focused on the role of polyphenols, naturally occurring compounds, in several pathogenic processes in many diseases, with special emphasis on cardiovascular, cancer and neurodegenerative disorders. One of the most well-known polyphenol is resveratrol, a widely distributed natural stilbenoid. The numerous experiments suggest that it possess beneficial properties such as anti-cancer, anti-inflammatory, blood-sugar-lowering and cardiovascular effects. It is supposed to trigger mechanisms that counteract aging-related effects and to have an interesting activity in neuroprotection - mainly through counteraction forming peptide aggregates and presumed sirtuins activation. Our research is

concentrated on effect of derivative of resveratrol – piceatannol and less known prenylated flavonoid – xanthohumol. Both exert beneficial effects on health, including antibacterial, antioxidant, and anti-inflammatory properties. However there are still not many data confirming these statements.

Taking into account the above considerations, we decided to investigate the effects of piceatannol and xanthohumol on hydrogen peroxide-induced oxidative stress in hippocampal cells (mHippoE-18). In order to determine the concentrations of polyphenols for further research the mHippoE-18 cells were treated with piceatannol or xanthoumol in concentrations ranging from 2.5 μM to 50 μM. The concentration of added hydrogen peroxide to cells was established in previous studies, it was 0.03 mM.

After 24 hours of incubation only with polyphenols we observed increased cell viability. To further research following concentrations were selected: for piceatannol in concentration range 2.5 – 50 μM and for xanthohumol in range 5 – 30 μM. In further experiments cells were preincubated with polyphenols for 3 hours and then hydrogen peroxide was added at a concentration of 0.03 mM. Comparing cell viability, we observed significantly increase in cells treated with both – piceatannol or xanthohumol and hydrogen peroxide, than in cells treated by oxidizing factor only. Furthermore, it was observed that the selected polyphenols affect on changes in the activity of GSH-dependent enzymes such as glutathione peroxidase, glutathione S-transferase and glutathione reductase.

Our data show that treatment with chosen polyphenols not only protect cells from hydrogen peroxide toxicity but also stimulates the proliferation of cells. In both cases, the chosen concentrations of tested compounds caused increase of viability about 30-40%.

In conclusion, piceatannol and xanthohumol seems to be promising, antioxidant agents. Investigated compounds indicate neuroprotective properties.

EFFECT OF RESVERATROL AND MELATONIN ON OXIDATIVE DAMAGE OF GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE AND LACTATE DEHYDROGENASE

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This work was financially supported by the National Science Center (Poland) (decision No. 2012/05/B/NZ1/00701)

Prevention of neurodegenerative diseases is one of the key challenges facing contemporary science. Despite substantial research in the field, the exact causes and development of these diseases at the molecular level have not been fully elucidated. One of the main causes of these diseases is oxidative stress coupled with a decreased capacity of antioxidative systems, which is noted especially in elderly persons. In the pathogenesis of neurodegenerative diseases, of fundamental importance are the processes of protein damage induced under oxidative stress. Probably one of the proteins involved in the neurodegenerative process is glyceraldehyde-3-phosphate dehydrogenase (GAPDH).
The study is designed to determine the influence of radiation generates reactive oxygen species (ROS) on the structure and function of glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase in order to elucidate the mechanisms of damage of this protein. Furthermore, the study examines the influence of antioxidants such as melatonin and resveratrol on the processes of oxidative damage of investigated proteins.
Aqueous solutions of dehydrogenases and of dehydrogenases in the presence of melatonin or resveratrol were exposed to ROS. Reactive oxygen species were generated by ir radiation of water solutions of proteins with X-rays in the atmosphere of air. The changes in the activity, content of free thiols and secondary structure were investigated in the presented study.
Based on the inactivation rate it was demonstrated that GAPDH is eight times more radiosensitive in comparison to LDH. The loss of enzymatic activity was coupled with the reduction of free –SH groups in the proteins exposed to ionizing radiation (significantly higher for GAPDH in comparison to LDH). Studied antioxidants: melatonin and resveratrol significantly decreased the inactivation rate, oxidation of free thiols and secondary structure changes under ionizing-radiation induced

oxidative stress conditions. In addition, the examined compounds were more efficient in preventing oxidative damage of LDH in comparison to GAPDH.
Based on the conducted experiments and bioinformatic analysis, we intend to find out which structural features of the proteins determine their vulnerability to ROS. Results of the studies will contribute to the understanding of the interactions of low-molecular weight antioxidants with oxidatively modified proteins.

NEW THERAPEUTIC APPROACHES FOR THE TREATMENT OF METASTATIC BREAST CANCER

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Breast cancer is the most commonly diagnosed malignant type of cancer among women and the second leading cause of death. Every year about half a million women diagnosed with metastatic breast cancer, die. In 2013 in Europe more than 400,000 new cases were diagnosed.

Until now metastatic breast cancer has been regarded as an incurable disease. However, in recent years, the number of cases of this type of tumor is gradually decreasing, and diagnosed patients tend to live longer. Improving the quality of life of patients and an increase of their survival rate are associated with the changes that have taken place not only in the philosophy of treating metastatic breast cancer, but also in the availability of new therapeutic approaches.

Treatment of breast cancer is mainly based on the commonly used methods of oncotherapy: surgical removal of cancerous changes, chemotherapy, radiation therapy and biological therapy. Shall also be used combinations of these methods. Expanding our knowledge in the field of molecular biology and mechanisms associated with carcinogenesis process, has allowed the development of targeted therapy. Previous studies have shown that the use of targeted therapy is beneficial for overall survival rate of patients, enhances the response to treatment and slows down the progression of the disease.

Newly developed methods for the treatment of metastatic breast cancer have two basic assumptions: *i)* improving therapy with cytotoxic drugs (designing new effective chemotherapeutic compounds and improvements in the distribution and prolonged circulation of drugs in the body, *ii)* development of more efficient methods of biological therapy (research focused on the use of receptors present on the surface of tumor cells, the use of tyrosine kinase inhibitors and the limit of the respective isoform overexpression of vascular endothelial growth factor VEGF). The challenge is to create the ideal combination of therapeutic agents and to determine their effective doses, the development of an effective treatment regimen suited to individual patient and minimizing the limitations of the multidrug resistance of tumor cells.

For the last few years we have observed an intensive development of nanotechnology science which possibilities and solutions are used in many fields of science. One of the possibilities offered by nanotechnological techniques is the design and synthesis of nanoparticles, acting as carriers of anticancer drugs called nanomedicines. Thereby it has been possible to develop new targeted anti-cancer therapy based on carbon nanotubes, dendrimers, quantum dots, liposomes, micelles, polymeric nanoparticles and nanoparticles containing metals as a new class of effective drug nanocarriers.

DIFFERENCES IN MITOCHONDRIAL PROTEINS EXPRESSION IN THYROID GLAND NEOPLASMS

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Introduction Multiple studies regarding the development and progression of cancer indicate defects of mitochondrial structure and function. To better understand basic mechanisms of tumor development and identify potential new biomarkers, we have performed nano-liquid chromatography mass spectrometry experiments on protein extracts from follicular adenoma and papillary thyroid carcinoma versus normal thyroid adjacent tissue.
Methods Homogenates from thyroid gross pathology sampled from operation theatre were obtained from two groups of patients, one with follicular adenoma (D, n=9) and another group with papillary thyroid carcinoma (P, n=9). Control (CD and CP) tissue samples adjacent to the tumors were analyzed from each specimen. The tissue homogenates were suitably processed for mass spectrometric analysis and their corresponding peptides were separated in 2-35% acetonitrile gradient using the EASY–nano liquid chromatograph. The high performance LTQ-Velos Orbitrap system was used to generate mass spectra using the Top 6 Data-Dependent method. The ratios of D/CD and P/CP protein abundance were processed by label free quantification with SIEVE 2.1 software. The general and in depth characterization of the various properties of proteins, annotation based on gene ontology (cellular component, molecular function and biological process) and the identification of the protein quantity alterations were extracted based on Protein Center software. The differentially expressed proteins were matched with KEGG databases to identify the over-represented signaling pathways.
Results and Discussion The homogenate comparative shotgun proteomic LC-MS experiments revealed a high plethora of proteins (3364 proteins).The Proteome Discoverer analysis of the raw data revealed 851±123 proteins in the tissue samples adjacent to the follicular adenoma (C_D), 1060± 253 proteins in the follicular adenoma samples (D), 934±235proteins in the control (C_P) and 1082±181proteins in the papillary cancer samples (P). Most of them are membrane (~12±0.4%), extracellular (~13±1%) and are involved in catalytic activity (~17±0.5%) and protein binding (~30±0.3%). From the total identified proteins, differences were observed between mitochondrial (D/C_D=1.53±0.65 and P/C_P=1.3±0.31) over-represented class proteins in neoplasm tissues versus control tissues. A pool of 504 identified mitochondrial proteins which were either up- or down-regulated in D and P groups versus control adjacent tissues, proved to be implicated in ~33 statistically significant over-represented signaling pathways. 28 proteins appeared to be differentially expressed in some of the papillary cancer samples, but not in follicular adenoma. These mitochondrial proteins may be valuable biomarker candidates for differentiate between the benign and malignant form of thyroid neoplasm. Studies are in progress to validate this hypothesis.
Acknowledgements The present work was supported by the Romanian Academy and Ministry of Education and Research grant PN-II/CNDI-UEFISCDI no. 153/2012 and 135/2012, POSDRU/159/1.5/S/ 137750.

DETERGENT RESISTANT MEMBRANE MICRODOMAINS ACTIN-DEPENDENT PATHWAYS IN EXPERIMENTAL HYPERLIPIDEMIA

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Introduction. Detergent resistant membrane (DRM) microdomains segregate important proteins involved in signaling pathways and vital cellular functions, such as transport, trafficking and cholesterol homeostasis. The aim of the study was to evaluate the alterations induced by hyperlipidemia and the statin treatment on DRMs actin-dependent signaling pathways using high performance mass spectrometry coupled to liquid nano-chromatography and bioinformatics tools.
Materials and Methods. DRM microdomains were isolated from lung tissue homogenates of a control group of Black C57 mice (C), a group of ApoE knockout mice (KO) that received a hyperlipidemic diet (A) and a group of ApoE KO mice that received a hyperlipidemic diet and a statin treatment (At). The DRMs were suitably processed for MS analysis and their corresponding peptides were separated in a 2-35% acetonitrile gradient using the Ultimate 3000 RSLC nano system (Dionex, now part of Thermo Scientific), before mass spectra were obtained using the LTQ-Velos Orbitrap hybrid mass spectrometry system (Thermo Scientific) using the Top 6 Data-Dependent method and CID fragmentation.
Results and Discussions. The Proteome Discoverer (Thermo Scientific) MudPIT analysis of the mass spectrometric raw data revealed a total of 1925 proteins, mostly of membrane, cytoskeletal and cytosolic origin, especially implicated in protein binding processes such as protein, nucleotide and metal ion binding, transporter and structural molecule activity and catalytic activity (Protein

Center). 654 of these proteins were found to be either up- or down-regulated in A and At groups versus C using label free quantitative analysis (Sieve, Thermo Scientific), based on the precursor intensity comparative evaluation. These proteins were evidenced in 13 statistically significantly over-represented signaling pathways, including *Regulation of actin cytoskeleton*, *Focal adhesion* and *Adherens junctions*, using Protein Center (Thermo Scientific) software platform computer analysis. The altered quantitative protein profile of the A and At groups relative to the control group was validated using the Principal Component Analysis (Sieve). For some proteins the expression alteration pattern was validated by immunodetection techniques.

Conclusions. Our study provides the basis for future functional studies concerning protein activities at the membrane cytoskeleton interface and dependency upon various stress factors including diet-induced hyperlipidemia.

Keywords. Detergent resistant membrane microdomains, actin cytoskeleton, mass spectrometry

Acknowledgements. The present work was supported by the Romanian Academy and Ministry of Education and Research grant PNII/CNDI-UEFISCDI 135/2012 and 153/2012], POSDRU/ 159/1.5/S/133391 and CARDIOPRO project ID:143, ERDF co-financed investment in RTDI for Competitiveness.

HMGB1-RAGE INTERACTION ACTIVATES BETA-CATENIN SIGNALING PATHWAY IN DIABETIC LUNG

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Introduction Type 1 diabetes is associated with an enhanced inflammatory state that can cause several health complications. The multifunctional proteins high-mobility group box 1 (HMGB1) and beta-catenin are important players of inflammatory process. HMGB1 is a nuclear DNA-binding protein with alarminactivity. The multifunctional protein beta-catenin has previously shown to regulate transcription of multiple genes involved in cellular proliferation, differentiation, apoptosis and inflammation. Since lung dysfunction in patients with diabetes is associated with systemic inflammation, the aim of this study was to investigate whether HMGB1 may activate the nuclear beta-catenin in experimental mouse model of type 1 diabetes.

Materials and Methods Blood samples and lung tissues of single transgenic mice Ins-HA+/–, TCR-HA –/ – used as control (C) and diabetic double transgenic mice Ins-HA+/ –, TCR-HA+/– (D) were harvested for biochemical, morphological, Western blot, immunoprecipitation and real time PCR analysis.

Results and Discussions After 8 weeks, D animals presented pathological values of serum glucose (435.45 ± 32.8 mg/dl) while C mice showed these values to be unchanged. Proteinand gene expression of HMGB1 were significantly increased (2-fold) in the Dgroup compared to control and was positively correlated with the HMGB1 values detected in serum of diabetic mice. Co-immunoprecipitation of HMGB1 and its receptor RAGE in diabetic mice co-exists with activation of both PI3K/ AKT1 and NF-kB signaling pathways. At the same time beta-catenin was increased in nuclear fraction (3.5 fold) while it was downregulated in diabetic plasma membrane fraction (2-fold). Interestingly, there was no difference of beta-catenin gene expression between the control and diabetic mice. Phosphorylation of beta-catenin at Ser552 was significantly higher in diabetic nuclear fraction, suggesting that AKT1 activation promotes beta-catenin nuclear translocation

Conclusions Taken together, the results reported support the novel concept that HMGB1 maintains inflammation through RAGE/AKT1/beta-catenin pathway in the diabetic lung.

Keywords HMGB1, beta-catenin, RAGE, AKT1, Type 1 diabetes

Acknowledgements Romanian Academy, Ministry of Education and Research grant PN-II-PCCA-2011-3 no. 135, 153/2012 and POSDRU/159/ 1.5/S/133391

Day 2: Developments in immunohistochemistry for diagnostic cellular pathology

Invited Speakers Abstracts

Emerging role of the pathologist and cancer biomarking in as applied to stratified medicine and targeted therapy

Professor Bharat Jasani, Cardiff University School of Medicine, Institute of Cancer & Genetics, UK

Stratified medicine is based on identifying subgroups of patients with distinct mechanisms of disease, or particular responses to treatments. This allows us to identify and develop treatments that are effective for particular groups of patients. Ultimately stratified medicine will ensure that the right patient gets the right treatment at the right time. In cellular pathology stratified medicine has begun to emerge as a strategy for biomarking of all cancers according to their distinct molecular subtypes targetable with specific drugs. The The pivotal role of biomedical scientists and pathologists in application of this strategy will be presented.

'Dying for a Tan'- immunocytochemistry in the assessment of photo damage and malignant melanoma.

Dr Guy Edward Orchard, Consultant Grade Biomedical Scientist/ Laboratory Manager, St. John's Institute of Dermatology, London

Immunocytochemistry (IMC) can be used to study the effects of ultra violet (UV) radiation on skin. Many are based on evaluation of cell proliferation (Ki67), apoptosis (bcl2) or oncogene expression (p53). Other markers are used to determine DNA damage (thymine dimmers). Langerhans cells assessed by CD1a, react strongly to UV insult and actively migrate out of the epidermis following sun damage. Repeated sun burn episodes, over time increases the likelihood of developing skin cancer later on in life in an almost exponential manner.

Molecular expression profiles of different types of thyroid carcinomas.

Dr. Monika Lamba Saini, Université catholique de Louvain, Brussels, Belgium

Papillary thyroid carcinoma (PTC) is the most common endocrine malignancy, accounting for 85 – 90% of all thyroid cancers. PTC also frequently carries several alterations in genes coding for proteins that activate the Mitogen-activated protein kinases (MAPK) signalling pathway, which plays a key role in the regulation of cell growth and differentiation. Mitogen-activated protein kinases (MAPKs) are signalling components that are important in converting extracellular stimuli into a wide range of cellular responses. Our study aims to describe the progress made in determining the role of MAPK signalling in thyroid cancers and also attempts to answer some of the open questions remaining in the field of MAPK signalling.

Application of the Nanotechnology in Immunohistochemical Staining Procedures

Dr Weiming Xu, CEO London Biotech Ltd and Department of Molecular Biology and Biotechnology, University of Sheffield, UK

Development of cancer biomarkers for early and rapid diagnosis is desirable as most tumour only be detected when they contain millions of cells that may already have metastasized. Current diagnostic techniques such as peroxidase-based immunohistochemical staining procedures are labour intensive, time consuming, expensive, in need of multiple amplifications and don't have multiplexing capability. Recent development on semiconductor nanocrystal quantum dots (QDs), has hold particular new promise as next generation of fluorescent probes. In this talk, I will present some of our latest findings on using the unfolded protein response (UPR) marker antibodies, such as anti- the glucose-regulated protein 78 (Grp78) antibody for prostate cancer and breast cancer detection and their potential usages for chemoresponsiveness and metastases in breast cancer tissue.

No Antibody? No Problem.

Mr Barry Lynch, Advanced Cell Diagnostics, Inc

Because over 70% of protein-coding genes have no reliable antibody for immunohistochemistry (IHC), research can come to a screeching halt while you wait for new antibodies to be developed. Whether your target is a novel gene with no commercial antibody available, a secreted protein with poor-quality antibodies for IHC, or a non-coding RNA our universal assay workflows and rapid probe design for any gene, eliminates the hassles of antibody screening, saving you precious time and effort, while delivering publication quality data today.

D-Sight, The full automated platform to analyze both nuclear and membrane Immunostaining. EGFR and Ki-67 quantification in pancreas pathologies

Dr Niccola Funel, University of Pisa, Division of Surgical Pathology, Pisa, Italy

Ki67 index (Ki67-I), is the percentage Ki67 immunoreactive cells, expressing tumor proliferation, with important clinical relevance in pancreatic neuroendocrine tumors (pNET) and to standardize its evaluation is extremely important. However this type of evaluation is currently done by subjective opinion of pathologist concerning the Area of Interest (AI). While EGFR quantification was recent associated with the

grading of Pancreatic Ductal Adenocarcinoma (PDAC), important for the prognosis of patients. We elaborated a new algorithm of analysis able to evaluate the protein expressions in tumor cells present in the selected area according to the pathologist's criteria.

The application of fluorescent confocal microscopy in corticosteroid-insensitive diseases

Dr Amir Hakim, Imperial College London and Royal Brompton Hospital, London, UK

Chronic obstructive pulmonary disease (COPD) is the fourth commonest cause of death worldwide. Corticosteroids are widely used in the treatment of COPD and severe asthma, however, in sharp contrast to mild-to-moderate asthmatics they provide little improvement in lung function and the underlying chronic inflammation. Recent evidence suggests glucocorticoid receptor nuclear translocation, essential for corticosteroid function, is impaired in severe asthmatics, which may contribute to corticosteorid-insensitvity. Several molecular biology tools, including flourescent confocal microscopy, have allowed us to furtner unravel the causes of corticosterod-insensitvity in COPD and severe asthma.

Oral Presentation Abstracts

EVIDENCE OF MTOR ACTIVITY DURING HUMAN SALIVARY GLAND ATROPHY

S BORZORGI[1], R HENLEY-SMITH[2] & GH CARPENTER[1]
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Introduction: salivary gland dysfunction is a surprisingly common ailment. 10-30 % of the general population suffer oral dryness due to lack of saliva. The main causes of the loss of saliva are Sjögren's syndrome, irradiation-damage and drug-induced hyposecretion. Common to all these causes is the atrophy of salivary glands. Although often considered a passive process of decay atrophy is in fact an active mechanism. Our recent work in animals has shown that mTOR (mammalian target of rapamycin) becomes activated in ligated salivary glands and coincides with autophagy (Silver, Proctor et al. 2010). Furthermore rapamycin treatment, which is a specific blocker of mTOR, delayed atrophy and halted autophagy although the gland eventually became resistant to rapamycin and reverted to the atrophic state (Bozorgi, Proctor et al. 2014). The present study sought evidence of aberrant mTOR activity in atrophic human salivary glands.
Methods; ten human submandibular gland samples were obtained with consent from the KHP Head and Neck biobank (London, UK). Samples were chosen with varying levels of fibrosis and fat replacement of parenchymal tissue. Tissue samples were homogenized and prepared for electrophoresis and immunoblotting. Part of the same biopsy sample were also sectioned for immunohistochemistry.
Results; all samples showed some degree of atrophy (dilated ducts, reduced acinar size, inflammatory infiltrates) even apparently healthy samples. Biochemically levels of phosphorylated 4E-BP1 and S6rp (both substrates of the mTOR kinase) were greatest in samples showing mild atrophy and least in the most atrophic samples. Levels of autophagy markers such as atg 3, 5 and LC3 were also greatest in the mildly atrophic samples and least in severely atrophic samples. Immunohistochemical analysis indicated most mTOR activity occurred in the acinar cells. In addition, levels of salivary proteins such as cystatin and carbonic anhydrase 6 whilst present in the mildly atrophic samples were not detected in the severely atrophic samples.
Conclusion: during the early stages of atrophy mTOR is most active and coincides with the highest levels of autophagy. When atrophy is severe and most acinar cells are replaced by fat cells and fibrotic deposits mTOR is no longer as active. The data aligns with the animal data and suggests that mTOR is driving the atrophic processes.
Bozorgi, S. S., G. B. Proctor and G. H. Carpenter (2014). "Rapamycin delays salivary gland atrophy following ductal ligation." Cell Death & Disease 5.
Silver, N., G. B. Proctor, M. Arno and G. H. Carpenter (2010). "Activation of mTOR coincides with autophagy during ligation-induced atrophy in the rat submandibular gland." cell death & disease 1(1).

RALOXIFENE PROTECTS AGAINST SEIZURES AND NEURODEGENERATION IN A MOUSE MODEL MIMICKING EPILEPSY IN POSTMENOPAUSAL WOMAN

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Epilepsy in menopausal women presents several challenges in the treatment including an increased risk of seizures due to hormone replacement therapy. We investigated the hypothesis if raloxifene, a selective oestrogen receptor modulator, could be employed to prevent behavioural seizures and morphological alterations in a mouse model mimicking epilepsy in postmenopausal women. Female mice were made ovotoxic by treatment with 4-vinylcyclohexenediepoxide (VCD) to mimic a postmenopausal state. They were then subjected to kainic acid (KA)-induced seizures and neurotoxicity, as assessed by microscopic examination of hippocampus, relevant to human temporal lobe epilepsy. VCD administration (for 15 days followed by a drug-free period of 30 days) induced ovotoxicity in mice as evidenced by reduced number of primary ovarian follicles. This was accompanied by a 62.4% reduction in serum estradiol levels. The bone mineral density of ovotoxic mice, however, remained unaffected. Raloxifene (8mg/kg) reduced the seizure severity score in both normal and ovotoxic mice and protected against degeneration induced by KA in the CA3, CA1 sub-fields and hilus of the DG. Hippocampal TGF-β3 levels were not affected by any of the treatments. We show the potential protective role of raloxifene in preventing seizures and neuronal damage in a mouse model mimicking epilepsy in postmenopausal women which was found unrelated to hippocampal TGF-β3. Raloxifene might represent a novel therapeutic option for postmenopausal temporal lobe epileptic woman.

Poster Presentation Abstracts

COMPARISON OF EXPRESSION PATTERNS OF NFκB SUNUBITS IN DIFFERENT RENAL CELL CARCINOMA SUBTYPES FROM A SINGLE SURGERY CENTRE

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Background: Renal cell carcinoma (RCC) is made up of several histological sybtypes of varying incidence. Approximate percentages are: clear cell RCC 80-85%; papillary RCC 10%; chromophobe RCC 5%; collecting duct RCC 1%; other RCC <1%. Patients with clear cell RCC have poorer prognosis compared with patients with papillary and chromophobe RCC. Renal oncocytoma, a benign neoplasm, occurs in around 5% of renal neoplasms. Molecular markers that indicate differences between the subtypes, and the grade and stage of the tumours, are needed. In this study, subunits of nuclear factor kappa B (NFκB) which plays an important role in the signal transduction system of RCC, were assessed.
Materials and methods: Our objective was to compare RCC and oncocytoma subtypes, both overall and stratified by tumour stage and grade, and compare NFκB subunits (p50, p52, p65, c-rel/relA and relB), using 101 patients treated with radical or partial nephrectomy at a single institution (Univ Malaya Medical Centre). Normal kidney samples of these patients were used as the controls. Paraffin embedded samples of RCC or oncocytoma were studied with immunohistochemistry using specific polyclonal antibodies of NFκB. Morphometry was used to determine intensity and localisation. Results were assessed with Spearman correlation and Pearson chi-square tests.
Results: There were 65% males in our study. Median age for males was 61.5 (range 83-39) and for females was 60.1 (range 79-39). All patients had localised disease at time of nephrectomy, however 20 patients (6 males) later developed disease progression (metastases). Expression of four NFκB isoforms (p50, p52, p65, c-rel) was higher in cancer specimens compared with control kidney ($p < 0.05$). RelB had negligible expression in normal kidney and kidney cancers. Tumour grade and stage may directly correlate with p65 expression intensity.
Conclusions: NFκB is believed to contribute to RCC development in its role as transcription factor for cancer-promoting molecules. Although only the p65 subunit appears to correlate with tumour

grade and stage, more studies are needed to define the relationship with NFκB subunits and, eventually, metastases and survival rates.

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Day 3: Morning session: Biomarkers

Implementation of personalized medicine into clinical pathology practice

Dr Gaynae Badalian-Very, Dana Farber Cancer Institute, Harvard Medical School, USA

Personalized medicine (PM) is the cornerstone of medical practice. This approach tailors treatments for specific conditions of the affected individual. The limitations of personalized medicine are defined by available technology and our understanding of the biology, physiology, and pathology of various conditions.

Scientists and physicians involved in PM intend to bring this individualized treatment to every corner of medical practice, but the applicability may be limited. Although recent achievements seem promising, we are still far away from applying PM to large populations. Limitations in science and technology, unsustainable cost of some approaches, and constrained budgets of health care providers have narrowed the distribution to a larger patient population. An integrated and revised approach in existing infrastructures is an absolute necessity to convert this potential to reality.

Companion diagnostic tests for targeted cancer treatment: advances and challenges

Professor Yao-Shan Fan, University of Miami Miller School of Medicine, Florida, USA

The new therapies targeting the biological signalling pathways represent a major progress in cancer treatment. Companion diagnostic testing has become a vital component of the daily pathology/oncology practice for personalized cancer treatment. This presentation provides 1) an overview on companion diagnostic tests on biomarkers including EGFR, KRAS, BRAF, PIK3CA, ALK, HER2 for common human cancers; 2) an update on practice guidelines on breast and lung cancers, 3) discussions on FDA approval of IVD and its impact on laboratory practice and 4) application of next generation sequencing as a clinical test for cancer treatment.

Diagnosing vitamin B12 deficiency using static and functional markers

Dr Agata Sobczyńska - Malefora, Viapath, St. Thomas' Hospital, London, UK

Vitamin B12 (cobalamin) is present only in foods of animal origin. Impaired vitamin B12 status, which may lead to clinical deficiency, is common in patient populations, especially in those older than 60 years and vegetarians/vegans. The timely detection and correction of vitamin B12 deficiency prevents anaemia, elevated homocysteine (possible thrombotic risk factor), potentially irreversible peripheral neuropathy, memory loss and other cognitive deficits. However, the prompt diagnosis of impaired vitamin B12 status has long been recognised as problematic. The current convention is to estimate the abundance of vitamin B12 using total serum vitamin B12, test which has a low sensitivity. Emerging evidence indicates that holotranscobalamin, the active fraction of B12, is a more reliable marker of impaired vitamin B12 status. Metabolic markers of status include homocysteine and methylmalonic acid.

Screening Serum Biomarkers on Cancer Patients: Using Mass Spectrometry to Answer Clinical Questions

Professor Aline M.A. Martins, Translational Medicine
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University Hospital - HUWC/UFC / Surgery Department Prof. Costa Mendes, 1608 - 3° Andar - Fortaleza – CE, Brazil

Serum markers are the ultimate frontier in clinical biomarker research, while proteomics tools are the perfect match technology in this cutting-edge area. In the field of Translational Medicine, noninvasive tools in the search for molecular targets, to monitor diseases and patient survival, remains a crucial challenge. Mechanistic insights about cancer biology progress and early changes in cell signal transduction are the key elements for successful management of worldwide burden pathologies. Nonetheless, organ biopsy is the gold standard for diagnosis cancer progression, a

major effort is been made to identify promising markers in body fluids, specially serum, as it represents a less aggressive procedure for searching and monitoring molecular signatures of tumor cells impair function.

Afternoon Session Histopathology and Cytopathology: Advances in research and techniques

Invited Speakers Abstracts

Pediatric Liver Tumors. Pearls and Pitfalls

Professor Consolato Sergi, Professor of Pathology and Adj. Professor of Pediatrics, University of Alberta, Canada

Hepatic tumors account for only about 1/20 of all intra-abdominal masses in children and primary hepatic neoplasms are only 0.5-2% of all pediatric malignancies. However, primary hepatic neoplasms are the third most common abdominal malignancy in childhood, after Wilms' tumor and neuroblastoma. Histologic patterns and classification concepts may be a challenge for the pathologist. The majority of liver tumors in children are, indeed, malignant, but 1/3 of the liver masses are benign and need to be carefully ruled out. Consequently, a differential diagnosis is paramount. Pearls and pitfalls would be of benefit for both pathologist in training and experienced pathologist.

Histopathologic diagnosis in the era of new and old yeast infections in critical ill surgical patients

Assistant Professor Paola Di Carlo, Department of Sciences for Health Promotion and Mother-Child Care, University of Palermo, Italy

Several antifungals are currently available for management of invasive fungal infections (IFI), but this exacerbating infectious disease still presents difficulties, especially in the case of opportunistic infection occurring in individuals with seriously impaired defense mechanisms or in multimorbid patients. Patients who undergo complex surgical procedures with or without immunosuppressive therapy are at increased risk for invasive fungal infections.

Supplemental procedures for diagnosis, such as indirect monitoring of pathophysiology of the infection, have become a more important and practical way for management of the disease. In this regard, histopathologic and/or cytopathologic examination can also provide insight into the diagnostic significance of some isolates such as have been shown some surgical and medical cases. Finally, the morphologic features of fungi difficult to Identify in infected tissue biopsy is of fundamental importance as it can show distinctive hyphae characteristics.

Additional training in mycology was requested by laboratories that examine clinical samples.

A New Age of Breast Cancer Diagnostics: Prognosis and Treatment Implications of Simultaneous Testing of Tumors and Cancer Stem Cells

Dr SuEllen Pommier, Associate Professor, Oregon Health & Science University, USA

Benign (SC) and malignant cancer stem/progenitor cells (BCSC) can be identified and collected by fluorescence-activated cell sorting (FACS) from fresh surgical specimens. The rarity of these cells in breast tumors hampers their detection during routine clinical pathological examination. However, we have shown that the estrogen receptor (ER) expression in cancer stem cells is negligible and differs from tumor of origin. We have also shown that tumors containing stem cell PI3K/Akt signaling mutations were significantly more likely to manifest nodal metastases. Genetic abnormalities are present in residual BSCS after neo-adjuvant chemotherapy and may be present in the blood.

In this talk, I will discuss how benign and malignant cancer stem/progenitor cells are identified and collected by fluorescence-activated cell sorting (FACS) from fresh surgical specimens. The rarity of these cells in breast tumors hampers their detection during routine clinical pathological examination, and thus they are not included in a final assessment. The hormonal and molecular statuses of the overall tumors dictate breast cancer treatment. However, the results of our studies demonstrate that malignant cancer stem/progenitor cells can differ in their mutations, gene expression and hormonal status from that of the tumor overall. These findings suggest that breast cancer stem/progenitor cells (BCSC) may be under treated and may be responsible for residual disease and recurrence. Through protein ligation assays and quantitative PCR, we have shown that the estrogen receptor (ER) expression in cancer stem cells is negligible, even in tumors that carry a

pathologic positive ER status. Similarly, genetic analysis showed that the majority of cancer stem cells and their tumors of origin carry discrepant oncogene abnormalities. We have also shown that tumors containing stem cell PI3K/Akt signaling mutations were significantly more likely to manifest nodal metastases. Recent work demonstrates that the frequency of specific cancer stem/progenitor cell sub-types found in ductal carcinoma differs by patient age at diagnosis.
Our most recent studies are evaluating the changes in cancer stem cell frequencies and associated genetic abnormalities that are present in residual disease after neo-adjuvant chemotherapy. Also, within this cohort of patients, we are measuring circulating cancer stem/progenitor cells in the blood. We have demonstrated that BCSC are present in the blood of certain patient populations and are capable of initiating new tumors in vitro. Taken together, we suggest that a comprehensive approach to tumor testing that includes cancer stem cell diagnostics will provide improved prognostic information and new directions for systemic and targeted therapies.

Role of cytopathology in steering targeted therapies in lung oncology patients

Dr Harman Sekhon, University of Ottawa, Ontario, Canada

Despite overall notable improvements in cancer therapeutics, worldwide lung cancer remains as the leading cause of cancer-related mortality. Although surgery is considered as the most effective method of treatment, only 30-40% of the cases present with resectable tumours. New molecular targets are being discovered and new generation of drugs are being formulated. However, optimal tissue acquisition and specific diagnosis is necessary to direct appropriate targeted therapy. The challenges are to met and solutions are to be designed to obtain proper diagnosis and molecular profile using minimally invasive techniques. The cytopathology is the trend that needs to be refined and optimized to address the future issues.

Talk to be confirmed

Mr Barry Lynch, Advanced Cell Diagnostics, Inc

Using array tomography to study synapse degeneration in human brain.

Dr Tara L Spires-Jones, Reader and Chancellor's Fellow, The University of Edinburgh, Scotland

Synapses, the connections between neurons in the brain, are key to learning and memory, and the degeneration of synapses is a driving factor in neurodegenerative diseases such as Alzheimer's disease. Postmortem studies of synapses in human brain are difficult due to the axial resolution limit of light microscopy. We have adapted a new technique called array tomography for use in human postmortem brain tissue. Array tomography overcomes the axial resolution problem by embedding tissue in resin and cutting ribbons of ultrathin serial sections. These sections can be stained and imaged with standard immunofluorescence techniques. After imaging, antibodies can be stripped off and the sections re-probed for other proteins. This allows imaging of multiple markers at tens of thousands of synapses. Using this technique, we have found that amyloid beta and apolipoprotein E are present at synapses in Alzheimer's disease brain and further that apolipoprotein E4, a genetic risk factor for the disease, is associated with increased toxic amyloid beta at synapses. Understanding the molecular changes at synapses will be important to develop treatments for neurodegenerative diseases and more broadly could be important to treating psychiatric and developmental brain disorders.

Oral Presentation Abstracts

APPLICATION OF DIGITAL CYTOLOGY FOR GYNECOLOGICAL CYTOLOGY - GEORGIAN EXPERIENCE

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Objective: The study aimed evaluation of the effectiveness of digital cytology for gynecological cytology practice and compare it with routine cytology diagnostic under the conditions of Georgia.
Materials and Methods: Gynecological cytology cases (n=550) were taken from the clinical laboratory. Cases were diagnosed routinely by one of four certified cytologists who provided cytology diagnoses. Digital images were obtained on all cases and were evaluated as computer images by a panel of cytologists.

Results: There was 96% concordance in average between routine versus digital images diagnostic. Intracytologists concordance averaged 97.5%. Image sharpness and quality were rated "good" and "excellent" in 97% cases. With respect to image color, 96% of the images were rated as "excellent" or "good".
Conclusion: Digital cytology for gynecological cytology diagnostic produces images of adequate quality and diagnostic concordance rates.

TANYCYTIC EPENDYMOMA: PRESENTATION OF A RARE CYSTIC DISEASE VARIANT AND REVIEW OF LITERATURE

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Background: Tanycytic ependymoma is a rare variant of ependymoma and guidelines for the treatment of patients with this disease are yet not established.
Objective: To conduct a systematic review of all reported cases from the literature to survey management options for patients with tanycytic ependymoma.
Methods: We performed a comprehensive Pubmed- and hand literature search to identify reported patients with pathologically confirmed tanycytic ependymoma. Signs and symptoms, radiological and specific pathological findings as well as treatment modalities and outcomes were recorded.
Results: 41 studies involving a total of 60 patients were included in this review. Most of the tanycytic ependymomas occurred in the spinal cord (45%), followed by lesions located in upper intracranial sites (41,7%) and only a few at the cervicomedullary junction (3,3%). There was no clear gender predelection (female to male ratio 1:1,3), and mean age at diagnosis was 34 ± 17 years. Defined follow-up periods for patients with tcE were only documented in 34/60 cases (56,7%) with a mean follow-up of 22,2 months. 28 reported cases (46,7%) had no recurrence of tumor after treatment over a mean follow-up of 25,6 months. Complete resection of the tumor without further additional therapy was the treatment of choice in most cases (30%) adjuvant postoperative radiotherapy was applied in 7 cases (11,7%) In 9 reported cases of tcE (15%), the treatment was not documented. One case of this rare disease is illustrated in detail by us.
Conclusion: This review on tanycytic ependymomas supports surgery as the initial treatment of choice for this rare tumor. Radiotherapy was taken into consideration when total gross resection could not be achieved and yielded prolonged progression-free survival. Given the benign nature of this subtype of ependymoma, aggressive treatment modalities such as chemotherapy are usually not indicated.

Poster Presentation Abstracts

TUMOR BUDDING: A PROMISING HISTOPATHOLOGIC PROGNOSTICATOR FOR ESOPHAGEAL CANCER

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Background: The presence of single cancer cell or small cluster (<5 cells) at the tumor invasive front (IF) has been defined as tumor budding (TB) and it showed a strong prognostic ability in many cancers including head and neck cancer, colorectal cancer, breast cancer and lung cancer. Here, we meta-analyze the studies evaluated TB in esophageal cancer aiming to summarize their findings.
Methods: We systematically reviewed the literature using the following databases: Scopus, PubMed, and Web of Science. The searching was limited to publication in English language. To calculate the hazard ratio (HR) and 95% confidence interval (CI), we conducted statistical meta-analysis.
Results: The total of 127 articles was retrieved. Only 11 articles have studied TB in esophageal cancer (esophageal squamous cell carcinoma and adenocarcinoma). In the meta-analysis, TB turned

out to be a significant prognosticator for esophageal cancer in both overall survival with hazard ratio (HR) of 2.4 (95 % CI: 1.7-3.4; P=0.0008), and for disease-free survival with HR of 3.2 (95 % CI: 2.1-4.8; P=0.002).
Conclusion: TB reveals the biological behavior of esophageal cancer and it is a promising prognosticator for survival. TB should be evaluated and involved in the routine pathologic report of esophageal cancer.

THE USE OF COMPUTER ASSISTED MEASUREMENTS FOR CELL COUNTING: COMPARISON OF METHODS, SPEED AND ACCURACY.

Williams MR & Acton P

One of the most persistent limiting factors in histological analysis is the time taken for researchers to make the appropriate measures. Whilst automatic cell counting has become viable in fields such as flow cytometry and fluorescent staining, computerized counting of histological- and immunochemical-stained cells has made limited progress. The rate-limiting factor in these studies is often the requirement of a trained operator to manually tag and analyse images.

We have addressed this limitation through a different route. Image analysis often requires additional factors such as use of grids or fixed regions, randomization of counting zones and magnification, and current software is built to give this functionality but only in manual form. Our in-house software, Segmentum™, has ben developed to automate these additional processes whilst leaving the actual cell counting and analysis up to the operator.

This presentation demonstrates the significant improvements in speed of measurement using this software with no increase in measurement error, making this type of research easier for experts in the field and practical for other pathological researchers under intense time pressure.

p16/INK4a METHYLATION AND PROTEIN EXPRESSION IN MENINGIOMAS GRADE I (NON RECURRENT VERSUS RECURRENT). ALTERATIONS OF THE RETINOBLASTOMA TUMOUR SUPPRESSOR PATHWAY

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AIMS:
Methylation inactivates tumor suppressor genes (TSG). The p16INKa cell cycle regulatory protein, control the Rb TSG pathway.
Meningiomas Grade I, recur after resection in 5% of cases at 5 years and 19% in long-term.
To get insights into the possible role of Rb TSG alterations in grade I meningiomas, we studied p16INK4a methylation & protein expression in 140 specimens: 29 non recurrent & 57 recurrent in one, two or three times.
Methods:
Methylation PCR by bisulfate modification, followed by sequencing of CpG islands & p16INKa immunostaining.
RESULTS:
p16INK4a was methylated in 37.9% of non-recurrent meningiomas, and 38.8% of the first biopsy of recurrent cases, increasing to 52.3 % in successive recurrences.
p16INK4a methylation & protein loss happen in 54.7% of cases. p16INK4a loss with unmethylated promoters occur in 52.9% of cases.
CONCLUSIONS:
Alterations of the Rb TSG pathway as shown by p16INK4a methylation & protein loss of expression, may have a pathogenic role in meningiomas.
Loss of p16/INKA protein expression associated with unmethylated promoter could be due to loss of heterozigosity or point mutation.
p16INK4a methylation increase along recurrences, suggests a possible role in tumor progression.

p14/ARF METHYLATION AND PROTEIN EXPRESSION IN MENINGIOMAS GRADE I (NON RECURRENT VERSUS RECURRENT). ALTERATIONS OF THE p53 TUMOUR SUPPRESSOR PATHWAY

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AIMS:
Methylation inactivates tumor suppressor genes (TSG). The p14/ARF cell cycle regulatory protein, control the p53 TSG pathway.
Meningiomas Grade I, recur after resection in 5%, of the cases at 5 years and 19% in long-term.
To get insights into the possible role of p53 alterations in grade I meningiomas, we studied p14/ARF methylation & protein expression in 140 specimens: 29 non recurrent & 57 recurrent in one, two or three times.

METHODS:
Methylation PCR by bisulfate modification, followed by sequencing of CpG islands & p14/ARF immunostaining.

RESULTS:
p14/ARF was methylated in 13.8% of non-recurrent meningiomas and 9.6% of the first biopsy of recurrent cases, increasing to 19.6 % in successive recurrences.
p14/ARF methylation & protein loss was observed in 18.8% of cases. p14/ARF loss with unmethylated promoters occur in 17.6% of cases.

CONCLUSIONS:
Alterations of the p53 TSG pathway as shown by p14/ARF methylation & protein loss of expression, may have a pathogenic role in meningiomas.
Loss of p14/ARF protein expression associated with unmethylated promoter could be due to loss of heterozigosity or point mutation.
p14/ARF methylation increase along recurrences, suggests a possible role in tumor progression.

SALIVARY GLANDS OF FEMALE TICKS *RHIPICEPHALUS SANGUINEUS* LIKE A POTENTIAL SOURCE OF MOLECULES WITH INHIBITORY ACTION: *IN VIVO* STUDY WITH WALKER 256 TUMOR CELLS

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This study evaluated the effects of one or two injection of different concentrations (0.2 and 0.04 µg/µL) of extracts obtained from the salivary glands of female ticks *Rhipicephalus sanguineus* (Acari: Ixodidae) (Latreille, 1806) also called "brown dog tick" fed for 2 days on the rabbit hosts on the morpho-physiology of the leg musculature of female Wistar rats inoculated with Walker 256 tumor cells. The number of leukocytes and the creatinine levels were quantified for individuals from all groups. The results of both histology and electron microscopy revealed that a single, low-concentration injection of the extract (0.04 µg/µL) was more effective in containing tumor invasion and caused less 'collateral damage' to the muscle tissue, which was the object of this study. The results also revealed that creatinine levels were higher in rats subjected to both one and two injections of the extract at a concentration of 0.04 µg/µL than in those subjected to one and two injections of the extract at the higher concentration (0.2 µg/µL), suggesting that in the first group, injection of the extract contributed to maintaining the integrity of the muscle tissue. With regard to the number of leukocytes, the results suggested that in all the inoculated rats (Walker 256 cells), there was a significant increase in the total number of leukocytes. The inoculated rats that received both one and two injections of the extract at a concentration of 0.2 µg/µL experienced a significant increase in the number of leukocytes compared with those inoculated but not exposed to the extract; this result can be explained by the fact that beyond the tumor cells, the extract itself acted to boost the defense response at this concentration. However, inoculated rats subjected to

injections (one and two) of the extract at a concentration of 0.04 µg/µL showed a significant decrease in the total number of leukocytes compared with rats that were only inoculated and those inoculated and injected with the extract at a concentration of 0.2 µg/µL. These results reinforced that the extract at the 0.04-µg/µL concentrations not only acted more effectively to inhibit Walker 256 tumor cells but also did not act as a stressor, because the number of leukocytes was lower. Therefore, the data obtained here indicated that the same molecules or a pool of molecules produced by the salivary glands of ticks belonging to this species have the ability to inhibit tumor growth, while minimizing 'collateral damage' to the body.

Keywords: Ticks, salivary gland extracts, Wistar rats, muscle, tumor inhibition

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