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Posters

Irina V Panyutin et al., J Cell Sci Ther 2017, 8:1 (Suppl)
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Effect of ionizing radiation on the proliferation of human embryonic stem cells

Irina V Panyutin, Sonia A Holar, Ronald D Neumann and **Igor G Panyutin**
National Institutes of Health, USA

We analyzed growth rates of seven hESC lines by measuring area of individual colonies. The doubling time averaged over all the colonies varies from 18.9 to 28.7 hours. We studied effect of 0.2 and 1.0Gy exposure on proliferation of these hESC lines. All cell lines showed similar reaction to IR, i.e. the number of cells dropped within 24-48 h; after that they recover and grow with the same rate as the sham-irradiated cells. The Relative Cell Survival (RCS) i.e. the fraction of cells in the irradiated samples relative to the sham-irradiated cells varied from 0.6 to 0.8 after 0.2Gy and from 0.1 to 0.2 after 1Gy IR. The RCS correlates directly with the doubling time, i. e. the faster cells grow the more radiosensitive they are. The doubling times and areas of individual colonies varies significantly for all cell lines. For all cell lines except WA22 we found no correlation between colony size and growth rate; however for several cell lines (H1, WA13, WA19) smaller colonies were more radiosensitive than the larger ones.

Biography

Irina V Panyutin has earned her Doctoral degree in Epidemiology from Moscow State Medical Academy in 1980 and has worked as an Epidemiologist at a Disease Control Station in Moscow, Russia. After coming to USA, she worked as a Research Scientist at Braton Biotech Inc., Rockville, Maryland from 1992 to 1996. Since 1996, she has been with the NIH, Nuclear Medicine Department. Her research interests include study of the effect of ionizing radiation on human embryonic stem cells survival, proliferation and differentiation.

ipanyutinv@mail.nih.gov

Notes:

Alfatih A A Aboagla et al., J Cell Sci Ther 2017, 8:1 (Suppl)
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Lysophosphatidic Acid Acyltransferase2 (LPAT2) enhances abscisic acid response and plays a positive role in osmotic stress in rice

Alfatih A A Aboagla and **Hong Yueyun**
Huazhong Agricultural University, China

Lysophosphatidyl acyltransferase (LPAT) is a pivotal enzyme controlling the metabolic flow of lysophosphatidic acid into different phosphatidic acids in diverse tissues. Recent results begin to shed light onto the involvement of *LPAT2* in response to ABA, water deficits, and salinity. We examined and characterized putative *LPAT2* gene in rice (*Oryza sativa*). *LPAT2* transcript is existing in all tissues tested with relatively higher in leaves and roots, and was induced by salt, drought and ABA treatment, suggesting its roles in plant growth and stress response. Moreover, *LPAT2* is localized to the endoplasmic reticulum (ER) membrane, implicati-ng its role in lipid metabolism and signaling. Additionally, *LPAT2* might be essential for gametophyte or embryo development as homozygous mutant for a T-DNA insertion in *LPAT2* coding region fails to recover in rice. Using a knockdown mutant *lpat2* and its genetic complementation revealed that *LPAT2* is important for plants to adapt osmotic stress. Reduced *LPAT2* conferred plants and seeds were more sensitive to ABA treatment and were less tolerant to salt and drought stress. The results suggest that *LPAT2* plays a positive role in ABA response and osmotic stress tolerance. The role of *LPAT2* in osmotic stress is mediated by ABA response as shown that the *lpat2* mutant exhibited more water loss from leaves when supplied ABA under salt stress. The ABA responsive gene RAB18 was less induced by ABA and salt treatments in the *lpat2* mutant. The result suggests that *LPAT2* enhance ABA response to promote plants osmotic stress tolerance. PA produced by *LPAT2* activity might be also important for ABA response. PA supplementation is capable of restoring ABA sensing and salt stress tolerance as WT performance. The effects of *LPAT2* on plant stress tolerance might be dual effects of PA, enhanced ABA response and enhanced growth. Our study reveals novel interactions among ABA, *LPAT2* and PA and provides insight into progresses in agronomic traits and adaptive growth through the manipulation of these pathways in rice.

Biography

Alfatih A A Aboagla has completed his MSc from Huazhong Agricultural University, Wuhan, P. R. China. Currently, he is doing his PhD in Microbiology (Bioengineering).

2725873001@qq.com

Notes:

Arshak R Alexanian, J Cell Sci Ther 2017, 8:1 (Suppl)
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Manipulation of adult stem cells plasticity with modulators of chromatin modifying enzymes

Arshak R Alexanian
Cell Reprogramming & Therapeutics LLC, USA

Development of safe and effective technologies for turning one adult stem cell type into another will open up remarkable opportunities for regenerative medicine. It is now well established that epigenetic regulation of gene expression is one of the key mechanisms that is involved in the regulation of cellular stemness, lineage commitment, differentiation and maintenance of these states, and DNA and histone covalent modifications play a key role in these processes. Thus, modulators of chromatin modifying enzymes could be efficient tools for manipulating cell plasticity. Recently, we were able to convert human mesenchymal stem cells (hMSCs) into neural-like cells by exposing them to chromatin modulating agents and neural inducing factors. In this study, we demonstrated that epigenetically manipulated hMSCs grown in specific differentiation conditions generated different cell types, such as smooth muscle and different neural cell types. Interestingly, chromatin modifying agents also promoted cell lineage switches when cells were exposed to another differentiation condition while in their early stages of commitment. These data suggest that the plasticity of adult stem cells can be manipulated by the modulators of chromatin modifying enzymes when combined with specific differentiation factors in appropriate culture conditions.

Biography

Arshak R Alexanian is currently the Chief Scientific Officer at Cell Reprogramming & Therapeutics LLC and an Adjunct Associate Professor in the Department of Medicine at the Medical College of Wisconsin (MCW). Previously, he held faculty positions in the Departments of Neurosurgery at MCW (2000-2013) and in the Departments of Anatomy and Neurobiology, as well as in Biochemistry and Molecular Biology, at Colorado State University (1997-2000). He has received training at universities and centers worldwide, including the Pasteur Institute and University of Montpelier in France, University of Saarland in Germany, Institute of Biochemistry in China and Russia and Colorado State University, where he gained extensive experience in the fields of Biochemistry, Molecular Biology, Cell Biology (stem cell biology) and Neurosciences. The areas of interest of his research are the epigenetic regulation of cell fate commitment and differentiation, development of cell reprogramming technologies to produce different neuronal and glial cell types and elucidation of the therapeutic effect of these specialized cell types in several neurological disorders.

aalexan@cellrtherapeutics.com

Notes:

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A targeted drug delivery system for selective deliver of insulin-like growth factor-1 to infarcted myocardium to improve stem cell survival

Bin Wang[1,2], **Ji Zhou**[1], **Yuan Tang**[2], **Andrew C Issekutz**[3] and **Mohammad F Kiani**[1]
[1]Widener University, USA
[2]Temple University, USA
[3]Dalhousie University, Canada

Stem cell therapy for treating MI has been widely studied, but the clinical applications of these studies have been disappointing, since current stem cell therapy has shown poor survival and engraftment of the stem cell in the diseased tissue. In this study, a novel approach was employed to improve the engraftment and viability of transplanted mesenchymal stem cells (MSCs) by selectively delivering insulin-like growth factor 1 (IGF-1) to the infarcted myocardium. One week after the MI surgery, immunoliposomes containing IGF-1 were infused into rats via. tail vein and MSCs were injected intramyocardially around MI area. Left ventricular fractional shortening (FS) was measured as an index of heart contractility. The combination of targeted IGF-1 and MSCs treatment significantly improved the FS function (2.5% gain) during 3 weeks (no treatment: 8% FS loss, targeted IGF-1: 4% FS loss, MSCs treatment 8% FS loss). Immunochemical staining shows that both IGF-1 alone and IGF-1+MSCs treatment facilitated vessel regrowth into the MI area, and much stronger stem cell fluorescence in the IGF-1+MSCs treatment group compared to the MSCs alone treatment group, indicating that IGF-1 treatment greatly improved the survival of the transplanted stem cells. The combination of targeted IGF-1 and stem cell transplantation results in a larger recovery in cardiac function compared to either IGF-1 or stem cell treatment alone. This recovery is probably achieved by targeted IGF-1 improved the stem cell survival and the subsequent stem cell therapy in the damaged myocardium.

Biography

Bin Wang has completed his PhD from Temple University in 2007 and Postdoctoral studies from Medical College of Georgia. He is the Assistant Professor of Widener University. He has published more than 30 papers in reputed journals and has been serving as an Editorial Board Member of several reputed journals.

bwang@widener.edu

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Accepted Abstracts

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Study on the diagnostic and prognostic aspects of bone marrow microenvironment components in Non-Hodgkin's lymphoma before and after therapy

Amira Hanafi Soliman
Cairo University, Egypt

Objectives: The main goal of the study is the evaluation of the stromal cells of bone marrow microenvironment (BMM) in bone marrow trephine biopsy (BMTB) and fibronectin, tumor necrosis factor- alpha (TNF-α), L-selectin of bone marrow (BM) plasma in Non-Hodgkin's Lymphoma (NHL) patients, before and after therapy.

Material & Methods: A total of 80 de novo NHL patients which were divided as B-cell lymphoma 64/80 (80%), comprising follicular cell lymphoma (FCL) 32/80 (40%) patients, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) 12/80 (15%) patients, and diffuse large cell lymphoma 20/80 (25%) patients and T-cell lymphoma, which constituted 16/80 (20%) of patients, all diagnosed as T-Lymphoblastic lymphoma. Patients were evaluated before and after therapy, and compared to 25 BM donors as control group. BMTB and BM aspirate were taken for morphological assessment of stromal cell. Plasma of BM samples was examined for TNFα, L-selectin, which were tested by ELISA technique, and Fibronectin by Radial immunodiffusion (RID).

Results: BM stromal cells comprising reticular macrophages and fibroblasts were increased in 53.3% of NHL at diagnosis. BM Fibronectin levels were decreased, while BM TNFα and L-selectin were higher at diagnosis in comparison to CR ($p<0.05$) and control ($p<0.05$). In NHL, elevated values of BM TNFα and BM L-selectin were associated with signs of aggressive disease, including, extra nodal sites were increased (>1), detectable B cell-symptoms, high grade NHL, signs of BM and CNS invasion, high International prognostic index (IPI) ($p<0.05$).

Conclusion: BMM components, TNFα, L-selectin and fibronectin in NHL can be useful in evaluating disease activity, extent and response to treatment and as prognostic markers according to (IPI).

amirasoliman777@hotmail.com

Crosstalk signals between transplanted neural stem cells, the host niche and dopaminergic neurons via. astrocytes trigger dopaminergic nigrostriatal neurorestoration in Parkinsonian mice

Bianca Marchetti[1, 2]
[1]University of Catania Medical School, Italy
[2]OASI Institute for Research and Care on Mental Retardation and Brain Aging, Italy

Within their specialized germinal niches, populations of local astrocytes instruct neural stem/progenitor cells (NSCs) via. complex cell-cell interactions and signaling cascades, which include the activation of the Wnt/β-catenin pathway, a signalling system required for specification and neurogenesis of midbrain dopaminergic (mDA) neurons, the pivotal neuronal population that degenerates in Parkinson's disease (PD) and in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD. Recently, we uncovered that the midbrain aqueduct (Aq)-periventricular regions (PVRs) SVZ act as a natural niche for mDA progenitors. Accordingly, mDA neuron death induced by the neurotoxin MPTP, promotes an early astrocyte-dependent activation of these Aq-PVR-DA progenitors, but a lack of appropriate niche environmental signals restrict their neurogenic potential and compromise neuronal survival/rescue. Given that transplanted NSCs possess intrinsic capacity to ameliorate the injured microenvironment and to rescue dysfunctional neurons, here we used adult green fluorescent protein $(GFP)^+$ NSCs as a graft source for unilateral transplantation above the subtantia nigra (SN) of MPTP mice. Remarkably, grafted GFP-NSC survived within the SN, in situ. Spatio-temporal analyses showed a significant protection/restoration of SN-TH^+ cell bodies. Additionally, GFP^+-NSCs were seen to accumulate at the Aq-SVZ niche, where they induced a profound remodelling of host $GFAP^+$ astrocytes and β-catenin over-expression thus suggesting activation of astrocyte-dependent Wnt signaling. Increased β-catenin expression was also observed in SN-repairing neurons together with a robust striatal reinnervation, thereby uncovering a critical role of NPC crosstalk with the host niche and DA neurons via astrocytes for DA neuroprotection and neurorestoration, with implications for cell-based therapies for PD.

biancamarchetti@libero.it

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Relationship between anesthetic-induced toxicity and NMDA receptor-mediated calcium influx in developing neurons

Cheng Wang, Fang Liu, Tucker A Patterson, Merle G Paule and William Slikker
National Center for Toxicological Research/Food and Drug Administration, USA

Ketamine is a non-competitive NMDA receptor antagonist and is used as a general anesthetic. Recent data suggest that anesthetics can cause neuronal damage when exposure occurs during development. To elucidate the underlying mechanisms associated with ketamine neurotoxicity, neural cells were harvested from the forebrain of newborn rats and neural stem cells were isolated from gestational day-16 rats. To determine the effect of ketamine on developing neurons and undifferentiated neural stem cells, cultures were exposed to 10 μM ketamine for 24 hours. Ketamine exposure resulted in elevated NMDA receptor (NR1) expression in primary cultures, and enhanced damage of developing neurons including those differentiated from the neural stem cells. However, the viability and proliferation rate of neural stem cells were not significantly affected after ketamine exposure. Calcium imaging data indicated that 50 μM NMDA did not cause a significant influx of calcium in typical neural stem cells; however, it has produced an immediate elevation of intracellular free $Ca2^{+}$ $[Ca^{2+}]i$ in neurons differentiated from the same neural stem cells. These findings suggest that prolonged exposure of developing neurons to ketamine produces an increase in NMDA receptor expression, which allows for a higher/toxic influx of calcium into neurons once ketamine is removed from the system, leading to neuronal cell death likely due to elevated reactive oxygen species generation.

cheng.Wang@fda.hhs.gov

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Application of neural stem cells to assess general anesthetic-induced neurotoxicity

Fang Liu and Cheng Wang
National Center for Toxicological Research/Food and Drug Administration, USA

Every year, approximately 6 million children in US and 2% of pregnant women in North America undergo general anesthesia. There is an increasing concern about the potential adverse effects of anesthetics on the developing brain. Neural stem cells (NSCs) are able to recapitulate most critical events of CNS development *in vivo* and, therefore, represent a valuable *in vitro* model for evaluating xenobiotic-induced developmental neurotoxicity. The potential toxic effects of ketamine and propofol and two commonly used pediatric anesthetics were examined using NSCs and NSC-derived neural cells. NSCs were harvested from gestational day (GD) 16 rat brain and confluent cell cultures were exposed to either ketamine or propofol at different doses and durations, followed by systematic evaluation of cytotoxicity. At clinically-relevant doses, propofol resulted in a significant reduction of NSC viability and proliferation rate, whereas ketamine did not show such effects. The different results may be attributed to the different mechanisms through which they cause neurotoxic effects: ketamine-induced neuronal damage was detected after NSCs differentiated. These data suggest that anesthetic-induced neurotoxicity depends upon the concentrations of drugs used, the durations of exposure, the receptor subtype activated and the developmental stages at the time of exposure.

Fang.Liu@fda.hhs.gov

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Social collaboration network analysis of animal-derived regenerative implantable medical devices: An overview based on Chinese literature from databases of CNKI, WANFANG DATA and VIP

Wang Hong-man and Shu-Yang
Peking University, China

Background: Tissue engineering involves many disciplines such as biology, material science, medicine and engineering, so, the collaboration among different research departments is becoming an important factor to enhancing research outputs with the rapid development of related sciences and technologies.

Objective: To draw the visualizing map of collaboration network of animal-derived regenerative implantable medical devices based on tissue engineering technology and describe its evolving process and current situation.

Methods: 2518 Chinese literatures about the animal-derived regenerative implantable medical devices based on tissue engineering technology published before 31st December 2014 were searched in CNKI, WANFANG DATA and VIP. Subsequently, social network analysis was conducted on those literatures by utilizing UCINET software and SASI developed by Peking University.

Results & Conclusion: The collaboration network of the animal-derived regenerative implantable medical devices has evolved from scattered to single-core dominated, and then to a core-edge one, characterized by increasing and extensive collaborations as well as decreasing network density and centralization. The core units from 2010 to 2014 include Tsinghua University, General Hospital of Chinese PLA and Affiliated Nanfang Hospital of South Medical University. Also, plenty of edge institutes exist. In conclusion, edge institutes should expand their scope of cooperation, while core institutes should improve their cooperation sustainability. Furthermore, cooperation among enterprises, research institutes and clinical hospitals should be strengthened to promote the industrialization of tissue engineering technology.

yxb100191@sohu.com

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In silico discovery and rational prediction of differential peptide mimetic active inhibitors against LINE1 and LINE2 conserved retro-transposition mechanism on the CD133/AID/APOBEC cancer stem cell derived protein motif like binding domains by annotating tetrahedral meshes in a QM Index Dynamic Unified Theorem

John Ioannis Grigoriadis
Biogenea Pharmaceuticals Ltd, Greece

Identification and solution structure of a highly conserved C-terminal domain within ORF1p is required for retro-transposition of long interspersed nuclear element-1. Retrotransposons constitute almost half of the human genome and are considered to be one of the major driving forces in the evolution of eukaryotic genomes. They are classified into two major types, long terminal repeat (LTR) retrotransposons, which include retroviruses and non-LTR retrotransposons. The non- LTR retrotransposon LINE1 (L1) and LINE2 (L2) clades, which are widespread among vertebrates, differ in two important structural and functional characteristics. Second, unlike the L1 reverse transcriptase that can mobilize other RNA species, the L2 enzyme is specific for its own 3' UTR. Furthermore, while both L1 and L2 elements are present in fish, amphibians and reptiles, only the L1 retrotransposon clade has greatly expanded in mammals, reaching 17% of the human genome. In contrast, the L2 retrotransposons are inactive in placental mammals, with only highly defective copies present in the human genome. It has also been shown that an elevated expression of the stem cell marker CD133 was associated with Line-1 demethylation in hepatocellular carcinoma. That relationship of Line-1 demethylation and the CD133 expression of cancer stem cells were discussed in hepatocellular carcinoma (HCC). Cancer cells are also characterized by expression of active LINE-1 elements (L1s, long interspersed nuclear elements-1). Several million small- like poly-pharmacophore molecules will be designed in-silico in a single HTS campaign within the cell populations for screening could easily invalidate an entire campaign. As a result in this scientific drug discovery approach we have introduced an in silico discovery and rational prediction of the solution structure of differential peptide mimetic active inhibitors of LINE1 and LINE2 conserved retro-transposition mechanism in the host defense stem cell marker CD133/AID/APOBEC by identifying QM Fragment LINE1 and LINE2 conserved retro-transposition mechanism utilizing URLs 3D structure precision utilities of QM Based Structure CD133/LINE-1 AID/APOBEC Protein/Ligand Complexes on Model derivation and symbolic representation of LINE1-AID to the retro-transposition locus. We finally, generated in-silico experimental procedures and network specifications of stem cell marker CD133/APOBEC3s LINE-1 retro-transposition physiological targets based on a Line-1, Line-2 QM index dynamic unified theorem for multiple entities as an efficient and versatile tool for the lead structure generation and optimization of a dynamic simulated in-silico druggable database of experimentally measured effects of mutations on structurally defined protein-ligand complexes of transposable elements for assessing and comparing protein–small molecule affinities by predicting binding affinities and scoring values on simulated importing and annotating tetrahedral meshes in reverse transcriptase LINE1 assays and L1 constructs for the discovery of chemical probes for fragment-based drug discovery by binding homology search according to ligand and receptor similarity to or better than other binding-homology methods with higher computational efficiency online.

biogeneadrug@gmail.com

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Genetic changes at chromosomal and DNA level during long term cultivation of hES cells

Jose Inzunza
Karolinska Institutet, Sweden

Human embryonic stem cells (hESCs) are important research tools in studies of the physiology of early tissue differentiation. In addition these cells are regarded as a promising approach to generate transplantable cells for the treatment of several diseases, and therefore offer an immense potential as a source of cells for regenerative medicine. However the possible ability of these cells to produce tumors *in vivo* presents a major impediment for this achievement. hESCs can obtain growth advantages *in vitro* by acquired mutations. The mechanisms that may influence chromosome modification in hESCs are not well known. We have performed a comparative *in vitro* and *in vivo* study on hESC lines produced in our laboratory to see if there are changes also during *in vivo* growth. *In vivo* differentiated cells and *in vitro* cultured hESCs were analyzed by using first comparative genome hybridization (CGH) and second a high-resolution Affymetrix SNP 6.0 array revealing DNA copy number variations. We were able, for the first time, to identify an aberrant X chromosome both *in vitro* and *in vivo* in one out of the 3 hESC line, we decteted an amplification of the whole X chromosome, possibly due to mosaicism of XY and XX cells. In the other hESC line, array results showed small amplifications and gains. The third hESC line was less altered, but contained also a new gain verified by fluorescent in situ hybridization in a teratoma in 21% of the cells. These results indicate that mutations occur during the *in vivo* differentiation process as well as *in vitro*. The potential of precancerous mutations in in-vivo conditions is important to consider for safety measures, and underlines the necessity to remove all pluripotent stem cells from the differentiated cell population that will be transplanted.

jose.inzunza@ki.se

Cutting edge concepts in the use of stem cell and PRP injections in an office setting

Joseph Purita
Institute of Regenerative and Molecular Orthopedics, USA

The presentation concerns PRP and stem cell (both bone marrow and adipose) injections for musculoskeletal conditions in an office setting. Indications are given as to which type of cell and technique to use to accomplish repair. Stem cells, both bone marrow derived (BMAC) and adipose, are used for the more difficult problems. PRP injections are utilized for the less severe problems. Indications are given when to use stem cells verses PRP and when to use both. The newest concepts in stem cell science are presented. These concepts include the clinical use of MUSE cells, exosomes, and blastomere like stem cells. Basic science of both PRP and stem cells are discussed. This presentation defines what constitutes an effective PRP preparation. Myths concerning stem cells are dispelled. One myth is that mesenchymal stem cells are the most important stem cell. This was the initial interpretation of Dr. Arnold Caplan the Father of Mesenchymal Stem Cell Science. Dr. Caplan now feels that MSCs have an immunomodulation capacity which may have a more profound and immediate effect on joint chemistry and biology. We now learn in the talk that the hematopoietic stem cells are the drivers of tissue regeneration. Also, discussed are adjuncts used which enhance the results. These therapies include supplements, LED therapy, lasers, electrical stimulation, and cytokine therapy. The scientific rationale is presented for each of these entities as to how they have a direct on stem cells.

jpurita@aol.com

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Generation of high quality iPS cells (super iPS cells) using linker histone H1foo

Keiichi Fukuda, Akira Kunitomi and Shinsuke Yuasa
Keio University School of Medicine, Japan

Embryonic Stem Cells (ESCs) are a hallmark of ideal pluripotent stem cells. Epigenetic reprogramming of induced pluripotent stem cells (iPSCs) has not been fully accomplished. iPSC generation is similar to somatic cell nuclear transfer (SCNT) in oocytes, and this procedure can be used to generate ESCs (SCNT-ESCs), which suggests the contribution of oocyte-specific constituents. Here, we show that the mammalian oocyte-specific linker histone H1foo has beneficial effects on iPSC generation. Induction of H1foo with Oct4, Sox2, and Klf4 significantly enhanced the efficiency of iPSC generation. H1foo promoted *in vitro* differentiation characteristics with low heterogeneity in iPSCs. H1foo enhanced the generation of germline-competent chimeric mice from iPSCs in a manner similar to that for ESCs. These findings indicate that H1foo contributes to the generation of higher-quality iPSCs.

kfukuda@a2.keio.jp

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Faster engraftment dependent to better mobilization

Mehrdad Payandeh
Kermanshah University School of Medicine, Iran

Although the process of HSPC mobilization has been used to benefit patients for close to 30 years, the mechanisms governing HSPC mobilization remain incompletely understood. This gap in knowledge has hampered the development of safer and more effective agents to mobilize HSPCs clinically in several key regulators of mobilization, most prominently the CXCL12/CXCR4 axis. [FDA] approval in 2008 of the CXCR4 antagonist plerixafor for the mobilization of CD34+ cells in patients with non-Hodgkin lymphoma and multiple myeloma (MM) prior to autologous HSPC transplantation. From the perspective of a clinician caring for patients undergoing autologous or allogeneic HSPC transplantation, one could argue that we already possess adequate means to procure HSPCs, either through simple bone marrow harvesting procedures or through stem cell mobilization techniques developed and used successfully over the past 30 years. Perhaps the agents we currently have at our disposal are good enough. But 10% to 15% of patients with lymphoma and MM do not mobilize HSPCs adequately and these patients need better mobilizing strategies. In this study, we described details of more than 30 patient with different disorders consist of NHL, MM, AML, ALL and HL transplant in the KUMS bone marrow transplantation center (west or Iran) during 2014-2016. Engraftment was done in equal or less than 10 days in these patients. Specific mobilization is important factor for fast engraftment in patients. Additionally, there are a number of important emerging clinical indications for HSPC mobilization where safer, more effective and more rapid means to procure HSPCs would be highly desirable. The most common method used to procure HSPCs from peripheral blood of patients: cyclophosphamide followed by G-CSF. Although certainly clinically relevant, it would be of interest to determine whether there are differences in HSPC phospho-proteomic profile following other stimuli including agents blocking the CXCR4/CXCL12 interaction or antagonists of VLA-4/VCAM1, among other agents, particularly because some data suggest that although HSPC mobilization may be the final common end point, mobilization methods may differ substantially in the manner in which they affect the bone marrow microenvironment. The importance of this article lies not only in the unleashing of another pathway regulating HSPC mobilization. Hopefully, just the beginning of the search for novel targets will improve the lives of our patients.

mehrnoush.aeinfar@gmail.com

Amniotic fluid: A new approach to biologics and regenerative treatments

Michael P Zahalsky
Author Affiliation

Regenerative medicine has often been associated with the process of replacing or reproducing tissues, human cells or organs back to their origin function. Recently, these treatments have involved the use of stem cell technology to treat and manage chronic diseases such as diabetes, heart failure and degenerative nerve, bone and joint conditions. Amniotic fluid is particularly appealing because it contains various proteins, cytokines and a multitude of growth factors that are believed to facilitate angiogenesis while simultaneously preventing inflammation and swelling. ProFlo is amniotic fluid that is minimally manipulated from an FDA cleared tissue bank. We believe that since it is from the genitourinary system, it can be used for homologous use on the genitourinary system. Patients diagnosed with Erectile Dysfunction were injected with ProFlo amniotic fluid and their responses were evaluated using a Penile Doppler to measure pre and post peak systolic velocities. The International Index of Erectile Function Questionnaire (IIEF-5) was also used to evaluate any changes in erectile function. Overall, results were very encouraging and we observed statistically significant changes in PSV values and IIEF responses. Several patients were satisfied with their treatments, reporting an improvement of erections with minimal to no discomfort. Based on our preliminary findings, ProFlo appears to be a possible alternative for men with erectile dysfunction. The use of amniotic fluid to treat erectile dysfunction is still being evaluated, preliminary results are encouraging but more research needs to be conducted to evaluate its effectiveness and overall safety.

kelly@urostembiologics.com

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A self-assembly peptide nanofiber scaffold containing laminin motif induces cellular survival, proliferation, migration, attachment, and neuronal differentiation: An *in vitro* study

Sajad Sahab Negah[1, 2] **and Ali Gorji**[1, 2, 3]

[1]Mashhad University of Medical Sciences, Mashhad, Iran

[2]Shefa Neuroscience Research Center, Iran

[3]Westfalische Wilhelms-Universitat Munster, Germanyt

Tissue engineering has created significant approaches toward the purposes of regeneration of damaged tissues. A general approach to construct artificial tissues needs artificial extracellular matrices (ECMs) with functions similar to the native ECMs. Considerable attempt has been made to combine biologically active molecules into the self-assembling peptide in order to improve cells growth, survival, and differentiation. In this study, RADA4GGSIKVAV (R-GSIK) was designed as a three-dimensional (3D) nano-fiber scaffold. The cell adhesion, viability, proliferation, migration, and differentiation of rat embryonic neural stem cells (NSCs) were investigated. The characterization of the R-GSIK shows an open porous structure and a suitable surface area available for cell interaction. R-GSIK promoted in terms of the cell adhesion, viability, proliferation, and migration. Additionally, the R-GSIK could enhance NSCs to differentiate into neuron cells. Moreover, the NSCs injected within R-GSIK had a lower glial differentiation rate than in the puramatrix. Based on our results, it might be concluded that R-GSIK holds great promise for central nervous tissue repair.

sajad.sahab2012@gmail.com

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The effectiveness of treatment of patients with luminal form Crohn's disease mesenchymal stromal cells of the bone marrow – 7 years of observation

Knyazev O[1], Kagramanova A[1], Fadeeva N[1], Boldyreva O[1], Lishchinskaya A[1], Ruchkina I[1], Babayan A[1], Kirova M[1], Konoplyannikov A[2], Noskova K[1], Orlova N[1] and Parfenov A[1]

[1]Moscow Clinical Research Center, Russia

[2]Medical Radiological Research Center, Russia

Introduction: Anticytokine therapy with anti-TNF-alpha drugs contribute to the achievement of stable remission of Crohn's Disease (CD). For the treatment of CD Mesenchymal Stromal Cells (MSCs) are also used.

Objective: To examine the long-term efficacy (7 years) therapy of Mesenchymal Stromal Cells (MSCs) from the bone marrow of patients with luminal Crohn's Disease (CD).

Materials & Methods: 80 patients with luminal form CD (terminal ileitis, colitis and ileokolit) were divided into two groups. The first group of patients aged 19 to 58 years old (Me-29) (n=34) received the culture of MSCs under the scheme (0-1-2-3, then every 26 weeks). The second group of patients with CD (n=46) aged 20 to 62 years (ME-28) received standard anti-inflammatory therapy with 5-aminosalicylic acid (5-ASA), glucocorticosteroids (GCS) and immunosuppressive (IS). Evaluation of the effectiveness of therapy on the level of the index of activity of Crohn's Disease (CDAI<150 point) was carried out at 12, 24, 36, 48, 60, 72 and 84 months after initiation of therapy.

Results: Among the patients in 1-st group, relapse in the 12 months of observation occurred in 4/36 patients (11.76%). In 2-nd group, relapse occurred in 5/46 (10.8%) (p=0.82). After 24 months in the 1-st group of patients receiving MSC, relapse occurred in 6/34 (17.6%). In the 2-nd group of patients relapse occurred in 19/27 (41.3%) (p=0.044). After 36 months in 1-st group patients with a relapse of the disease was in 11/34 (32.3%). In the 2-nd group relapse was 29/46 (63.1%) (p=0.01). After 48 months in 1-st group, receiving MSCs, relapsed in 15/34 (44.1%). In the 2-nd group relapse was in 33/46 (71.7%) (p=0.023). After 60 months in the 1-st group relapse was in 19/34 (55.9%). In the 2-nd group relapse was 40/46 (86.9%) (p=0.004). After 72 months in 1-st group relapse was 25/34 (73.5%). In 2-nd group relapse of the CD occurred in 45/46 (97.8%) (p=0.001). After 84 months in 1-st group relapse CD occurred in 29/34 (85.3%). In the 2-nd group of patients CD relapse occurred in 46/46 (100.0%) (p=0.011).

Conclusions: MSCs transplantation helps to maintain a long-term clinical remission in patients with luminal crohn's disease compared with GCS/IS therapy.

oleg7@bk.ru

Exposure to excess phenobarbital negatively influences the osteogenesis of chick embryos

Yu Yan

Jinan University, China

Phenobarbital is an antiepileptic drug that is widely used to treat epilepsy in a clinical setting. However, a long term of phenobarbital administration in pregnant women may produce side effects on embryonic skeletogenesis. In this study, we aim to investigate the mechanism by which phenobarbital treatment induces developmental defects in long bones. We first determined that phenobarbital treatment decreased chondrogenesis and inhibited the proliferation of chondrocytes in chick embryos. Phenobarbital treatment also suppressed mineralization in both *in vivo* and *in vitro* long bone models. Next, we established that phenobarbital treatment delayed blood vessel invasion in a cartilage template, and this finding was supported by the down-regulation of vascular endothelial growth factor in the hypertrophic zone following phenobarbital treatment. Phenobarbital treatment inhibited tube formation and the migration of human umbilical vein endothelial cells. In addition, it impaired angiogenesis in chick yolk sac membrane model and chorioallantoic membrane model. In summary, phenobarbital exposure led to shortened lengths of long bones during embryogenesis, which might result from inhibiting mesenchyme differentiation, chondrocyte proliferation and delaying mineralization by impairing vascular invasion.

283329554@qq.com

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Nitromedicine: a new concept

Knyazev O[1], Kagramanova A[1], Fadeeva N[1], Boldyreva O[1], Lishchinskaya A[1], Ruchkina I[1], Babayan A[1], Kirova M[1], Konoplyannikov A[2], Noskova K[1], Orlova N[1] and Parfenov A[1]

Salaheldin Halasa, Praveen R Arany and Michael R Hamblin

Nitromedicine is a new medical treatment paradigm, focused on increasing nitric oxide (NO) bioavailability and modulating redox-signaling pathways combined with phototherapy, electrotherapy and stem cell therapy. It has been known since the discovery of the biological role of NO in the 1980s, that supplying NO donors such can have many beneficial effects in different conditions by stimulating stem cells and modulating the immune response, but there also exists a substantial risk of side-effects with long-term use. Excess NO can inhibit mitochondrial metabolism by binding to cytochrome c oxidase (CCO) and can also produce reactive nitrogen species (Peroxynitrite) by interacting with reactive oxygen species (ROS). To avoid these potential damaging side-effects we propose to combine the use of NO donors with three additional components. Firstly we believe that addition of antioxidants such as hydrogen sulfide donors, polyphenols and vitamins can neutralize ROS and RNS. Secondly we believe that application of appropriate wavelengths and dosages of light (blue, red or near infrared depending on the exact condition being treated) will dissociate NO from CCO (and other storage sites) thus restoring mitochondrial ATP production and stimulating healing in many situations. Thirdly delivering electrons to the body might help to saturate the free radicals with electrons, eliminate underlying oxidative stress, stabilize mitochondria, prevent further formation of pathological free radicals and increase the nitric oxide bioavailability. This combination therapy may be applied to treat a large variety of oxidative stressed related diseases such as degenerative diseases, immunological diseases, chronic infectious diseases, cancers and a broad range of unmet medical needs involving chronic inflammation with an emphasis on pain management.

info@nitromedicine.com

A unique network involving CXCR4 and CXCR7 coordinates cardiac lineage specification and mobilization of induced pluripotent stem cells

Sima T Tarzami

Howard University College of Medicine, USA

An adult heart has an intrinsically limited capability to regenerate damaged myocardium. Human embryonic and induced pluripotent stem cell (hESC/hiPSC)-based therapies offer a unique strategy for developing cell replacement treatments for numerous disorders including cardiac diseases. The present study identifies a unique signaling network, SDF-1/CXCR4/CXCR7, that regulates cardiac lineage differentiation and migration in human induced pluripotent stem cells (hiPSCs). The fact that SDF-1 binds to CXCR4 and CXCR7 raises a concern on how to distinguish the potential contribution of the SDF-1/CXCR7 pathway from SDF-1/CXCR4 pathway in all the processes that were previously attributed to SDF-1/CXCR4. Therefore, we set these studies to disseminate the role of the SDF-1/CXCR4/CXCR7 network in cardiogenic lineage differentiation and migration of hiPSCs with the premise that their improved recruitment could translate into therapeutic benefits. Using lentiviral vectors to ablate CXCR4 and/or CXCR7 expression, hiPSC-derived cardiomyocytes (hiPSC-CMs) were tested for phenotypic and functional properties due to gene knockdown. Gene expression confirmed cardiomyocyte phenotype of differentiated hiPSCs, although reduction of CXCR4 and CXCR7 expression resulted in delayed cardiac phenotype. Only knockdown of CXCR4 reduced the spontaneous beating of hiPSC-CMs. Knockdown of CXCR4 and CXCR7 differentially altered calcium transients and β-adrenergic response in hiPSC-CMs. In engineered cardiac tissues, depletion of CXCR4 or CXCR7 had opposing effects on developed force. The transendothelial migration response to SDF-1 was suppressed by knockdown of either CXCR4 or CXCR7. In contrast, in a trans-well chemotaxis assay, only depletion of CXCR4 reduced hiPSC migration in response to SDF-1 indicating that both CXCR4 and CXCR7 have distinct roles in the SDF-1/CXCR4/CXCR7 axis as network coordinators of cardiogenic induction and mobilization of hiPSCs. We contend that gaining further insight into the molecular nuances of this phenomenon will provide new insights for optimization of the cardiac repair potential of cell-based therapies.

sima.tarzami@howard.edu

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Role of point of care pharmacist in patient receiving oral chemotherapeutic agents

Naureen Wajid
American Hospital Dubai, UAE

Background: Oral chemotherapeutic agents have been conceptualized as a convenient, less toxic form of therapy that is preferred by the patients. However many safety issues related to chemotherapeutic agents are appreciated. Safety issues which include lack of check and balance to avoid medication errors, drug interactions, side effects, administration issues, lack of patient adherence and shift of responsibility for managing a potential complicated oral regimen from Oncologists, nurses and Pharmacists to the patient and caregivers. As a result of these factors Oncology Pharmacist can be utilized as Point of Care Pharmacist (PCP) and can be consulted to identify drug related problems (DRPs) and to provide patient counseling.

Objectives: To evaluate the 1) role of Point of Care Pharmacist (PCP) service provided to the patients receiving Oral Chemotherapeutic Agents 2) Number of DRPs identified by the PCP and 3) Type of recommendations made for management of DRPs.

Study Design: This is prospective observational study. PCP can help the patient with everything to get the Oral Chemotherapy to start and provides the cost estimate for insurance, corporate and self payers. PCP can help in designing standard order forms for Oral Chemotherapeutic agents which includes all the information including diagnosis, cycle number, and body surface area and dosing calculations. PCP met with patient receiving oral chemotherapeutic agents and takes the Patient medication history (PMH), check for drug-drug, drug-food interactions, and provides patient counseling and patient education materials. Complete pre and post counseling questionnaire to capture the understanding of their oral chemotherapeutic agents.

Methodology: PCP RECEIVES PROTOCOL → PROVIDE COST ESTIMATE → MEDICATION PROCUREMENT → PCP RECEIVES CONSULT → PRE –COUNSELLING QUESTIONNAIRE → PCP COMPLETES PMH , INTERACTION CHECKING ,COUNSELLING & PROVIDING PATIENT EDUCATION MATERIALS →POST COUNSELLING QUESTIONNAIRE → RECOMMENDATIONS

Intended outcomes: The intended outcomes are as follows: Peace of mind for physicians, nurses and patients by expert support from point of care pharmacist, standard order forms for oral chemotherapy in order to keep the cycle track, reducing medication errors by multiple checking of order forms from oncologists, PCP and nurses, helps in resolving tough administration issues e.g. IV to oral switching, can be crushed or not, can be given through nasogastric tubes, extemporaneous compounding options etc., identifies drug interactions, communicate to oncologists and document the recommendations, reduction in DRPs and to improve understanding of oral chemotherapy by the patients.

Conclusion: The study will suggest that the consult service of PCP for oral chemotherapeutic agent is beneficial and should be continued.

naureenwajid786@gmail.com