EuroSciCon

DAY 1

Scientific Tracks & Abstracts

International Conference on

BIOTECHNOLOGY, BIOMARKERS & SYSTEMS BIOLOGY

March 04-05, 2019 | Amsterdam, Netherlands

DAY 1

March 04, 2019

Sessions

Biotechnology | Bioengineering | Nanobiotechnology | Bioinformatics | Biochemistry | Molecular Biology | Genetic Engineering | Biomarkers & Research | Circulating Biomarker Classes | Breastcancer Biomarkers

Session Chair
Vasiliev Ilie
World Academy of Medical Sciences, Netherlands

Session Co-Chair
Hitoshi Sohma
Sapporo Medical University Center for Medical Education, Japan

Session Introduction

Title: **Engineering the biosynthesis of artemisinin**
Wim J Quax, University of Groningen, The Netherlands

Title: **Generation and characterization of some UnaG mutant**
Numan Eczacioğlu, Karamanoğlu Mehmetbey University, Turkey

Title: **Personalized and regeneration medicine require a coagulum-OMICs model**
James Andrew Henry, Atlantis BMS Limited, United Kingdom

Title: **Modification of nutritional properties of microalgae for Artemia breeding**
Zeynep E Çakmak, Istanbul Medeniyet University, Turkey

Title: **A Current Update of Metallothionein and its Isomers**
N Thirumoorthy, PPG College of Pharmacy, India

Title: **Incorporation of genetic markers in diagnostic criteria of osteoarthritis knee**
Sachin Avasthi, RML Institute of Medical Sciences, India

Title: **Development of circulating biomarkers for early and advanced breast cancer**
Ana Pedro, University of Hull, United Kingdom

Title: **Improvement of selenomonas ruminantium β-xylosidase thermal stability by replacement of free buried cysteines**
Ehsan Dehnavi, Gene Transfer Pioneers (GTP) research group, Iran

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Wim J Quax et al., Biochem Mol biol J 2019, Volume:5
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ENGINEERING THE BIOSYNTHESIS OF ARTEMISININ

Wim J Quax, Ingy I Abdallah, Hegar Pramastya and Dan Xue
University of Groningen, The Netherlands

Terpenoids represent the largest class of natural products with a diverse array of structures and functions. Many terpenoids have reported therapeutic properties such as antimicrobial, anti-inflammatory, immunomodulatory and chemotherapeutic properties making them of great interest in the medical field. Terpenoids suffer from low natural yields and complicated chemical synthesis; hence there is a need for a more sustainable production method. Metabolic engineering using biosynthetic mevalonate and non-mevalonate pathways provides an excellent opportunity to construct microbial cell factories producing terpenoids. The complexity and diversity of terpenoid structures depends mainly on the action of the terpene synthases responsible for their synthesis. Amorpha- 4, 11-diene synthase (ADS) cyclizes the substrate farnesyl pyrophosphate to produce amorpha- 4, 11-diene as major product. This is considered the first committed and rate-limiting step in the biosynthesis of the antimalarial artemisinin. Here, we utilize a reported 3D model of ADS to perform mutability landscape guided enzyme engineering. A mutant library of 258 variants along sixteen active site residues was created and then screened for catalytic activity and product profile. This allowed for identification of the role of some of these residues in the mechanism. The mutability landscape also helped to identify variants with improved catalytic activity. H448A showed ~4 fold increase in catalytic efficiency and the double mutation T399S/H448A showed that kcat has improved by ~5 times. This variant can be used to enhance amorphadiene production and in turn artemisinin biosynthesis. Our findings provide the basis for the first step in improving industrial production of artemisinin and they open up possibilities for further engineering and understanding of ADS.

Biography

Wim J Quax was appointed as Professor of Pharmaceutical Biology at the University of Groningen in 1998. He has developed an extensive research group focussing on directed evolution and protein design technology for researching pharmaceutically relevant proteins. One of his focus areas are enzymes catalysing the synthesis of natural products. He has published >300 peer reviewed papers and book chapters and he is Inventor of >30 patents. He is the former Scientific Director of the Groningen Research Institute for Pharmacy (GRIP).

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GENERATION AND CHARACTERIZATION OF SOME UNAG MUTANT

Numan Eczacioğlu[1], Yakup Ulusu[1], Isa Gökçe[2] and Jeremy H Lakey[3]

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[2]Gaziosmanpaşa University, Turkey
[3]Newcastle University, UK

UnaG protein from Japan eel (*Anguilla japonica*) is a novel fluorescent protein with binding domain that acquires fluorescence when bound to unconjugated bilirubin (UC-BR). In this study, several point mutations (F17M, N57D, N57E, N57R, L41F, Y99F_Y134W, Y99M_Y134M, and W9F_W103F) were made on the UnaG nucleotide sequence via using a method for sequence and ligation independent cloning (SLIC). The aim of the mutations on UnaG is to figure out the change in fluorescence properties. The new mutagenic vector was transformed into the commercial competent cells (*E. coli* Mach1) by using heat shock at 42 °C for 2 minutes. Transformed cells were grown on and selected from the LB agar plate with ampicillin. (1:1000). The DNA sequencing results show that all these mutations have done correctly. The expression of the mutant proteins was made in the pTOLT expression system by inducing with IPTG. Cells were collected with high speed centrifugation. Before disrupting the cells, lysozyme enzyme was added to make break up the cells easier, some protease inhibitors (phenylmethylsulfonyl fluoride, benzamidine) were added for the protection from proteases of the protein and DNase and RNase were added on the cell pellet to avoid the DNA and RNA contaminations. Ultracentrifugation was applied on the cell lysate. Ni-NTA affinity chromatography system was used to get the pure mutant proteins from supernatant. SDS-PAGE and semi-dry Western blot were applied on the protein for the qualitative analyse. The pure protein bands were observed on the SDS-PAGE gel image. Additionally, the spectroscopic features of purified mutant proteins were measured after adding fresh UC-BR on fluorescence spectrophotometer. Excitation and emission spectra of the mutant proteins are similar; even so they have different fluorescence intensity at the same concentration. This study suggests that mutant UnaG proteins can be used to detect UC-BR level of cells/tissue.

Biography

Numan Eczacioğlu has started his PhD at Karamanoğlu Mehmetbey University, Turkey and still continues. Also, he is working as a Research Assistant at Bioengineering Department of the same university. He is the part of Tubitak and British Council Newton Katip Celebi Fund bilateral cooperation program with collaborate Newcastle University and Karamanoğlu Mehmetbey University.

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PERSONALIZED AND REGENERATION MEDICINE REQUIRE A COAGULUM-OMICS MODEL

James Andrew Henry
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Background: In 2017, a program on patient blood management was posted to the National External Quality Assurance Scheme conference for hemostasis and thrombosis [1]. This subsequent coagulum-OMIC framework is a standard for predictive value within personalized and regeneration medicine. An OMIC model is a foresight by the author of this program to achieve OMIC flow [View Fig. 1.]. This model sustains the success of Coagulum-OMICS when supported with the ISO 9000 series. [2] [3] [4].

Study: ISO 9001 and 9004 are powerful tools to identify and define good practice in an OMIC model. ISO 9001 is a process based standard and an ideal standard for OMIC interfaces. The greater challenge in haemostasis and thrombosis is the end to end process involves several parts of healthcare under different clinical management or vendor arrangements. The flexibility of ISO 9004 makes it an ideal tool to access Coagulum-OMICs and sustain the success of personalised and regeneration medicine.

Program Development: A strategy for Research, Family, Organ and Acute Coagulum-OMICs commences biphasic policy objectives for genomics as a primary care with viscoelastic science, coagulum and platelet proteomics [Fig. 2]. Model OMIC development, resources, performance review and innovation, become learned. OMIC teams self-assess the Coagulum-OMICs to identify conformity with the model. Regional committees are supported by a joint working group on quality assurance to manage or improve OMICs

Conclusion: A model for blood coagulum-OMICs is a benchmark to sustain excellence in the future of biological systems. The agility of Coagulum-OMICS to transverse primary and secondary care with genomic [pharma] pre-exams and viscoelastic or proteomic exams makes it a perfect learning initiative, self-assessment tool and governance program. The caveat is a need for expertise to sustain the success of coagulum-OMICs, in situ, with personalised and regeneration medicine.

Biography

James Henry completed his Master of Science (Upper Merit) in 2009 from Middlesex University U.Kin Molecular Pathology. Also he completed his Master of Science (Distinct) in 2014 from University of Greenwich U.K in Patient Blood Management Quality Systems. In 2014 a Patient Blood Management program was overseen by a U.K national governance representative, sponsored by an anesthetic lead and edited by an MHRA inspector who stated "this program is suitable for the NHS". In 2017 that program was posted to the National External Quality Assurance Scheme and then to the British Blood Transfusion Society. In 2018, ISO published the "Quality of an organization — Guidance to achieve sustained success". The author of "Blood Coagulum-OMICs" has developed a model for hemostasis and thrombosis genomic pre-exams and a viscoelastic & proteomic examination to improve predictive value in personalized and regeneration medicine.

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Zeynep E Çakmak et al., Biochem Mol biol J 2019, Volume:5
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MODIFICATION OF NUTRITIONAL PROPERTIES OF MICROALGAE FOR ARTEMIA BREEDING

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[2]Ege University, Turkey

Artemia (brine shrimp) is used as a live-feed stuff for seed fish in fish hatcheries and aquarium fisheries. Nutritional properties of Artemia are in close relationship with the nutritional facts of the microalgae it is fed by. In this study, 20 different microalgae and cyanobacteria (indigenous strains from *Dunaliella, Isochrysis, Phaeodactylum, Tetraselmis, Nannochloropsis, Spirulina, Synechocystis, Synechococcus, Chlamydomonas, Chlorella, and Scenedesmus* genus) were supplied to *A. franciscana* as food source and growth characteristics of *A. franciscana* and were followed during 10 days of growth. Seven microalgae strains were selected for Artemia breeding and dry weight, total protein, starch and lipid contents of microalgae and *A. franciscana* were recorded. Then, microalgae were exposed to N-, S-, P-deprivation and high salt stress for 5 days of incubation. Total lipid, protein and carbohydrate contents of those strains were recorded and 5-days stress exposed microalgae were supplied to *A. franciscana* as only food source. Lastly total lipid, protein and carbohydrate content of *A. franciscana* was followed during 10 days of growth. In most cases, feeding *A. franciscana* with D. tertiolecta was superior to other strains studied.

Biography

Zeynep Elibol Çakmak has completed her PhD in 2013 from Kırıkkale University. She has been working as an Academics Instructor in Bioengineering Department of İstanbul Medeniyet University, Istanbul, Turkey. She has published more than 15 papers in reputed journals. Her focus lies in the field of Microalgal Biotechnology. Nowadays, she has been working on a project regarding alteration of nutritional properties of microalgae for increased nutritional value of Artemia as fish food source.

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A CURRENT UPDATE OF METALLOTHIONEIN AND ITS ISOMERS

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This protein has several interesting biological effects including detoxification of heavy metals like mercury and cadmium, homeostasis of essential metals including copper and zinc, antioxidation against reactive oxygen species, protect against DNA damage, oxidative stress, cell survival, angiogenesis, apoptosis, as well as increase proliferation etc,. Numerous studies have been demonstrated increase focus on the role of MT in various biological systems in the past three decades. The studies on the role of MT were limited with few areas like apoptosis and antioxidants in selected organs even fifty years after its discovery. We now acknowledge the exploration of MT isomers such as MT-I, MT-II, MT-III and MT-IV in various biological systems and disease conditions like diabetic, kidney dysfunction, sclerosis, cancer, bone growth retardation, neuro toxicity etc in different organs (heart, CNS, kidney, etc) were established in recent years to research further.

Introduction: This article is an attempt to focus some of its important and current finding of isomers of metallothionein role in the biological system to explore in many new areas. The metallothionein (MT) was first isolated in 1957 from the cortex of horse kidney as a cadmium binding protein [1]. This protein was first reported by Kagi and Vallee in 1960 and by Kojima in 1976 as cysteine-rich (33 mol %), low molecular weight (7 kDa), heat-stable and metal binding protein.

Discussion: There are at least ten known closely related metallothionein proteins expressed in the human body. In humans, large quantities are synthesized primarily in the liver and kidneys, however they have been found at a number of other sites as well. Its production is dependent on availability of the dietary minerals zinc and selenium, and the amino acids histidine and cysteine present in the body. This article conclude that, many independent groups of investigators found direct casual relationships between MT and pathophysiology but more pronounced reasons among those was endogenous and exogenous stimuli including glucocorticoids, interferon, interleukin-1, progesterone, vitamin D3 endotoxins, serum factors, heavy metals, storage of metal ions and regulation of cellular zinc etc. may trigger the expression of MT in human and animal's body.

Biography

N. Thirumoorthy is working as professor cum director in PPG College of pharmacy Coimbatore India. He has 19 years of teaching and 8 years of research experience in his field. He completed his PhD at the age of 31 years from Jadvapur University . He has more than 30 papers in reputed journals at national and international level and has been serving as an editorial board member of repute. He is guiding students of both M.Pharm and Ph.D projects topics under various Universities in Tamilnadu. he received many awards particularly the best teacher award of the Tamilnadu Dr MGR Medical University couple of years back.

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INCORPORATION OF GENETIC MARKERS IN DIAGNOSTIC CRITERIA OF OSTEOARTHRITIS KNEE

Sachin Avasthi

RML Institute of Medical Sciences, India

Osteoarthritis knee is one of the most prevalent orthopaedic disorders worldwide. In Indian subcontinent, the prevalence of this disorder is estimated to be in the range of 22% to 39%. Many studies have been conducted for confirmation of association of genetic factors with clinical and radiological features of osteoarthritis knee. Nearly all have proven role especially in association with CALM-1, ESR-alpha, GDF-5 gene etc. Still we are diagnosing such a majorly prevalent disorder on the basis of clinical and radiological criteria only. We conducted a case control study in between for confirmation of the same and found statistically significant results. 120 cases and 120 controls were enrolled. Clinical and radiological features were noted and clinical scoring was done using VAS, WOMAC and Lequesne's scoring system. Genetic polymorphism in relation to intronic region of CALM 1 gene was studied by DNA extraction, PCR and RFLP method. Statistical analysis was done using Stata software. CALM-1 gene intronic SNP (rs3213718) was found in significantly higher number in cases than controls (p value=.0022). Logistic regression has also proved significant association of occurrence of Single nucleotide polymorphism (SNP) with disease. We concluded that CALM 1 gene intronic SNP (rs3213718) is present in Indian Population. The target SNP significantly affects the disease as the difference between cases and controls is highly significant (p value=.0022). We further discussed that there have been multiple such publications confirming the role of genetic factors in causation and progression of osteoarthritis knee. These criteria should be incorporated as biomarkers in diagnosis of osteoarthritis knee.

Biography

Sachin Avasthi is an Associate Professor and Head in Department of Orthopaedic Surgery, DR RML Institute of Medical Sciences, Lucknow. His qualifications are MBBS, MS, PhD. He has an experience of 19 years in the field of Orthopaedic Surgery after completing his graduation. He has more than 120 publications to his credit which include scientific papers, abstracts and book chapters. He has achieved ISAKOS scholarship which is a prestigious association of sports injury. He is an achiever of Health Icon Award by the State Government of UP in India. He has guided more than 45 students in their Postgraduate thesis work. He is Member of many national and international associations such as AO Trauma, IOA etc.

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DEVELOPMENT OF CIRCULATING BIOMARKERS FOR EARLY AND ADVANCED BREAST CANCER

Ana Pedro[2,3], Rod Tucker[1,2], Michael J Lind[3] and Barbara Guinn[3]

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[3]University of Hull, UK

Early work uncovered HCG1745306 isoform CRA-a, and histone H1.2 as potential specific plasma biomarkers for the identification of patients with early ER+ (estrogen receptor-positive) breast cancer. However, while these markers were absent in controls, the results were obtained from only two patients and therefore require verification in a larger patient cohort. Moreover, in another preliminary study, we identified potential extracellular vesicles (EV) factors which might serve as biomarkers for predictive and diagnostic purposes in metastatic breast cancer. Plasma samples from seven different metastatic and non-metastatic ER+ breast cancer patients were collected, EV were isolated and their protein content analyzed by mass spectrometry and FunRich analysis. In this study, we found several putative plasma EV biomarkers for metastatic ER+ breast cancer prediction and diagnosis, such as serum amyloid A1, known to promote widespread metastasis in a breast cancer animal model. In conjunction with academic and clinical colleagues from the Department of Biomedical Sciences at the University of Hull and Castle Hill Hospital, we propose to examine the pathophysiological role of the proteins found in the original works using tissue samples from patients with a confirmed diagnosis of breast cancer compared to adjacent benign breast tissues. If the initial results are confirmed, we will determine if these markers can be identified in whole blood samples from a larger cohort of patients with breast cancer. These projects have the potential to identify blood biomarkers for early breast cancer improving the specificity of mammography, allowing patients to be selected for auxiliary imaging based on the presence of specific biomarkers in the blood and to identify with certainty which early precursor lesions will progress to malignancy. Also, they have the potential to be used to identify both early breast cancer and whether the disease has metastasized to other sites. If confirmed in a large patient cohort, the biomarkers could be isolated and incorporated into a non-invasive test such as a lateral flow device that could be used for the detection of patients with early and metastatic breast cancer and to identify the sites to which the cancer has metastasized. This would help clinicians decide which patients will benefit of adjuvant therapy. If the original findings cannot be replicated, we will search for suitable biomarkers in urine or saliva samples or plasma samples using 2D gel electrophoresis and the scioDiscover platform.

Biography

Ana Pedro working as Pharmacist in Rowland's Pharmacy, she works in the development of test kits for circulating biomarkers for early and advanced breast cancer to be commercialized in community pharmacies. Also undertaken community clinical pharmacy research work and looking forward to develop PGDs and educational materials to pharmacists and bring overseas pharmacists to work in UK.

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IMPROVEMENT OF *SELENOMONAS RUMINANTIUM* β-XYLOSIDASE THERMAL STABILITY BY REPLACEMENT OF FREE BURIED CYSTEINES

E Dehnavi, M Aghaeepoor and A Akbarzadeh
Gene Transfer Pioneers (GTP) research group, Iran

Cysteine is an intriguing and enigmatic amino acid; while it is one of the least abundant amino acids in the structure of proteins, but it is frequently observed in functionally important sites of proteins such as catalytic, regulatory and cofactor binding sites. Moreover, its physio-chemical classification as a hydrophobic or polar residue is arguable. Whereas it has polar thiol group, free Cys in proteins is often buried and surrounded by a hydrophobic environment. It has been shown that both removal and insertion of Cys can lead to increase protein thermo-stability. Since *Selenomonas ruminantium* β-D-xylosidase (SXA) has four free cysteines, it used as a model. To characterize the role of cysteine residues in the structure, function and stability of SXA, we prepared and evaluated wild-type and four cysteines deficient SXA proteins. Buried cysteine residues, were replaced with valine using QuikChange site-directed mutagenesis. In comparison with the wild-type, the Km values remained relatively constant while the kcat decreased in mutants. The optimal pH and temperature were similar in the wild-type enzyme and its variants. The C101V and C286V displayed higher thermal stability than the wild-type at 55 and 60 °C. Secondary and tertiary conformational changes using circular dichroism and fluorescence spectroscopy revealed that changing a buried cysteine to a hydrophobic residue could lead to an increase in thermal stability with minimal perturbation of the overall wild-type protein structure. In addition to experimental methods, the stability of WT SXA and C101V and C286V mutants at 333 K was also studied by MD simulation. Our theoretical data had a good agreement with the experimental results.

Biography

E Dehnavi has graduated in Biochemistry in 2015 from Tarbiat Modares University, Tehran Iran. His doctoral dissertation was conducted in consultation with Prof Khajeh and examines the use of protein engineering methodology for improving kinetic properties of hemicellulosic enzymes. He has been working on the expression of *Selenomonas ruminantium* Xylosidase in yeast *Pichia pastoris*. Moreover, he worked to improve the thermal stability of some industrial enzyme by site-directed mutagenesis. Currently, he is a Team Leader in Gene Transfer Pioneers research group, the company is active in the field of biotechnology where optimization of protein expression of some industrial enzymes such as endoglucanase, xylosidase and phytase through protein engineering and producing more effective expression vectors is done. His current research is increasing enzymatic saccharification yields through cellulase and hemicellulase enzymes protein engineering. He has published 10 papers in reputed journals.

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DAY 2

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A MODEL AND GUIDE FOR BLOOD COAGULUM-OMICS

James Andrew Henry

Atlantis BMS Limited, UK

The NHS will be a world-leading healthcare organization to predict and diagnose inherited and acquired disease, and to personalize treatment and intervention. This program was designed to validate and quality manage the introduction of Blood Coagulum-OMICS and verify genomic, viscoelastic, and proteomic predictive value for hemostasis and thrombosis.

Background: In 2014, a Patient Blood Management program was overseen by a national governance representative, sponsored by an anesthetic lead and edited by an MHRA inspector who stated "this program is suitable for the NHS". In 2017, that program was posted to the hemostasis and thrombosis, National External Quality Assurance Scheme and then to the British Blood Transfusion Society, in the UK.

Study: The conclusion read as "scientific specialists are now firmly planted in the realms of clinical effectiveness, interfacing clinicians on the governance board. We must now accelerate the PBM Quality Assurance network to control risk from genomic and proteomic explosions in personalized medicine. Quality assures our technological advances from end to end of the surgical examination phase and control our pharmacological breakthroughs in support of healthcare clinicians.

Program Development: On the 4th of July 2017, Professor Dame Sally, the Chief Medical Officer of the UK called on the NHS to provide access to genomic sequencing, as standard. This followed studies that realised genome models to pre-empt a bleed or thrombotic event. Meanwhile coagulation and fibrinolysis elasticity reference ranges to monitor a clinical event or target a therapy are developing, at a time when coagulation proteomics have passed proof of concept.

Conclusion: This second program on Blood Coagulum-OMICS was designed to stop the bleed and thrombotic event by improving the predictive value in pre-examination and examination phases. A program for Blood Coagulum-OMICS is a minimum standard for haemostasis and thrombosis and requires consideration by the International Organisation for Standardisation.

ISO. STD	REVISED	INTERNATIONAL ORGANISATION FOR STANDARDISATION: TITLE
10005	2005	QMS – Guidelines for Quality Plans
14155	2011	Clinical Investigation of Medical Devices for Human Subjects – GCP *
15189	2012	Medical Laboratories – Requirements for Quality and Competence
27001	2013	Information Security Management Systems - Requirements
13485	2016	Medical Devices – QMS – Requirements for Regulatory Purposes
31010	2009	Risk Management – Risk Assessment Techniques
18113	2009	Specific Requirements for Info. Supplied by the Manufacturer of IVD reagents
14791	2012	Risk Management for Medical Devices
22870	2016	Point of Care Testing (POCT) – Requirements for Quality and Competence
17043	2010	Conformity Assessment – General Requirements for Proficiency Testing
9000	2015	QMS – Fundamentals and Vocabulary
90011	2011	Guidelines for Auditing Management Systems
17011	2017	Conformity Assessment General Requirements for Accrediting Conformity

***Excludes IVD Medical Devices [This excludes viscoelasticity. An application to spectral proteomics is unknown]**

Table 1. List of ISO standards in a Blood Coagulum-OMICS program

Biography

James Henry completed his Master of Science (Upper Merit) in 2009 from Middlesex University U.Kin Molecular Pathology. Also he completed his Master of Science (Distinct) in 2014 from University of Greenwich U.K in Patient Blood Management Quality Systems. In 2014 a Patient Blood Management program was overseen by a U.K national governance representative, sponsored by an anesthetic lead and edited by an MHRA inspector who stated "this program is suitable for the NHS". In 2017 that program was posted to the National External Quality Assurance Scheme and then to the British Blood Transfusion Society. In 2018, ISO published the "Quality of an organization — Guidance to achieve sustained success". The author of "Blood Coagulum-OMICs" has developed a model for hemostasis and thrombosis genomic pre-exams and a viscoelastic & proteomic examination to improve predictive value in personalized and regeneration medicine

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PROTEOME PROFILING IN THE VASCULAR SMOOTH MUSCLE CELL IN RESPONSE TO BRADYKININ AND LEPTIN

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[1]American University of Beirut, Lebanon
[2]Texas Tech University, USA
[3]Qatar University, Qatar

Background: Atherosclerosis is the leading cause of cardiovascular diseases and a worldwide health burden imposing a considerable health toll. Vascular fibrosis and inflammation in the vessel walls are among the risk factors of atherosclerosis development, characterized by vessel stiffness and subsequent loss of elasticity. Bradykinin (BK) and leptin have been previously shown to be involved in the development of atherosclerosis through their effect on vascular fibrosis and inflammation processes.

Aim: In this study, we investigate the effect of BK and leptin on the global protein profile in vascular smooth muscle cell (VSMC), employing the LC-MS/MS technique and systems biology analysis, to gain insight into the different pathways modified by BK and leptin, and the diseases and biological pathways they are involved in, and to search for a candidate molecule(s) that would serve as a biomarker for the progression of vascular injury.

Results: In our study, we identified 1837 proteins in the control samples. Among these proteins, BK modified 70 (3.8%) and 120 (6.5%) proteins compared to controls after 24 and 48 hrs, respectively. BK induced the expression of the leptin receptor, TGFβ and COX1 in VSMC by promoting vascular fibrosis and inflammation. On the other hand, leptin modified 189 (10.2%) and 127 (6.5%) proteins compared to controls after 24 and 48 hrs, respectively. For instance, leptin induced the expression of collagen IV suggesting a role of leptin in the development of vascular fibrosis. Furthermore, leptin reduced cofilin expression, confirming the role of leptin in actin remodeling. Finally, pathway analyses indicated that the activation of MAPKs and AKT pathways to be a common mediator between BK and leptin signaling.

Conclusion(s): BK stimulation showed a proteomic pattern favoring vascular fibrosis, inflammation and the involvement of the leptin pathway. On the other hand, leptin stimulation induced ECM proteins and reduced actin remodeling proteins. These findings point to a possible interaction between BK and leptin pathways in VSMC to promote vascular injury.

Biography

Moustafa Al Hariri is Co-ordinator of Department of Emergency Medicine Research Unit at the American University of Beirut Medical Center (AUBMC). He has graduated with a PhD degree in 2017 from the American University of Beirut. After Graduation, he has joined the Department of Emergency Medicine at AUBMC to coordinate and manage the clinical and biomedical research activities in the department. His research work since included oversea and supervise the quality of research activities in the department, search for funding opportunities for the projects, and increase the research visibility of the department research activities.

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EFFECT OF PROPEPTIDE ALTERATION ON THE EXPRESSION OF RECOMBINANT HUMAN FACTOR IX IN DROSOPHILA S2 CELL LINE

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Production of biologically active human vitamin K-dependent proteins (VKDPs) in heterologous hosts is challenging, due to insufficient carboxylation. In VKDPs, an N-terminal propeptide containing recognition site for γ-glutamyl carboxylase (GGCX) is required for carboxylation. The weak-binding of the propeptide to GGCX, increases the carboxylation rate of the protein. The human prothrombin (hPT) is highly carboxylated and its propeptide affinity to GGCX is 10-fold weaker than that of the human factor IX (hFIX). To study the function of the hPT propeptide on the carboxylation efficiency of hFIX in Drosophila cell, we generated three constructs, based on a Drosophila-specific expression vector, carrying a chimeric hFIX cDNA next to the hPT pre-pro sequence, a mutant hFIX cDNA carrying an R-9N substitution in its propeptide, and a normal hFIX cDNA. The three constructs were subjected for transient expression analysis of hFIX in a Drosophila cell line, by performing coagulation test, ELISA and γ-carboxylation assay, on the cultured media after various post transfection time. Based on the results obtained, the functional impact of the hPT propeptide on the hFIX γ-carboxylation, in a distantly related host, was addressed. Our finding suggested the saturation of Drosophila GGCX, as a result of accumulation of hFIX in endoplasmic reticulum. These results demonstrated the functional importance of amino acid at position -9 in a mammalian derived propeptide on the expression efficiency of its cognate protein.

Biography

Samira Bahrami has completed her MSc in the field of Biochemistry from Tehran University. Her thesis was about Studying the expression hFIX, when it's signal/propetide is replaced with that of the human propetide. This study was supported by a grant (Project No. 372) from the National Institute of Genetic Engineering and Biotechnology (NIGEB) of Iran. She has worked at the NIGEB as a Research Assistant for about two years. Now, she is a PhD candidate in Molecular Medicine at Shahid beheshti University of Medical Sciences, Tehran, Iran. Her PhD thesis is about Assessment of protein expression pattern in muscle-invasive bladder tissue using 2-DE and MS techniques. This study is performing for the first time in Iran and is supported by a grant from the National Institute for Medical Research Development (NIMAD).

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EFFICIENT GENOME-WIDE ASSOCIATION STUDIES AND POST-GWAS INTEGRATIVE ANALYSES FOR HUMAN CANCER AND NEURODEGENERATIVE DISEASES

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It is evident that in etiologies of human complex diseases, genetic factors play some important roles. Genome-wide association study (GWAS) is a standard technique to identify heritable genetic basis of complex diseases. In relation with GWAS, there exist some challenges in selecting input samples completely randomly, to biologically describe GWAS results, to translate them into clinical benefits and to compare germ line variants achieved from GWAS with somatic mutations in creating, development and treatment of human complex diseases. Likelihood-based statistical methods are robust in estimating linkage disequilibrium when factors like non-randomness and population structures exist. Then the results of GWAS can be used for post-GWAS analyses to predict multiple biological components like genes, non-coding RNAs and transcription factor binding sites in association with complex diseases. An integrative analysis seeks to pool information from multiple GWAS results, somatic mutations and genetic drug targets of human complex disorders and the results of such analysis can provide new insight into the genetic and treatments of complex diseases. This presentation is prepared from the viewpoint that the robust statistical methods can be applied to arrive at valuable results from GWAS and that primarily genetic information derived from GWAS is subject to further post-GWAS analysis to provide more biologically informative results in relation with genetics of human complex diseases that can be applied to real time clinical applications. Then the results of such analyses can be used to discuss and compare human cancers and neurodegenerative diseases from a genetic perspective. We concluded that in spite of the differences between human cancers and neurodegenerative diseases, the roles of germ line and somatic mutations in creating, developments and treatments of those two kinds of human complex diseases are similar.

Biography

Zahra Mortezaei has completed her Undergraduate in Mathematics from Amirkabir University of technology (Tehran Polytecnique), Iran and studied for M Phil degree in Mathematical physics at University of Nottingham (UK). She then completed her PhD in Bioinformatics at University of Birmingham (UK) and the University of Tehran (Iran). She is working as Bioinformatician at human genetic research centre in Iran.

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CREATION OF A TECHNO-MEDICAL PLATFORM FOR EMERGENCY CONSULTATION OF INVESTIGATIVE AND THERAPEUTIC ORTHOPAEDICS IN THE FORM OF AN ANDROID APPLICATION

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Shri Ramswaroop Memorial Group of Professional Colleges, India

Orthopaedic disorders are diverse and many a times we get coexisting pathologies existing in a same patient at the same time. Therapeutic modalities need to be planned such that we can address all the pathologies at the same time. This needs a lot of varying investigations and biomarker assessments to be done in a single sitting. In emergency situation, when the life and limb salvage procedures are being done on the patient, the treating team might miss to recall some of the investigative procedures. Our aim was to make an android application which assists the emergency orthopaedic team for complete management of the patient. We created an android application on the basis of iterative water fall model. The main objective of iterative development is to build the system incrementally, starting from the partial system features and gradually adding more features until the entire system is completed. The model passed through various phases like system and software requirements, architectural and detailed design, Coding, testing etc. Initially the data flow diagram was made which passed through level 1 assessment followed by entity relationship diagram. We delivered a working android application which will help orthopaedic surgeons and the attendants of patients to understand the type and seriousness of ailment they are suffering from. The treating team will not miss any investigation or therapeutic need after making diagnosis of the patient.

Biography

Priya Shukla has done her schooling from Canossa Girl's Inter College and Loyola Public School. She is a promising B Tech Graduate with aspirations to serve patients utilizing her technical knowledge. She is a Young Scientist Achiever and her scientific project has been sanctioned by government institutes for funding.

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MIXTURE OF ALKALOIDS AFFECTS MMP9 PROTEIN EXPRESSION IN AN INFLAMMATORY *IN VITRO* MODEL

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Background: Matrix metalloproteinas-es (MMPs) are a large family of ubiqui-tously expressed zinc-dependent enzymes with proteolysis activities. They are ex-pressed in physiological situations and pathological conditions involv-ing inflammatory processes, Epithelial to Mesenchymal Transition (EMT), neuronal injury and cancer. There is also evidence that MMPs regulate inflammation in tumor microenvironment, which plays an im-portant role in cancer progression. Looking at both inflammatory and neuronal damag-es, MMP, specially MMP-2 and MMP9 are involved in both processes and their modulation seems to be regulated by two major actors as tumor necrosis factor alpha (TNF-alpha) and interleukin 6 (IL-6). The BV-2 cells (microglial cells of mouse) were been used as *in vitro* model fto simulat both inflammatory and neuronal injury pathologies. In these models, MMP9 seems to be involved in cellular migration throughout inflammatory activation in de-pending manner. Leonurine, an alkaloid derived from Herbal Leonuri, seems to af-fect the induced inflammatory expression in BV-2 cells, while, the effects of alka-loids against MMP9 seem not to be demonstrated in BV-2 cells. Nevertheless, Ukrain (UK) a mixture of alkaloids had demonstrated to regulate the MMP9 ex-pression. Aim of this study was to investi-gate the role of alkaloids against MMP9 in BV-2 cells.

Materials & Methods: The immortalized murine BV2 cell line (ATCC Cell Line Collection, Milan, Italy) was cultured in RPMI 1640 medium with phenol red (nvi-trogen) supplemented with 10% fetal bo-vine serum (FBS), 1% penicillin-streptomycin (Invitrogen), and 1% gluta-mine (Invitrogen). Cultures were grown at 37 °C in 5% CO2 until 50% confluence. BV-2 cell culture was used to investigate the MMP9 expression by ELISA test and for Immunofluorescence (IF) assay. BV2 mouse microglial cells were seeded in 12-well plates, in order to obtain three differ-ent experiments for UK concentration 5 µM. The inflammatory stimulation was induced by lipopolysaccharide (LPS). We used BV-2 treated with UK alone, as con-trols. In all experiments the cells were treated for 24 hrs. ELISA tests: the medi-ums were harvested for ELISA analyses of MMP9. 2) IF Analyses. BV2 mouse mi-croglial cells were seeded in 8-well Cham-ber Slides (CS) (Lab-Tek1 Chamber Slide™ system, Nalge Nunc International, Naperville, IL, US), putting in 5000 cells/well in a 650 µ L final volume. CS was prepared in order to obtain three dif-ferent experiments in triplicate. After treatments, cells were fixed directly on the slides by Carnoy's solution for 10 min and the chamber slide wells were removed by mechanical support following manufactur-er's instructions. The IF for MMP9 protein detection was performed using a monoclo-nal primary antibody anti-MMP9, followed by Fluorescent secondary antibody. The nuclei of cells were counterstained using a DAPI solution.

Results: We performed the calibration curve of MMP9 and we tested the presence of MMP protein in BV-2, before the treatments. The MMP9 protein expres-sion was present inside BV-2 before the chemical treatment. The MMP9 expression was down regulated in both cultures LPS+UK and UK with respect to their controls. In particular, we showed that MMP9 concentration gone down during UK treatment (p=0.0001). Indeed, looking at IF profiles, the levels of MMP9 decreased drastically with respect to those observed in their respective controls.

Conclusions: There is increasing evidence that mixture of alkaloids can affects MMPs protein expression not only in cancer, but in other *in vitro* models. Additional precise information on the MMP interaction with other protein might open novel therapeutic treatments for inflammatory diseases and cancer blocking overexpressed actions of MMPs

Biography

Wassil Nowicky, Director of "Nowicky Pharma" and President of the Ukrainian Anti-Cancer Institute (Vienna, Austria). Has finished his study at the Radiotechnical Faculty of the Technical University of Lviv (Ukraine) with the end of 1955 with graduation to "Diplomingeniueur" in 1960 which title was nostrificated in Austria in 1975. He is the Inventor of the anticancer preparation on basis of celandine alkaloids "NSC-631570". He is an Author of over 300 scientific articles dedicated to cancer research. He is a real Member of the New York Academy of Sciences, Member of the European Union for Applied Immunology and of the American Association for scientific progress, Honorary Doctor of the Janka Kupala University in Hrodno, Doctor "honoris causa" of the Open international university on complex medicine in Colombo, Honorary Member of the Austrian Society of a name od Albert Schweizer. He has received the award for merits of National guild of pharmacists of America. the award of Austrian Society of sanitary, hygiene and public health services and others.

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