This meeting draws together international experts to discuss current techniques and research involved in cellular and molecular pathology.

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](#)

This abstract book will be finalised two weeks before the event [www.regonline.co.uk/path2015](http://www.regonline.co.uk/path2015)

#Path2015
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**Invited Speakers Abstracts**

**The challenge of early cancer detection**  
*Professor Ian A Cree, Yvonne Carter Professor of Pathology, Warwick Medical School, Clinical Sciences Building, University Hospitals Coventry and Warwickshire, London, UK*

Early cancer detection results in improved outcomes and can reduce the cost of treatment of each patient. However, screening can be expensive, and the dangers of over-investigation and over-treatment are well known. Screening programmes are only viable for a few common cancer types, yet those with rare cancers often present late and have poor outcomes. Each of the hallmarks of cancer is accompanied by biomarkers that can be measured in blood, so it is theoretically possible to detect cancer from a single blood sample by measuring multiple biomarkers. The development of an inexpensive, blood-based, generic cancer screening strategy capable of detecting a range of cancer types would be game-changing. Our concept is that initial testing using biomarkers with high sensitivity could be followed by reflex testing of the same sample with more specific markers within a defined algorithm.

**Dematitis herpetiformis is, first and foremost, a cutaneous disease**  
*Professor Marian Dmochowski Poznan University of Medical Sciences, Poznan, Poland*

There still is a tendency to understand dermatitis herpetiformis (DH), where autoimmunity intertwines with inflammation most likely on multiple cutaneous pathways, in the context of celiac disease (CD). Key players in DH skin seem to be activated neutrophils (producing inflammation/even autoinflammation) and epidermal transglutaminase moving, as a result of yet unknown triggers operating, from cornified envelope to the dermal-epidermal junction area (responsible for predominantly IgA1-mediated autoimmunity). Lumping DH and CD together may be misleading and research efforts should focus on exploring features that are as unique to DH as possible instead of those shared by both DH and CD.

**The aneuploidy paradox in malignant tumours: let us consider parasexual processes**  
*Dr.h.m Jekaterina Erenpreisa, Latvian Biomedical Research & Study Centre, Riga, Latvia*

Aneuploidy in cancer is usually considered both as a consequence and cause of the genome instability, stochastically leading to re-replication followed by gain and loss of chromosomes during mitotic aberrant divisions. This concept does not explain the aneuploidy paradox, a contradiction between addiction of malignant proliferating tumours to aneu-polyploidy, which is anti-proliferative by its essence. The data will be analysed viewing cancer as an evolutionary pre-programmed event, which can use several parasexual processes providing reset of immortality in a life-cycle-like process. It deals with segregations and fusions of the whole genomes, while losses and gains of individual chromosomes represent only secondary, partially counterbalanced events.

**IKK alpha is a novel target for ER positive breast cancer**  
*Dr Joanne Edwards, BSc, PhD, FRCPath, Senior Lecturer, Wolfson Wohl Cancer Research Centre, Institute of Cancer Sciences, University of Glasgow, Garscube Estate, Glasgow, Scotland, UK*

The NF-κB pathways regulate transcription of genes involved in various processes, many of which are hallmarks of cancer. We have observed that high IKKα expression was associated with poorer disease-free survival in patients with ER positive tumours. In an independent ER positive cohort, it was also associated with cancer specific survival and quicker time to recurrence on tamoxifen. Silencing IKKα impacted on cell viability and apoptosis in ER positive MCF7 cells but not in ER negative MDA-MB-231 cells. This suggests the NF-κB pathways are involved in the progression of ER positive breast cancer and indicate IKKα is a potential target.

**Cardiovascular pathology from tobacco smoke**  
*Dr Aurelio Leone, Independent Researcher, Castelnuovo Magra, Italy*

Evidence indicates that tobacco smoke toxics, primarily carbon monoxide and nicotine, play a strong and adverse effect on heart and blood vessels. Several types of damage of these structures, which may be considered as target organs of smoking, have been described and well documented. Initially, a functional, but transient alteration related to a reduced tolerance to exercise and endothelial dysfunction with increased heart rate and systolic blood pressure may be seen. On the contrary, chronic and prolonged exposure to smoking causes an irreversible damage characterised by atherosclerotic alterations mainly affecting coronary, cerebrovascular and carotid arteries as well as ischemic alterations of the heart muscle. A specific pattern of experimental pathology from smoking described in the animals is the smoke cardiomyopathy, which, usually, develops heart failure as an effect of intracellular alterations of the enzymatic respiratory chains.
Imaging in cancer immunology: Phenotyping of multiple immune cell subsets in-situ in FFPE tissue sections
Ms Roslyn Lloyd, Quantitative Pathology Applications, PerkinElmer, USA
Examples of this new methodology will be presented: the simultaneous labelling, analysis and validation of CD4, CD8, CD20, PD-L1, Foxp3, cytokeratin and DAPI in breast cancer; and CD8, CD34, PD-L1, FOXP3 and DAPI in head and neck squamous cell carcinoma. Each example will show the application of the multiplexed staining, per-cell quantitation and cellular phenotyping from multispectral images of FFPE tissue sections, as well as methods to explore the spatial distributions of the phenotyped cells in and around the tumour.

Functional role of microRNAs in alcoholic liver diseases
Dr Fanyin Meng, Assistant Professor, Digestive Disease Research Center, Scott & White Hospital, Texas A&M HSC College of Medicine, Texas, US
The function of microRNAs (miRNAs) during alcoholic liver disease (ALD) has recently become of great interest in biological research. ALD associated miRNAs play a crucial role in the regulation of liver-inflammation and fibrosis agents. Certain miRNAs show a positive correlation in up-regulation of liver fibrosis signaling when they are exposed to ethanol and LPS. miR-34a and miR-21 are the critical mediators in ethanol/LPS activated survival signaling during ALD. In this talk, I will present our recent findings regarding the experimental and clinical aspects of functions of specific miRNAs, focusing mainly on inflammation, cell survival liver fibrosis in human alcoholic disorders.

Diagnostic dilemmas in lupus anticoagulant detection
Dr Gary Moore, Consultant Biomedical Scientist, Head of Diagnostic Haemostasis & Thrombosis Laboratory, London, UK
Lupus anticoagulants (LA) belong to the heterogeneous group of autoantibodies termed antiphospholipid antibodies (APA). Antiphospholipid syndrome is diagnosed when APA persistence accompanies arterial or venous thrombosis, or pregnancy morbidity. Paradoxically, LA are associated with thrombosis in vivo but anticoagulation in vitro, arising from their ability to inhibit phospholipid-dependent coagulation assays. LA detection is based on inference by performing screening tests to detect clotting time prolongation, mixing tests to evidence inhibition, and confirmatory tests for phospholipid-dependence. Diagnostic dilemmas arise from issues such as choice and number of screening tests, antibody dilution in mixing tests, data manipulation and interpretation, and interfering factors.

An actionable molecular test as cost-effective risk predictor of oral cancer progression
Dr Catherine F. Poh, University of British Columbia, BC Cancer Agency, Canada
Early detection of at-risk oral lesions followed by effective intervention is the key to control oral cancer. Watchful waiting is no longer cost-effective for the management of oral premalignant lesions. Loss of heterozygosity (LOH) at specific chromosomal loci has shown to be a validated risk indicator for cancer progression. However, the technique used is radio-isotope-based electrophoresis with costly, labor-intensive, and time barriers. We developed a comparable lab-friendly actionable test with advantages in cost, time, and objectivity. Using health state transition modelling, this proposed test provides a cost-effective strategy to triage patients into risk groups with different management regime in Canada.

Endothelial Dysfunction in Diabetic Heart; from Phenotypic Changes to Associated Molecules
Dr Lucia-Doina Popov, Ph.D., biochemist. Head of Pathophysiology and Pharmacology Department of « N. Simionescu » Institute of Cellular Biology and Pathology of the Romanian Academy
In diabetic myocardial capillaries, the quiescent phenotype of endothelial cells (ECs) is converted to new dysfunctional phenotypes. This talk will discuss endothelial dysfunction (ED), under three perspectives: (i) fine structure changes, (ii) molecules involved, and (iii) potential alleviation. Electron microscopy data assess ECs activation, and phenotype-specific cellular modifications. The ECs proliferative phenotype frequently displays centrioles presence; associated molecules are activated MAPK, VEGFR1/R2, inflammation and mTOR pathways, and augmented expression of NADPH oxidases; this phenotype can be regulated by exploitation of the anti-VEGF-VEGFR system, by mTOR inhibitors, and by miRNA-320,-503 and 221/22. The ECs biosynthetic phenotype shows abundant rER indicative for synthesis of extracellular matrix components, endothelin, and NO among others; modulation of this phenotype may be achieved by L-arginine, BH4, and eNOS transcription enhancers AVE9488 and AVE3085 or statins. Beyond ECs vasoconstrictor phenotype stays the biosynthesis of PGH2, TXA2, ROS, angiotensin II, ET-1, and activation of PKC; this phenotype may be mitigated by Alamandine, and inhibition of dipeptidyl peptidase 4. The molecules engaged in the ECs inflammatory phenotype are cytokines, adhesion molecules, chemokines, transcription factors, and endocan; diet supplementation by Mn2+, and miRNA-126 are among the modulators of this phenotype. The ECs pro-thrombotic phenotype exposes a luminal surface adhesive to circulating cells associated with generation of PAI-1, thromboxane and vWF; the anti-thrombotic strategies consist in anti-platelet drugs, MoAb targeting platelet GPIbβ, and in the switch of integrin signaling
Although it is found in the mtDNA databases. MitoChip analysis is a quantitative assessment of myofiber compositions could be a powerful tool with ranges observed in the CCA cell lines, 28 were non-agressive high demand, during aging or diseases. Quantitative assessment of myofiber composition, often recognized by myosin heavy chain (MyHC) gene expression. Four major myofiber contractile composition, which can be categorized based on metabolic and function/dysfunction is still an open issue.

**Patient-derived Xenografts Reveal that Intraductal Carcinoma of the Prostate Is a Prominent Pathology in BRCA2 Mutation Carriers with Prostate Cancer and Correlates with Poor Prognosis**

Professor Gail Risbridger, Research Director, Monash Partners Comprehensive Cancer Consortium (MpCCC), Monash University, Victoria, Australia

**Background:** Intraductal carcinoma of the prostate (IDC-P) is a distinct clinicopatho-logic entity associated with aggressive prostate cancer (PCa). PCa patients carrying a (BRCA2) germline mutation exhibit highly aggressive tumours with poor prognosis. Objective: To investigate the presence and implications of IDC-P in men with a strong family history of PCa who carry a BRCA2 pathogenic mutation. Design: Patient-derived xenografts (PDXs) were generated from BRCA2 mutation carriers and showed increased incidence of IDC-P (42%, n = 33, p = 0.004) vs sporadic PCa cases (9%, n = 32). WG-CNA confirmed that the genetic profile of IDC-P from a matched (primary and PDX). BRCA2 carriers and BRCAX patients with IDC-P had significantly worse prognosis than BRCA2 carriers and BRCAX patients without IDC-P (HR: 16.9, p = 0.0064 and HR: 3.57, p = 0.0086). Conclusions: PDX’s revealed IDC-P in patients with a germline BRCA2 mutation or BRCAX classification, identifying aggressive tumours with poor survival even when the stage and grade of cancer at diagnosis was similar. Further studies are warranted to confirm its prognostic significance in sporadic PCa cases.

**Diffuse axonal injuries**

Professor Mårten Risling, Karolinska institutet, Department of Neuroscience, Stockholm, Sweden

Traumatic brain injuries (TBI) can be focal and or diffuse. Acceleration TBI can result in injuries that range from mild to severe. Diffuse axonal injury in the white matter is (DAI) an important component in the lesions from acceleration induced TBI. In histological sections DAI can be detected by immunohistochemistry for the amyloid precursor protein (APP). Serum biomarkers and DTI can assist the diagnosis in TBI patients. The presentation will describe how experimental TBI models can be used to define injury criteria and define biomarkers for DAI.

**Tissue Pathology and Pathways in Chronic Human Lymphatic Insufficiency**

Dr Stanley G. Rockson, Stanford University School of Medicine, Stanford, CA, USA

Lymphedema is a morbid disease characterized by chronic limb swelling and adipose deposition. Although it is clear that lymphatic injury is necessary for this pathology, the mechanisms that underlie lymphedema remain largely unknown. Our recent work in both human chronic lymphedema and in murine models of the human disease has begun to explicate the inflammatory substrate of the disease. These insights are beginning to suggest routes to the discovery of biomarkers for the disease. Pathogenetic insights will increasingly lead to development of effective pharmacological interventions.

**High-throughput myofiber histology in disease and aging conditions**

Dr Vered Raz, Department of Human Genetics, Leiden University Medical Centre, Leiden, Netherlands

Muscle function is in part dictated by myofiber composition, which can be categorized based on metabolic and contractile composition, often recognized by myosin heavy chain (MyHC) gene expression. Four major myofiber types have been described in adult muscles and a switch in MyHC composition is induced by changes in physical demand, during aging or diseases. Quantitative assessment of myofiber compositions could be a powerful tool to describe changes in muscle function in diseases, aging and exercise conditions. Moreover, the evaluation of therapeutical treatments to delay muscle weakness would be more accurate. High-throughput fluorescent-based histology procedure will be presented.

**Mitochondriome and Cholangiocellular Carcinoma**

Professor Consolato Maria Sergi, Department of Lab. Medicine and Pathology, University of Alberta, Canada

Somatic mitochondrial DNA (mtDNA) mutations have been found in several cancers. Some of these malignancies contain changes of mtDNA, which are not or, very rarely, found in the mtDNA databases. MitoChip analysis is a strong tool for investigations in experimental oncology and was carried out on three cholangiocellular carcinoma (CCA) cell lines (HuCCT1, Huh-28 and OZ) with different outcome in human and a Papova-immortalized normal hepatocyte cell line (THLE-3). Real time quantitative PCR, western blot analysis, transmission electron microscopy, confocal laser microscopy, and metabolic assays including L-Lactate and NAD+/NADH assays were used. Among 102 mtDNA changes observed in the CCA cell lines, 28 were non-synonymous coding region alterations resulting in an amino acid change. Thirty-eight were synonymous and 30 involved ribosomal RNA (rRNA) and transfer RNA (tRNA) regions. We found three new heteroplasmic mutations in two CCA cell lines (HuCCT1 and Huh-28). Interestingly, mtDNA copy number was decreased in all three CCA cell lines, while complexes I and III were decreased with depolarization of mitochondria. L-Lactate and NAD+/NADH assays were increased in all three CCA cell lines. MtDNA alterations seem to be a common event in...
CCA. This is the first study using MitoChip analysis with comprehensive metabolic studies in CCA cell lines potentially creating a platform for future studies on the interactions between normal and neoplastic cells.

**New Decalcification Methods Applied to Bone and Teeth Tissues using Nanomaterials**  
Dr. Gerardo Rivera Silva, University of Monterrey, NL, Mexico  
Decalcification of teeth and bone is a crucial phase during histological processing of these tissues. The effect of decalcifying substances on the tissue and its staining characteristics are significant considerations, which impact the selection of decalcifying agents. The purpose of this study was of modifying the Brown-Breen stain and developing a new method to decalcify bone and teeth tissues in a period of 5-10 days using nanomaterials.

**Day 1:**

**Oral Presentation Abstracts**  
Oral presentations will be added after the submission deadline

**APPLICATION OF CISH FOR HPV GENOTYPING**  
Kldiaivili E.  
75 Kostava str., 0171 Tbilisi, Georgia  
Objective: The study aimed evaluation of the effectiveness of CISH for HPV genotyping on cervical smears and compare the results of genotyping with cytopathology findings of routine screening.  
Materials and Methods: Gynecological cytology cases (n=1000) were taken from the clinical laboratory. Cases were diagnosed routinely by application of LBC method. Patients with diagnosed atypia were recalled for obtaining of material for HPV genotyping. This has been performed by usage of CISH methodology.  
Results: There has been revealed 92% concordance in average between genotyping results and cytopathology findings of routine screening.  
Conclusion: CISH is effective and easy for implementation method for HPV genotyping on cervical smears.

**HOW MUCH RESEARCH DO UK ROUTINE BIOMEDICAL LABORATORIES SUPPORT ( SURVEY REPORT )**  
A survey was carried out to see how much Research UK Biomedical Laboratories support.  
It is fair to say that every NHS Trust and most sizeable Private Hospitals will have an associated Pathology laboratory performing diagnostic healthcare testing. They will all on occasion have a role in supporting Clinical or other Research Studies. This can range from one Doctor or Scientist’s personal interest through to assistance with Large Formal Clinical Trials.  
This is understood but a number of questions might arise. How much actually goes on? How much is it a benefit or burden to the routine lab? And what sort of additional Quality Management arrangements should be put in place?  
The Internet and other investigations yielded little information so a 23 question survey was drawn up. In this presentation I will aim to describe the methodology and results of a comprehensive UK wide

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Tel: 0151 430 2180
Day 2:

Oral Presentation Abstracts

PATHOLOGICAL STUDIES IN GOATS FED ACACIA SALIGNA RAISED IN THE EGYPTIAN DESERT

Soad M. Nasr1*, Mohamed I. Dessouky2, Fathy A. Ibrahim3, Adel Bakeer4 and Hamdy Soufy1
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Abstract

Many efforts have been made to assess the nutritional value of the natural vegetation resources such as Acacia saligna which remain even green during all the year, tolerate salinity and drought seasons. The present experiment was conducted to study the pathological changes of internal organs (liver, kidney, heart, spleen, rumen, reticulum, abomasum, small intestine, adrenal gland and lung) of goats fed Acacia saligna (as forage) for 50 weeks. Twenty adult dry Barki goats randomly divided into two equal groups. The first group (control) was fed on Berseem hay ad. Libitum. The second group fed on Acacia for 50 weeks. All groups were offered barley (100% of their energy maintenance requirements). Tissue specimens (liver, kidney, heart, spleen, rumen, reticulum, abomasum, small intestine, adrenal gland and lung), were obtained from slaughtered control and experimental animals at the 26th and 50th weeks of treatment for histopathological investigations. Phytochemical screening of Acacia revealed presence of tannins, alkaloids, flavonoids and saponins in different amounts. No mortality occurred during the experimental period. Post mortum findings revealed congestion was noticed in the hepatic tissue as well as in the corticomedullary junction of kidneys after 26 weeks of feeding Acacia. Twenty-four weeks later, the hepatic tissue was atrophied and had shrinkage capsule. The spleen was smaller in size and pale anaemic in colour. Erosions with hyperemia were noticed in the abomasal mucosa. The intestinal wall was oedematous and congested. Histopathological observations recorded that after 26 weeks, cytomegalic hepatocytes with mild portal inflammatory reaction were noticed. Periglomerular inflammatory reaction and mild myocarditis were noticed with hyalinized stromal splenic tissue. There was focal abomasitis, ruminitis, reticulitis and catarrhal enteritis. Fifty weeks later, hepatic and renal hemorrhages were observed. There was mild hyalinization in stromal tissue of spleen with mild myocarditis. Non suppurative subacute abomasitis, ruminitis, reticulitis and catarrhal enteritis were observed in association with hyperemic adrenal glands. It was concluded that goats could depend on Acacia saligna as roughage without any serious effect for less than 26 and the histopathological changes of internal organs showed increase in severity by prolonged feeding of this plant.

Day 3:

Oral Presentation Abstracts

Oral presentations will be added after the submission deadline

ADENOCARCINOMA ARISING IN BARRETT’S OESOPHAGUS

Zakaria Eltahir, P Griffith, G Jenkins
The Royal Marsden Hospital, Translation GI & Lymphoma Research Laboratory, Histopathology Department, Fulham Rd, London, SW3 6JJ

Abstract:

In the last few decades adenocarcinoma incidents at the lower oesophagus have been steadily increasing while the oesophageal carcinoma incidents are decreasing. Barrett’s Oesophagus is the only known precursor to the oesophageal adenocarcinoma. There are no clear clinical or molecular signs for which patient might progress and develop this type of cancer.

Our aim was to investigate the cellular proliferation and apoptosis among a group of 200 patients’ with different stages of the disease for possible biomarker/molecular significance. Underlining disease subjective gastric reflux juice was also to be investigated. Prevention of cancer development is another goal on our project agenda.
Methodologies applied include: In-vitro by looking into the role of bile acids in inducing DNA damage, also chemo-prevention of popular diet ingredients. In-vivo morphological identification of apoptosis, Histological immunostaining, gene expression and a pilot study.

Our findings showed that: Apoptosis resistance was obvious in Barrett’s oesophagus cancer histological series. Bile acid (DCA) is genotoxic and it induces DNA damage in oesophageal cell lines and genetic abnormalities. The genotoxicity we have seen is caused via ROS pathway. Other bile type could prevent DNA damage such as UDCA. Antioxidants could restore oesophageal cellular stability at an early stage of the disease. Apoptosis frequency was declining significantly during cancer progression. Significant differences were noted between early histology and late histology, (p-value < 0.0003). Anti-apoptotic proteins (Bcl-xl, XIAP) were increasingly expressed during disease development, differences between early histology and late histology were significant (p-value < 0.01 and p-value < 0.02 respectively). Bcl-xl and XIAP mRNA increased 4-fold when normal and cancer tissues compared but showed no significance differences (p-values = 0.08, and p = 0.43 respectively).

Conclusions: We have provided further evidence that bile acids (DCA) play a major role in oesophageal pathogenesis. Indeed bile acid induces DNA damage, interferes in tissue recovery and regulates malignant development as well as cell death signals. Apoptotic resistance may drive neoplastic development in the oesophagus and BCL-XL may be a prime target for therapy.

**Poster Presentation Abstracts**

Poster abstracts will be finalised weeks before the event

**THE EXPERIENCE OF BRINGING GENOMIC RESEARCH DEVELOPMENTS EXPEDITELY INTO THE ROUTINE LABORATORY**

The experience of bringing genomic Research Developments expedite into the Routine Laboratory will be described

A summary report on the experience of pushing work through on an expedite basis from the Research Laboratory into the Routine Lab will be described

The challenges, the pitfalls and also strategies and advice and tips to making it work will be described

The context here is the UK 100 000 Genome Project and Government initiatives to advance Genetic Medicine from Research to Routine Practice

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**HOW MUCH RESEARCH DO UK ROUTINE BIOMEDICAL LABORATORIES SUPPORT (SURVEY REPORT)**

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This is understood but a number of questions might arise. How much actually goes on? How much is it a benefit or burden to the routine lab? And what sort of additional Quality Management arrangements should be put in place?

The Internet and other investigations yielded little information so a 23 question survey was drawn up. In this presentation I will aim to describe the methodology and results of a comprehensive UK wide
Pancreatic duct glands (PDGs) are crypt-like invaginations off the pancreatic ductal tree, embedded in mesenchyme, and form an anatomically distinct compartment with molecular features similar to the gastric, ileal and colonic crypt stem cell niches. PDGs, like ileal crypts, have zones of increased replication, consistent with a transit amplifying zone, and a predominance of exocrine epithelial cells with a minority of endocrine cells.

The PDG compartment has been proposed as a stem cell niche, potentially serving as a source of new endocrine cells. However, when stimulated, it might give rise to pancreatic intraepithelial lesions (PanINs) and is thus of interest as a candidate site for the origin of pancreatic cancer. The ongoing beta cell apoptosis in T2DM may serve as a source of chronic intra-pancreatic inflammation linking T2DM with an increased risk for pancreatic cancer.

In order to assess the activity of the PDG compartment in T2DM, we evaluated human pancreas from brain dead organ donors with T2DM and non-diabetic controls. We found that both the abundance of PanIn lesions and proliferation within PDGs was increased in T2DM.

Given the apparent importance of the PDG compartment in T2DM, we next set out to characterize the molecular signature of PDGs. To date, the molecular characterization of PDGs has revealed Pdx-1, nestin, mucin-6, Hes1 and Ngn3 expression. However, the approaches used relied on candidate immunohistochemistry or RT-PCR in rats or mice, with the potential for non-specific staining and bias by selection that this introduces. We used Laser Capture Microdissection (LCM) to procure high quality RNA from PDGs. We then characterized the transcriptional profile of these samples using RNA sequencing (RNA-seq) to establish the molecular identity of the PDG compartment in an unbiased manner. We used these data 1) to compare the molecular signature of PDGs to that of multiple tissues; 2) to identify possible markers overrepresented in the PDG compartment; and 3) to compare PDG samples from a rat model of type 2 diabetes (HIP rat) compared to wild type controls, with the goal of identifying the signaling networks and pathways altered in PDGs in the context of diabetes.

Our unbiased genetic expression studies by RNA-seq of PDGs reveal a molecular signature intermediate between the exocrine and endocrine pancreas as well as the well-defined stem cell niche in the testes, consistent with a pancreatic stem cell niche. The genes activated in HIP rats vs controls are consistent with inflammatory and regenerative signaling pathways, raising the possibility that islet inflammatory signals in type 2 diabetes chronically stimulate repair pathways in this putative stem cell niche, and might result in neoplasia in the long term.
NATURAL ISOLATED IMMUNE PEPTIDES FROM THE HONEYBEE, APIS MELLIFERA JEMENITICA: A POSSIBLE NATURAL TREATMENT OF HUMAN SALMONELLOSIS


Zoology Dept., College of Science, King Saud University, Saudi Arabia

Abstract:
Human Salmonellosis is a food borne disease and currently considered as an increasing public health problem in Saudi Arabia. Resistance of the causative bacteria, Salmonella enteritidis to therapeutic treatment, is currently emerging as a global health problem. We have conducted this study to test fractions of purified antibacterial immune peptides isolated from the local honeybee, Apis mellifera jemenitica, as natural antibiotics against human Salmonellosis. The adherence of S. enteritidis to human gut epithelial cells is an essential prerequisite to infection and toxin production, and thus, the effects of 6 purified antibacterial peptides fractions (A, B, C, D, E and F) on the attachment activity of S. enteritidis to the human intestinal epithelial cells were tested in vitro. Data revealed that the peptide fractions A, B, C and D have significantly decreased the adhesion of S. enteritidis to epithelial cells (p<0.001), but the peptide fractions A and B showed the highest adhesion reduction. Comparatively, the effect of fraction B is more adverse than that of A (p=0.02). Moreover, All purified peptide fractions also showed detectable growth inhibition zone activities against Micrococcus luteus, Escherichia coli and Paenibacillus larvae as standard bacterial strains. The susceptibility of S. enteritidis to the six antibacterial immune peptide fractions was determined by Kirby-Bauer Disk Diffusion method. The peptide fraction B that showed the highest antibacterial activity compared with ciprofloxacin antibiotic standard could be proposed as a suitable candidate for the treatment of human Salmonellosis. Characterization of the anti-Salmonellosis immune peptides are now underway, which may lead to synthetic production of a potential natural antibiotic against this disease.

Keywords: Adhesion; epithelial cells; Apis mellifera jemenitica, purified peptides, Salmonella.

GAP JUNCTION CX43 EXPRESSION AND LOCALIZATION IN HEALTHY AND ULCERATIVE AGLANDULAR GASTRIC MUCOSA OF HORSES

Vanía Pais Cabral Castelo Campos1; Carla Bardi Belle2; Maria Lucia Zaidan Dagli3; Aliny Antunes Barbosa Lobo Ladd4; Aparecida Joana Moreto5; Juliana Shimara Pires Ferrão6, Francisco Javier Hernandez-Blazquez4

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2. Department of Internal Medicine of the School of Veterinary Medicine and Animal Sciences, University of São Paulo (FMVZ/USP);
3. Department of Pathology, FMVZ/USP;
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The presence of gap junctions and cell adhesion proteins is poorly investigated and understood both in normal and ulcerative epithelial gastric mucosa of horses. This study is the first report of the presence and distribution of connexin 43 (Cx43) in the equine stomach. The Cx43 was studied in the aglandular plicate border (margo plicatus) of healthy gastric mucosa (10 horses) or in individuals showing ulcerative or erosive lesions (13 horses). The samples were obtained at abattoirs. Cx43 was always expressed in the gastric squamous stratified epithelium, however its intraepithelial distribution differs in healthy stomachs and stomach areas showing a lesion. In healthy stomachs the Cx43 was primarily found at the cell membranes and in the cytoplasm of cubic cells of the intermediate layer and was seldom found in the basal cell layer (perinuclear localization) and in the surface layers (at cell membrane). The borders of ulcerative lesions and the bed of erosive lesions frequently displayed the Cx43 in the basal cells, both in the cytoplasm and at the cell membrane forming gap junctions. In shallow and medium-erosive lesions Cx43 was also found in the most superficial epithelial layers. The lower expression of Cx43 at the cell membrane and cytoplasm of basal cells of healthy tissue suggests low gap junction activity at this layer and may be related to its higher cell division activity to maintain the integrity of the stratified epithelium, as it is known that Cx43 inhibits cell division. The high presence of gap junctional Cx43 at the cell membranes of the intermediate epithelial layer in both healthy and injured gastric mucosa indicates a high cellular communication activity in this layer, which may be necessary for the synchronization of the intercellular processes in this region. The Cx43 increased expression in the basal epithelial layer of injured mucosa and its cell membrane localization becomes interesting when we consider that in skin studies this Cx43 behavior implies delayed healing processes. Thus the higher presence of Cx43, a protein that inhibits cell proliferation, at the cell membranes of epithelial basal cells next or under injuries may be related to the process of relapses of ulcerative lesions in horses. Again, as is the case in skin, interfering with the expression of Cx43 may be an option to...
Several intrinsic and extrinsic etiological factors are involved in the incidence of ulcerative lesions in the gastric mucosa. Here we investigated the incidence and distribution of ulcerative lesions of the gastric mucosa (aglandular and glandular regions) of sixteen horses. The samples were obtained at abattoirs and a macroscopic morphological analysis was carried out. The stomachs were removed and an incision was done along the greater curvature of the stomach, which was washed and examined by a veterinarian pathologist. The stomachs of 15 horses showed ulcerative lesions. Sixty percent (60%) (9/15) had ulcerative gross lesions in both aglandular and glandular mucosal regions; 33.33% (5/15) only in the aglandular mucosa and 6.66% (1/15) only in the glandular region. Considering only the ulcerative lesions present in the aglandular mucosa, 100% (14/14) of the stomachs showed lesions next to the margo plicatus region, mainly in its ventral region; 50% of the stomachs showed lesions next to the cardiac ostium (7/14) and 42.8% of the stomach had lesions in the fundus region (6/14). It is known from the literature that morphological and functional differences between the equine aglandular and glandular stomach regions interfere with the incidence of ulcerative lesions, which is higher in the aglandular mucosa. We verified that the ulcerative lesions were more frequent in the region of the margo plicatus, which is the transition area between the aglandular and glandular mucosa. The higher occurrence of ulcerative lesions in this region is probably due to the topographic characteristics of the more ventral positioning of the margo plicatus compared to the cardiac ostium and the stomach fundus, allowing a greater exposure of the covering epithelium to the gastric juice. This anatomical proximity of the margo plicatus to the acid producing glandular region of the equine stomach may favor the higher incidence of ulcerative gastric lesions in the margo plicatus region.

This work was approved by the Ethics Committee on the use of animals (CEUA-FMVZ/USP) and the first author is recipient of a Post Doctoral fellowship grant (n. 2014/18635-5) from FAPESP (São Paulo Research Foundation) in the University of São Paulo Post Doctoral Program.
pharmacophore were.

The statistically curing of several side effects of hydroxyurea therapy have been evaluated the effect of piceatannol on neuroblastoma cells (N2a mouse model to evaluate the therapeutic ability of obtained results also indicate disturbance in the functioning of the antioxidant system in Neuro. In conclusion, piceatannol increases cellular cytotoxicity and inhibits the proliferation of neuroblastoma cells.

polyphenol caused a decline in the percentage of viable cells. We observed that longer time of incubation with polyphenol increases its activity. Reverse effect we observed when we examined superoxide dismutase activity in N2-a cells. Staining with annexin-V and flow cytometry analysis indicate that piceatannol induces apoptotic processes. Furthermore longer time of preincubation with polyphenol caused a decline in the percentage of viable cells.

In conclusion, piceatannol increases cellular cytotoxicity and inhibits the proliferation of neuroblastoma cells. Obtained results also indicate disturbance in the functioning of the antioxidant system in Neuro -2a cells after treatment with piceatannol and enhanced apoptotic processes.
THE EFFECT OF THE FIRST- AND SECOND-GENERATION OF ANTIPSYCHOTIC DRUGS ON SH-SYSY BRAIN CELLS AND THEIR TOXICITY.

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Antipsychotic drugs are primarily used to manage several psychiatric disorders, including schizophrenia, bipolar mania, and related mental illnesses. The present study examined the effect of the first and second generation of antipsychotic drugs on neuronal and non-neuronal cells. The toxicity of both-generation of antipsychotics was tested in both the SH-SYSY brain cell line and the COS7 kidney cell line. According to the LC50 values for each of chlorpromazine, trifluoperazine and olanzapine the neurotoxicity of the two classes in SH-SYSY exceeded their common cytotoxicity in COS7 cells, indicating that neuronal cells are at greater risk of cell death with low concentrations of antipsychotics at micro molar comparing to non-neuronal cells. Details study looked at the mechanism of cell death indicates that both apoptosis and necrosis play roles.

THE POSSIBILITY OF GRAY-ZONE ANALYSIS

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Aim: The Aim of this report is to try the Gray Zone analysis of Sudden Infant Death Syndrome (SIDS) from comparison with classical SIDS cases.

Material & Methods: The material analyzed was found in the Japanese Pathology and Autopsy Report from the Japanese Society of Pathology (January 1982 to December 1986). A $\chi^2$ test & factor analysis (the principle factor method with Varimax rotation) were performed to find the difference Classical SIDS and Gray zone SIDS in each autopsy finding.

Results: 1) Using $\chi^2$ test, a lymph tissue enlargement was found to have a high statistical value in Classical SIDS. Congestion, thymus enlargement, pulmonary oedema, adrenal gland atrophy, lymph tissue enlargement and neonate were recorded with higy factor loading in Classical SIDS by factor analysis. 2) Using $\chi^2$ test, a pneumonia, premature baby and cardiomegaly were recorded with high statistical value in Gray Zone SIDS. Asphyxia, congestion, atelectasis, pulmonary emphysema, adrenal gland atrophy, premature baby and thymus hypoplasia, cardiac malformation and ectopic hemopoesis were recorded with high factor loading in Classical SIDS by factor analysis. Thymus enlargement and adrenal gland atrophy were recorded in the third factor of Gray Zone SIDS having rather high negative factor loading using factor analysis.

Conclusion: Compared with the autopsy findings of SIDS in Wales Institute of Forensic Medicine almost at the same period, the second factors of SIDS such as thymus enlargement & lymph tissue enlargement found in Japanese data has been lost in Walish data. This fact might suggest the difference of expert view of pathologists in each country. As for SIDS, this trial should be repeated using nowadays data from the prospect of rather new guideline of Japanese SIDS Research Society. However, the establishment of the method of the gray zone analysis could contribute to revise the nowadays system of health promotion & criminal investigation of cause of death etc.

IMMUNOHISTOCHEMISTRY OF BINDING PROTEIN

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AIM: At the time of diagnosis of SIDS, we must discriminate external factors & internal factors. For this purpose, we have proposed the immunohistochemistry (IHC) of binding proteins. As one trial, we could suggest the IHC of TATA-binding protein applied to SIDS & non-SIDS cases.

Materials & methods: A total of 38 infants, including 26 cases of SIDS, died under 6 months of age, in a cohort of 27,000 infants studied prospectively to characterize their sleep-wake behavior. The frequency & duration of sleep apnea was analyzed. Brainstem material was collected & immunohistochemistry of TBP was carried out. The density of TBP-positive neurons was measured quantitatively. Correlation analyses were carried between the density of TBP-positive neurons and the data concerning sleep apnea.
Results: One SIDS-specific positive correlation occurred between the density of TBP-positive neurons in the dorsal raphe nucleus of the midbrain and the duration of central apnea (p=0.049) and two SIDS-specific negative correlation between the density of TBP-positive neurons in the pars compacta and dissipata of the pedunculopontine tegmentum nucleus (PPTNc, PPTNd) in the midbrain and the duration of apnea (p=0.035).

Conclusions: The significant correlations between the findings of TBP-positive neurons in the midbrain arousal pathway and the characteristics of sleep apnea in SIDS victims in in agreement with the both association of apnea & arousal phenomenon in pathophysiology of SIDS.

LONG-TERM CULTURE OF MESENCHYMAL STROMAL CELLS ISOLATED FROM WHARTON’S JELLY
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Mesenchymal stromal cells (MSCs) represent a population of adult stem cells characterized by special characteristics - the ability to self-renew, plasticity, regeneration and immunological properties, the ability to migrate to the site of injury, inflammation or a tumor. MSCs have been found in different tissues of human body, including bone marrow, dental pulp, muscles, adipose tissue and umbilical cord. Umbilical cord contains two arteries and one vein surrounded by Wharton’s jelly, a gelatinous substance made largely from mucopolysaccharides which protects these vessels. MSCs derived from Wharton’s jelly represent a population of primitive stromal cells. Wharton’s jelly is unique and readily available source of stem cells that are not loaded with the loss of telomerase activity, have a shorter time of dividing and a large expansion potential.

In our study we focused on the comprehensive analysis of biological properties of stem cells isolated from Wharton’s jelly. Cells were obtained by the enzymatic digestion (0.1% collagenase type I) as well as by the explanting method. Cells were analyzed by flow cytometry to assess expression of surface antigens. Real time PCR was used to analyze expression of Tp53 and Bcl2.

Using flow cytometry we observed changes in phenotype of MSCs. Positivity for markers of MSCs CD90, CD73 and CD105 decreased with increasing height of the passage, while in 1st passage the positivity was 80.8%, in 11th passage it was only 1%. At the same time using gene expression analysis we observed decrease in the expression of the Tp53 while expression of Bcl2 rises in 9th passage.

The result of our study is finding out that with increasing length of the culture of MSCs isolated from Wharton’s jelly these cells lose their stemness.

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PARALLEL TRANSCRIPTOME AND PROTEOME ANALYSIS OF EA.hy926 CELLS UNDER STRESS CONDITIONS INDUCED BY NANOMATERIALS
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In the last decades the field of nanotechnology becomes the main focus of numerous research centers over the world. Nanomaterials are being used in rapidly increasing applications and many products in nano-scale have already been launched on the market. However, despite of new, unique and alternative properties of nanomaterials comparing to their bulk counterparts, the level of risk towards human health is still unclear and not fully examined. In the view of above, the use of commercial products in biomedical applications raises big concerns – due to the investigated short- or long-term toxicity of nanoparticles and potential hazardous influence on human body. In the present study, we investigated the influence of three commercially available
nanoproducts: polyamidoamine dendrimers (PAMAMs) of 4.0 generation, silver nanopowders (SNPs) and multi-walled carbon nanotubes (MWCNTs) on transcriptome and proteome of EA.hy926 endothelial cell line.

Cells were cultured in DMEM medium at 37°C in a 5% CO2 atmosphere and exposed to 24h contact with the studied nanoparticles. Total RNA and proteins were isolated from the cells with TRI Reagent. For transcriptomic analysis Agilent SuperPrint G3 Human GE 8x60K V2 oligonucleotide microarrays were used. Total RNA form EA.hy926 cell was purified, labelled and hybridized for 17h at 65°C with a rotation speed 10 rpm. Gene Spring GX 12.6.1 Software was used to analyze gene expression data. For assessment of the whole proteome of endothelial cells 2D-DIGE electrophoresis was performed. Samples were labeled with Cy3, Cy5, isofocused on 18cm long DryStrip gels (pH 4-7) using IPGphor III (GE) and separated in second dimension in Ettan DALTsix system (GE). Obtained data were analyzed with ImageMaster 2D Platinum 7.0 DIGE. Proteins under changed expression were analyzed with MALDI-TOF/TOF spectrometer (UltrafleXtreme Smartbeam II, Bruker Daltonics). Identification was performed via MASCOT search engine against Swiss Prot database.

Microarray full genome analysis showed dysregulation of expression level of numerous genes. Incubation of nanoparticles with cells caused changes in expression of 1271 genes (1130 up-regulated, 141 down-regulated) for MWCNT, 431 genes (322 up-regulated, 109 down-regulated) for PAMAM and 299 for SNP (250 up-regulated, 49 down regulated). Moreover 16 genes under altered expression were common to all examined nanoparticles. DIGE analysis revealed changes in the expression of a number of distinct proteins. In response to nanoparticle incubation, among almost 900 proteins expressed in cells, statistically significant changes were addressed to SNP for 100 proteins (74 down-regulation, 26 up-regulation), to MWCNT – 41 proteins (31 down-regulation, 10 up-regulation) and to PAMAM dendrimers – 50 proteins (29 down-regulation, 21 up-regulation). It was also found that only 10 proteins correlated with changes in expression of 16 genes.