13th International conference on

Pathology and Molecular Diagnosis

June 26-27, 2017
San Diego, USA

Posters

Elena Kleymenova et al., J Clin Exp Pathol 2017, 7:2 (Suppl)
DOI: 10.4172/2161-0681-C1-035

Expression of G-protein coupled receptors in the basal region of the gastrointestinal epithelium

Elena Kleymenova, William Benson Jr and **Jean-Louis Klein**
GlaxoSmithKline, USA

G-protein coupled receptors (GPCRs) regulate gastrointestinal food intake making them attractive targets for therapeutic interventions of the metabolic syndrome and type II diabetes as well as nutritional control. Basal insulin release and insulin-mediated glucose uptake and dispensation are in part controlled by fatty acids. FFAR1-3 are among principal receptors to free fatty acids and have been proposed as chemo sensors of short/medium (FFAR1) and long (FFAR2-3) free fatty acids in the gut content. Operating in concert with FFARs, GPR 119 is believed to act as a chemo sensor of locally fat-derived molecules in the gut lumen. The luminal chemo sensing hypothesis was largely based on expression of these GPCRs in the gastrointestinal enteroendocrine cells. By IHC, we have also detected expression of FFARs and GPR119 in the basal compartment of enteroendocrine cells. In addition, we have observed expression of these GPCRs at the basolateral aspect of the cell plasma membrane of human and rodent enterocytes. GPR 39, a member of ghrelin/neurotensin receptor subfamily involved in Zn-mediated insulin secretion and gastric emptying was also detected by IHC in enteroendocrine cells and enterocytes at the basolateral aspect of the cell plasma membrane. In polarized CaCo-2 cells used to *in vitro* model gastrointestinal nutrient uptake GPR 39 was expressed at the basolateral aspect of the cell plasma membrane as well. Expression of FFARs, GPR119 and GPR 39 at the basal region of the gastrointestinal epithelium highlights the complexity of the food intake regulation and the need for revision of the luminal chemosensing model. The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents. All studies were conducted in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals and were reviewed by the Institutional Animal Care and Use Committee either at GSK or by the ethical review process at the institution where the work was performed.

Biography

Elena Kleymenova holds Master's Degree in Physics from M Lomonosov Moscow State University (Russia) and PhD in Biology from NN Blochin Cancer Research Center of the Russian Academy of Medical Sciences (Russia). She has conducted Post-doctoral Research at MD Anderson Cancer Center in Texas and continued her studies as a Research Associate at Hamner Institute for Health Sciences, North Carolina. She has authored more than 25 articles in reputed peer-reviewed Life Science journals and served on several NIH Extramural Researches review panels. Currently, she is a Senior Scientific Investigator at the pharmaceutical company, GlaxoSmithKline where she is involved in molecular profiling of new drug targets.

elena.v.kleymenova@gsk.com

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Chien Kuo Tai et al., J Clin Exp Pathol 2017, 7:2 (Suppl)
DOI: 10.4172/2161-0681-C1-035

Genome-wide methylation analysis of tissue DNA in oral squamous cell cancer patients

Chien Kuo Tai, Wei-Ru Liu and **Yu-Fen Li**
National Chung Cheng University, Taiwan

This study explores the association between the genome-wide DNA methylation status and the occurrence of oral squamous cell cancer (OSCC). A case-control study design was applied to 34 tissue samples from 26 OSCC patients and 8 non-cancer participants. Whole-genome DNA methylation profile of the tissues was measured by Infinium HumanMethylation450 BeadChip, and the methylation levels were presented as β values. Normalization and batch effect adjustment were applied for processing these β values. We defined Δβ as the difference in the mean β values between the cancer and non-cancer groups. Probes with |Δβ| greater than 0.2 were considered for further analysis. The area under Receiver Operating Characteristic curve (AUC) was used to evaluate the discrimination ability of each probe. We also defined the differentially methylated regions (DMRs) of OSCC which may minimize potential artifacts due to random methylation alterations in single probes. Results: Among ~485,000 probes in the BeadChip, ~25,000 probes showed |Δβ| greater than 0.2 and AUC greater than or equal to 0.9. Hierarchical cluster analysis showed that the top 500 most significant probes can correctly distinguish OSCC and normal tissue samples. We identified four genes: BHLHE23, GSX1, MIR124-3, and SVIP with a great accuracy to detect OSCC tissues. We also identified 280 DMRs that included 1,097 hyper-methylated probes with 446 probes located on gene bodies (40.7%). Conclusion: This study shows that there is a strong relationship between OSCC and DNA methylation. DNA methylation can be promising epigenetic biomarkers for the detection of OSCC.

Biography

Chien Kuo Tai completed his PhD from USC Pathology and is currently working as a Professor at National Chung Cheng University, Taiwan.

write2ck@gmail.com

Notes:

Miranda Li et al., J Clin Exp Pathol 2017, 7:2 (Suppl)
DOI: 10.4172/2161-0681-C1-035

Impact of substance use on salivary cytokine levels in healthy female individuals

Miranda Li, Hazel Huang and **Anna-Barbara Moscicki**
University of California, USA

Substance (tobacco, alcohol) use and various inflammatory diseases (periodontitis, oral lichen planus, leukoplakia) induce chronic inflammation, which is a mechanism for head and neck squamous cell carcinoma (HNSCC). Analysis of salivary cytokine levels reveals abnormal cytokine production, which, if detected early enough, could improve treatment and survival rates for HNSCC. The correlation between substance use and cytokine levels has not been well researched. This pilot study examines the correlation between substance use (tobacco, alcohol, marijuana) and cytokine levels (IFN-α, IL-10, IL-12, IL-13, MIP-1α, TNF-α, IL-4, IL-6, IL-8, IL-1α, IL-1β) in 71 healthy women (25-32 years old). Luminex-based multi-analyte MILLIPLEX™ MAP Human Cytokine/Chemokine Magnetic Bead Kits (Millipore Corp., Billerica, MA) and MAGPIX® imaging technology was used to analyze the saliva samples. There was a statistically significant difference in cytokine interleukin (IL)-1β levels between the control group (n=24, SD=26.90) and the tobacco/light alcohol user group (n=21, SD=164.14), $p \leq 0.05$. There was also a statistically significant difference in cytokine IL-8 levels between the control group (n=24, SD=236.58) and the tobacco/heavy alcohol user group (n=18, SD=295.26), $p \leq 0.01$. These results suggest that young women who use tobacco and alcohol heavily are already showing signs of chronic inflammation that make them at risk for HNSCC later on. With more research, a saliva-based test could be a cost-effective tool in assisting early diagnosis of head and neck cancers through promising associations between substance use and pro-inflammatory cytokines.

Biography

Miranda Li is an Honors High School Student who dedicates most of her free time to research.

mirandali62@gmail.com

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13th International conference on

Pathology and Molecular Diagnosis

June 26-27, 2017
San Diego, USA

Accepted Abstracts

J Clin Exp Pathol 2017, 7:2 (Suppl)
DOI: 10.4172/2161-0681-C1-035

Chromosome microarray analysis - Changing the landscape of clinical cytogenetics

M Anwar Iqbal
University of Rochester Medical Center, USA

The conventional technique of G-banded chromosome analysis reliably detects large chromosomal abnormalities and rearrangements at a minimum size of about 3-10 Mb, and requires dividing cells. Its main limitation is that smaller chromosomal deletions or duplications may be overlooked. Fluorescence in situ hybridization (FISH) was developed to rapidly detect smaller chromosomal abnormalities with locus-specific probes, but one must clinically suspect a specific diagnosis associated with a particular chromosome or chromosomal region to request the appropriate probe. Array-based comparative genomic hybridization (aCGH) developed as a method to examine the entire genome for copy number changes caused by deletions, duplications, or whole chromosome aneuploidy. It improved resolution over conventional G-banded karyotype in detecting much smaller chromosomal abnormalities, as small as 50 to 100 kb, and does not require dividing cells. It has become a first-line diagnostic tool for the detection of chromosome abnormalities at both macro and micro level in postnatal, high-risk pregnancies and in products of conception samples. Application of these technologies in cancer research has produced a wealth of useful information about copy number alterations (CNAs), Loss of heterozygosity (LOH) and mutations of specific genes and their implications in cancer classification, disease progression, therapy response, and patient outcome. There is an increasing interest in the genetic diagnostic community in applying this new technology for cancer diagnosis. Our experience on more than 4000 cases performed using the aCGH, and aCGH and SNP arrays in postnatal, prenatal and cancer will be presented.

anwar_iqbal@urmc.rochester.edu

Drug-responsive chromatin structures and the underlying genetic alterations in leukemia

Jason X Cheng
University of Chicago Medical Center, USA

Chemical modifications of DNA/histone play an important role in organization of human chromatin into distinct structural domains that control gene expression, stem cell differentiation and tumorigenesis. Drugs that target various chromatin modifiers have become one of the promising treatments for many types of cancer including solid tumors and hematologic malignancies such as myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). However, most, if not all of the cancers treated with epigenetic drugs eventually develop drug resistance and render epigenetic drugs ineffective in cancer patients. The mechanisms underlying the selectivity and efficacy of epigenetic-modifying drugs are still unknown. Therefore, a major challenge in today's cancer treatment is to unravel the mechanisms of drug resistance and to develop strategies to prevent or reverse drug resistance in various types of cancer. In this study, we developed a new method to simultaneously measure 5-methylcytosine (5-mC) and hydroxymethylcytosine (5-hmC). CDMIAs revealed significantly drug-responsive changes in 5-mC/5-hmC at the promoters of differentiation/lineage-controlling genes such as PU.1/SPI1. Immunoprecipitation experiments demonstrated lineage-specific, drug-sensitive interactions between the PU.1/SPI1 and GATA1 transcription factors and the DNA/histone modifying complexes. ChIP-seq and chromatin conformation capture (3C) showed that distinct chromatin structures at the gene locus in a lineage-specific manner. Importantly, novel mutations in TET2, TET3, DNMT3L and PU.1/SP1 were revealed by genome-wide sequencing and confirmed by Sanger sequencing. These mutations correlated with the altered interactions between PU.1/SPI1 and the DNA/histone modifying complexes and predicted the responses to epigenetic modifying drugs. Examination of clinical specimens from patients with MDS confirmed the presence of distinct lineage/differentiation-specific chromatin structures. These results demonstrate the importance of functional genomics in the pathogenesis of MDS and leukemia and may identify novel therapeutic targets.

Jason.Cheng@uchospitals.edu

J Clin Exp Pathol 2017, 7:2 (Suppl)
DOI: 10.4172/2161-0681-C1-035

The role of diagnostic molecular pathology in the era of targeted therapy

Abeer A Bahnassy
Cairo University, Egypt

In the era of targeted therapy, the assessment and evaluation of solid tumors in pathology is becoming more and more based on a combination of the histopathology and the molecular analysis of tumor tissues or liquid biopsy. In addition, the choice of treatment protocols is increasingly based on the molecular features of the tumor as a consequence of the rapid development of new cancer treatments that specifically target aberrant proteins present in tumor cells. Not only the number of patients eligible for targeted precision medicine, but also the number of molecular targets per patient and tumor type is rising. Therefore, diagnostic molecular pathology has attained much attention in the last few years. It is of utmost importance to determine the relevant molecular aberrations present in tumors for diagnostic, prognostic or predictive purposes. However, this is faced with several challenges. First, the molecular pathology lab has to meet the challenge of doing the required molecular tests using the limited amount of tumor tissues embedded in paraffin after formalin fixation in short turnaround time. Second, the choice of the detection method is critical, since the analytical methods should provide accurate, reliable and cost-effective results. Third, the validation of the test procedures and results is essential. In addition, participation and good performance in internal (IQA) and external quality assurance (EQA) schemes is mandatory. However, in spite of all these obstacles, molecular pathology is increasingly becoming an integral part of the diagnostic workup of most solid tumors, as well as, in determining prognosis and response to treatment. The list of molecular tests for breast, lung, colon, stomach, bone and soft tissue tumors are continuously increasing.

chaya2000@hotmail.com

The effect of surface functionalization upon the cellular uptake characteristics of upconversion nanocrystals

Libing Fu[1], **Bingyang Shi**[1], **Dayong Jin**[2] and **Roger Chung**[1]
[1]Macquarie University, Australia
[2]University of Technology Sydney, Australia

Recently, the investigation of the interactions between nanomaterials and biological systems (known as nano-bio interactions) has spurred tremendous research interest in the field of nanotechnology. To improve the therapeutic potential of the nanoparticle (NP)-based vehicles for the intracellular delivery, it is crucial to systematically study the fate of NPs with uniformity of particle size, shape and surface charge, which are desired for elucidating the effects of these properties on cell uptake and bio-distribution. Anthanide-doped upconversion nanoparticles (UCNPs) provide a novel BBB delivery approach, as their shape/size/surfaces are tunable. Furthermore, these nanoparticles have excellent detection characteristics such as background free, photo stable, and deep tissue penetration. In this work, we compared a series of upconversion nanoparticles (UCNPs), including original UCNPs, OA-free UCNPs, DNA-modified UCNPs, SiO2-coated UCNPs and PEG-conjugated UCNPs to analyze the principle factors that facilitate the transport of nanoparticles into the mouse NSC-34 motor neuron cells. It is found that UCNPs cellular uptake is mainly dependent on the dispersity in cell culture media. The surface charge plays an important role during this procedure as well. Specifically, PEG-conjugated UCNPs showed the most excellent cell uptake ability among these five types of UCNPs. While, the original UCNPs were primarily found attached on the cell membrane, because they formed aggregation in the cell culture media. The cytotoxicity of the UCNPs in NSC-34 cells demonstrated that the PEG-conjugated UCNPs possessed minimal cellular viability. Through this work, the results highlight the potential application of constructing a multifunctional UCNPs nano-composite with integration of brain drug delivery, diverse biomolecule monitoring and deep tissue imaging.

libing.fu@students.mq.edu.au

J Clin Exp Pathol 2017, 7:2 (Suppl)
DOI: 10.4172/2161-0681-C1-035

Study of possible relation between maternal serum resistin and insulin resistance in patients with pre-eclampsia

Alyaa Ahmed El-Sherbeny, Manal Mohsen, Eman Abdelmoniem Algohary, Safeya Hamdy Hassan, Emad Abd El-mohsen Abd El-hadi and Abdel Latif Galal El-kholy
Ain Shams University, Egypt

Introduction: In humans resistin antagonizes the effects of insulin on glucose metabolism in liver and skeletal muscle, interacts with and reinforces inflammatory pathways and may promote endothelial cell activation. Increased resistin levels have been associated with obesity, insulin resistance, metabolic syndrome, type 2 diabetes and increased cardiovascular risk

Objectives: Our study aimed to investigate the utility of maternal serum resistin in women with pre-eclampsia compared to normal pregnant women and its relation to insulin resistance.

Methods: The study was conducted on ninety (90) females, divided into two groups: - Group I: Pre-eclampsia (n=60) and Group II: Healthy pregnant Control (n=30). All individuals were subjected to the following after an informed oral and written consent: Full history taking, clinical examination with special emphasis on edema, blood pressure measurement and Maternal body mass index (BMI); Index (weight (kg)/height^2 (m^2)), determination of gestational age according to the date of the last menstrual period and confirmed by first trimester ultrasound. Laboratory investigations including CBC, AST, ALT, BUN, creatinine, HOMA-IR and serum resistin were performed.

Results: Statistical comparison between pre-eclamptic patients (Group I), and the healthy control group (Group II) regarding the different studied parameters revealed a highly statistically significant increase in the patients group than the control group regarding SBP, DBP, BMI, CRE, AST, ALT, 50 g oral glucose challenge test (GCT), FBG, fasting insulin, HOMAIR and resistin. On the contrary, there was a highly statistically significant decrease in the patients group than the control group regarding HB.

Conclusion: In this study, it was found that elevated serum resistin levels could be associated with exaggerated insulin resistance in patients with pre-eclampsia. Further studies are needed to clarify the role of resistin in the patho-physiology of preeclampsia and insulin resistance.

draliaa78@yahoo.com

J Clin Exp Pathol 2017, 7:2 (Suppl)
DOI: 10.4172/2161-0681-C1-035

Role of liquid based cytology vs. conventional cytology in FNAC of abdominal masses

Archna Rautela
Army College of Medical Sciences, India

The study was conducted to assess the role of Thin Prep, one of the FDA approved Liquid based cytology techniques in the diagnostic cytology work up of abdominal masses. We evaluated a total of 30 patients presenting with abdominal masses. The aspirate material was processed by conventional technique and by Thin Prep method and also rinsed into cell block fluid. The slides prepared from both the methods were compared by two independent pathologists. They were evaluated by comparing adequacy, cellularity, architectural pattern, cellular morphology preservation and background. Findings suggested that cellularity was more often higher in conventional smears than on Thin Prep slides (p value=0.025). Architectural pattern preservation was better on conventional smears (p value=0.001). Cytoplasmic preservation was better on conventional smears (p value=0.001), but difference in preservation of nuclear details was not statistically significant. The background in smears prepared by Thin Prep slides were significantly cleaner than direct smears (p value=0.001). Non epithelial elements like mucin and neurofibrillary tangles were better preserved on direct smears (p value=0.001), but diagnostic accuracy for both the methodologies showed no statistically significant difference (p value=0.226). The Liquid based cytology techniques utilize expensive equipment, reagents and they also generate certain morphological artifacts in slides with which a cytologist needs to get familiar. On using alone they might not consistently provide any added benefit in the work up of such lesions and should be employed as an adjunct to conventional smears. They may be preferred in situations where material needs to be transported or is required for ancillary tests.

drarchnarautela@gmail.com

Vitamin B_{12} and folate deficiency status in a strict lacto-vegetarian population of Tharparkar

Suresh K Langhani[1], **M Akber Agha**[2] and **Loung V Umedani**[3]
[1]Jinnah Sindh Medical University, Pakistan
[2]Dow University of Health Sciences, Pakistan
[3]King Saud Bin Abdul Aziz University of Health Sciences, KSA

Vitamin B12 and folate are essential for maturation of the red blood cells. B12 is only found in animal products, while folate is abundant in plants. Strict lacto-vegetarians are at high risk to develop vitamin B12 deficiency. After an ethical approval of Dow University of Health Sciences (DUHS), we carried a randomized, cross sectional, descriptive and analytic study at a Tharparkar village to observe the prevalence and subsequent hematological parameters due to deficiencies of these vitamins in 200 subjects (100 strict lacto-vegetarians, compared with 100 non-vegetarians). After a physical examination the blood samples were collected and sent to DUHS lab for serum B12 and folate levels and complete blood counts. The data were analyzed descriptively and statistically by SPSS 17 to calculate the Odds Ratios and p-Values. The mean age of strict-vegetarian group was 30.5 years (± 8.3) and non-vegetarian as 30.1 years (± 9.2). Male to female ratio was 3.4:1.0. Vit-B12 deficiency was found in 83% strict-vegetarian and in 66% of non-vegetarian group, low folate 7% in vegetarian versus 23% non-vegetarians and anemia in 36% vegetarians versus only 20% in non-vegetarian group. Definite high MCV was found in 30% vegetarians and 26% in non-vegetarians. Thrombocytopenia and leucopenia were unremarkable. It is concluded that vitamin B12 deficiency is predominantly found in the strict-vegetarians who also displayed alarming levels to produce neuropathy. The levels of folate were normal in the studied groups. Vitamin B12 supplementation is recommended in the high risk areas of Tharparkar.

mukhi_suresh@yahoo.com

J Clin Exp Pathol 2017, 7:2 (Suppl)
DOI: 10.4172/2161-0681-C1-035

Sputum but not blood periostin levels correlate with sputum eosinophil counts in asthma

Marie-Chantal Larose, Anne-Sophie Archambault, Véronique Provost, Jamila Chakir, Michel Laviolette and **Nicolas Flamand**
Université Laval, Canada

Objective: Periostin is increasingly recognized as a biomarker, notably in atopic dermatitis. Recent studies underscored that serum periostin was one of the best biomarkers of severe asthmatics with persistent airway eosinophils and symptomatically uncontrolled despite high doses of corticosteroids. However, given that the role of periostin to identify eosinophilic asthma is still debated, we evaluated whether blood and sputum periostin levels link to sputum eosinophil counts and asthma severity.

Methods & Measurements: Blood and induced sputum samples were obtained from healthy and asthmatic (mild, moderate, severe non-eosinophilic, severe eosinophilic) subjects, and blood samples from atopic dermatitis subjects. Human bronchial epithelial cells (BECs) obtained from healthy subjects and mild or severe eosinophilic asthmatics were treated with IL-13. Periostin and CCL26 levels were quantitated by ELISA.

Main Results: In sputum, eosinophil counts, CCL26 and periostin levels significantly correlated. Plasma periostin levels were similar in healthy and asthmatic subjects. In asthmatics, they correlated with sputum eosinophil counts in severe eosinophilic asthma, but weakly in the whole group. Of note, BECs of severe eosinophilic asthmatics released greater amounts of periostin than those of mild asthmatics and healthy subjects. Blood eosinophil counts did not correlate with plasma periostin levels and showed a slightly better correlation with sputum eosinophil counts.

Conclusions: Except for severe eosinophilic asthma, plasma periostin levels weakly correlated with sputum eosinophil counts and could not predict the degree of bronchial eosinophilic inflammation. Both sputum periostin and CCL26 levels showed better correlation with sputum eosinophil counts.

Biography

Marie-Chantal Larose has completed her Master's from Laval University. She is completing her Doctoral studies at the Laval University, under the supervision of Dr. Michel Laviolette and Dr. Nicolas Flamand.

marie-chantal.larose@criucpq.ulaval.ca