Poster Presentations

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Sendai virus recruits cellular villin to remodel actin cytoskeleton during fusion with hepatocytes

Sunandini Chandra
University of Delhi, India

Reconstituted Sendai viral envelopes (Virosomes) are well recognized for their promising potential in membrane fusion mediated delivery of bioactive molecules to liver cells. Despite the known function of viral envelope glycoproteins in catalyzing fusion with cellular membrane, the role of host cell proteins remains elusive. Here, we used two-dimensional differential in-gel electrophoresis (2D-DIGE) to analyze hepatic cells in early response to virosome-induced membrane fusion. Quantitative mass spectrometry together with biochemical analysis revealed that villin, an actin-modifying protein, is differentially up-regulated and phosphorylated at Threonine-206 (T206), as an early molecular event during membrane fusion. We found that villin influences actin dynamics which, in turn, promotes membrane mixing through active participation of Sendai viral envelope glycoproteins. Modulation of villin in host cells also resulted in a discernible effect on the entry and egress of progeny Sendai virus. Taken together, these results suggest a novel mechanism of regulated viral entry in animal cells mediated by host factor villin.

Speaker Biography

Sunandini Chandra is trained in the field of virus-host interactions, especially in the field of viral fusion with host cell membrane. After a Master's in Biotechnology, she recently completed her Doctoral research work in Sendai virus-host cell interactions, with special emphasis on the role of actin modifying proteins in fusion. Her work employed proteomic approaches and is the first to result in identifying a host cell protein- villin, implicated in virus induced membrane fusion with the host cell membrane. This work provides an insight into the mechanism of membrane fusion mediated entry of enveloped viruses and may be exploited for therapeutic interventions for other related pathogenic viruses. Also, this information could be exploited to improve fusion efficiency of Sendai virosomes, for efficient targeted delivery to liver cells.

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Long-term effect of policosanol on the functional recovery of non-cardioembolic ischemic stroke patients: A one year study

Julio C Fernández[1], Javier Sánchez[3], José Illnait[2], Rosa Mas[1], Sarahí Mendoza[1], Lilia Fernández[1], Meilis Mesa[2], Hermys Vega[2], Pablo Reyes[4] and **Dalmer Ruiz[4]**

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Introduction: Stroke is a leading cause of mortality and disability. Policosanol has been effective in brain ischemia models. Clinical studies suggested that policosanol (20 mg/day) + standard aspirin (AS) therapy had benefits versus placebo + AS given for 6 months to patients with recent non-cardioembolic ischemic stroke. The objectives of this study investigate whether policosanol, added to AS therapy within 30 days of stroke onset, is better than placebo + AS for the long-term recovery of non-cardioembolic ischemic stroke subjects.

Methods: This study was randomized, double-blind, placebo-controlled. Eighty patients with a modified Rankin Scale score (mRSs) 2 to 4 were randomized, within 30 days of onset, to policosanol/AS or placebo/AS, for 12 months. The primary outcome was mRSs reduction; the secondary outcome is the increase of Barthel Index (BI). Low-density lipoprotein-cholesterol (LDL-C) reduction and high-density lipoprotein-cholesterol (HDL-C) increase were collateral outcomes.

Results: Eighty patients (mean age: 69 years) were randomized. Policosanol/AS decreased significantly mean mRSs from the first interim check-up (1.5 months) ($p<0.0001$ vs placebo/AS). The treatment effect did not wear off, even improved, after long-term therapy ($p<0.0001$ versus placebo/AS). More policosanol/AS (35/40, 87.5%) than placebo/AS (0/30, 0.0%) achieved mRSs ≤1 ($p<0.0001$). Policosanol/AS increased significantly BI, lowered LDL-C and increased HDL-C versus placebo/AS, Treatments were well tolerated. There were 12 withdrawals, three due to fatal adverse events, all happened in the placebo/AS groups.

Conclusions: Long-term (12 months) administration of policosanol/AS given after suffering non-cardioembolic ischemic stroke was shown to be better than placebo/AS in improving functional outcomes at 3 and 12 months when used among patients with non-cardioembolic ischemic stroke of moderate severity.

Speaker Biography

Julio C Fernández is a Senior Investigator in Clinical Trials Unit, National Centre for Scientific Research, Havana city, Cuba. He has completed his BSc in Pharmaceutical Sciences from Havana University Cube in 1996. He was awarded with PhD in Pharmaceutical Sciences in 2003. He has published more than 130 publications and presented more than 100 papers in various scientific events. His research interest mainly focuses on clinical trials phase I-IV of different natural products: Policosanol, Abexol, Prevenox, Palmex.

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Lipid-lowering and antioxidant effects of policosanol in diabetic patients: A pilot study

Julio C Fernández[1], José Illnait[1], Lilia Fernández[1], Sarahí Mendoza[1], Ambar Oyarzábal[1], Sonia Jiménez[1], Meilis Mesa[2] and **Ernesto López[2]**
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Introduction: Coronary artery disease is the major complication and leading cause of death among patients with diabetes mellitus. Oxidative stress and dyslipidemia plays an important role in the pathogenesis and complications of diabetes. Policosanol is a mixture of primary aliphatic alcohols purified from sugar cane wax. The objective of this randomised, double-blinded and placebo-controlled pilot study was to investigate the effect of policosanol treatment on lipid profile and plasma oxidative variables in diabetic patients with hypercholesterolemia.

Methods: Thirty diabetic patients of both sexes, aged 50 to 70 years were enrolled in the study. Fifteen patients were treated with policosanol (10 mg/day) and 15 with placebo for 12 weeks. The primary efficacy variable was to significant reduced low-density lipoprotein-cholesterol (LDL-C) values. Plasma oxidative markers were secondary variables.

Results: Baseline characteristics were well matched in both groups. After 12 weeks policosanol produced significant reductions of LDL-C and total cholesterol and increase in high-density lipoprotein-cholesterol. In addition, serum malondialdehyde significantly decreased and the total antioxidant capacity of the plasma increased. There were no significant changes in any of the variables in the placebo group. Treatments were safe and well tolerated. No patient withdrew from the study.

Conclusions: Policosanol treatment favorably modified lipid profile and plasma oxidative variables in diabetic patients. Further studies should expand more data on the effects of policosanol treatment in diabetic patients.

Speaker Biography

Julio C Fernández is a Senior Investigator in Clinical Trials Unit, National Centre for Scientific Research, Havana city, Cuba. He has completed his BSc in Pharmaceutical Sciences from Havana University Cube in 1996. He was awarded with PhD in Pharmaceutical Sciences in 2003. He has published more than 130 publications and presented more than 100 papers in various scientific events. His research interest mainly focuses on clinical trials phase I-IV of different natural products: Policosanol, Abexol, Prevenox, Palmex.

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Human c-Cbl and Cbl-b proteins are more highly expressed in the thymus compared to the testis

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Background & Objectives: c-Cbl and Cbl-b are two members of the Cbl family proteins, with a crucial role of downregulation of tyrosine kinase receptors. They act as E3 ubiquitin ligases and are multivalent adaptor proteins, making them important in maintaining homeostasis in the body. This study investigated the expression level in thymus and testis in normal conditions.

Methods: The expression level was assessed by immunochemistry of tissue microarrays of normal thymus and testis biopsies.

Results: Cbl-b and c-Cbl proteins were found to be highly expressed in normal testis and thymus, indicated as yellowish-brown granules in the cytomembrane and cytoplasm compared to controls. The c-Cbl appears to be more highly expressed than the Cbl-b in the thymus, while c-Cbl appears slightly stronger than Cbl-b in the testis. The thymus was found with a higher grade compared to testis.

Conclusion: In this work, we concluded that in normal condition, thymus tissue expresses more Cbl family proteins (c-Cbl and Cbl-b) than the testis tissue in humans.

Speaker Biography

Mazo Kone received basic training in biology of cancer, physiopathology of metabolic diseases, infectious diseases and many more. However, he quickly developed an interest for the molecular biology of cancer, physiology of the cell and infectious diseases. He worked on the oncogenic properties of Human c-Cbl and Cbl-B as Master's project work. Currently, he is doing a Ph.D in Cell Biology and Genetics at the University of Ibadan in Nigeria. His research is on congenital infections in pregnancy both in Mali and Nigeria. In general, his research works are focused on molecular biology of cancer and infectious diseases. He is the Leader of Rachetes Algeria since 2012, the promoter and the manager of The Biomedical researcher project. He is a Lecturer in Mali (Universite Scientique Libre de Bamako). He is also a writer and an author in personal development. He likes research, teaching, reading and communication.

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A study on effect of anti-tuberculosis drugs on liver function

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University of Rwanda, Rwanda

A study is aimed to assess the effect of anti-tuberculosis drugs on liver function among the patients who attended King Faisal Hospital during the period of two years. A sample of 165 tuberculosis patients receiving the standard treatment of TB drug during the period of two years was done, only 22 patients for whom liver function test was done before and during treatment with anti-tuberculosis (TB) drugs were considered. The hepatotoxicity consisted of high concentration of ALT (Alanine Amino Transferase)/ AST (Aspartate Amino Transferase), Jaundice or high concentration of total bilirubin. The result revealed that among 22 patients, 68.5% had ALT/AST at normal range at the beginning of treatment with anti TB drugs. After 3 months of anti TB therapy the record showed that 45% of them has ALT beyond the normal range and after 6 months of therapy the ALT level showed high frequency of liver dysfunction up to 79%. It is essential to monitor liver function from beginning of therapy with anti-tuberculosis drugs.

Speaker Biography

Liliane Uwimana is working as a Pharmacist in Pharmacie Royale and has completed her Bachelor's degree from University of Rwanda. She has worked in King Faisal Hospital as Pharmacist where she did her research on Effect of Anti Tuberculosis Drugs on Liver Function.

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Co-regulation of the Glycine max soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor (SNARE)-containing regulation occurs during defense to a root pathogen

Keshav Sharma
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Heterodera glycines, also known as Soybean Cyst Nematode (SCN) is a major pathogen of soybean (Glycine max), causes nearly a billion-dollar loss in U.S. every year (Wrather et al. 2001; Wrather and Koenning, 2006; Smolik and Draper, 2007; Koenning and Wrather, 2010). Efforts to combat SCN include production of resistant soybean varieties, use of nematicides, improved crop rotation and bio-control methods (Wrather et al. 1984; Chang et al. 2011). However, effective control has not been achieved yet. Study of host plant interactions at the cellular level is important as it may provide new species-specific means of controlling SCN (Klink et al. 2007). We are conducting various molecular approaches to find actual cellular mechanism of host resistance. Closer study of infected cells in resistant variety G. max [Peking/PI548402] and susceptible variety G. max [Williams 82(PI518671)] through laser microdissection have resulted various unique genes present in G. max [Peking/PI548402] (Klink et al. 2007; 2009). Overexpression of these genes in susceptible cultivar G. max [Williams 82(PI518671)] have resulted resistance by inducing incompatible reaction and RNA interference of these genes in resistant genotypes resulted susceptible reaction, thereby inducing compatible reaction (Matsye et al. 2012; Pant et al. 2014). In this approach we have overexpressed the components of the Soluble N-ethylmaleimide-sensitive fusion (NSF) Attachment Protein (SNAP) REceptor (SNARE) complex that helps in docking of the vesicles to the membrane and subsequent release of the vesicular contents to the apoplast (Jahn and Fasshauer et al. 2012; Matsye et al. 2012; Pant et al. 2014). There are many proteins that play significant role in this process however, the core components of this study are syntaxin 121 (SYP121), Synaptosomal-associated protein 25 (SNAP-25), Synaptotagmin (SYT), Synaptobrevin (SYB), Secretion 1/mammalian uncoordinated-18 ([Sec1]/Munc18) and N-ethylmaleimide-sensitive fusion protein (NSF). Syntaxin 121, G. max homolog of Saccharomyces cerevisiae, Suppressor of sec1 (SSO1) known as PENETRATION1 (PEN1) in Arabidopsis thaliana, (Collins et al. 2003) function in resistance to Heterodera glycine. Co-expression of SYP121 with SNARE homologs results elevated transcripts in infected cells inducing resistance reaction.

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Histopathological changes in male Wistar rats maintained on a water-based *Sutherlandia frutescencs* extract

Nicolas John Wickens
Nelson Mandela University, South Africa

In this study, a standardized 46-week chronic drinking water toxicity protocol was used to elucidate the toxic potential of *Sutherlandia frutescens* using histopathologic, morphometric and transmission electron microscopic analysis. In this study, the histopathologic changes in the duodenum, heart, kidney, liver, lung, pancreas and spleen of male Wistar rats was evaluated. Fifty-four rats were randomly divided into four groups: Group 1–Normal diet control (ND control), n=7, Group 2–Normal diet + plant extract (ND+p), n=9, Group 3–High fat diet control (HFD control), n=19. Group 4–High fat diet + p (HFD + p), n=19. In the high fat group male Wistar rats were fed ±55 g/day of a specialized high fat diet over a 46-week period to induce obesity and an insulin resistant state. The treatment groups (groups 2 and 4) received a dose concentration of a tea extract of the *S. frutescens* plant in their drinking water daily. This study showed that the consumption of *S. frutescens* significantly reduces weight gain per week in male Wistar rats on a chronic high fat diet ($p \leq 0.001$ vs. HFD control group). *S. frutescens* appears to propagate periportal and centrilobular glycogen storage in rat hepatocytes in the experimental groups as exemplified by a significantly ($p \leq 0.0001$ vs. control groups) increased incidences of Periodic Acid Schiff (PAS) positive staining. *S. frutescens* also reduced intracellular lipid accumulation as made evident by the significantly lower incidence of epicardial adipose tissue (EAT), hepatic steatosis and pancreatic interstitial fat. Obesity was associated with increased fibrotic lesions such as myocardial perivascular fibrosis, centrilobular hepatic fibrosis and pancreatic periductal fibrosis. In this study, pulmonary infection was equally prevalent in all rats. Despite the complex histopathology in all groups' unique histopathology such as a conservative PMNL infiltration, substantial intra-alveolar oedema and focal arterial wall hypertrophy in the control groups was highly suggestive of Sendai viral infection. However, histopathologic evidence in the treatment groups, suggested chronic recurrent viral infection with superimposed *Mycoplasma pulmonis* bacterial infection. The impact of advanced suppurative pulmonary infection was widespread and exemplified by increased lesion incidences of spontaneous murine progressive cardiomyopathy (MCP) and spontaneous chronic progressive nephropathy (CPN) among others. In conclusion, *S. frutescens* administered for 46 weeks to male Wistar rats significantly lowered intracellular lipid accumulation and obesity associated myocardial, renal, hepatobiliary, pulmonary and pancreatic histopathology. Moreover, duodenal, cardiovascular, hepatobiliary, pulmonary, renal, pancreatic and splenic tissue did not show histopathologic evidence of direct plant extract associated carcinogenicity or toxicity.

Speaker Biography

Nicolas John Wickens has completed his Doctorate from the Nelson Mandela University in South Africa. He is a Lecturer of Pathology and Histology in the Department of Medical Laboratory Sciences in the Faculty of Health Sciences. After his pre-med studies at the University of Stellenbosch, he went on to complete a Master's degree in Medical Laboratory Sciences at the Nelson Mandela University, where he was offered a post as Lecturer in the department. During his Doctorate studies, he investigated the histopathology found in male Wistar rats after chronic consumption of an extract of the plant *Sutherlandia frutescencs* which is used in South Africa by the indigenous people to lower blood sugar in patients with type 2 diabetes.

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Estimation of phytochemicals and evaluation of their antimicrobial & antioxidant activity In *Nardostachys jatamansi*

Mohammad Sajid
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Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are nonessential nutrients, meaning that they are not required by the human body for sustaining life. It is well-known that plant produces these chemicals to protect themselves but recent research demonstrate that they can also protect humans against diseases. *Nardostachys jatamansi* is incense, a sedative and an herbal medicine said to fight insomnia, birth difficulties and other minor ailments. The medicinal properties of the plant are due to the anti oxidants present in the plant. My Present studies deals with the identification of phytochemicals and there quantitative estimation, antimicrobial activity of *Nardostachys jatamansi* and its important medicinal qualities provided one does not play around with poisonous plants they are totally free of harmful side effects - unlike the modern drug industry

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The constitution of the cancer tissues

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The growth of cancers depends on the formation of blood vessels that provide the supply of nutrients and oxygen and the nervous system acts as a crucial part of cancer microenvironment. The angiogenesis and vasculogenesis are essential during cancer progression. Recently, several studies have reported that glioblastoma stem cells are able to give rise to tumor vascular endothelial cells (ECs) and vascular pericytes for tumor vascularization. However, there is no evidence to show that the tumor stem cells from other kinds of tumors including carcinoma produce endothelial cells to constitute the functional blood vessels in cancer. In addition, recent evidences have demonstrated that the stimulation of both cancer growth and metastasis by members of nervous system such as neurons. How the nervous system built in cancer tissues is unknown. We transplanted the cancer stem cells that were isolated from the patients with gastric and colorectal carcinoma into nude mice via subcutaneous and intraperitoneal injections to produce human cancer xenografts. Then the innervations, angiogenesis were examined and the origins of the neural cells and endothelial cells were determined in cancer tissues. A single cancer stem cell from the cancer tissues of human patients could generate neurons including sympathetic and parasympathetic cells to take part in the nervous system in cancer tissues. Knocking down the neural generating capabilities of the human cancer stem cell inhibited the growth of tumors. Next, we show that cancer stem cells of the human colorectal carcinoma (CoCSCs) give rise to vascular endothelial cells and compose the vasculatures in cancer tissues. NuMA+ EC incorporated blood vessels were functional. Our data demonstrate that neural cells and endothelial cells originated from human cancer stem cells constitute blood vessels and nerve system for the cancer progression in the cancer tissues.

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Biogelx: Designer gels for cell culture and bioprinting

Elia Lopez-Bernardo, Eleanore Irvine, Rein Ulijn and **David Lightbody**
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Biogelx is a biomaterials company that designs tunable peptide hydrogels, offering artificial tissue environments to cell biologists for a range of cell culture applications. The hydrogels are highly tunable, cell-matched biomaterials, capable of revolutionizing the way cell biologists control and manipulate cell behavior in the laboratory. This is of direct relevance to fundamental cell research, including the study of stem cell biology and disease models within academic and medical labs. Biogelx's hydrogels also have a potentially dramatic impact on harnessing the capabilities of 3D bio-printing; where they are being used as the 'bio-ink' in the printer. Biogelx offers a range of hydrogel platforms that are three dimensional (3D), 99% water and have the same nanoscale matrix structure as human tissue. This gives control back to the cell biologist, as the gels can be tuned to meet the needs of any given cell type. This presentation showcases the underlying chemistry of Biogelx's peptide hydrogels, highlighting the range of chemical and mechanical modifications that can be implemented within the gels, in order to address a wide range of cell based applications. Some examples of academic and industrial collaborative work shall also be presented, including how the gel tunable properties, can be used to influence the differentiation pathway of stem cells.

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Vangl2 regulates membrane-protrusive activity in migrating gastrula cells

Anna Love and **Jason Jessen**
Middle Tennessee State University, USA

Our lab works to determine the mechanism whereby the planar cell polarity (PCP) protein Vang-Like 2 (*Vangl2*) regulates cell migration during embryogenesis. We focus on the gastrula stage of zebrafish development as the cells naturally undergo PCP-dependent migration. Loss of *Vangl2* in trilobite mutant embryos results in a strong convergence and extension phenotype characterized by shortened and broadened body axes. Here, both ectodermal and mesodermal cell populations fail to polarize. Previous data established migrating *vangl2* mutant cells lack directionality and meander compared to wild type. We have also shown *vangl2* mutants have increased matrix metalloproteinase activity and decreased fibronectin extracellular matrix (ECM). We hypothesize defective cell-ECM interactions underlie the *vangl2* mutant phenotype. Using time-lapse confocal imaging, we have now analyzed the membrane protrusive activity of ectodermal cells from wild-type and PCP mutant embryos. Our current data suggest *vangl2* mutant ectodermal cells exhibit increased membrane protrusive activity and have significantly fewer polarized protrusions. Our data suggest filopodia are concentrated at the trailing edge in wild-type cells, while *vangl2* mutant cells have more filopodia at the leading edge. We also found that *vangl2* mutant ectodermal cells have reduced directness compared to wild type. To determine the requirement for fibronectin during protrusion formation, we used morpholinos to knockdown fibronectin protein expression in wild-type embryos. The data showed that fibronectin morpholino injected cells exhibited increased formation of non-polarized membrane protrusions similar to *vangl2* mutant cells, suggesting defective cell-ECM interactions contributing to at least a portion of the mutant phenotype. Our preliminary studies suggest decreased *Vangl2* protein localization to filopodia and larger membrane protrusions. Together, our data suggest a model whereby *Vangl2*-dependent regulation of cell-ECM interactions is required to suppress inappropriate proper membrane protrusive activity.

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Brain lipid binding protein (BLBP) regulates the proliferation of astrocytes *in vitro*

Haoming Li, Jianbing Qin and **Guohua Jin**
Medical School of Nantong University, China

The aim of the study is to explore the function of brain lipid binding protein in the proliferation of rats' astrocytes *in vitro.* The *BLBP* overexpressed adenovirus or small interference RNA (siRNA) was used to transfect into astrocytes. 3 days later, the gene and protein expression of *BLBP* was detected, the proportion of Ki67 and EdU positive cells was measured and the cell cycle was investigated by flow cytometry methods. After treatment of *BLBP* adenovirus, the expression of *BLBP* gene was elevated, the exogenous expression of *BLBP* could be expressed in astrocytes, the proportion of Ki67 and EdU positive cells was also increased, as well as the proportion of cells at S phase. After treatment of *BLBP* siRNA, the expression of *BLBP* gene was decreased, the proportion of Ki67 and the cells at S phase was reduced as well. In conclusion, besides as a fatty acids transporter, *BLBP* also regulates the proliferation of astrocytes *in vitro*.

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Stem cells from exfoliated deciduous teeth, a way for pulp and dentin regeneration (an animal study)

Shirin Shahnaseri and **Seyed Amir Mousavi**
Isfahan University of Medical Sciences, Iran

Dental pulp tissue has the potential to regenerate dentin in response to stimulations. Thus, stem cell therapy has considerable a promise in pulp dentin regeneration. The aim of this study is an in-vivo evaluation of pulp's stem cells capacity in pulp and dentin regeneration in dogs. To isolate stem cells, one Iranian mixed-breed, 5-months dog was used. The deciduous tooth was extracted. Pulp of the tooth was isolated and exposed to type 1 collagenase enzyme. Isolated cells were cultured on Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic. Polyglycolate (PGA) scaffolds were prepared and sanitized in 75% ethanol and seeded with 4×104 cells. Twenty anterior and premolar dogs' teeth underwent shallow pulpotomy. Then all teeth were divided to three groups. Twelve teeth transplanted with seeded scaffolds and then cavities were filled with MTA and Amalgam. Control groups consisted of four teeth with unseeded PGA restored with MTA and Amalgam and four teeth with only MTA and Amalgam. Eight weeks after transplantation, samples were histologically analyzed. Mann Whitney U test was used to compare inflammation, calcific barrier and hyperemia and Chi-square test to compare necrosis and Odontoblastic layer formation. There was no significant difference between 3 groups except for calcified barrier type between group 1 and 2, Dentin like matrix, collagen fibers and small vessels observed in the cavity in group using stem cells. The results of the study suggest the possibility of pulp and dentin regeneration with stem cells in damaged teeth.

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Differentiation of human messenchymal stem cells into vascular endothelial cells

Chengen Wang, Min Yang, Yuan Li, Huihui Liu, Zeyin Liang, Yue Yin, Xiaoqiang Tong, Li Song, Guochen Niu, Ziguang Yan, Bihui Zhang and **Yinghua Zou**
Peking University First Hospital, China

Objective: The objective of the study is to investigate the differentiation of bone marrow mesenchymal stem cells (MSCs) into vascular endothelial cells *in vitro*.

Methods: MSCs were isolated from bone marrow by attaching growth and identified by flow cytometry. MSCs were seeded in inducing medium supplemented with VEGF, bFGF, EGF and IGF to differentiate into endothelial cells. The differentiated cells were identified by measuring surface marks (CD31 and CD34) on the 2nd, 6th, 10th and 14th day. qPCR was performed to detect the expression of vWF and Flk-1 on mRNA level. The functional behavior of the endothelial cells from MSCs was tested by tube formation assay *in vitro* on matrigel gel.

Results: The primary MSCs demonstrated spindle-shaped morphology and expressed stem cells marker. After two days induction, CD31 and CD34 began to express in 7.27% cells. The expression of CD31 and CD34 increased gradually during inducing period and reached a peak on the fourteenth day with 57.6% positive cells. qPCR demonstrated that the expression of vWF and Flk-1 was significantly higher in induced MSCs. The induced MSCs could form vessel-like structures on matrigel gel.

Conclusion: MSCs can differentiate into functional endothelial cells after induced by VEGF, bFGF, EGF and IGF and form vessel-like structures *in vitro*, suggesting that induced MSCs will be ideal seed cells for the treatment of lower extremity atherosclerotic occlusive disease.

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Alternative therapy of corneal endothelial dysfunction using skin-derived precursors

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Purpose: Explore the feasibility of differentiating skin stem cells into corneal endothelial cell-like cells (CEC-like cells) to cure corneal endothelial dysfunction.

Methods: Human skin stem cells were cocultured with corneal endothelial cells (CECs) through transwell coculture system to obtain CEC-like cells. CEC-like cells were identified by immunofluorescence, real time RT-PCR, western blotting. Dil-labeled CEC-like cells were transplanted into the rabbit's corneal endothelial dysfunction models to detect the cell function *in vivo*. Histological examination of corneas was performed to detect CEC-like cells attachment.

Results: CEC-like cells could be derived from skin stem cells and they had similar morphology and characteristic to CECs. They expressed major markers of CECs, such as Na+/K+ ATPase alpha 1, zonula occludens-1 and other functional markers. The expression levels of differentiation transcription factors FoxC1 and Pitx2 were also significantly upregulated compared with skin stem cells. CEC-like cells were transplanted into the rabbit's corneal endothelial dysfunction models, their corneal transparency and the thickness recovered while the control groups remain opaque. Histological examination showed Dil-labed CEC-like cells covered nearly full Descemet's membrane and expressed Na+/K+ ATPase in CEC-like cells injected group while almost no cells were detected on Descemet's membranes in control group.

Conclusions: This protocol enables efficient production of CEC-like cells from skin stem cells and these CEC-like cells have therapeutic effect in corneal endothelial dysfunction model. The renewable cell source and novel deriving method may lead to potential applications in cell replacement therapy for corneal endothelial dysfunction.

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Low-dose DHA-induced astrocyte proliferation can be attenuated by insufficient expression of BLBP *in vitro*

Jianbing Qin, Guohua Jin and **Haoming Li**
Nantong University, China

Docosahexaenoic acid (DHA) is an n-3 long chain polyunsaturated fatty acid (PUFA) that is involved in a wide range of cellular processes in human cells. Brain lipid binding protein (BLBP) exhibits a high affinity for n-3 PUFAs, especially DHA, but the precise functional contributions of DHA and BLBP in astrocytes are not clear. We analyzed cell viability and the ratio of Ki67 positive cells after manipulating DHA and/or BLBP levels in cultured astrocytes and found that 10 μM (low-dose) DHA stimulated proliferation of astrocytes, whereas this proliferative effect could be attenuated by downregulation of BLBP. Moreover, we found that astrocyte proliferation was directly regulated by BLBP independently of DHA. Taken together, low-dose DHA-induced astrocyte proliferation was disturbed by insufficient BLBP; and besides acting as a fatty acid transporter, BLBP was also involved in the proliferation of astrocytes directly.

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The gap junction as a biological rosetta stone: Implications of evolution stem cells to homeostatic regulation of health and disease in the barker hypothesis

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The discovery of the gap junction structure, its functions and the family of the connexin genes, has been basically ignored by the major biological disciplines. These connexin genes code for proteins that organize to form membrane-associated hemi-channels, connexons, co-join with the connexons of neighboring cells to form gap junctions. Gap junctions appeared in the early evolution of the metazoan. Their fundamental functions, (e.g., to synchronize electrotonic and metabolic functions of societies of cells and to regulate cell proliferation, cell differentiation and apoptosis), were accomplished via integrating the extra-cellular triggering of intra-cellular signaling and therefore, regulating gene expression. These functions have been documented by genetic mutations of the connexin genes and by chemical modulation of gap junctions. Via genetic alteration of connexins in knock-out and transgenic mice, as well as inherited connexin mutations in various human syndromes, the gap junction has been shown to be directly linked to many normal cell functions and multiple diseases, such as birth defects, reproductive, neurological disorders, immune dysfunction and cancer. Specifically, the modulation of gap junctional intercellular communication (GJIC), either by increasing or decreasing its functions by non-mutagenic chemicals or by oncogenes or tumor suppressor genes in normal or initiated stem cells and their progenitor cells, can have a major impact on tumor promotion or cancer chemoprevention and chemotherapy. The overview of the roles of the gap junction in the evolution of the metazoan and its potential in understanding a system view of human health and aging and the diseases of aging will be attempted.

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Oct4 expression in adult human stem cells: Evidence in support of the stem cell theory of carcinogenesis

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The *Oct3/4* gene, a POU family transcription factor, has been noted as being specifically expressed in embryonic stem cells and in tumor cells but not in cells of differentiated tissues. With the ability to isolate adult human stem cells, it became possible to test for the expression of *Oct3/4* gene in adult stem cells and to test the stem cell theory of carcinogenesis. Using antibodies and PCR primers, we tested human breast, liver, pancreas, kidney, mesenchyme and gastric stem cells, the cancer cell lines HeLa and MCF-7 and human, dog and rat tumors for *Oct4* expression. The results indicate that adult human stem cells, immortalized non-tumorigenic cells and tumor cells and cell lines, but not differentiated cells, express Oct4. *Oct4* is expressed in a few cells found in the basal layer of human skin epidermis. The data demonstrate that adult stem cells maintain expression of Oct4, consistent with the stem cell hypothesis of carcinogenesis.

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Metformin represses self-renewal of the human breast carcinoma stem cells via inhibition of estrogen receptor-mediated *OCT4* expression

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Metformin, a type II diabetic treatment drug, which inhibits transcription of gluconeogenesis genes, has recently been shown to lower the risk of some diabetes-related tumors, including breast cancer. Recently, cancer stem cells have been demonstrated to sustain the growth of tumors and are resistant to therapy. To test the hypothesis that metformin might be reducing the risk to breast cancers, the human breast carcinoma cell line, MCF-7, grown in 3-dimensional mammospheres which represent human breast cancer stem cell population, were treated with various known and suspected breast cancer chemicals with and without non-cytotoxic concentrations of metformin. Using *OCT4* expression as a marker for the cancer stem cells, the number and size were measured in these cells. Results demonstrated that TCDD (100 nM) and bisphenol A (10 mM) increased the number and size of the mammospheres, as did estrogen (10 nM E2). By monitoring a cancer stem cell marker, *OCT4*, the stimulation by these chemicals was correlated with the increased expression of *OCT4*. On the other hand, metformin at 1 and 10 mM concentration dramatically reduced the size and number of mammospheres. Results also demonstrated the metformin reduced the expression of *OCT4* in E2 & TCDD mammospheres but not in the bisphenol A mammospheres, suggesting different mechanisms of action of the bisphenol A on human breast carcinoma cells. In addition, these results support the use of 3-dimensional human breast cancer stem cells to screen for potential human breast tumor promoters and breast chemopreventive and chemotherapeutic agents.

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Melatonin decreases estrogen receptor binding to estrogen response element sites on the *OCT4* gene in human breast cancer stem cells

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Cancer stem cells (CSCs) pose a challenge in cancer treatment, as these cells can drive tumor growth and are resistant to chemotherapy. Melatonin exerts its oncostatic effects through the estrogen receptor (ER) pathway in cancer cells; however, its action in CSCs is unclear. Here, we evaluated the effect of melatonin on the regulation of the transcription factor *OCT4* (Octamer Binding 4) by estrogen receptor alpha (*ERα*) in breast cancer stem cells (BCSCs). The cells were grown as a cell suspension or as anchorage independent growth, for the mammospheres growth, representing the CSCs population and treated with 10 nM estrogen (E2) or 10 µM of the environmental estrogen Bisphenol A (BPA) and 1 mM of melatonin. At the end, the cell growth as well as *OCT4* and *ERα* expression and the binding activity of *ERα* to the *OCT4* was assessed. The increase in number and size of mammospheres induced by E2 or BPA was reduced by melatonin treatment. Furthermore, binding of the *ERα* to *OCT4* was reduced, accompanied by a reduction of *OCT4* and *ERα* expression. Thus, melatonin treatment is effective against proliferation of BCSCs *in vitro* and impacts the ER pathway, demonstrating its potential therapeutic use in breast cancer.

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What roles do colon stem cells and gap junctions play in the left and right location of origin of colorectal cancers?

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This Commentary examines an important clinical observation that right-sided colorectal cancers appear less treatable than the left-sided cancers. The concepts of the initiation/promotion/progression /process, the stem cell hypothesis, the role gap junctional intercellular communication, cancer cells lacking GJIC either because of the non-expression of connexin genes or of non-functional gap junction proteins and the role of the microbiome in promoting initiated colon stem cells to divide symmetrically or asymmetrically are examined to find an explanation. It has been speculated that embryonic-like lesions in the ascending colon are initiated stem cells, promoted via symmetrical cell division, while the polyp-type lesions in the descending colon are initiated stem cells stimulated to divide asymmetrically. To test this hypothesis, experiments could be designed to examine if right-sided lesions might express *Oct4A* and *ABCG2* genes but not any connexin genes, whereas the left-sided lesions might express a connexin gene, but not *Oct4A* or the *ABCG2* genes. Treatment of the right sided lesions might include transcriptional regulators, whereas the left-sided lesions would need to restore the posttranslational status of the connexin proteins.

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Molecular characterization of calreticulin: A biomarker for temperature stress responses of the giant tiger shrimp *Penaeus monodon*

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In crustaceans, calcium signaling plays important roles in growth, reproduction and molting. Calreticulin (CRT) is a main protein involved in calcium homeostasis of eukaryotes. The full-length cDNA of CRT in the giant tiger shrimp (*Penaeus monodon*), identified by RACE-PCR, was 1682 bp in length, containing an ORF of 1221 bp corresponding to a deduced protein of 406 amino acids. Genomic sequence of PmCRT spanned 3006 bp, composing of 4 exons (85, 119, 187 and 830 bp) and 3 introns (411, 231 and 120 bp). Semi-quantitative RTPCR revealed that PmCRT in hemocytes of juvenile *P. monodon* was up-regulated at 0 and 1 h post treatment (hpt) at 35°C for 3 h (Pb0.05). However, expression levels of PmCRT in gills and hepatopancreas after the temperature stress (0-48 hpt) were not significantly different (n=3 for each group; PN0.05). Quantitative real-time PCR confirmed the expression profile of PmCRT in hemocytes and illustrated that this transcript was up-regulated at 0 and 3 hpt for approximately 25 folds (n=5; Pb0.05), reduced to about 5 folds between 3 and 12 hpt (Pb0.05) and returned to the baseline level at 24 and 48 hpt (PN0.05). Recombinant PmCRT was successfully expressed *in vitro* and exhibited an ability to form a complex with recombinant endoplasmic reticulum protein 57 of *P. monodon* (rPmERp57). Results from this study strongly suggested that PmCRT can be regarded as a biomarker for temperature stress responses in *P. monodon.*

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A conceptual integration of extra-, intra- and gap junctional-intercellular communication in the evolution of multi-cellularity and stem cells: How disrupted cell-cell communication during development can affect diseases later in life?

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An attempt will be made to provide a short conceptual review to integrate, from an evolutionary perspective, how the emergence of gap junctional intercellular communication helped to bring about multi-cellularity and new adaptive phenotypes. This new fundamental biological function of the metazoans was needed to provide homeostatic control of new cellular functions of an interacting society of different cell types existing in a 3-dimensionalunit. Changing paleo-physics- and -chemistry of the earth led to single celled organisms that metabolized sugar via glycolysis and survived via symmetrical cell division and occasional mutations. With the appearance of oxygen-producing phytoplankton, the single cell organism, the mitochondrion, symbiotically- fused with a primitive cell to form the first multi-cellular organism, which could metabolize glucose via oxidative phosphorylation. The new society of adherent cells developed new strategies for adaptive survival. New genes and phenotypes included: growth control, differentiation, programmed cell death; senescence; regulation of gene expression-"epigenesis"; germline and somatic stem cells; asymmetrical cell division; and anoxic stem cell niches. The evolutionary development of the normal human organism, starting from a single "toti-potent" stem cell to the mature, reproductive and self-aware being, consisting of over 100 trillion cells, of which 200 different cell types and having three major functional cells- (organ-specific stem cells; their progenitor derivative cells; and their differentiated daughters), could only come about by a delicate homeostatic integrated feedback system of extra-, intra- and gap junctional inter-cellular communication (GJIC). Since GJIC occurs in all organs, any disruption of the three forms of cell communication mechanisms by genetic or epigenetic factors, particularly during embryonic, fetal and neonatal periods, could lead to alteration of risks to diseases later in life (i.e., the Barker hypothesis). Chronic disruption of these signaling mechanisms in the adult organs could also lead to several kinds of chronic, stem cell-based diseases, diabetes, cancer, atherogenesis and premature aging.

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Calcium signaling-related genes in *Penaeus monodon* respond to abiotic stress and pathogenic bacteria common in aquaculture

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Stress of cultured species is a common problem, affecting the host health, in aquatic farming. It occurs when interactions among the three elements – host, environment and pathogens, are not in balance, particularly under intensive culturing conditions. Hosts respond to stress by triggering cellular Ca2+ signaling to activate many downstream pathways for their functions in order to mitigate the stress effect. Calreticulin (CRT), Calnexin (CNX) and Endoplasmic reticulum protein 57 (ERp57) are involved in Ca2+ signaling in stress response mechanisms and are essential for survival and viability of organisms under stress conditions. In this study, the full length cDNA of CNX and ERp57 in the giant tiger shrimp (*Penaeus monodon*) were identified. Amino acid sequences deduced from both cDNA showed significantly high similarity with those present in other crustaceans. One and two forms of PmCNX and PmERp57 transcripts were found, respectively. For PmERp57, its genomic sequence was additionally identified. It spanned 8257 base pairs (bp), composing of10 exons (84–226 bp long) and 9 introns (93–2787 bp long). Both genes were expressed in all examined tissues of both juvenile and broodstock *P. monodon*. Effects of Vibrio harveyi and abotic stress commonly found in aquaculture on expression of PmCRT, PmCNX and PmERp57 were examined in hemocytes by quantitative real time PCR. High temperature, hypo- and hyper-osmosis and *V. harveyi* infection induced expression all of the three genes ($P<0.05$). In contrast, analysis of hypoxia effect on expression of the Ca2+ signaling-related genes, which was firstly studied in aquatic species, showed to suppress their expression ($P<0.05$). Results from this study suggested that PmCRT, PmCNX and PmERp57 are related to stress response mechanisms and immune system of *P. monodon*. Their expression is therefore a potential candidate for markers to detect or monitor the early sign of shrimp's stress and immunity changes in farming.

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Transforming stem cell research to cardiovascular remodeling

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Cardiovascular diseases (CVDs) remain the foremost reason of mortality and debility accounting for 31% of deaths worldwide. Currently, regeneration of damaged cardiac tissue with functional cardiomyocytes via stem cell therapy represents an effective approach in CVDs treatment. In this study, differentiation of cardiosphere derived cells (CDCs) to cardiomyocytes was performed in search of enhanced cardiovascular regeneration therapeutics. Cardiac Progenitor Cells (CPCs) from rat heart were propagated by explant culture and CDCs were derived. Cardiac explant outgrowth cells (CEOCs) and CDCs were characterized by Immunofluorescence, flow cytometry and reverse transcriptase PCR. Further, CDCs were treated with an optimized concentration of 10µM 5-Azacytidine for 24 h followed by supplementation with 10-4M ascorbic acid for 14 days. Extent of differentiation was analyzed by immunofluorescence and quantitative real-time PCR (qRT-PCR). In the results, flow cytometric analysis has demonstrated that 30.23% of CEOCs were positive for the c-kit marker, specific to CPCs. Gene expression analysis showing high expression of *GATA4*, *Nkx2.5* and *CD90* markers suggested enhanced cardiac lineage commitment of CDCs in comparison to CEOCs. Immunofluorescence results confirmed that 5-Aza+AA treated CDCs expressed cardiomyogenic markers i.e. α-sarcomeric actinin and Nkx2.5. qRT-PCR analysis revealed relative up-regulation of Nkx2.5, *GATA4* and *α-MHC* markers in 5-Aza+AA treated CDCs while Wnt markers, Wnt 3a, β-catenin and cyclin D1 were down regulated. Generation of spontaneous beating of 5-Aza+AA treated CDCs further reinforces that 5-Aza+AA efficiently differentiated CDCs. The cardiomyogenic potential of CDCs indicates that they can serve as an effective cellular therapeutic as well as an ideal candidate for the treatment of cardiovascular disorders.

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