ROYAN International Twin Congress

22

22nd Congress on Reproductive Biomedicine
September 1 - 3, 2021

17th Congress on Stem Cell Biology & Technology
September 4 - 5, 2021

September 1- 5, 2021 Tehran- Iran

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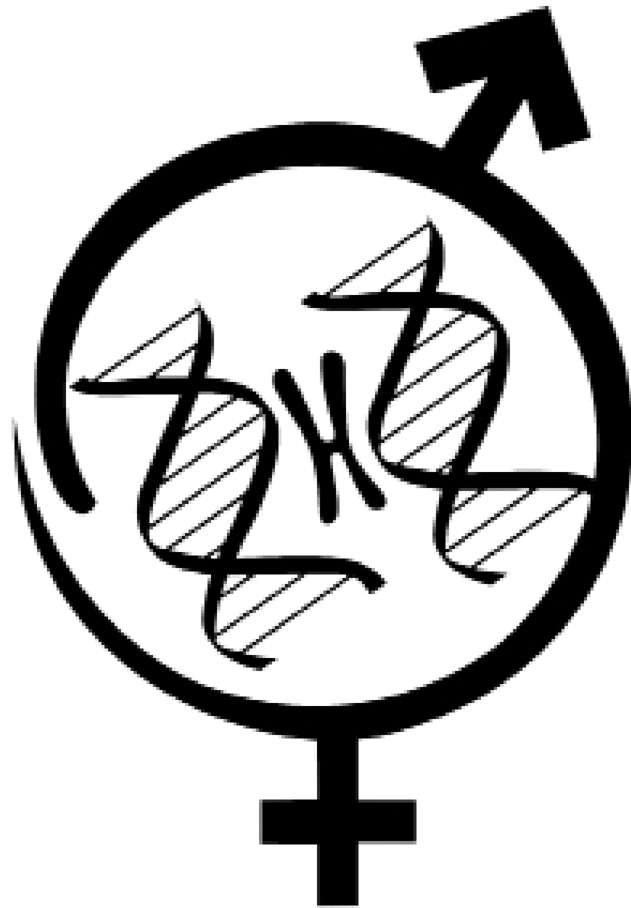
Abstracts of

Royan International Virtual Twin Congress

17th Congress on Stem Cell Biology and Technology
4-5 September 2021



Royan Institute

Cell Science Research Center

Tehran, Islamic Republic of Iran

Abstracts of the 17th Congress on Stem Cell Biology and Technology (2020)

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Royan International Virtual Congress (2021)

Congress Chairperson

Fereshteh Karamali

Dear Colleagues,

It is our great pleasure to welcome you to the 17th International Virtual Congress on Stem Cell Biology and Technology (ICSCBT), 3-5 September 2021.

The ICSCBT is an international annual gathering to feature a global line-up of scientists and clinicians in order to grow the community working on stem cells, developments in basic and translational research.

The aim of ICSCBT is to bring us together for scientific and friendly discussion on our impediments and innovation, in the hope of broadening and sharing our margins of understanding in the field of stem cell, to facilitate the transition from bench to market.

22th International congress on Reproductive Biomedicine will be held virtually and paralleled to this event (1-3 September 2021).

Royan institute was established in 1991 by the late Dr. Kazemi Ashtiani. As a pioneer institute, Royan Institute for Stem Cell Biology and Technology (RI-SCBT embraced stem cells basic and translational studies, developmental biology and regenerative medicine.

On behalf of the organizing committee, I welcome you to the 17th Congress. We are excited to get connected with you in ICSCBT 2021 to learn, share and network.

Kind regards
Fereshteh Karamali, PhD
Chairperson of 17th Royan International Virtual Congress on Stem Cell Biology & Technology

Invited Speakers

Is-1:: Chicken Chimera for Testing Human Pluripotent Stem Cell Potency

Akhlagh Pour A

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Human pluripotent stem cells (hPSCs) which display two distinct states of pluripotency, naïve and primed, emerge capability to differentiate into widespread lineage diversities under proper conditions. Chimera formation has been extensively accepted as a stringent test to evaluate the functionality of these hPSCs. However, this strategy lacks an appropriate animal model recipient for these hPSCs. Here, we report that the developing chicken embryo is a permissive and potent niche that can be used to show the pluripotency potential of hPSCs. In the matched developmental stage approach, fluorescence microscope imaging and PCR analysis results both showed that the host chick embryo provided a suitable microenvironment for naïve-like and primed hPSCs to survive and undergo differentiation. Importantly, primed hPSCs that were injected into the host embryos in the non-developmental stage match approach could generate chimeras, whereas the naïve-like hPSCs did not have an adequate contribution. Therefore, we suggest that chick embryo chimerism can be used to identify different pluripotent states in hPSCs. Our findings show that this is an appropriate model to study the biological potential of hPSCs and understand the early mechanisms that regulate differentiation.
Keywords: Naïve and Primed Human Pluripotent States, Chimera Formation, Chick Embryo

Is-2

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Is-3: Artificial Intelligence in Chemical Biology - From Differentiating Cells to Drug Discovery

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Is-4: Controversial Role of Dexamethasone on Mesenchymal Stem Cells Differentiation Toward Osteogenesis and Chondrogenesis

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Objective: Dexamethasone is a synthetic glucocorticoid, widely employed in *in vitro* differentiation protocols. In the case of bone marrow mesenchymal stromal cells (BMSCs), it is present in all differentiation cocktails to commit cells towards the classical mesenchymal lineages, i.e., osteogenic, chondrogenic, and adipogenic differentiation. In the field of bone research, dexamethasone has been often used as the sole inducer of cell commitment for human BMSCs. However, the activation of the glucocorticoid receptor induces a multiplicity of cellular effects, both genomic and extra-genomic. It is known that dexamethasone inhibits terminal differentiation of osteoblasts and has a strong pro-adipogenic effect; moreover, one of the main risks of long-term clinical administration of glucocorticoids is the development of osteoporosis and osteonecrosis. This raised the question whether dexamethasone is the right tool to study differentiation mechanisms *in vitro*. We have previously demonstrated that dexamethasone alters non-coding RNA expression during early stages of osteo- and chondrogenesis, an effect that was independent of the differentiation status. The aim of the study was to clarify the effect of dexamethasone during early osteogenic commitment of human BMSCs, with a particular attention to the three main lineage-specific transcription factors: RUNX2, SOX9, and PPARG.
Materials and Methods: Human BMSCs were isolated from the bone marrow of at least 15 donors. Cells were induced to osteogenic differentiation using a combination of concentrations of dexamethasone and time points of exposure. Gene expression analysis, Alizarin Red/Oil Red O staining, and computational methods were used to analyse the effects of dexamethasone on cell differentiation.
Results: Results showed a dose-dependent effect of dexamethasone in the repression of SOX9 expression (hence improving osteogenic differentiation), and in increasing PPARG levels, with the consequence of the formation of pre-adipocyte-like cells within mineralizing cultures. RUNX2 gene expression levels were largely unaffected by dexamethasone. Then, we have introduced dexamethasone in a computational model describing the interaction between RUNX2, SOX9, and PPARG. We used approximate Bayesian computation (ABC) simulations to estimate the parameters of the theoretical model that could best fit the experimental results about the three transcription factors levels. The simulations reproduced the experimental trends, but they also predicted a lower RUNX2 activity compared to experimental gene expression data, indicating that we need to integrate further signalling components.
Conclusions and future perspectives: Despite dexamethasone is effective in inducing osteogenic differentiation of human BMSCs through SOX9 downregulation, it can also induce a simultaneous off-target adipogenic differentiation in a subset of cells. This warrants further studies to improve our models of *in vitro* osteogenesis. This work raises the urgent need to find new strategies to induce SOX9 downregulation but excluding PPARG upregulation.

Is-5: Engineering Biomaterial-Cell Interactions

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Biomaterial-cell interaction is one of the most interesting subjects of research in tissue engineering and regenerative medicine. Biomaterials commonly interact with the cells and influence their fate both directly and indirectly. In this manner, polymeric materials are an important class of biomaterials that categorized as synthetic and natural. Here, we will review different physicochemical and biological cues of polymeric materials and their effects on the cell fate.

Is-6: Microfluidic Technologies for Stem Cell Analysis

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Stem cell research breakthrough depends severely on throughput and precision of stem cell acquisition as well as downstream assays high controllable condition. According to increasing demand for purified stem cells, separation and analysis technologies need to be improved to meet with current requirements.
The prerequisite step in almost all cell-based experiments is cell isolation. Although there are fully developed well-known techniques for this aim including Fluorescence activated cell sorting (FACS), Magnet-activated cell sorting (MACS), density gradient centrifugation, field flow fractionation (FFF), and dielectrophoresis (DEP), stem cell research was not completely satisfied by these techniques. The main challenges and drawbacks of mentioned conventional cell separation techniques are multiple manipulation for sample preparation, time consuming and aggressive separation manner which affect stem cells desired fate, viability and quality.
Microfluidic technology provides distinguished proportional features for scientists, considering their specific needs in various applications. Microfluidics benefit from their special intrinsic properties such as high controllability, low sample volume, low biohazard, high precision and reliability, possibility of real-time screening and multifunctionality. All these privileges make it a promising adoptable tool corresponding to desired purposes.
In this speech first, emerging stem cell separation methods based on microfluidic platforms are reviewed. Proceeding to this review, the microfluidic devices for analysis will be discussed and finally, multiplexed integrated microchips as next-generation bioreactors will be introduced.

Is-7: Bottom-Up Synthetic Embryology for Understanding Early Human Development

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Bottom-Up Synthetic Embryology for Understanding Early Human Development Early human development remains mysterious and very difficult to study. Recent advances in mammalian embryology, stem cell biology, organoid technology, and bioengineering have contributed to a significant interest in bottom-up, synthetic stem cell-derived models of human development (or embryoids). The controllability and reproducibility of human embryoids coupled with the ease of genetically modifying stem cell lines, the ability to manipulate culture conditions and the simplicity of live imaging make them robust and attractive systems to disentangle cellular behaviors and signaling interactions that drive human embryogenesis. In this talk, I will describe our effort in using human pluripotent stem cells (hPSCs) to develop tractable experimental models of the peri-implantation embryonic development and neurulation. The peri-implantation human embryoids developed by us recapitulate key early post-implantation developmental landmarks successively, including pro-amniotic cavity formation, amniotic ectoderm-epiblast patterning, primordial germ cell specification, and development of the primitive streak with controlled anteroposterior polarity. I will further discuss an hPSC-based neuroectoderm patterning model to recapitulate the formation of the neural plate and another more recently developed, patterned neural tube model with fully defined anterior-posterior and dorsal-ventral axes. This patterned human neural tube model is capable of capturing neuronal pattering along two orthogonal axes in a three-dimensional tubular geometry, the hallmark of neural tube development *in vivo*.

Is-8: Chondrocyte and Stem Cell-Based Cartilage Regeneration: Effect of Biomaterial and Biomechanics

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Chondral and osteochondral lesions are commonly encountered in clinical practice, yet the restoration of functional hyaline cartilage remains challenging. Cell and biomaterial assisted cartilage regeneration strategies hold significant promise and have widely been investigated in preclinical and clinical studies. Mechanical stimuli, applied *in vitro* for tissue engineering or *in vivo* through physical rehabilitation therapy, play a crucial role for functional cartilage restoration. Various *in vitro* studies have shown that mechanical compression and shear promotes the development of a cartilaginous extracellular matrix and a low-friction surface in chondrocytes seeded biomaterial constructs. Furthermore, chondrogenic differentiation of bone marrow derived mesenchymal stem cells can be achieved by joint specific mechanical cues without the need for exogenous growth factor supplementation. Biomaterials that facilitate the recruitment of endogenous cells are under investigation as cell-free (osteo)chondral implants. We compared different hyaluronan and fibrin-based hydrogels regarding their ability to allow chemoattractant guided migration of mesenchymal stem cells. Both *in vitro* and *in vivo* studies confirmed the superior behaviour of a fibrin-hyaluronan hydrogel for cell infiltration and
neocartilage formation. Finally, the chemoattractant containing hydrogel was tested in an osteochondral defect explant model

under joint mimicking mechanical load in our bioreactor. This lecture will highlight (i) the use of a bioreactor mimicking joint kinematics for preclinical *in vitro* studies; (ii) the relevance of mechanical stimuli for functional cartilage regeneration and (iii) the influence of the biomaterial composition on cell-based and cell-free cartilage repair. (osteo)chondral implants. We compared different hyaluronan and fibrin-based hydrogels regarding their ability to allow chemoattractant guided migration of mesenchymal stem cells. Both *in vitro* and *in vivo* studies confirmed the superior behaviour of a fibrin-hyaluronan hydrogel for cell infiltration and neocartilage formation. Finally, the chemoattractant containing hydrogel was tested in an osteochondral defect explant model under joint mimicking mechanical load in our bioreactor. This lecture will highlight (i) the use of a bioreactor mimicking joint kinematics for preclinical *in vitro* studies; (ii) the relevance of mechanical stimuli for functional cartilage regeneration and (iii) the influence of the biomaterial composition on cell-based and cell-free cartilage repair.

Is-9: *In Vitro* Morphogenesis Model for Intra-Hepatic Bile Duct Development

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During embryo development, the bipotent hepatoblasts in the septum transversum mesenchyme (STM) differentiate into hepatocytes and biliary cells that form the intra-hepatic bile ducts. The cell fate decisions are governed by the microenvironmental cues in the STM and the portal mesenchyme. The formation of ductal plate induced by the interactions with the portal vein and the dilations control biliary fate decision and development of intra-hepatic bile ducts. In our mesenchymal ECM sandwich culture, which mimics the STM invasion of the hepatoblasts, adult liver stem cells undergo morphogenesis into tubular biliary structures and the early events mirror spatiotemporal regulation of SOX9 observed during liver development. Using this model, we are exploring the mechanisms that govern spatiotemporal regulation of SOX9 and the dilations events observed *in vivo*. These mechanisms provide critical insights to understanding the deregulated regeneration in chronic liver diseases and might provide avenues for therapeutic intervention.

Is-10: Dysregulation of the Cap-Dependent mRNA Translation in Cancer

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Precise spatio-temporal regulation of gene expression in response to changes in environmental conditions is indispensable for maintenance of cell homeostasis. The mRNA translation machinery plays a key role in this process and therefore is tightly regulated through several, at times overlapping, mechanisms that modulate its efficiency and accuracy. Due to their fast rate of growth and metabolism, cancer cells require an excessive amount of mRNA translation and protein synthesis. However, unfavorable conditions, such as hypoxia, amino acid starvation, and oxidative stress, which are abundant in cancer, as well as many anti-cancer treatments inhibit mRNA translation. Cancer cells adapt to the various internal and environmental stresses by employing specialised transcript-specific translation to survive and gain a proliferative advantage. I will highlight some of our recent findings on the mechanisms of regulation of cap-dependent mRNA translation, including the key element in mediating the microRNA-induced translational silencing and RNA helicases, which enables the growth and survival of cancer cells.

Is-11: Olfactory Ensheathing Cell Transplantation to Repair Spinal Cord Injuries: Stimulating Cells to Improve Efficacy

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Objective: Olfactory ensheathing cell transplantation to treat spinal cord injury is a promising therapeutic approach. However, regeneration outcomes are limited and the therapy needs improving. One of the main barriers of cell transplantation is that the vast majority of cells do not survive more than 1-2 days after transplantation. Therefore strategies that enhance cell survival may improve therapeutic outcomes. As first step toward improving olfactory ensheathing cell cell survival and efficacy, we have screened compounds to identify potential drugs that can stimulate the activity of olfactory ensheathing cells.
Materials and Methods: Primary mouse olfactory ensheathing cells were assayed with various natural products and synthetic compounds, and effects on cell proliferation, migration and phagocytic activity were examined. Compounds that were active against olfactory ensheathing cells were tested for efficacy on the related glial cell Schwann cells.
Results: A range of different compounds were found to significantly stimulate the proliferation, migration and/or phagocytic activities of olfactory ensheathing cells. Some natural compounds were highly specific in their efficacy, and displayed considerably different efficacy against the related glial cell Schwann cell. The drug liraglutide modules olfactory ensheathing cell migration partly by altering the extracellular matrix production by the cells.
Conclusion: Select stimulation of activities of olfactory ensheathing cells which are therapeutically useful for spinal cord repair can be achieved by natural and synthetic compounds.

Is-12: Retinal Progenitor Cells for Treatment of Retinitis Pigmentosa

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Retinitis pigmentosa (RP) is a severely disabling degenerative disease of the retina that has proven notoriously resistant to treatment. A rare but classic example of a neural degeneration, RP is representative of a broad area of significant unmet medical need. Yet despite the daunting clinical picture, there is a clear rationale as to why it might be possible to achieve a beneficial

effect in this condition. RP is a rod-cone dystrophy in which the defective genes are predominantly specific to rod photoreceptors and only rarely expressed by RPE cells or cones. Cone death is therefore a knock-on effect that might be further delayed via neurotrophic rescue, even without correcting underlying genetic errors or replacing degenerating rod cells, thus prolonging sight in these patients. Our approach is based on the use of allogeneic human retinal progenitor cells (hRPCs), injected into the vitreous cavity, from which location the cells spontaneously secrete endogenous neuroprotective factors that spread to the retina. The treatment is performed under topical anesthesia, without surgery or immunosuppression. Clinical studies to date include an initial phase 1/2a trial, a masked and randomized 2b study, together with additional studies examining the safety of repeat dosing. Data collected to date are consistent with a high level of tolerability for the allogeneic RPC product and provide evidence of a functional signal, thereby laying the groundwork for an upcoming phase 3 trial in RP.

Is-13: Blood Vessel Formation and Bone Regeneration Potential of Human Adipose Stem Cells in Combination with Calcium Phosphate Ceramics for Jaw Bone Augmentation

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Bone substitutes are used as alternatives for autologous bone grafts in patients undergoing maxillary sinus floor elevation (MSFE) for dental implant placement. However, bone substitutes lack osteoinductive and angiogenic potential. Addition of adipose stem cells (ASCs) may stimulate osteogenesis and osteoinduction, as well as angiogenesis. We aimed to evalutate 1) the potential additive effect on bone regeneration by the addition of freshly isolated, autologous but heterologous stromal vascular fraction (SVF), which is highly enriched with adipose stromal/stem cells when compared to native adipose tissue, and 2) the vascularization in relation to bone formation potential of the ASC-containing SVF of adipose tissue, seeded on two types of calcium phosphate carriers, within the human MSFE model, in a phase I study. Autologous SVF was obtained from ten patients and seeded on β-tricalcium phosphate (n = 5) or biphasic calcium phosphate carriers (n = 5), and used for MSFE in a one-step surgical procedure. After six months, biopsies were obtained during dental implant placement, and the quantification of the number of blood vessels was performed using histomorphometric analysis and immunohistochemical stainings for blood vessel markers, i.e., CD34 and alpha-smooth muscle actin. Bone percentages seemed to correlate with blood vessel formation and were higher in study versus control biopsies in the cranial area, in particular in β-tricalcium phosphate-treated patients. This study shows the safety, feasibility, and efficiency of the use of ASCs in the human MSFE, and indicates a pro-angiogenic effect of SVF, providing the first step toward a novel treatment concept that might offer broad potential for SVF-based regenerative medicine applications.

Is-14: Glioblastoma Treatment with Engineered Exosomes

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Is-15: A fully-synthetic polyplex-in-hydrogel system for microRNA-92a inhibitor delivery to improve localized angiogenesis

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Objective: Therapeutic angiogenesis, which involves the generation of new vessels by delivery of specific factors, is essential to achieve repair in ischemic or injured tissues. MicroRNA-92a (miR-92a), a member of the miR-17-92 cluster, serves as a negative regulator of endothelial function and angiogenesis in ischemic conditions. While its mechanistic role in angiogenesis is known, a safe and efficient delivery of miR-92a inhibitor/s should be accomplished for its clinical translation.
Materials and Methods: In the current study, we have modified 1.8 kDa-branched polyethylenimine (PEI1.8) with a bile acid, deoxycholic acid (DA), and subsequently used resulting conjugates (PEI1.8-DA) to deliver a locked nucleic acid (LNA)-based miR-92a inhibitor (LNA-92a) *in vitro* and *in vivo*.
Results: The PEI1.8-DA conjugate successfully delivered LNA-92a into endothelial cells isolated from human umbilical vein (HUVECs), which led to a substantial increase in the expression levels of the integrin subunit alpha 5 (ITGA5), the sirtuin-1 (SIRT1) and Krüppel-like factors (KLF) KLF2/4, formation of capillary-like structures and migration of HUVECs *in vitro*. Down-regulation of miR-92a following the polyplex-mediated delivery of LNA-92a, also resulted in significantly improved capillary density in a chicken chorioallantoic membrane (CAM) model. Finally, sustained release of PEI1.8-DA/LNA-92a polyplexes from an in situ forming, biodegradable hydrogel based on clickable poly(ethylene glycol) (PEG) macromers, led to a substantial increase in formation of new capillaries and mature vessels in the subcutaneous tissues of mice.
Conclusion: Our data demonstrate the potential of PEI1.8-DA conjugates as non-viral polymeric platforms for safe and efficient delivery of LNA-92a to improve angiogenesis both *in vitro* and *in vivo*. We suggest click PEG hydrogel as a promising platform for localized retention and sustained presentation of PEI1.8-DA polyplexes for different therapeutic targets.

Is-16: Advanced 3D Printed Microfluidic Technologies for Stem Cell Research

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Scaling down the high-cost and demanding facilities into a tiny, multi-functionalized microchip has revolutionized research areas and is named microfluidics. Microfluidics is a science that allows the manipulation of minuscule fluid samples, ordinarily in the range of microliters, within networks of channels ranging from tens to hundreds of micrometers. Microfluidic systems are promising tools for the advancement of stem cell research with evident benefits. Improvements such as reduced reagent consumption, deeper analysis, higher sensitivity, modeling of *in vivo* microenvironments, rapid processing, detailed spatial resolution, process integration, and automation in stem cell biology have been achieved over the past three decades.
In recent years, additive manufacturing, in particular, 3D printing, has gained significant traction, being named the third industrial revolution. Due to the expanding use of microfluidic systems in laboratories, 3D printing has emerged as an alternative method to the traditional, costly fabrication process. The ability to fabricate structures ranging from a few microns to several centimeters is a complex process that can only be accomplished by taking advantage of 3D printing methods. Rapid prototyping provides an opportunity to adopt a "fail fast and often" strategy, motivating the researchers to utilize 3D printers in the field of microfluidics.
The modularization of microfluidic devices using additive manufacturing enables researchers to fabricate integrated microfluidic devices for various applications. Among all these different applications, research in stem cell biology has shown significant promise. Not long ago, microfluidics was a burgeoning technology on the fringe of practical applications; now, it is coming of age in the field of stem cells. Thus, microfluidics is being to recognize its enormous potential for research in stem cell biology. In this presentation, I am pleased to present the use of advanced 3D printed microfluidic technologies for stem cell research.

Is-17 Blastoids: Modeling Blastocyst Development and Implantation

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Is-18: Sound-Induced Morphogenesis of Multicellular Systems for Rapid Orchestration of Vascular Networks

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Morphogenesis, a complex process, ubiquitous in developmental biology and many pathologies, is based on self-patterning of cells. Spatial patterns of cells, organoids, or inorganic particles can be forced on demand using acoustic surface standing waves, such as the Faraday waves. This technology allows tuning of parameters (sound frequency, amplitude, chamber shape) under contactless, fast and mild culture conditions, for morphologically relevant tissue generation. We call this method Sound Induced Morphogenesis (SIM). In this work, we use SIM to achieve tight control over patterning of endothelial cells and mesenchymal stem cells densities within a hydrogel, with the endpoint formation of vascular structures. Here, we first parameterize our system to produce enhanced cell density gradients. Second, we allow for vasculogenesis after SIM patterning control and compare our controlled technology against state-of-the-art microfluidic culture systems, the latter characteristic of pure self-organized patterning and uniform initial density. Our sound-induced cell density patterning and subsequent vasculogenesis requires less cells than the microfluidic chamber. We advocate for the use of SIM for rapid, mild, and reproducible morphogenesis induction and further explorations in the regenerative medicine and cell therapy fields.

Is-19: Engineering a Model to Study Viral Infections: Bioprinting, Microfluidics, and Organoids to Defeat Coronavirus Disease 2019 (COVID-19)

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While the number of studies related to severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) is constantly growing, it is essential to provide a framework of modeling viral infections. Therefore, this review aims to describe the background presented by earlier used models for viral studies and an approach to design an "ideal" tissue model for SARS-CoV-2 infection. Due to the previous successful achievements in antiviral research and tissue engineering, combining the emerging techniques such as bioprinting, microfluidics, and organoid formation are considered to be one of the best approaches to form in vitro tissue models. The fabrication of an integrated multitissue bioprinted platform tailored for SARS-CoV-2 infection can be a great breakthrough that can help defeat coronavirus disease in 2019.
Keywords: Severe acute respiratory syndrome-related coronavirus 2, Coronavirus disease 2019, Coronavirus, Bioprinting, microfluidics, Organoids, Tissue models, Viral infection, Body-on-a-chip

Is-20: *In vitro* Derivation of Oocytes from Stem Cells: The State of the Art, or Artificial Egg, Two Decades Later"

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Is-21: Novel Therapeutic Approaches for COVID19

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To date, there is no licensed treatment or approved vaccine to combat the coronavirus disease of 2019 (COVID-19), and the number of new cases and mortality multiplies every day. Therefore, it is essential to develop an effective treatment strategy to control the virus spread and prevent the disease. Here, we summarized the therapeutic approaches that are used to treat this infection. Although it seems that antiviral drugs are effective in improving clinical manifestation, there is no definite treatment protocol. Lymphocytopenia, excessive inflammation, and cytokine storm followed by acute respiratory distress syndrome are still unsolved issues causing the severity of this disease. Therefore, immune response modulation and inflammation management can be considered as an essential step. There is no doubt that more studies are required to clarify immunopathogenesis and immune response; however, new therapeutic approaches including mesenchymal stromal cell and immune cell therapy showed inspiring results.

Is-22: Cancer Nanotechnology and Personalized Medicine: Recent Advances and Future Direction of Cancer (Stem) Cells with RNA Delivery Systems

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The emergence of nanoscience and nanotechnology has created many hopes for the treatment of various diseases such as cancer through the introduction of novel strategies. By benefiting from this technology, an anti-cancer drugs could be administered to the body without body negative reaction, could easily be dissolved in the body fluids and being transported throughout the body via systematic circulation. Nanotechnology could also tackle and regulate the pharmacokinetics (PK) and pharmacodynamics (PD) features of drug formulations leading to an improved efficacy. However, we still observe that with such promising technologies, we have not had enough success to conquer cancer. It seems that cancer cells show resistance against these treatment strategies. We believe that using the same technology (nano drug delivery systems) and carrying the genes such as RNA that could regulate the cells' molecular pathways, cancer could be defeated more easily. In other words, we had addressed not only the external environment of cancer cell, but also the internal microenvironment. This new technology could be considered as the basis of personalized medicine, and in more advanced form, RNA loaded nanocarriers could be promising in regulating the cancer stem cells leading to a decrease in cancer cases through preventing its recurrence.

Oral Presentations

O-1: Improvement of Chondrogenesis by Kartogenin Loaded in Exosome from Human Bone Marrow Derived Mesenchymal Stem Cells In Vitro

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Objective: Rheumatoid arthritis (RA) is a systemic autoimmune disease that is characterized by synovial tissue inflammation and also cartilage destruction. Despite the cell therapy progression, there are fundamental gaps in RA that need to be effort including the inadequate de-novo cartilage formation. On the other hand, exosomes (Exo)-derived mesenchymal stem cells (MSCs) are the promising alternative of MSCs for future clinical approaches as nano-carriers for transporting proteins, miRNAs, and small-molecules (SMs). In addition, using effective biological components and SMs have substantially improved clinical outcomes. For instance, Kartogenin (KGN) is an SM that induces chondrogenesis of MSCs in vitro and *in vivo*; Although short half-lives and high doses needed for SMs are disadvantages of KGN. Hence, the main aims of the current study are the use of EVs for safe drug delivery that has more efficiency and durability to improve the chondrogenesis in vitro and then to understand the efficacy and durability of Exos as carriers of SMs for CIA in the rat model.
Materials and Methods: MSCs were isolated from bone marrow stroma and identified by the expression of cell surface markers (CD90, CD105, CD73, CD34, and CD45) and also osteogenic/adipose/chondrogenic differentiation. Subsequently, to isolate Exo we used collected condition media of passage-3 cells from which exosomes were isolated by differential ultracentrifugation and were characterized. The purified-Exo were determined by the BCA protein assay and the DLS analysis for the future experiment. To create Exo-KGN, electroporation subsequently was used to load KGN inside Exo. Then, high-performance liquid chromatography (HPLC) was used to evaluate the KGN release of Exo. To investigate the uptake of Exo-KGN, after culturing cells in 24 well cultural dishes, exosomes, and cells that were labeled before with calcine dye and PKH26, respectively was added to cells and then at 1, 12, and 24 hours, the cells were examined by fluorescent microscope. The result of the chondrogenic effect of Exo-KGN was evaluated after 21 days compared to MSCs treated with chondrogenic induction media and KGN without Exo. The real-time polymerase chain reaction (RT-PCR) analysis was applied to evaluate the expression of chondrocytes genes (Collagen II, Aggrecan, and Collagen X). Finally, Histological and immunohistological (IHC) analysis was used to investigate the differentiation to cartilage.
Results: Results showed the concentration of 1.5-2×103 µg EVs harvested from MSCs using the BCA method. The size of EVs calculated with the DLS technique was between 70-150 nm. The MTT result of days 3, 5, and 7 showed that 100 µg/ml concentration of KGN is nontoxic for the treated cells compared to the control group. The uptake of Exo by cells was confirmed after 24 h and also the KGN release of Exo was detected by chromatography technique. The effect of Exo on chondrogenesis was also assessed in 4 groups of Exo-KGN, Exo, KGN, and chondrogenic media after 21 days. As a result, the Exo-KGN showed the most expression of COL II and Aggrecan genes. Exo-KGN significantly induced the chondrogenesis in cells compared to the control group (**$P<0.01$, ***$P<0.001$). In addition, the CoL II and Aggrecan expression was confirmed by IHC.
Conclusion: We investigated that Exo-KGN increases chondrogenesis similar to the KGN group (without Exo). More importantly, the use of KGN was reduced significantly in Exo-KGN compared to the KGN group. Taken together, exosomes are an ideal vehicle for drug delivery as well as for future therapeutics application.
Keywords: Chondrogenesis, Mesenchymal Stem Cells, Exosomes, Kartogenin, Carrier

O-2: Distribution and Population of Key Cells Responsible for Regeneration in Chronic Kidney Disease

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Objective: Chronic kidney disease (CKD) is a health care problem that is increasing rapidly. The main cause of renal regeneration in CKD seems to be due to the population of renal progenitor cells (RPCs), mesenchymal/stromal stem cells (MSCs), and endothelial progenitor cells (EPCs). To better understand CKD and non-CKD renal tissue regeneration, the RPCs, MSCs, and EPCs were histologically tracked in human kidneys.
Materials and Methods: Fifty participants were selected randomly and were assigned to the non-CKD and CKD groups in Bushehr. The glomerular filtration rate (GFR) of patients was calculated using the MDRD formula. Two groups (n=5) were sampled. People with non-CKD and CKD were biopsied. The samples were evaluated with immunohistochemistry for anti-CD133, anti-CD105, anti-CD24, and anti-CD34 antibodies and were visualized by 3, 3-diaminobenzidine (DAB) staining. The images were analyzed using the cell counting plugin by ImageJ and an independent sample t-test by IBM SPSS Statistics 26 software. The data were considered statistically significant at $p\leq0.05$.
Results: The number of RPCs were detected by CD24 and CD133 positive cells in CKD patients which the number of CD133 positive cells was higher than the patients with normal kidneys ($p<0.001$). The number of the renal MSCs in CKD was biologically more than the normal kidney, however, this difference was not significant ($p>0.05$). The number of CD34 positive cells indicating EPCs in CKD was higher than the normal kidney ($p<0.001$).
Conclusion: RPCs and EPCs in patients with CKD have more regenerating roles than MSCs comparing to the non-CKD kidneys in humans.
Keywords: Chronic Kidney Diseases, Renal Progenitor Cells, Mesenchymal/Stromal Stem Cells, Endothelial Progenitor Cells

O-3: Interferon Beta Attenuates Tauopathy and Recognition Memory Deficits in A Rat Model of Alzheimer's Disease: Activation of PI3K Signaling Pathway as Underlying Mechanism

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Objective: Alzheimer’s disease (AD) is a prevalent, age-related neurodegenerative disease characterized by a cognitive decline as well as accumulation of hyperphosphorylated tau tangles in the brain. It has been revealed that disruption of the phosphatidylinositol-3 kinase (PI3K) signaling pathway contributes to tau hyperphosphorylation and memory deficits. Recently, interferon-beta (IFNβ), an approved drug for treating multiple sclerosis, has been received increasing attention in neurodegenerative diseases. However, the molecular mechanisms involved in the protective effects of IFNβ in AD are less known. In this study, we aimed to investigate the effects of IFNβ on the recognition memory performance, hyperphosphorylated tau level, and the expression of PI3K signaling pathway components in a rat model of AD.
Materials and Methods: The lentiviruses expressing mutant human amyloid protein precursors were injected bilaterally to the rat hippocampus. From day 23 after virus injection, rats were intranasally treated with IFNβ (68,000 IU/rat) every other day until day 50. Recognition memory performance was evaluated by a novel object recognition test on days 46-49. The expression levels of phospho-tau and total tau in the hippocampus were measured by western blotting. The mRNA expression levels of PI3K, AKT, and mTOR were assessed by quantitative polymerase chain reaction (qPCR).
Results: Intranasal administration of IFNβ ameliorated recognition memory deficits in AD rats. Interestingly, a decrease in hyperphosphorylated tau level in parallel to increased expression of PI3K, AKT, and mTOR were identified in the hippocampus of IFNβ-treated AD rats.
Conclusion: These results showed pro-cognitive and anti-neurodegenerative effects of IFNβ in AD, with an emphasis on activation of the PI3K-AKT-mTOR signaling pathway as a downstream effector.
Keywords: Alzheimer’s Disease (AD), Tauopathy, Recognition Memory, Interferon Beta (IFN-B), Phosphatidylinositol-3 Kinase

O-4: N-Acetylcysteine Attenuates Dissociation-Induced Apoptosis in Human Embryonic Stem Cells Through Nrf2 Pathway

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Objective: Human embryonic stem cells (hESCs) can proliferate endlessly through self-renewing which present great potential in regenerative medicine. hESCs are highly susceptible to cell death upon matrix detachment via a process referred to as anoikis. Extracellular matrix (ECM) detachment results in increased reactive oxygen species (ROS) production which leads to increased lipid peroxidation. In this study, we investigated the effects of N-acetylcysteine (NAC) on the apoptosis of hESCs and its possible mechanism.
Materials and Methods: The enzyme activity including Malondialdehyde (MDA) and Glutathione peroxidase (GPX4) were measured in dissociated hESCs. The expression of nuclear factor erythroid 2-related factor 2 (Nrf2) and GPX4 were detected using Western blotting. In the next step, hESCs were treated with NAC, and enzyme activity was measured. Moreover, Nrf2 and GPX4 expression were measured in the presence of NAC.
Results: Our data showed that MDA level increased after dissociation in hESCs in a time-dependent manner while GPX4 activity decreased. The amount of MDA decreased in NAC-treated-hESCs compared to the control. Also, GPX4 activity increased in NAC-treated hESCs in comparison to the control group. Moreover, GPX4 and Nrf2 expression decreased in dissociated hESCs compared to the control. Treatment with NAC increased GPX4 and Nrf2 expression.
Conclusion: In conclusion, our data showed that dissociation leads to the increasing of MDA consequently apoptosis induction. Antioxidant supplement such as NAC results in diminishing the MDA and GPX4 activity. Our findings suggest activation of the Nrf2 pathway will help develop new strategies for the treatment of dissociated hESCs.
Keywords: Human Embryonic Stem Cells, Nrf2, Cell Death, Lipid Peroxidation, N-Acetylcysteine

O-5: Towards Cardiac Regeneration: Structural and Functional Maturation of Re-Shaped Induced Pluripotent Stem Cell-Derived Cardiomyocytes

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Objective: Human pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) morphologically and functionally resemble immature embryonic rather than adult CMs. While adult ventricular CMs present membrane invaginations called transverse (t-) tubules, hiPSC-CMs lack such specific microarchitecture resulting in inefficient Ca2+ handling and force production. Many efforts are currently made to experimentally induce structural and functional maturation in these cells to advance their use for future clinical applications. This project focuses on the functional improvement of hiPSC-CMs by inducing structural and molecular remodeling during culture. We hypothesize that the combination of three-dimensional (3D)-reshaping of single cells and expression of the scaffolding protein Bin1, a primary candidate for the development of t-tubules, can synergistically change the microarchitecture of iPSC-CMs by triggering membrane invaginations to improve Ca2+ homeostasis and EC-coupling in these young CMs.
Materials and Methods: Briefly, hiPSCs were differentiated into CMs, seeded into 3D micro-scaffolds of a specific shape, and transfected with adeno associated virus 6 with or without

the gene encoding BIN-1. Structural changes were examined by confocal live-cell imaging and immunostainings. Functional adaptations were assessed by measuring Ca2+ currents and Ca2+ transients using the whole-cell voltage-clamp technique in combination with laser-scanning confocal microscopy in the line-scan mode, respectively.
Results: Structural analysis revealed strong membrane invaginations of the sarcolemma in 3D-shaped Bin1-expressing cells, indicative of early, yet unstructured tubular network formation. EC-coupling analysis revealed faster Ca2+ transient kinetics, suggesting maturation at the level of cytosolic Ca2+ handling.
Conclusion: Our data indicate that 3D-reshaping and Bin-1 expression not only remodel the microstructure of CMs but can also associate with functional maturation.
Keywords: hiPSC-CMs, 3D-Reshaping, Bin-1, T-Tubules, Maturation

O-6: Comparison of Cytotoxicity Effect of Activated NK Cells on The GBM Cells in 2D and Neurosphere Culture Models

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Objective: Glioblastoma multiforme (GBM) is the most aggressive and mortal human malignancy. Today, NK cell therapy is one of the new strategies for GBM treatment. This study aimed to compare the cytotoxicity effect of activated NK cells on GBM cells in monolayer (2D culture) and neurosphere cul-tures.
Materials and Methods: We used the human GBM cell-line U251 and cultured them in both monolayer (2D) and neurosphere (CSC enrichment) culture models. Then CD56+ 16+ NK cells were isolated from healthy donor PBMC by BD FACS Aria II and stimulated overnight by IL-15 (10 ng/ml). The ex-pression of the CD133 stemness marker was evaluated on the neurospheres by flow cytometry. In the next step, activated NK cells were co-cultured with GBM cells with a 3:1 (E: T) ratio in both models. After 48 hours, cytotoxicity of NK cells was evaluated using Propidium iodide (PI) staining by flow cytometry.
Results: The purity of NK cells was 92% after cell sorting. The expression of the CD133 marker on the neurospheres was 80% that confirms neurospheres contain cancer stem cells. Finally, the cytotoxicity of activated NK cells on the GBM monolayer cells and neurospheres was 22.65% and 45%, respectively.
Conclusion: In conclusion, activated NK cells have a cytotoxicity effect on both monolayer and neurosphere culture mod-els. It was showed that the cytotoxicity effect of activated NK cells on neurospheres is higher than 2D culture, and there is a significant difference between the two study groups. Since neurospheres have cancer stem cells, we suggest NK cells may have a potent cytotoxicity effect on the neurospheres. However, confirmation of this result requires more studies.
Keywords: NK Cells, Glioblastoma Multiforme (GBM), Neurosphere, 2D Culture, Cytotoxicity

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O-7: Crayoprotective Effects of Pentoxifylline on Spermato-gonial Cells Following Transplantation into Tortion Model

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Objective: Preserving the spermatogonial stem cells (SSCs) for long periods during the treatment of male infertility using stem cell banking systems and transplantation is an important issue.
Materials and Methods: Therefore, this study was conducted to develop an optimal cryopreservation protocol for SSCs using 10 mM pentoxifylline (PTX) as an antioxidant in the ba-sal freezing medium. Testicular torsion - a mouse model for long-term infertility- was used to transplant fresh SSCs (n=6), fresh SSCs treated with PTX (n=6), cryopreserved SSCs with basal freezing medium (n=6), and cryopreserved SSCs treated with PTX (n=6). Eight weeks after germ cell transplantation, samples were assessed for proliferation, through evaluation of dx4 and Id4 markers, and differentiation via evaluation of C-Kit and Sycp3, Tnp1, Tnp2, and Prm1 markers.
Results: According to morphological and flow cytometry results, SSCs can form colonies and express ID4, α6-integrin, and β1-integrin markers. We found positive influence from PTX on proliferative and differentiative markers in SSCs transplanted to azoospermic mice. In the recipient testis, donor SSCs formed spermatogenic colonies and sperm.
Conclusion: Respecting these data, adding pentoxifylline is a practical way to precisely cryopreserve germ cells enriched for SSCs in cryopreservation, and this procedure could become an efficient method to restore fertility in a clinical setup. However, more studies are needed to ensure its safety in the long term period.
Keywords: Male Infertility, Pentoxifyllin, Testicular Torsion, Transplantation, Spermatogonial Stem Cells

O-8: Non-Obstructive Azoospermia Treatment by Mesenchymal Stromal/Stem Cells: A Non-Randomized, Open-Label Phase I Clinical Trial

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Objective: Non-obstructive azoospermia is the primary reason for resistance to male infertility therapy. Mesenchymal stromal/stem cell (MSC) therapy has been recognized as a new therapeutic approach for male infertility in in vitro and *in vivo* models. The present clinical trial aimed to develop a novel approach for treating non-obstructive azoospermia with MSCs.
Materials and Methods: Nineteen patients from 24 to 48 years old with non-obstructive azoospermia were enrolled in a non-randomized, open-label phase I clinical trial (IRCT registration number: IRCT20190519043634N1). Total testosterone, LH, FSH, and inhibin levels as well as spermogram were assessed before and after the interventions. Then, bone marrow-derived MSCs (BM-MSCs) were harvested from the iliac crest of each patient, and autologous transplantation was done after 14-days of culture. During testicular micro-TESE, these MSCs were injected into the testicular reticulum and the spermatic cord. All patients were followed six months after MSCs transplantation. Data were assessed by paired sample t-test and $p<0.01$ was considered significant.
Results: The mean±SD of total testosterone level before treatment was 6.8±5.9 ng/mL. After transplantation testosterone level increased to 10.0±5.2 ng/mL ($p=0.002$). In 6 (31.6%) patients, testosterone level returned to normal value and in 3 (15.8%) patients, the value remained within the normal range. The FSH level in all patients was 28.6±16.6 mIU/mL before transplantation. After treatment, the mean FSH level decreased to 28.6±16.6 mIU/mL ($p<0.001$), while in 9 (47.4%) patients FSH level returned to normal value. The LH level in patients was 10.3±8.0 mIU/mL before transplantation. After treatment, the mean LH level decreased to 8.4±5.1 mIU/mL ($p=0.03$). The LH level increased in 9 patients, of which in 6 it returned to normal after treatment. The level of inhibin B before treatment in all patients was lower than the normal value (7.2±4.3 pg/mL). After treatment, it was noted to increase by 17.5±6.9 pg/mL ($p<0.001$). In 4 (21.1%) patients, it returned to normal. Finally, in 4 patients (21.1%) 6 months after treatment, sperm cells were found in the spermogram, which were cryopreserved for subsequent preparation for IVF.
Conclusion: The therapeutic efficacy of BM-MSCs in non-obstructive azoospermia according to the hormonal profile and spermogram data has been shown in this clinical trial.
Keywords: Azoospermia, Mesenchymal Stromal, Stem Cell, Cell Therapy

Poster Presentations

Ps-1: NK Upraise in The Dark World of Cancer Stem Cells

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Objective: One of the obstacles in treating different cancers, especially solid tumors, is cancer stem cells (CSCs) with their ability in resistance to chemo/radio therapy. The efforts for finding advanced treatments to overcome these cells have led to the emergence of advanced immune cell-based therapy (AICBT). Today, natural killer (NK) cells have become the center of attention since they have been proved to show appropriate cytotoxicity against different cancer types as well as the capability of detecting and killing CSCs.
Materials and Methods: Attempts for reaching an off-the-shelf source of NK cells have been made which resulted in the emergence of chimeric antigen receptor natural killer cells (CAR-NK cell). The CAR technology has then been used for generating more cytotoxic and efficient NK cells, which has increased the hope for cancer treatment.
Results: Since utilizing this advanced technology to target CSCs have been published in few studies, the present study has focused on discussing the characteristics of CSCs, which are detected and targeted by NK cells, the advantages and restrictions of using CAR-NK cells in CSCs treatment, and the probable challenges in this process.
Keywords: Natural Killer (NK) Cell, Cancer Stem Cells (CSC), Chimeric Antigen Receptor (CAR), Immunotherapy, Immune Cell-Based Therapy

Ps-2: Reduced Apoptosis of Neural Stem Cells Using Uncoated-SPIONs

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Objective: Nowadays brain and spinal cord injuries are common disorders. Recent reports indicate improvement using neural stem cell transplantation because these cells are able to differentiate into various types of cells in the central nervous system, including neurons, astrocytes, and oligodendrocytes. But apoptosis is a common problem of transplantation and a substance that reduces apoptosis is important. Superparamagnetic Iron Oxide Nanoparticles (SPION) can be easily internalized into the cells. They can be used in the fields of biomedicine, cell tracking, gene transfer, and others.
Materials and Methods: In this study, bone marrow mesenchymal stem cells were isolated from rats femurs and cultured in the DMEM medium with 10% FBS. After 4 passages, flow cytometry confirmed the property of the cells. They were cultured in the DMEM medium supplemented with B27, bFGF, and EGF in order to generate the neurospheres, then they were harvested and cultured in the neurosphere medium with 5% FBS to produce neural stem cells. The latter was characterized by immunocytochemical techniques. They were cultured at different durations (24, 48, and 72 hours) in a medium containing uncoated SPIONs.
Results: The entry of SPIONs into the cells was confirmed with Prussian Blue stain. Determination of apoptosis was performed by Annexin-PI kit and Viability by Trypan blue.
Conclusion: Statistical analysis showed that apoptosis was significantly increased in the uncoated SPIONs as compared with the control group.
Keywords: Neural Stem Cells, Superparamagnetic Iron Oxide Nanoparticles, Apoptosis

Ps-3: Prion-like Characteristic of P-tau: Propagation of Pathogenicity During Tauopathy Process

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Objective: Abnormal accumulation of p-tau is associated with several neurodegenerative diseases; collectively termed tauopathies. According to the prion-like propagation hypothesis, tauopathies and other neurodegenerative disorders are reminiscent of prion diseases. The notion that a misfolded protein is the cause of prion disease is now extensively accepted. This misfolded protein is termed “propagon”. It uses to define proteins that can transmit misfolding *in vitro* or *in vivo*. It seems that among propagons, the misfolded form of Tau protein is involved in molecular mechanisms of various "Tauopathies", including Alzheimer's disease. Lately, studies have shown that phosphorylated tau at Th231 exists in two distinct cis and trans conformations; in which that cis pT231-tau is neurotoxic and acts as an early driver of tauopathy in many neurodegenerative disorders. It remains unknown, how tau pathology spreads during tauopathies. Here we use an *in vitro* model to study the prion-like propagation of p-tau.
Materials and Methods: We cultured SH-SY5Y and studied the effect of exogenous p-tau on them by immunofluorescence staining and immunoblotting.
Results: We showed cis but not trans; p-tau seeding reflects tauopathy propagation in cultured neural cells. We found that extracellular cis p-tau can induce intracellular tau pathology and spread.
Conclusion: These results have supported the prion-like propagation hypothesis. Our findings would open new windows toward the tauopathy molecular mechanism, especially p-tau

spreading. The results would have profound clinical implications in fighting devastating neurodegenerative disorders.
Keywords: Seeding, Templating, Spreading, Prion-like Propagation

Ps-4: Providing mRNA-miRNA Biomarker Panel Based on Liquid Biopsy for Early Detection of Alzheimer's Disease

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Objective: Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by the accumulation of amyloid plaques and neurofibrillary tangles in the brain, approximately 24 million people are currently affected by the disease. Because of the high incidence of AD, there is an urgent need to develop new strategies to diagnose and treat AD. However, the full spectrum of molecular factors that contribute to AD pathogenesis is not known. Noncoding (nc) RNAs, including microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs), regulate gene expression at the transcriptional and post-transcriptional levels in various diseases, serving as biomarkers and potential therapeutic targets. There is rising recognition that ncRNAs have been implicated in both the onset and pathogenesis of AD. Our study aimed to identify key differentially expressed genes (DEGs) and miRNAs (DEmiRNAs) which can serve as potential biomarkers for diagnosis and therapy of Alzheimer's disease (AD).
Materials and Methods: We performed miRNA and mRNA integrated analysis (MMIA) to identify DEGs and DEmiRNAs of AD. We conducted MMIA of AD based on 1 miRNA dataset and 1 mRNA dataset derived from the Gene Expression Omnibus (GEO) database; 112 DEGs and 50 DEmiRNAs were obtained. The AD-specific DEmiRNAs-targets interaction network was contrasted and performed receiver operating characteristic curve analysis of the respective DEmiRNA biomarkers.
Results: DEGs of AD were significantly enriched in AD. 32 DEGs regulated by 8 DEmiRNAs, including miR-4781-3p, miR-26b-3p, miR-101-3p, has-let-7g-5p, miR-15a-5p, miR-1468-5p, miR-3127-3p. These 8 miRNAs were the top 8 miRNAs covering most DEGs.
Conclusion: In conclusion, our study demonstrated the importance of miRNAs in Alzheimer's disease development. Future studies will evaluate some of these miRNAs as biomarkers for early disease detection and develop therapeutic strategies to clinically control Alzheimer's disease progression.
Keywords: Alzheimer Disease, Biomarker, Early Detection, mRNA-miRNA, Biological Markers

Ps-5: Ameliorative Effects of Zinc Against Morphine Induced Testicular Cells Toxicity Through P53 and P-Akt Mediated Pathways in Male Rat Model

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Objective: Zinc deficiency induces apoptosis via p53and caspase-dependent pathways in human neuronal precursor cells and exacerbates diabetic down-regulation of Akt expression and function in the testis. The purpose of this study was to explore the effects of morphine and pretreatment with zinc on p53 and p-Akt protein levels in rat testis.
Materials and Methods: Male Wistar rats were divided into three groups (n = 5/group): Control (normal saline 0.2 mL; IP, for 21 consecutive days); morphine (3 mg/kg; IP, for 21 consecutive days); and morphine pretreated with zinc (zinc 5 mg/kg; IP, 1 h before morphine injection, for 21 consecutive days). In the end, the rats from every group were sacrificed under ketamine (60 mg/kg) and xylazine (10 mg/kg) anesthesia. Testis samples were collected for western blot analysis.
Results: In morphine-treated rats, p53 protein levels were significantly increased compared with the control group ($P<0.01$). Also, p-Akt protein levels decreased in morphine-treated rats compared with the control group, but this decrease was not significant. In the pretreatment group, there was a significant decrease in p53 protein levels and a significant increase in p-Akt protein levels, compared with the morphine groups ($P<0.05$).
Conclusion: The present data indicated the protective effect of zinc against morphine-induced testicular cell toxicity via p53/Akt pathways at *in vivo* models and suggested the clinical importance of zinc on infertility among chronic opioid users and addicted men.
Keywords: Morphine, p53, p-Akt, Testis, Zinc

Ps-6: Bioinformatics Analysis Suggests That miR-378g Might Inhibit Gastric Cancer Cells: A Study Based on The TCGA Database

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Objective: Gastric cancer (GC) is one of the most prevalent cancers worldwide. MicroRNAs (miRNAs) play substantial roles in the development of GC. Here, we aim to analyze miRNA expression profiles to identify poorly-expressed miRNAs in GC tissues that might inhibit GC growth.
Materials and Methods: miRNA- and mRNA-sequencing data of advanced GC patients (stages III and IV) were downloaded from The Cancer Genome Atlas (TCGA) project. The obtained data were analyzed using R programming to determine the differentially expressed mRNAs/miRNAs (absolute $logFC > 1.7$; adjusted p value < 0.05). miRNA target prediction was performed using TargetScan, miRDB, and miRWalk. miRNA validated targets were obtained from miRTarBase, and enrichment analyses were carried out using Enrichr.

Results: Our results indicated that 19 miRNAs were down-regulated while 177 miRNAs were up-regulated in the advanced stages of GC, with the former reportedly serving as tumor-suppressors in several cancer types including GC. Importantly, five of the down-regulated miRNAs (i.e. miR-202-5p, miR-378g, miR-548ba, miR-6507-5p, and miR-6510-3p), which we found to potentially resist tumorigenesis, have not yet been analyzed experimentally in the GC cells. Notably, some of these miRNAs act as anti-cancer agents in certain types of cancer. We have chosen miR-378g for subsequent functional analysis since our data suggests it as a better candidate for suppressing GC.
Conclusion: miR-378g is poorly expressed in GC tissues and might suppress GC cell growth if overexpressed.
Keywords: microRNA, Tumor Suppressor, Gastric Cancer Cells, Malignancy

Ps-7: Valproic Acid Promotes Rat Adipose-Derived Stem Cells to Neuron-Like Cells by Neurosphere Cultivation Assay

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Objective: Adult mesenchymal stem cells had multilineage differentiation potential to differentiate into several cell types showing their potential for regenerative medicine and could differentiate into neuron-like cells induced by special induction media. Valproic acid (VPA) which is well known as a histone deacetylase (HDAC) inhibitor, was an ideal candidate due to neural differentiation. This research aimed to perform VPA-induced neural differentiation of adipose-derived stem cells (ADSCs) by neurosphere cultivation assay.
Materials and Methods: ADSCs were isolated from rat inguinal and pararenal regions. Then using neurosphere culture, cells differentiated to the neural stem/progenitor cells (NS/PCs). NS/PCs were treated with different concentrations of VPA to differentiate into neuron-like cells at specific time points (48 and 72 hours). After induction, neuronal differentiation of ADSCs was evaluated using immunocytochemistry and real-time reverse-transcription polymerase chain reaction (RT-PCR) techniques.
Results: Our data showed that ADSCs were immunoreactive to CD90, CD29, CD49d, CD44, CD105, and CD99 without any immunoreactivity for CD106 and CD31. In addition, these cells expressed the genes of Nanog, Oct4, and Sox2. Both Neurosphere and NS/PCs were immunoreactive to Nestin, NF-68, and NF-200 and also were able to express Nanog, Sox2, NeuroD1, Oct4, Musashi1, and Nestin genes. Moreover, these cells were unable to express the MBP gene, but ADSCs could differentiate into neural-like cells *in vitro* in the presence of 1μM VPA for 72h. Neural-like cells were immunoreactive to CNTF, NGF, BDNF, GDNF, NT4, and NT3.
Conclusion: In general, our results conclude that VPA can differentiate ADSCs into neural-like cells.
Keywords: Adipose-Derived Stem Cells, Valproic Acid, Neuronal Differentiation, Neuron-Like Cells, Neurosphere Cultivation Assay

Ps-8: Cytotoxicity of Gold Nanoparticles Complexes with Retinoic Acid Macromolecules

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Objective: Gold nanoparticles (AuNPs) have been suggested as helpful medical vehicles in the field of regenerative medicine due to their unique optical properties.
Materials and Methods: The current study examined the direct conjugation ability of retinoic acid (RA) with AuNPs and the effects of AuNPs-RA complex on cell viability of human adipose-derived stem cells (hADSCs).
Results: The TEM and FE-SEM assessment revealed that the synthesized AuNPs were spherical with an average diameter of 36.5 nm. Infrared spectroscopy was performed to identify different functional groups in RA, AuNPs, and AuNPs-RA complex solutions. The conjugation efficiency of RA in AuNPs was analyzed with UV-Vis absorption spectroscopy and the optimal concentration of RA for prepared AuNPs-RA complex was 0.14 μM. In the MTT assay, an RA concentration of 66 μM caused a 50% inhibition of cell viability and AuNPs were not cytotoxic for concentrations below 5 μg/ml.
Conclusion: These data, confirmed the direct conjugation ability of RA with AuNPs.
Keywords: Human Adipose Derived Stem Cells, Retinoic Acid, Gold Nanoparticles, Cell Viability

Ps-9: Involvement of Nerve Growth Factor and Glial Cell Derived Neurotrophic Factor in Neurogenic and Prosurvival Effects of Interferon Beta in Alzheimer's Disease

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Objective: Alzheimer's disease (AD) is the most common neurodegenerative disorder. Hippocampal neurogenesis impairments and neurodegeneration contribute to AD-related memory loss. In previous studies, we found that treatment with interferon-β (IFNβ) improves spatial memory in rats with AD, at least partly by promoting neurogenesis and inhibiting apoptosis in the hippocampus. However, the molecular mechanisms involved in neurogenic and anti-apoptotic effects of IFNβ in AD are unknown. It has been previously indicated that nerve growth factor (NGF) and glial cell line-derived neurotrophic factor (GDNF) are involved in neuronal survival and maintenance of neural stem cells as well as their proliferation and differentiation into neurons.
Materials and Methods: The AD model was induced by bilateral injection (3μl; 109 TU/ml/side) of lentiviruses express-

ing human amyloid protein precursor with Swedish and Indiana mutations in the hippocampus of adult male rats. Intranasal administration of IFNβ (1µg/kg dose) was started from day 23 after virus injection and continued every other day to day 50. The mRNA expression levels of NGF and GDNF in the hippocampus were measured by quantitative polymerase chain reaction (qPCR). Furthermore, Nissl staining was used to assess neuronal survival.

Results: Treatment with IFNβ attenuated neuronal degeneration in the CA1, CA3, and DG regions of the AD rat hippocampus when compared with the control group. In addition, sustaining the survival of hippocampal neurons, IFNβ increased mRNA expression levels of NGF and GDNF in the AD rat hippocampus.

Conclusion: These results confirmed that growth factors and neurotrophic factors may participate in neurogenic and pro-survival effects of IFNβ in an AD-like neurodegenerative context.

Keywords: Alzheimer's Disease, Neural Stem Cells, Nerve Growth Factor, Glial Cell Line-Derived Neurotrophic Factor, Neuronal Survival

Ps-10: Selection of Appropriate Specific Surface Antigen of Pancreatic Cells for Antibody Production: A Bioinformatic Analysis

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Objective: Important applications of antibodies that bind to specific tissue and cells include 1. Conjugating of antibodies to immunomodulatory molecules (cytokines) for local regulation of the immune system in autoimmune diseases. 2. Conjugation of antibodies with detectors such as fluorescent, radioactive and magnetic resonance imaging for non-invasive imaging to assess and diagnose diseases *in vivo*. 3. Isolation of specific cells using flow cytometry to custom pure and homogeneous cell populations. 4. Utilize as a receptor in chimeric antigen receptor-T cells (CAR-T cells). The first step of tissue-specific antibody production is the selection of appropriate specific surface antigens.

Materials and Methods: For this aim, we compared the gene expression profiles of pancreatic cells as well as other tissues. Genes that are more expressed in pancreatic cells and their proteins are enriched were classified. Protein atlas and UCSC databases were used for this purpose. Also, the raw data obtained from microarray in two separate studies of pancreatic cells isolated from rats (Accession N: GSE47174) and human pancreas (Accession N: GSE30803) in the GEO database were applied. Finally, the preferential expression of the genes was confirmed by a review of the literature.

Results: Sharing between genes encoding cell transmembrane-secretory proteins in humans (4796 genes) with genes with preferential expression in the pancreas (276 genes), revealed 18 genes including AQP12B, BRSK2, CRF2-9, FBXO35, NEPH3, TALK-1, CUZD1, ZNT8, Fbw12, NPHN, ZAP75, GRP-R, DAP, SQLE, TMED6, G6PC2, IDDM1, and PTPRN.

Conclusion: Based on the result, there are 18 membrane proteins in the pancreas that are potential candidate targets for specific antibody production.

Keywords: Surface Antigen, Pancreatic Cells, Antibody Production

Ps-11: Design and Fabrication of Ion-Exchange Sulfonated Poly (Ether Sulfone) Nanofibers for Bone Regeneration

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Objective: Despite significant progress in the field of bone regenerative medicine during the years, current therapies still have many limitations. Bone tissue engineering is a promising method for the treatment of bone defects. Designing a functional scaffold with mechanical, morphological, electrical, and chemical properties similar to the natural bone extracellular matrix is the first step for stimulating bone regeneration. Ions such as calcium and strontium play an important role in the regulation of cellular behaviors. Recent studies showed that ion-loaded scaffolds can stimulate the proliferation and osteogenesis of stem cells. One of the newest approaches for ion delivery to cells is using ion-exchange polymers as the carrier. In this study, cation-exchange nanofibers were fabricated for enhancing bone regeneration.

Materials and Methods: Sulfonated poly(ether sulfone) (SPES) was prepared using chlorosulfonic acid as the sulfonating agent. Three SPES with different sulfonation degrees were prepared (SPES1, SPES2, SPES3). Poly(ether sulfone) (PES) and SPES1-3 nanofibers were fabricated through electrospinning. The nanofibrous scaffolds were characterized using scanning electron microscopy, acid–base titration, and cell culture study.

Results: Sulfonation of PES was confirmed by titration. The nanofibers were homogenous and beadless. It was found that by increasing the degree of sulfonation, the diameter of SPES nanofibers decreased. In addition, the viability of cells cultured on the nanofibrous scaffolds was found to be excellent.

Conclusion: Ion-exchange nanofibers are promising candidates for bone tissue engineering scaffolds. The designed SPES nanofibers can be used for delivering different cations such as calcium to the cells and enhance bone regeneration.

Keywords: Ion-Exchange Polymer, Electrospun Nanofibers, Sulfonated Poly(ether sulfone), Bone Regeneration, Tissue Engineering

Ps-12: Differentiation Potential of Adipose Tissue-Derived Mesenchymal Stem Cells into Germ Cells With and Without Growth Factors

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Objective: One of the best resources for regenerative medicine is mesenchymal stem cells (MSCs). In recent years, researchers have been able to differentiate these cells into a variety of cell types such as germ cells. Nowadays, efforts are being made to find the best cellular source and application of different growth factors and nutrients, for the improvement of differentiation efficacy. This study aimed to culture the adipose tissue-derived MSCs (AT-MSCs) with and leukemia inhibitory factor (LIF), glial cell line-derived neurotrophic factor (GDNF), epidermal growth factor (EGF), and retinoic acid (RA), and investigate their impact on the differentiation of these cells into germ cells.
Materials and Methods: MSCs were separated from adipose tissue of mice, and characterized by flow cytometry. The cells were cultured in different conditions, including MSCs grown in the presence of the growth factors, MSCs without the growth factors, MSCs cultured with combined growth factors and RA, and MSCs cultured with RA. After 2 weeks, the gene expression of c-Kit, Gcnf, Mvh, and Scp3 and the protein expression of c-Kit and Gcnf, were assessed by real-time polymerase chain reaction (PCR) and western-blot, respectively.
Results: Scp3 was overexpressed in the groups supplemented with RA ($P<0.01$). The expression of c-Kit and Mvh in the growth factor supplemented groups was increased ($P<0.01$).
Conclusion: The use of the growth factors for the long-term culture of stem cells can be beneficial. However, to promote germ cell differentiation, the growth factors might be used by other meiosis inducer factors.
Keywords: Epidermal Growth Factor, Germ cells, Glial Cell Line-Derived Neurotrophic Factor, Leukemia Inhibitory Factor, Mesenchymal Stem Cells

Ps-13: CoCl2 Induced Hypoxia Enhances Osteogenesis Effect of Human Adipose-Derived Stem Cells in Partial Osteotomy of Tibia in Rat Model

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Objective: Bone fracture is one of the most prevalent clinical issues in the world. For bone formation during bone fracture repair, mesenchymal stem cells (MSCs) are the source of osteogenic cells. MSC migration is also essential for the treatment of bone diseases. One way to improve MSC migration is culturing cells under hypoxic conditions. Cobalt Chloride (Cocl2) is a common chemical inducer of hypoxia. Cocl2 has positive effects on MSCs migration and homing.
Materials and Methods: In this study, osteoporosis was induced by bilateral ovariectomy (OVX) and partial osteotomy in the tibia was made after 4 months. Adipose-derived mesenchymal stem cells (AdMSCs) were pre-conditioned with 200 μM cobalt chloride for 48 h. Twenty-four rats have been distributed into three groups: group 1 (OVX+ Osteotomy+ phosphate buffer saline (PBS)), group 2 (OVX+ Osteotomy+ hypoxia preconditioned AdMSCs), group 3 (OVX+ Osteotomy + normoxia preconditioned AdMSCs). At 4 and 8 weeks, sampling was performed to evaluate the expression of Runx2, OPG, and OC proteins by Western blotting.
Results: The results showed that in the group receiving hypoxic cells in both weeks 4 and 8, the expression of Runx2, OPG, and OC proteins was significantly increased compared to the sham and normoxic groups ($P = 0.000$).
Conclusion: The present study shows that CoCl2 induced hypoxia enhances the osteogenesis effect of human adipose-derived stem cells.
Keywords: CoCl2, Human Adipose-Derived Stem Cells, Osteotomy, Osteogenesis

Ps-14: Application of Quantum Dots for *In Vivo* Molecular Imaging of Stem Cells

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Objective: Tracing and visualization of stem cell fate and engraftment using conventional tomographical imaging modalities is limited due to some of their disadvantages such as the short half-life of radiotracers and toxicity of silver nanoparticles in nuclear medicine and computerized tomography scan (CT scan), respectively. Also, visualization of stem cell dynamic behavior using bioluminescent imaging due to the mutational risk is worrying
Materials and Methods: Quantum dot-based cellular imaging is expected to contribute to the progression of regenerative medicine, due to their well-known characteristics such as emitting in the extended region of the electromagnetic spectrum. Fluorescence imaging of stem cells labeled with quantum dots in the extended region of the spectrum could provide better temporal and spatial resolution due to a decrease in photon scattering, tissue absorption, and autofluorescence.
Results: These quantum dot-based fluorophores hold great promise for the real-time and non-invasive visualization of stem cell engraftment, fate, and cellular dynamics in a living body with outstanding spatial and temporal resolution.
Conclusion: While each imaging modality has its limitation for cell tracking, *in vivo* molecular imaging using quantum dots has demonstrated continual advancements.
Keywords: Imaging, Cell Tracking, Molecular Imaging, Quantum Dot, Engraftment

Ps-15: The Effect of Exosomes Secreted from Bone Marrow Tissue Derived Mesenchymal Stem Cells in The Treatment of Osteoarthritis in The Mouse Model

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Objective: Extracellular vehicles (EVs) are nanoscale intercellular messengers secreted from cells to deliver biological signals. Today, EVs have become a new field of research in regenerative medicine and are considered as potential therapies to control inflammation, wound healing, and enhance and improve

healing in many diseases. Given the global burden of osteoarthritis (OA) as the fastest-growing health condition and one of the major causes of physical disability in the aging population, researches to establish EVs as therapeutic products can meet the basic clinical needs in the management of OA.
Materials and Methods: In this *in vivo* experimental study, mesenchymal stem cells (MSCs) were isolated from human bone marrow (BM) tissue and cultured in the laboratory until passage 3. Finally, these cells secreted exosomes were isolated from their conditioned medium. Ciprofloxacin-induced OA mice were divided into 3 groups: OA, sham (phosphate buffer saline (PBS) intraarticular injection), and BM-MSCs-Exo (exosome intraarticular injection). To evaluate the changes, the expression of sox9, aggrecan, collagen I, and II (COLII) genes using real-time polymerase chain reaction (PCR), histological analysis, and immunohistochemically (IHC) studies were performed.
Results: The IHC results showed that although the expression of COLII as a specific marker of hyaline cartilage was lower in the BM-MSCs-Exo group than in the control group, in comparison with the sham and OA groups, the expression of this protein was significantly higher. Other evaluations (gene expression and histology) have provided similar data.
Conclusion: It can be generally concluded that the injection of exosomes derived from BM-MSCs has a promising therapeutic effect on osteoarthritis.
Keywords: Exosome, Mesenchymal Stem Cells, Bone Marrow, Mouse Model

Ps-16: Activation Induction of Natural Killer Cells in Patients With AML as Adoptive Immunotherapy

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Objective: Background. Acute myeloid leukemia (AML) is a heterogeneous disease, the therapies of which have currently shown elevated toxicity and a high rate of relapse. Limited activity of NK cells due to increased expression of inhibitory markers and decreased expression of activating markers in AML patients' derived NK cells has restricted the development of NK cell immunotherapy. Research in immune checkpoints, such as PD-1/PD-L1 has renewed the interest in immune-based cancer therapies due to their ability to prevent immunosuppression against tumors. In this study, cytotoxicity was induced in NK cells using immune checkpoint blockers with the aim of fighting against AML.
Materials and Methods: Enriched NK cells from AML patients were activated with interleukin-15 (10ng/ml) and PD1-Blocker (0.5 μg/ml), and the cells were assayed for cytotoxic function, immunophenotyping, and gene expression.
Results: NK cells surface markers immunophenotyping resulted in an increase in the expression of the activating receptors such as NKP30 (3 fold) and NKP46 (1.5 fold) and a decreased expression of inhibitory receptors such as NKG2A (5 fold) and PD-1 (2.1) at 48 hours after treating with Pd-1 blocker and IL-15. Upregulation of genes such as TRAIL and FasL and releasing of IFN-γ were also observed.
Conclusion: In the present study, a method for activating AML patient-derived NK cells with cytotoxic properties encountered with tumor cells was developed.
Keywords: AML, Natural Killer Cells, Immnonotherapy

Ps-17: Chronic Kidney Disease Reflects Profound Neurodegeneration in The *In Vitro* System

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Objective: Chronic Kidney Disease (CKD) is a prevalent disease, which may cause different complications, such as neurodegeneration in the brain. The main pathogenic factor playing part in CKD complications is high concentrations of circulating uremic toxins. Despite extensive considerations, it remains uncertain how CKD results in neurodegeneration.
Materials and Methods: We employed an *in vitro* CKD model. We isolated primary neurons from 17-day-old embryonic mice cerebral cortex. Hyperuremia was induced by 6 different concentrations of urea (50 – 100 - 150 – 200 – 250 – 300 mM) for 8 days in cultured neurons. Live and dead study was performed using FDA-PI to detect neuronal cell death. Moreover, we employed immunostaining with AT180 to study the tauopathy process in the stressed-out neurons
Results: We found prominent neurodegeneration in cultured neurons treated with 300 mM (30.33±24.58) and 200 mM (41.6±28.43) urea compared to the control group (63.940±7.37) after 2 days. Furthermore, we observed time-dependent cell death in the treated neurons with the 50 mM group (63.940±7.37 on day 2 and 28.21±2.02 on day 8). We also observed profound tau pathology in the urea-treated neurons, confirmed by AT180 staining
Conclusion: We herein showed that urea can cause tau pathology and neuronal cell death. Our findings unravel the molecular mechanism of central nervous system destruction upon CKD and would have profound clinical implications for fighting the devastating disorder.
Keywords: Chronic Kidney Disease, Hyperuremia, Neurodegeneration, Urea, Tau Pathology

Ps-18: Transforming Growth Factor Beta Can Enhance Chondrogenesis of Adipose-Derived Stromal Cells on Poly(E-Caprolactone)/ Cartilage-Derived Matrix /Fibrin Hybrid Constructs Prepared Via Sandwich Model

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Objective: Scaffolds based on electrospun fibers play a critical role in cartilage tissue engineering (CTE). Incorporation of extracellular matrix (ECM) derivatives to synthetic polymers by electrospinning, improves cell viability and differentiation and provides ideal mechanical properties. The Transforming growth factor-beta (TGF-β) family is a key regulator of mesenchymal stem cell (MSC) differentiation for chondrogenesis. The objective of this study was to fabricate hybrid constructs using composite electrospun scaffolds based on poly (ε-caprolactone) (PCL) and cartilage-derived matrix (CDM) and fibrin hydrogel via sandwich techniques to improve the viability and differentiation of human adipose-derived stem cells (ADSCs) for CTE applications.

Materials and Methods: Initially, PCL-CDM electrospun mats were fabricated. Fibrin/ ADSCs hydrogel was seeded on PCL- CDM mats and arranged layer-by-layer using the sandwich technique. Real-time reverse-transcription polymerase chain reaction (RT-PCR), were performed to examine the expression of collagen types II and X, SOX9, and aggrecan. The production of glycosaminoglycan (GAG) was also tested *in vitro* by Toluidine blue stain and biochemical assay in the cultured scaffolds.

Results: The findings demonstrated that incorporation of CDM in PCL fibers results in improved cell viability. The expression of collagen types II and X, SOX9, and aggrecan in the group consisting of TGF-β are significantly higher than the control group (PCL-CDM). Hematoxylin and eosin staining showed that the sandwich method resulted in homogenous cell seeding.

Conclusion: The RT-PCR, biochemical and histological results, showed that incorporation of the CDM into PCL/fibrin sandwich scaffolds and use of TGF-β stimulated ADSCs chondrogenesis and produced the products which increased expression of chondrogenic genes.

Keywords: Electrospun Fibers, Growth Factor ,Hybrid Scaffolds, Cartilage, Tissue Engineering

Ps-19: Preparation and Characterization of Poly (3-Hydroxybutyrate)/Chitosan Hybrid Scaffolds With Glucosamine Sulfate Release Capability

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Objective: Three dimensional (3D) structures created via electrospinning are generally used as an engineered extracellular matrix (ECM) to regenerate damaged or lost tissues. This feature distinguishes nanofibers from other scaffolds by the fact that nanofibers have a high potential for modification to promote cell growth and function. Simple physical hybridizing synthetic polymers with natural biopolymers and other biomolecules, then converting the hybrids into nanofibers will offer a more facile and cost-effective route for modifying and tailoring the nanofibers.

Materials and Methods: In this study, multiwalled carbon nanotubes (MWCNTs) and glucosamine sulfate (GAS) with 5 and 7% weight ratios were added to poly (3-hydroxybutyrate)-chitosan (PHB-CS) solution (100:20) to obtain an electrospinning solution for the production of cartilage scaffolds. The use of GAS as one of the key components in the ECM of natural cartilage tissue has an important role in the development of scaffold applications.

Results: Our characterization results showed that the addition of 5% GAS into polymer solution has been increased average fibers diameter, increased tensile strength, and improved hydrophilicity of scaffolds. Moreover, 50% GAS was slowly released from PHB-CS/MWCNT-5% GAS fibers over 4 days which may subsequently induce chondrogenesis for cartilage tissue engineering.

Conclusion: These results are exciting as they indicate the feasibility of creating bioactive hybrid nanofibers as a delivery system and tissue replacement for meeting new challenges in regenerative medicine, particularly in relation to cartilage reconstruction.

Keywords: Poly (3-Hydroxybutyrate), Glucosamine Sulfate, Cartilage, Drug Delivery

Ps-20: Size-Based Isolation of Extracellular Vesicles from Dental Pulp Stem Cells Using Lab-on-Chip Approach

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Objective: Extracellular vesicles (EVs) are cell-derived nanoscale vesicles that are involved in intracellular communication and the transportation of biomarkers. Recently, dental pulp stem cells have demonstrated a promising neuroprotection potential through a paracrine manner, which is carried out by EVs. However, effective isolation of EVs is still challenging and isolation methods determine the composition of enriched EVs and their subsequent biological and functional effects.

Materials and Methods: We designed a microchip to capture and detect small EVs, which are derived from stem cells from apical papilla. To confirm the enrichment criteria of the proposed microchip, a two-dimensional numerical simulation incorporating the dominant trapping forces has been performed.

Results: We showed that trapping forces, which are dominant for the applied experimental conditions, work through a size-depended mode as predicted by theory.

Conclusion: EVs' manipulation using active forces in a microfluidic device has appeared to be a reliable and sensitive diagnosis and trapping technique.

Keywords: Extracellular Vesicles, Stem Cells from Apical Pa-

pilla, Lab-on-Chip

Ps-21: Establishing CRISPR-Cpf1 Mediated Knockout TCR Human T Cell Line

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Objective: Genetically engineered T cell therapy has been tested for various types of cancers and it has shown considerable therapeutic potential, both for solid tumors and blood cancers. The development of effective chimeric antigen receptor (CAR) constructs can be done through screening experiments in simpler models such as the Jurkat T cell line. However, the presence of endogenous TCR in these T cell lines can interfere with CAR construct signaling, since the CAR construct triggers a TCR-like signal through its CDTζ domain.
Materials and Methods: In this research, to establish a T cell line suitable for screening CAR constructs in the absence of endogenous TCR signaling, we used CRISPR/Cpf1 gene-editing system to knock out the TCRα chain in a Jurkat T cell line. To target the TRAC locus that encodes the constant region of TCRα, three crRNA was designed and constructed. The efficiency of this gene-editing system was assessed in two levels: genome sequence and protein expression by DNA sequence analysis and flow cytometry respectively.
Results: The results showed that two of three designed crRNAs against TRAC locus introduced indel mutations in the constant region of TCRα and prevented the functional TCR expression at the cell surface. Furthermore, no T cell activation was detected after TCR stimulation of the TRAC-knockout cells.
Conclusion: These findings showed that CRISPR/Cpf1 is an appropriate gene-editing system to remove endogenous TCR from the surface of the Jurkat T cell line which is a valuable means for the screening and development of CAR constructs. Moreover, the crRNAs designed in this study can be applied to remove endogenous TCR from the surface of primary T cells for the manufacture of allogeneic engineered T cell therapy.
Keywords: CRISPR-Cpf1, TRAC Locus Knock-Out, CAR Constructs Screening, Knockout TCR Human T Cell Line

Ps-22: The Effect of TAK242 Loaded in Exosome Derived from Mesenchymel Stem Cells on Decrease of TLR4 in IFN-Y- Prime MSCs *In Vitro*

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Objective: Recently, mesenchymal stem cells (MSCs)-derived exosomes (Exos) are the most efficient and safe delivery tools for therapeutic approaches. Exosome-encapsulated drugs, miRNAs, and small molecules (SMs) have been identified as a new approach in systemic diseases treatment. Using of effective biologic components and SMs has been reported to substantially improve clinical outcomes. For instance, TAK242 is considered as a TLR4 inhibitor that has been used in clinical trial studies for inflammatory disease such as Rheumatoid Arthritis (RA); Although the short half-lives of SMs are the issue of a challenge that requires frequently repeated in cultured media for *in vitro* usage or several injections for clinical utilization. Hence, the main aims of this study are to find an optimal dose of TAK242 for anti-inflammatory cytokines reduction *in vitro* and find the efficacy and durability of Exos as a carrier of SMs for CIA in rat models.
Materials and Methods: MSCs were isolated from bone marrow stroma. Then, isolated cells differentiated toward bone/cartilage/adipose tissue. The phenotype of differentiation examined by the quantitative real-time polymerase chain reaction (qRT-PCR) technique and cell surface markers (CD105, CD73, CD90, CD44, CD34, and CD45) were detected using flow cytometry. Then, differential ultracentrifugation was used to isolate Exos from condition media collected from passage-3 cells. The purified-Exo were determined by BCA protein assay and DLS analysis for the future experiment. The effective concentration of TAK242 was measured by MTT. Subsequently, electroporation was used to load TAK242 inside Exo. Then, high-performance liquid chromatography (HPLC) was used to evaluate the release of TAK242 from Exo. To measurement the uptake of Exo-TAK242 by cells, Exos and cells were labeled with calcine dye and PKH26 respectively and were added to cells and followed at 1, 12, and 24 hours by fluorescent microscope. The result of anti-inflammatory cytokines reduction by Exo-TAK242 was evaluated after 24-48 hours of being added to IFN-Y-prime cells in comparison with nonprime MSCs. The enzyme-linked immunosorbent assay (ELISA) analysis was applied to evaluate the anti-inflammatory cytokines reduction genes (IL-4, TGF-B, and IL-10). Real-time PCR analysis was used to investigate the expression level of anti-inflammatory cytokines genes. Finally, the expression of TLR4, MYD88, and TRAF6 genes was analyzed by the immunohistochemistry method.
Results: Results showed the concentration of 1.5×103 μg of EVs collected from MSCs using the BCA method. The size of EVs is considered with the DLS technique is between 70-150 nm. The MTT result on days 3, 5, and 7 showed that 100 ng/ml concentrations of TAK242 are nontoxic and also cause to reduction of anti-inflammatory cytokines in primed cells. The uptake of Exo by cells was confirmed after 24 h and also the TAK242 release of Exo was distinguished by chromatography technique. The effect of Exo-TAK242 on the reduction of anti-inflammatory cytokines was also measured in 3 groups of Exo-TAK242, Exo, and TAK242 after 48 hours. By the ELISA results the Exo-TAK242 causes a significant reduction of anti-inflammatory cytokines compared to the control group (**P<0. 01, ***P<0.001). In addition, real-time PCR confirmed the significant reduction of anti-inflammatory cytokines genes.
Conclusion: We investigated that Exo-TAK242 reduces anti-inflammatory cytokines expression, more importantly, the use of TAK242 reduced significantly in Exo-TAK242 compared to the TAk242 group. Taken together, the exosome is an ideal vehicle for drug delivery as well as for future therapeutics applications.
Keywords: Anti-Inflammatory Cytokines, Mesenchymal Stem Cells, Exosomes, TAK242, Carrier

Ps-23: Human Testicular Cells Display Immunomodulatory Feature Similar to Mesenchymal Stem Cells

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Objective: Previously similar to some other reports, we have shown that cells cultured from human testicular sperm extraction (TESE) biopsies display multipotent mesenchymal stem/stromal (MSCs) characteristics. On the other hand, it has been reported that MSCs modulate immune response by secreting cytokines in both *in vitro* and *in vivo* models. The secretion of anti-inflammatory molecules and the modulation of the immune system have made MSCs a suitable tool for the treatment of various disorders. Recently it has reported that human Sertoli cells display similar immunomodulatory features to MSCs. Here, in this study we have compared the level of the immunomodulatory gene expression profile of Yazd human testicular derived cells (YhTCs) in different passage numbers (P3-P9).
Materials and Method: YhTCs were thawed and then cultured with DMEM+20%FBS from passage 2+1 (P3) to passage 2+7 (P9). The MSC characteristic of the cells was assessed using flowcytometry for MSC markers and hematopoietic stem cells (HSCs). Then, using Q-RT-PCR technique, the expression level of immunomodulatory genes in different passages was assessed. In this study, the expression level of COX1, TGFβ, IL6, and VCAM in YhTCs were analyzed.
Results: Flowcytometry data indicates that YhTCs display MSC markers by expressing CD105, CD44, CD73, and CD90 in different passage numbers (P3, P6 and P9) while they didn't express HSC markers CD34, and CD45. Q-RT-PCR data showed that cells express immunomudulatory genes in all three passage numbers (P3, P6 and P9) while the level of the expression of the immunomodulatory genes increased in the highest passage number (P9).
Conclusion: Our findings confirm the previous report by showing that YhTCs display immunomodulatory features in different passage numbers. Moreover, we have shown that this feature in passage dependent and increased in the highest passage number of the cells (P9). Further studies are needed to prove the safety and the effectiveness of these cells and their products such as conditioned medium, microvesicles, and exosomes in autoimmune disorders treatment.
Keywords: Human Testicular Cells, Immunomodulatory, Immunotherapy, Mesenchymal
Stem/Stromal Cell

Ps-24: Increased Osteogenic Differentiation Efficiency of hMSCs by Oxaloacetate

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Objective: The bone is a dynamic tissue with peculiar properties such as regeneration capacity and high strength. Although the regeneration process was performed by osteoblasts and osteoclasts, some bone defects and injuries caused by trauma, infections, and surgeries are too severe to repair automatically. Since human mesenchymal stem cells (hMSCs) have a critical role during remodeling processes, it is believed that finding an appropriate inducer is crucial for the treatment of osteogenic diseases and disorders. The present paper studied the effect of oxaloacetate on osteogenic differentiation of hMSCs *in vitro*.
Materials and Methods: First, the most favorable concentration of oxaloacetate was measured using the MTT assay and next, acridine orange (AO) was carried out to analyze the cell morphology in various concentrations of oxaloacetate. The osteogenic differentiation of hMSCs incubated with the osteogenic medium containing ascorbic acid, dexamethasone, and β-glycerophosphate was then assessed using alkaline phosphatase (ALP) activity test and Alizarin Red S on the 7th and 14th days.
Results: The results of osteogenic differentiation tests showed that oxaloacetate at the 1 µM dose increased the ALP enzyme activity during the induction as well as a mineralized matrix in differentiated cells *in vitro*.
Conclusion: In conclusion, oxaloacetate may have beneficial properties and could be a potential candidate for osteogenic differentiation of hMSCs.
Keywords: Human Mesenchymal Stem Cells, Osteogenic Differentiation, Oxaloacetate

Ps-25: Modeling A Microfluidic Platform for Label-Free Isolation of Mesenchymal Stem Cells from The Umbilical Cord Blood

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Objective: Mesenchymal stem cells have been wildly used in regenerative medicine and cell therapy. Umbilical cord blood-derived mesenchymal stem cells possess particular properties in comparison to the stem cells extracted from other sources like bone marrow. Utilizing an isolation method that causes the least damage to cells and cell loss is critical for stem cell isolation.
Materials and Methods: In this paper, we introduce a novel label-free method for the isolation of mesenchymal stem cells from umbilical cord blood based on the size differences of existing cells. A microfluidic serpentine channel is modeled, analyzed, and optimized for the removal of undesired cells, such as red blood cells, platelets, and white blood cells. For analysis of the proposed platform, a set of numerical models were constructed to observe the particle trajectories of different cells in the microchannel.
Results: A majority of red blood cells and platelets were successfully removed from the cord blood with no need of lysing solution and good purity of selected stem cells was achieved at the product outlet.

Conclusion: Finally, to cut down the processing time of a standard cord blood unit, a parallelized platform of a specified microchannel is proposed. Hence, mesenchymal stem cells could be isolated with a processing time significantly lower than conventional methods. The results could be used as a guide for the fabrication of this platform and further investigations.
Keywords: Microfluidics, Mesenchymal Stem Cell, Umbilical Cord Blood, Label-Free Isolation

Ps-26: Investigation and Identification of The Function of MNPs as A Nano-Drug in Inhibiting CD44 via Notch Signaling Pathway in Pancreatic Cancer Cells

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Objective: The theory of cancer stem-like cell (or cancer stem cell, CSC) has been established to explain how tumor heterogeneity arises and contributes to tumor progression in diverse cancer types and can also act as a reservoir of cancer cells that may cause a relapse after surgery, radiation, or chemotherapy. Emerging evidence showed that nanoparticles effectively inhibit many types of CSCs by targeting various specific markers and also signaling pathways like Notch, Hedgehog, and TGF-β which are critically involved in CSC function and maintenance. This study aimed to find and introduce specific nanoparticle markers involved in the control of CSCs in pancreatic cancer (PC) via the Notch signaling pathway.
Materials and Methods: In this bioinformatics study, all of the nanoparticles involved in the Notch signaling pathway were collected from the literature studies and valid electronic databases such as KEGG. The gene expression profiles of gastric, colorectal, and pancreatic were obtained from Gene Expression Omnibus (GEO). Nanoparticles were screened based on state-of-the-art nanomedicine approaches screening of normal and cancer samples of patients with three of the CSCs in PC from the Nano work database. Nonhuman studies were deleted and finally, we studied regulatory networks of upregulated nanoparticles in cancer stem cells via the Notch signaling pathway based on biological systems analysis.
Results: After completing the final analysis we found that CD44, CD90, and CD133 via Notch signaling play an important role in tumor regulation in PC. Then we selected CD44 and their effects on Notch signaling because it plays an important role in tumor regulation in the colorectal CSC.
Conclusion: We confirmed the selective drug delivery potential of the magnetic NPs (MNPs) by killing CD44-positive cancer cells using CD44 negative non-tumorigenic cell lines via Notch signaling as a control in PC cell lines.
Keywords: Cancer Stem Cells (CSCs), Notch Signaling Pathway, CD44, Pancreatic Cancer (PC), Nanoparticles

Ps-27: Investigation and Identification of Expression and Function of Two Long Non-Coding RNA HOTAIR & H19 in Controlling Cancer Stem Cells in Gastrointestinal via The WNT Signaling Pathway

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Objective: In recent years, knowledge of the biology of cancer stem cells (CSCs) has been very effective and the precise and proper regulation of stem cell function is important for their bio-activity. The main WNT signaling pathway has roles in regulating them which mediate different stem cell properties including self-renewal, survival, proliferation, and differentiation. Also, molecular structures such as Long non-coding RNAs (lncRNAs) act as tumor inhibitors or oncogenes and will change the direction of the messenger. The purpose of this study is to find and introduce lncRNAs involved in controlling cancer stem cells in gastrointestinal (GI) cancers via the WNT signaling pathway.
Materials and Methods: In this bioinformatics study, all of the lncRNAs involved in the Wnt signaling pathway were collected from the literature studies and valid electronic databases such as KEGG and LncRNA Disease were screened based on high throughput technics like microarray screening of normal and cancer samples of patients with three of the CSC in GI cancers. Also, we studied regulatory networks of upregulated lncRNAs in cancer stem cells via the Wnt signaling pathway based on biological systems analysis.
Results: After the final analysis, we indicated that HOTAIR and H19 and their effects on Wnt signaling play an important role in tumor regulation in the gastric, colorectal, and pancreatic CSC.
Conclusion: HOTAIR and H19 are involved in the development and exacerbation of gastric, colorectal, and pancreatic cancer stem cells by their expression and also in their interaction with other non-coding RNAs directly and indirectly through Wnt signaling.
Keywords: Cancer Stem Cells (CSCs), WNT Signaling Pathway, HOTAIR, H19, GI Cancers

Ps-28: Neural Differentiation of Adipose-Derived Mesenchymal Stem Cells in The Presence of Physostigmine-Calcium

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Objective: In the last few decades, adipose-derived mesenchymal stem cells (AD-MSCs) have received a great deal of attention as potential strategies for treating neurological injuries and diseases due to their multipotency, easy accessibility, abundance, and low immunogenicity. These stem cells have commonly displayed various phenotypic characteristics of osteoblasts, adipocytes, endothelial cells, and neurons under

suitable stimuli *in vitro*. According to previous research, the calcium signaling pathway regulates the proliferation and differentiation of neural progenitor cells, as well as their contact with neighboring cells. In this paper, we studied the effect of a tertiary amine carbamate acetylcholinesterase inhibitor called physostigmine by measuring the calcium concentration on the differentiation of AD-MSCs into neural progenitors.
Materials and Methods: Initially, the ideal concentration of physostigmine was determined using the MTT assay as a quantitative test and acridine orange (AO) staining as a qualitative test. After 7 and 14 days of neural induction, calcium content was performed to estimate the effect of the physostigmine with and without calcium on differentiation of AD-MSCs in neural medium containing forskolin and 3-isobutyl-1-methyl-xanthine (IBMX). Finally, the morphology of differentiated cells was examined using optical microscopy at the mentioned point.
Results: Our findings show that physostigmine in the presence of calcium (physostigmine-calcium) not only increased the cellular calcium concentrations but also showed neural progenitor morphology in differentiated cells *in vitro*.
Conclusion: In conclusion, this investigation illustrated that physostigmine-calcium as a stimulant agent showed an improvement in neural differentiation of AD-MSCs.
Keywords: Adipose-Derived Mesenchymal Stem Cells, Calcium, Neural Differentiation, Physostigmine

Ps-29: The Regulatory Role of miR-146a in Breast Cancer Stem Cells

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Objective: Breast cancer is the most prevalent cancer worldwide and its incidence is increasing annually. Cancer stem cells (CSCs) constitute a small proportion of cancer cells in a tumor with the ability of self-renewal and tumorigenesis and the root of tumor recurrence. MiR-146a has been reported to play both oncogenic and tumor-suppressive roles in different cancers through the regulation of stemness and epithelial/mesenchymal factors. Therefore, we aimed to study whether it affects the CSC population in breast cancer.
Materials and Methods: Breast cancer cell lines, MDA-MB-231 and MDA-MB-468, were cultured and transfected with a miR-146a-5p mimic. Analysis of CSC population and cell cycle was performed through detection of CD24 and CD44 markers and PI staining using flow cytometry. In addition, expression of NANOG and OCT4A, and some epithelial to mesenchymal transition (EMT)-related markers including VIM, SNAI1, and ZEB1 were assessed by quantitative polymerase chain reaction (qPCR).
Results: The CD44+/CD24- population in both cell lines was relatively decreased in the miR-146a transfected group compared to the control. EMT-related genes were downregulated in both cell lines after transfection with miR-146a. NANOG expression was downregulated in both cell lines while OCT4A was upregulated in MDA-MB-468 cells after miR-146a transfection. The cell cycle analysis of both cell lines showed a partial cell cycle arrest at the G1 phase after miR-146a transfection.
Conclusion: Our results are indicating that exogenous induction of miR-146a may exert tumor-suppressive effects through cell cycle arrest, decreasing the CSC population and suppression of EMT genes. Notwithstanding, more evidence would be required through additional *in vitro* and *in vivo* experiments.
Keywords: Breast Cancer, miRNA, Cancer Stem Cells, Epithelial To Mesenchymal Transition (EMT), miR-146a

Ps-30: Bioinformatics Analysis of Gene Expression Profiles Reveals Pivotal Signaling Pathways and Genes Associated With Ion Channels in Acute and Subacute Spinal Cord Injury

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Objective: A neural network is a set of neurons connected by synapses that transmit their messages to each other. Factors such as the type of cells and the expression of ion channels are involved in these signaling. Spinal cord injury (SCI) can disrupt the generation, frequency, and intensity of cell signaling by affecting ion channels or lead to neurotransmitter release disorders. This study aimed to explore gene expression changes underlying SCI in rats by bioinformatics.
Materials and Methods: The differentially expressed genes (DEGs) in acute and subacute SCI were screened based on GSE464, GSE45006, GSE46988, GSE2599 microarray data downloaded from Gene Expression Omnibus. Pathway enrichment analysis for the identified DEGs was performed by Enrichr considering KEGG, Reactome, Wikipathway, and Gene Ontology databases. In addition, membrane proteins of DEGs were recognized using the Uniprot and transmembrane helices prediction (TMHMM) method. The RNA expression profile of some of the significantly changed genes has been confirmed by real-time polymerase chain reaction (PCR).
Results: We found in both acute and subacute phases, up-regulated DEGs mainly participated in apoptosis, angiogenesis, vesicle-mediated transport, immune and calcium-related pathways; while down-regulated DEGs were mostly involved in ion transport and glutamate related pathways. Furthermore, we found a set of genes that encode transmembrane proteins. Particularly, in both phases of SCI, genes related to the ion channels were downregulated like Scn1a, Kcnc3, Kcnk1, Gria2, Grik4, and Grm1.
Conclusion: Our results may provide novel insight into the relationship between ion channels and neural activity in SCI, which can help enlargement of strategies to enhance recovery following SCI.
Keywords: Spinal Cord Injury, Ion Channel, Bioinformatics, Signaling Pathway, Gene Expression

Ps-31: S14161 as A Novel PI3K Inhibitor Promoted The Autophagic Effects on AGS Cell Line

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Objective: Gastric cancer is the third-most disease of cancer-related death around the world. Due to the late detection of the disease, patients' survival rate is poor. Previous studies have shown that the use of small molecules due to their unique properties, including antitumor and anti-proliferative activity, can be promising in the treatment of cancer. The present study aimed to evaluate the therapeutic potential of S14161 small molecule in the treatment of AGS cell line by expression of genes involved in autophagy.
Materials and Methods: AGS cells were first seeded and treated with 2, 5, 10, 15, and 25 μM concentrations of S14161. Cell viability and autophagic changes were assessed by MTT assay and MDC staining. The expression of genes involved in autophagy was analyzed by the real-time polymerase chain reaction (PCR) technique. To analyze the results, SPSS software version 25 was used.
Results: In the MTT assay, IC50 of S14161 small molecule in AGS cells treated with this substance was 15 μM, indicating a dose-dependent cytotoxic effect ($P<0.05$). Morphological changes, including changes in nucleation, cell shrinkage, chromatin compaction by staining, observing autophagosome vacuoles are evidence of cell death by autophagy. The results of real-time PCR indicated increased expression of Beclin-1 and Lc3 autophagic genes and decreased expression of mTOR anti-autophagic gene in AGS cells. The 24-hour treatment with S14161 indicated autophagy induction.
Conclusion: Induction of autophagic cell death pathways in AGS cells by S14161 small molecule and it's suggested as suitable drug candidate for the treatment of gastric cancer.
Keywords: AGS, S14161, Beclin-1, Lc3, mTOR

Ps-32: The Effect of TPSF Small Molecule on The Induction of Autophagy in Ovarian Cancer

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Objective: Ovarian cancer which is a common type of cancer ranks the fifth leading cause of death among women. Previous studies have shown that the use of small molecules due to their unique properties, including anti-tumor and anti-proliferative activity, can be promising in the treatment of cancer. The aim of the present study was to evaluate the therapeutic potential of TPSF small molecule in the treatment of ovarian cancer by expression of genes involved in autophagy and also by inhibition of ERα in SKOV- 3 cancer cell lines.
Materials and Methods: SKOV- 3 cells were first cultured and treated with 5, 10, 15, 20, and 40 μM concentrations of TPSF. Cell viability and autophagic changes were assessed by MTT assay and MDC staining. The expression of genes involved in autophagy such as Beclin-1, Lc3, mTOR, and AKT was analyzed by the quantitative real-time polymerase chain reaction (qRT-PCR) technique.
Results: In the MTT assay, IC50 of TPSF small molecule in SKOV- 3 cells treated with this substance was 15 μM, respectively, indicating a dose-dependent cytotoxic effect ($P<0.05$). Morphological changes, including changes in nucleation, cell shrinkage, chromatin compaction by staining, and autophagosome vacuoles, are evidence of cell death. The results of qRT-PCR indicated increased expression of Beclin-1, Lc3 decreased expression of anti-autophagic, mTOR, and AKT genes in SKOV- 3 cells. The 24-hour treatment with TPSF indicated autophagy induction.
Conclusion: TPSF small molecule can induce autophagic cell death pathways in SKOV- 3 cells and it's suggested as suitable drug candidates for the treatment of gastric cancer.
Keywords: TPSF, Autophagy, Ovarian Cancer

Ps-33: The Effect of S14161 on Inducing Apoptosis in Gastric Cancer Cell Line

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Objective: Gastric carcinoma as a malignant epithelial tumor is one of the most common cancer types around the world. Previous studies have shown that the use of small molecules due to their unique properties, including anti-tumor and anti-proliferative activity, can be promising in the treatment of cancer. The present study aimed to evaluate the therapeutic potential of S14161 small molecule in the treatment of AGS cell line by expression of genes involved in apoptosis and also by inhibition of PI3K in AGS cancer cell line.
Materials and Methods: AGS were first seeded and treated with 2, 5, 10, 15, and 25 μM concentrations of S14161. Cell viability and apoptotic changes were assessed by MTT assay and Giemsa staining. The expression of genes involved in apoptosis was analyzed by the real-time polymerase chain reaction (PCR) technique.
Results: In the MTT assay, IC50 of S14161 small molecule in AGS cells treated with this substance was 15 μM, indicating a dose-dependent cytotoxic effect ($P<0.05$). Morphological changes, including changes in nucleation, cell shrinkage, chromatin compaction by staining are evidence of cell death. The results of real-time PCR indicated increased expression of the Bax apoptotic gene and decreased expression of the Bcl-2 anti-autophagic gene in AGS cells. The 24-hour treatment with S14161 indicated apoptosis induction.
Conclusion: In this study, we showed that S14161 small molecule can induce apoptotic cell death pathways in AGS cells and it's advised as a suitable drug candidate for the treatment of gastric cancer.
Keywords: S14161, Gastric Cancer, Apoptosis

Ps-34: Beta Amyloid Effects as A Stress Inducing Factor On Inflammatory Markers in Microglial Cells

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Objective: Microglial cells are resident immune cells in the central nervous system (CNS) that are responsible for a part of inflammatory responses inside the CNS. Recent evidences designate that they play a key role in the pathogenesis of neurodegenerative diseases such as Alzheimer's (AD) and Parkinson (PD). Amyloid beta (Aβ) itself, is an inducer of microglia activation and neuroinflammation, considered as a pathologic factor in the development of AD. Hyper activated microglia (M1) or bad microglia can involve in AD pathogenesis by releasing inflammatory mediators such as inflammatory cytokines, chemokines, and free radicals that are all known to exacerbate beta-amyloid (Aβ) toxicity. While, good microglia (M2) can play beneficial roles such as stimulating clearance of amyloid plaques. In this study, we want to evaluate effects of beta-amyloid on cell morphology differences of microglia shifting and relationship between ramification index that treated with several concentrations of beta-amyloid and gene expression of some inflammatory markers.
Material and Methods: In this study, microglia were isolated from the brains of 2-day-old male mouse pubs and treated with different concentrations of Aβ (0,50,100 nM) for 24 h. Then expression of some inflammatory markers was performed in protein and mRNA levels. Morphological assessment (calculation of ramification index) on cells was also performed using image j software. Pearson correlation coefficient (r) between ramification index and microglial inflammatory response (IL1β expression) was determined.
Results: The results showed that Aβ (50 nM) significantly increases the expression of inflammatory markers such as IL1β. Also, it affected cell morphology and decreased ramification index in comparison to control group, as the morphology of microglial cells changed from rod-shaped or branched to amoeboid form in the presence of beta-amyloid. Pearson correlation coefficient (r) analysis showed that r was -0.8867 and there was a significant inverse correlation between ramification index and microglial inflammatory response (IL1β expression).
Conclusion: According to the results of this study, when microglial cells were gone under Aβ stress, the expression rate of their proinflammatory cytokines increases and neuroinflammation occurs. Also, r index showed that there is a significant inverse correlation between morphology of microglia (ramification index) and its inflammatory state.
Keyword: Microglia Cell, Beta Amyloid, Inflammatory Markers, Ramification Index

Ps-35: Expression of A Cell Cycle Regulator, MEIS1, Increases During Maturation of Human Embryonic Stem Cell-Derived Cardiomyocytes

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Objective: The adult mammalian heart has limited regeneration capacity and the loss of cardiomyocytes is the major contributor to the pathogenesis of many cardiovascular diseases. Most cardiac ischemic conditions involve myocardial remodeling in which the dead cells are replaced by scar tissue. This limited regeneration mostly originates from the cell cycle arrest of postnatal cardiomyocytes, which is caused by the downregulation of cyclins and upregulation of cell cycle inhibitors. Among the important factors involved in cell cycle arrest, is a complex network of transcription factors and proteins, which either positively or negatively regulate the expression of cell cycle proteins. Meis1, a member of the TALE homeodomain transcription factors, along with Hoxb13 as its cofactor, has been identified to contribute to postnatal cardiomyocytes' cell cycle exit. The expression of Meis1 and Hoxb13 were upregulated in mouse cardiomyocytes soon after birth which coincided with cell cycle arrest.
Materials and Methods: In this study, we sought to investigate the expression of MEIS1 and HOXB13 at different time points of *in vitro* cardiogenic differentiation of human embryonic stem cells (hESC). We aimed to use this *in vitro* platform to mimic human heart development and get access to heart tissue throughout cardiogenesis. Quantitative real-time polymerase chain reaction (qRT-PCR) was used for gene expression analysis.
Results: Our results showed that the progress of *in vitro* cardiomyocytes differentiation coincided with a gradual increase in the expression of MEIS1 and HOXB13. MEIS1 expression was substantially higher in hESC-derived cardiomyocytes at day 40 of differentiation compared to day 7. Furthermore, the proliferation of differentiated cardiomyocytes was markedly decreased at late time points of differentiation.
Conclusion: In summary, our results may provide preliminary information on the possible relation between MEIS1/HOXB13 expression and the cell cycle exit of human cardiomyocytes.
Keywords: Adult Cardiomyocyte, Cell Cycle Arrest, Cardiomyocyte Differentiation, Transcription Factors

Ps-36: Optogenetics Stimulation Upregulates The Expression of Doublecortin and Microtubule-Associated Protein 2 in Human Dental Pulp Stem Cells

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Objective: Human dental pulp stem cells (hDPSCs), a promising source for autologous transplantation in regenerative medicine, are capable of differentiating into neural precursors. Optogenetics is a new approach for neuromodulation. In this technique, genetically modified cells expressing light-sensitive channels are stimulated by light. To consider whether the optical stimulation can promote hDPSCs differentiation into neurons, we investigated the expression levels of doublecortin (DCX)

and microtubule-associated protein 2 (MAP2) as immature and mature neuron markers, respectively.
Materials and Methods: The hDPSCs were isolated by mechanical enzymatic digestion from an impacted third molar and cultured in DMEM/F12. The cells were infected with lentiviruses carrying CaMKIIa-hChR2 (H134R). Opsin-expressing hDPSCs were plated at the density of 5 × 104 cells/well in 6-well plates and optical stimulation was conducted with blue light (470 nm) pulsing at 15 Hz, 90 % Duty Cycle and 10 mW power for 10 s every 90 minutes for 5 days. Furthermore, the expression levels of DCX and MAP2 in hDPSCs were examined by immunocytochemistry assay.
Results: Optical stimulation increased the expression levels of DCX and MAP2 in hDPSCs accompanied by morphological changes from spindle shape to neuron-like shape.
Conclusion: Our results revealed that optogenetics efficiently promotes neural differentiation of hDPSCs and it can be a promising candidate for cell-based therapy of neurodegenerative disease.
Keywords: Optogenetics, Human Dental Pulp Stem Cells, Doublecortin, Microtubule-Associated Protein 2

Ps-37: Improved Killing of Ovarian Cancer Stem Cell With Activated Natural Killer Cell

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Objective: Ovarian cancer stem cells as a small population of tumor cells are responsible for drug resistance, metastasis, and recurrence of tumors. However, the effect of the immune system on this type of tumor cell is unknown. Natural killer (NK) cells as the effector lymphocytes of the innate immune system can eliminate the tumor and the infected cells. However, the effect of NK cells on ovarian cancer stem cells is unknown. In the present study, we evaluate the effect of activated NK cells on ovarian cancer stem cells enriched in ovarospheres.
Materials and Methods: SKOV3 cell line was selected for two-dimensional (2D) and three-dimensional (3D) cultures (Ovaspheres). The NK cells were isolated from the peripheral blood of the healthy donors using CD56 enriching magnetic activation cell sorting (MACS) kit and were activated with IL-15 (10 ng/ml) and FOXO1 inhibitor (0.125 µM) for 24 hours and then co-cultured with SKOV-3 cells in 2D and 3D models at 1:3 ratio for 48 hours. The cell cytotoxicity was evaluated through live/dead flow cytometry.
Results: The cytotoxic effect of activated NK cells in 2D culture was about 8.98% in presence of IL15 and 16.34% with the IL15+FOXO1 inhibitor. The cytotoxic rate significantly increased to 37.73% and 51.11% for the IL15 and IL15+FOXO1 inhibitor, when spheroids were cultivated with activated NK cells.
Conclusion: According to the results, the NK cells were activated with a combination of IL15+FOXO1 inhibitor compared to the IL15 demonstrated a better cytotoxic effect on SKOV-3 cancer cells. However, it should be confirmed in a mouse model.
Keywords: Ovarian Cancer, Cancer Stem Cell (CSC), Natural killer Cell (NK), Immunotherapy

Ps-38: *In Vitro* Evaluation of High-Speed-Derived Extracellular Vesicles Derived from Clonal Bone Marrow Mesenchymal Stromal Cell Regard to Cutaneous Wound Healing

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Objective: Skin wound formation is an inevitable part of every single human in life that cures in most cases without intervention. Nonetheless, burnings, diabetic foot ulcers, pressure ulcers, and surgeries are distinguished situations that need substantial help to be healed completely. There are plenty of regenerative medicine therapies which include cell-based and cell-free types. Usage of cell secretomes such as extracellular vesicles (EVs) is one of the cell-free methods that has been widely studied in wound healing within the past decade. The purpose of this study is *in vitro* evaluation of High-Speed EVs (HS-EV) derived from clonal bone marrow-derived mesenchymal stromal cells (cBM-MSC) regard the cutaneous wound healing process.
Materials and Methods: We isolated cBM-EVs by 20000 × g centrifugation (HS-EVs) and characterized them based on MISEV2018 guideline, then we analyzed proliferation and migration analysis through growth curve test and scratch assay, respectively, with the use of human dermal fibroblasts (HDF). We also examined tube formation assay with HUVEC cells. All tests were treated with a 100 µg/ml dosage of MSC-HS-EV.
Results: MSC-HS-EV enhanced the rate of HDF proliferation and migration strongly. In addition, newly formation of angiogenesis was seen in MSC-HS-EV.
Conclusion: Taken together, our results indicated that HS-EVs can have significant effects on cellular healing processes. This subpopulation of EVs has shown cell-free therapeutic effects and being economic and convenient are considered as additional benefits.
Keywords: Wound Healing, Extracellular Vesicles, Mesenchymal Stromal Cell, Human Dermal Fibroblast

Ps-39: Therapeutic Effects of Human Adipose Mesenchymal Stem Cells and Their Secretomes on Retinal Degeneration in Rats

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Objective: Retinal degenerative diseases are major reasons for visual disability. Treatments based on Mesenchymal stem cells (MSC) have been promising. Some studies showed that most therapeutic effects of MSCs are rooted in their paracrine agents including their conditioned medium (CM) and exosomes. In this research, we studied the therapeutic potency of human adipose mesenchymal stem cells (hADSC) and their paracrine agents on retinal degeneration.
Materials and Methods: Retinal degeneration model was generated by injecting sodium iodate into Wistar rats. The effects of treatments were assessed by visual cliff test and real-time reverse-transcription polymerase chain reaction (RT-PCR) for retinal genes expression. Research therapeutic groups were phosphate buffer saline (PBS), hADSC, CM, exosome, and exosome+CM. All of them received two doses of therapeutic agent except the hADSC group which received one dose only. Therapeutic agents were injected intravitreally. Rats were sacrificed 6 weeks after receiving their first therapeutic dose.
Results: Best results of the visual cliff test were in the CM group. RT-PCR data analysis indicated that the expression of Opn1sw and Pax6 was higher in the CM group. Vsx2 expression was significantly high in the exosome+CM, exosome, and CM groups in order from high to low. The exosome and CM groups' Crx expression was more than the others and Nrl''s highest expression could be seen in the exosome group. Considering the gene expression and visual cliff test, the best therapeutic results came from the CM group.
Conclusion: The findings of our study showed that secretomes of hADSCs are better therapeutic agents than the cells themselves and it suggests that further experiments in this field should be more focused on cell-free therapies and stem cells secretomes.
Keywords: Mesenchymal Stem Cells, Retina, Exosome, Conditioned Medium

Ps-40: The Effect of Yazd Human Foreskin Fibroblasts #8 Derived Conditioned Medium on Wound Healing in Animal Model

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Objective: Recent studies have shown that conditioned medium collected from mesenchymal stem cells has a supportive effect on wound healing. In this study, the effect of Yazd human foreskin fibroblasts #8 derived-conditioned medium (YhFF#8-CM) on wound healing in rats is investigated.
Materials and Methods: For this purpose, 18 adult male rats were randomly divided into three groups: (1) control group, (2) sham group (DMEM-F12), and (3) test group (YhFF#8-CM). A 1cm×1cm full-thickness wound was created on the back of the rats. At days 4, 7, and 14 post-wounding the wound closure area of each wound was measured. Wound tissues were collected at days 7 and 14 post-wounding and stained with hematoxylin and eosin and Masson's trichrome for microscopic analysis.
Results: Wound closure area was increased in the test group (YhFF#8-CM) at days 4, 7, and 14 after wound creation compared to the control and sham groups. Histological studies indicated a significant increase in the rate of re-epithelialization, angiogenesis, number of cells, and collagen synthesis.
Conclusion: In sum, our data indicate that YhFF#8-CM can be considered as a novel cell-free therapeutic product for wound healing.
Keywords: Animal Model, Conditioned Medium, Human Foreskin Fibroblasts, Regenerative Medicine, Wound Healing

Ps-41: Fabrication and Characterization of A PTH1-34-Releasing PLA Scaffold

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Objective: Polylactic acid (PLA) as a biomaterial demonstrates some drawbacks that limit its applications. PLA is bioinert and lacks active surface functional groups which results in its inability to establish direct contact with the adjacent tissue. Therefore, one of the recently proposed solutions to this problem is the adsorption of bioactive molecules onto the surface of PLA. Parathyroid hormone (PTH) is composed of 84 amino acids and is responsible for the regulation of calcium homeostasis in the body and plays a crucial role in the production of bone tissue. PTH1-34 is a 34 amino acids long peptide derived from the N-terminal of PTH. PTH1-34 exerts anabolic effects on bone tissue by increasing osteoblast proliferation and viability and is an FDA-approved drug in the treatment of osteoporosis.
Materials and Methods: Accordingly, in this study, PLA scaffolds were fabricated by the salt leaching method and characterized using SEM and porosity measurements. The surface of the scaffolds was activated using two different methods to generate amine and carboxyl functional groups on the surface of the scaffolds. These functional groups were in turn, employed to covalently immobilize PTH1-34 on the PLA scaffolds using EDC/NHS as crosslinkers.
Results: The fabricated scaffolds possessed porous structures with 60-90% porosity and pore sizes of 200-600 μm. Additionally, the concentration of generated amine and carboxyl groups on each scaffold was determined to be 0.099±0.017 and 0.056±0.016 mol/scaffold, respectively.
Conclusion: Ultimately, the successful immobilization of PTH1-34 on the surface of the scaffolds was confirmed by

ATR-IR. The fabricated scaffolds could be good candidates for bone tissue engineering and delivery vehicles for PTH1-34 in the treatment of osteoporosis.

Keywords: Scaffold, Polylactic Acid, Parathyroid Hormone, Covalent Immobilization

Ps-42: Fabrication and Evaluation of A Conductive Polyaniline-Based Nanofibrous Scaffold for Neural Regeneration

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Objective: In the nervous system, cells exist in the fibrous, conductive, and active extracellular matrix (ECM) made of fibrillary proteins that regulate cellular behaviors. It is well-known that neurons have electrical activity and communicate through action potentials. In many central nervous system (CNS) damages, progressive neuron loss happens. Unfortunately, the nervous system has low regenerative capacity due to the small pool of neural stem cells and the inhibitory environment. In tissue engineering, the scientific strategy is to mimic the conductive and fibrous environment for neural proliferation and differentiation by designing functional scaffolds. Here, we aim to develop a conductive composite nanofibrous scaffold for neural regeneration.
Materials and Methods: PLLA was dissolved in chloroform. Equivalent amounts of polyaniline (PANi) and its dopant, camphor sulfonic acid, were dissolved in chloroform and dimethylformamide. The ultimate composite nanofibers of blended PLLA/PANi were produced by using the electrospinning technique. Different parameters of electrospinning were examined in the fabrication process.
Results: Our scaffold presented beadless, uniform, and porous nanofibers with approved conductivity as the nervous system. The concentration of the carrier polymer mainly affected the nanofiber pore size and fibrillary structure. The viability of the cells seeded on these scaffolds was excellent.
Conclusion: In conclusion, we produced the potential candidate to mimic neural ECM for neural regeneration. Noteworthy, this is an attractive platform to study exogenous electrical stimulation effects on neuron function or stem cell neural fate *in vitro*.

Keywords: Neural Regeneration, Tissue Engineering, Conductive Scaffolds, Polyaniline, Electrospun Nanofibers

Ps-43: The Secretome of Human Pluripotent Stem Cell-Derived Cardiomyocytes Suppresses The Growth and Invasion of Lung Cancer Cells

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Objective: The heart has a very limited repair capacity after birth, which is not sufficient during myocardial injury and ultimately results in functional inadequacy. This limitation mainly stems from cardiomyocytes' cell cycle arrest and lack of cell proliferation. On the other hand, the heart develops cancer only rarely, which might be due to its diminished proliferation capacity. Based on cardiomyocytes' cell cycle exit and the possibility for related signaling molecules in the heart microenvironment, we hypothesized that the heart tissue resistance to tumorigenesis might originate, at least partly, from the anti-tumor secretions of the cardiomyocytes. Therefore, we aim at investigating the possible tumor-suppressing effects of the secretome of cardiomyocytes differentiated from human pluripotent stem cells.
Materials and Methods: A549 lung cancer cells were cultured in RPMI media containing 10% fetal bovine serum. The cells were analyzed three days after treatment with a conditioned medium of human pluripotent stem cells-derived cardiomyocytes. Cell survival assays were performed using crystal violet staining and the MTS viability test. Cell cycle analysis was performed using flow cytometry.
Results: Our results indicated that the conditioned medium of human pluripotent stem cell-derived cardiomyocytes substantially decreased the viability of lung cancer cells. In addition, the secretome of cardiomyocytes inhibited the cell cycling of the cancer cells and suppressed their ability to migrate. Finally, these secretions significantly reduced the colony-forming capacity of the lung cancer cells.
Conclusion: The conditioned media of human pluripotent stem cell-derived beating cardiomyocytes decrease the growth and migration of lung cancer cells *in vitro*.

Keywords: Cardiomyocyte, Lung Cancer Cells, Tumorigenicity, Conditioned Medium

Ps-44: Identifying Significant Exosomal Serum Proteins in Metastatic Gastric Cancer Patients With In Silico Approach

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Objective: Gastric cancer is one of the most prevalent malignancies of the gastrointestinal tract. Exosomes are nanometer-sized vesicles and as important parts of the tumor microenvironment are involved in tumorigenesis, progression, and response to treatment. In this study, differentially expressed proteins (DEPs) of exosomal serum proteins have been selected and evaluated with in-silico approaches to identify the most important serum exosomal DEPs in gastric cancer patients.
Materials and Methods: A list of 40 up-regulated and 40 down-regulated exosomal serum proteins in gastric cancer patients in comparison with the exosomal serum protein content of normal individuals, were chosen from previous proteomics studies and their protein-protein interaction (PPI) network was drawn using string-db (https://string-db.org/) and Cytoscape v 3.8.2 and then the most important proteins in each group were selected based on the highest score of node degree and betweenness centrality criteria.
Results: Evaluating PPI networks of up-regulated and down-regulated exosomal proteins in patients with metastatic gastric cancer revealed that Hepatoglobin and Actin-alpha1 are the

hubs and bottleneck nodes of up-regulated and down-regulated exosomal proteins respectively. Determining the hub and bottleneck nodes of each group was performed based on choosing the node with the highest degree and betweenness centrality score.
Conclusion: Hepatoglobin and Actin-alpha1 were found to have significant roles among deferentially expressed proteins of serum exosomes in patients with metastatic gastric cancer. This finding could be helpful for future studies for evaluating these two proteins as potential targets for early diagnostic or therapeutic targets for gastric cancer.
Keywords: Gastric Cancer, Tumor Microenvironment, Nanovesicles, Exosome, Bioinformatics

Ps-45: Using of Natural Killing to Target Metastatic Ovarian Cancer; A Case Report Study

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Objective: There are currently no curable treatments for most metastatic tumors including ovarian cancer. Recently immunotherapy has generated a promising revolution worldwide due to its favorable toxicity profile and cleaning of tumor cells. Among different clinical setting, activated natural killer (NK) cell, has shown the natural ability to remove the cancer cells. We herein report the case of a haploidentical NK cell therapy.
Materials and Methods: A 61-year-old woman with a metastatic brain tumor and diffused peritoneal tumors from primary ovarian cancer were included in the study (with Golestan University of Medical Sciences ethics committee certificate: 10/183777). Before immunotherapy, we cultivated brain tumor biopsy to evaluate the cytotoxic effect of haploidentical NK cells on metastatic cells. NK cells were isolated from her son, activated 12 h with IL15, and then co-cultured with cultured metastatic cells for extra 48 hours. The patient received activated NK cells between 2 cycles of conventional chemotherapy. NK cells infused intra peritoneum (IP) (2x106/kg) and into the cerebral spinal fluid (CSF) (5x106).
Results: Our *in vitro* setting showed about 60% toxicity of activated NK cells on metastatic ovarian cancer cells. The infusion of cells (2 times, weekly) into the CSF and IP was safe and without any side effects. The MRI results showed tumor clearance in the peritoneal cavity and even brain post 8 months follow-up.
Conclusion: Activated NK cells from haploidentical donors could be a safe approach to target metastatic seeding in malignant ovarian cancer. However, the procedure should be confirmed in a higher number of patients.
Keywords: Ovarian Cancer, Cell Therapy, NK Cells

Ps-46: Evaluating The Co-Culture Effect of Mesenchymal Stem Cells With Interleukin-1 Beta-Induced Caco-2 Cell Line on The Expression of Inflammatory Genes *In Vitro*

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Objective: One of the most common inflammatory bowel diseases (IBD) is Crohn's disease (CD). It is now well established that conventional therapies in CD (surgery, medication, etc.) are inefficient and recent studies have shown that due to the anti-inflammatory effect of these cells, mesenchymal stem cells (MSCs) may play an alternative role in treating CD. In this study, we investigated the effect of the co-culture of MSCs and interleukin-1 beta-treated Caco-2 cell lines on the expression of inflammatory genes *in vitro*.
Materials and Methods: Mesenchymal stem cells were isolated from human bone marrow tissue (BM-MSCs) and adipose tissue (AD-MSCs). Finally, their conditioned medium was collected. The Caco-2 cell line was exposed to interleukin-1 beta at a concentration of 10 ng/ml for 24 hours. Human adipose- and bone-marrow-derived MSCs with a cell density of 30,000 cells and their conditioned medium were cultured on interleukin-1 beta-treated Caco-2 cells in each well. The expression of inflammatory genes of CDX2 and IL-12 were analyzed by the real-time polymerase chain reaction (PCR) method and the results were statistically analyzed by SPSS software.
Results: The obtained results showed a significant difference between CDX2 and IL-12 gene expression between AD-MSCs, BM-MSCs, and their conditioned medium. AD-MSCs were shown to have the least expression of CDX2 and IL-12 rather than BM-MSCs, and their conditioned medium.
Conclusion: According to the results of this study, it can be concluded that AD-MSCs can be used as an appropriate cell source for improving anti-inflammatory properties in CD.
Keywords: Mesenchymal Stem Cells, Interleukin-1 Beta, Caco-2, Inflammatory Genes, Co-Culture

Ps-47: To Enhance Safety and Efficiency of Mouse Embryonic Fibroblast Reprogramming into Renal Cells by Episomal Vectors and miR-92a

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Objective: Acute and chronic renal failure is a disease with an accelerating incidence rate all around the world. The survival of these patients depends on kidney transplantation or dialysis procedures. Therefore, access to new therapies such as transdifferentiation can be a promising solution to repair lost kidney cells. Our aim in this study was to increase the safety and efficiency of direct conversion of mouse embryonic fibroblasts to renal cells, using episomal vectors and miR-92a.

Materials and Methods: Fibroblasts were extracted from 12.5-day-old mouse embryos and cultured in a complete DMEM. Then plasmids were transfected by Yamanaka factors to these cells with electroporation. Cells were cultured in a reprogramming medium for four days, and then placed in a specific nephrogenic medium. On the ninth day of the protocol, miR-92a was transfected into cells. Up to the twelfth day, the cells underwent cellular and molecular evaluations. E-cad and zo-1 were used as general markers and Wt1, Lim1, and Pax2 as specific markers of renal cells.
Results: Our results indicate that our modified protocol for renal cell trans-differentiation, using episomal vectors and miR-92a. It can have more complete immunity than the viral method, which was confirmed by polymerase chain reaction (PCR) and non-expression of vectors in cells. Also, using miR-92a the efficiency of kidney cells was increased, which was confirmed by immunofluorescence and real-time PCR. Flow cytometry's data showed that the efficiency has increased by 4%.
Conclusion: Our modified protocol for renal cell trans-differentiation, using episomal vectors and miR-92a, increased differentiation efficiency, and this protocol is safer in comparison with former protocols, using viral vectors.
Keywords: Kidney Failure, Trans-Differentiation, miR-92a, Episomal Vector

Ps-48: A Sindbis Virus Replicase-Based Minicircle: A Novel Tool for Transdifferentiation

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Objective: Transdifferentiation has the potential of enormous impacts on regenerative medicine. Among all the utilized approaches for transdifferentiation, mRNA serves as a safe and efficient integration-free tool. However, the low stability of produced mRNAs by *in vitro* transcription (IVT) results in daily transfection that is costly and time-consuming. To overcome these limitations in this study, the Sindbis virus (SINV) replicase system was employed in combination with minicircle DNA technology to generate self-replicating mRNAs.
Materials and Methods: A fragment containing SINV replicase, subgenomic promoter (PSG), and EGFP sequences were cloned between attB and attP sites downstream of the eukaryote promoter in the structure of a parental plasmid (PP-EuP-REP-PSG-EGFP). The related minicircle (MC-EuP-REP-PSG-EGFP) was generated by induction of intramolecular recombination between attB and attP sites in PP-EuP-REP-PSG-EGFP cassette. Then, the resulted minicircle was transfected into HEK293T cells and EGFP expression was assessed using fluorescent microscopy and flow cytometry.
Results: PP-EuP-REP-EGFP and MC-EuP-REP-EGFP were successfully constructed. Correct orientation and accuracy of cloned fragments were confirmed using polymerase chain reaction (PCR), restriction digestion, and sequence analysis. The fluorescent signal of transfected HEK293T cells with MC-EuP-REP-EGFP was successfully detected using a fluorescent microscope and flow cytometry.
Conclusion: Our findings demonstrated that the constructed replicase-based minicircle enhanced the EGFP expression level for a long duration compared to a simple minicircle (MC-EuP-EGFP). This novel replicase-based minicircle produces a bicistronic-genomic RNA transiently that contains replicase and EGFP sequences. Expression of EGFP by this system resulted in a higher level and longer-term expression of the transgene in transfected cells in comparison to usual vectors. Accordingly, it seems that using this modified minicircle as a non-viral vector can improve the efficiency and safety of the transdifferentiation process.
Keywords: Sindbis Virus, Minicircle, Self Replicating RNA

Ps-49: UCB HSCS -Derived Functional NK Cells Against Tumors Through Harnessing IGF-1R and SMADS Signaling Pathways

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Objective: The natural killer (NK) cells differentiated from umbilical cord blood (UCB) hematopoietic stem cells (HSCs) may be more suitable for cell-based immunotherapy compared to the NK cells from adult donors. This is due to the possibility to choose alloreactive donors and potentially more robust *in vivo* expansion. However, the cytotoxicity of UCB-HSC-derived NK cells against cancer cells might be suboptimal. To overcome this obstacle, we attempted to generate NK cells with potent antitumor activity by targeting RAS/MAPK, IGF-1R, and TGF-β signaling pathways using IL-15, IGF-1, and SIS3 respectively.
Materials and Methods: The CD34 + cells were isolated from human UCB mononuclear cells through magnetic activation cell sorting (MACS) with a purity of (≥ 90%) and were subjected to differentiate into NK cells. After 21 days of induction with SFTG36 (SCF, FLt-3L, TPO, GM-CSF, IL-3, and IL-6), IS721 (IGF-1, SIS3, IL-7, and IL-21), and IL-15/Hsp70 media, NK cells phenotypes were studied and their cytotoxicity against K562 human erythroleukemia cells and SKOV3 ovarian carcinoma cells was analyzed.
Results: The NK cells induced in SFTG36/IS721 medium were selected for activation due to their higher expression of CD56 + 16 + CD3 - (93.23% ± 0.75) and mean fluorescence intensity (MFI) of NKG2D + (168.66 ± 20.00) and also a higher fold expansion potential (11.893 ± 1.712) compared to the other groups. These cells once activated with IL-15, demonstrated higher cytotoxicity against K562 (≥ 90%; P ≤ 0.001) and SKOV3 tumor cells (≥ 65%; P ≤ 0.001) compared to IL-15/Hsp70-activated NK cells.
Conclusion: The differentiation of *ex vivo* expanded CD34 + cells through manipulation of RAS/MAPK, IGF-1R and TGF-β signaling pathways is an efficient approach for generating functional NK cells that can be used for cancer immunotherapy.
Keywords: Cancer Immunotherapy, Cytotoxicity, Differentiation, Natural Killer Cells, Umbilical Cord Blood

Ps-50: Improving Immunomodulatory and Regenerative Properties of Bone Marrow Derived-Clonal Mesenchymal Stromal Cells by Priming

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Objective: The main therapeutic properties of mesenchymal stromal cells (MSCs) are through immunomodulatory and regenerative properties, especially paracrine factors. Therefore, we decided to improve these properties and use MSCs' secretions by several priming regimens applied on clonal MSCs that could eventually be developed to use in clinical trials.
Materials and Methods: MSCs at passage 10–15 were cultured in a serum-containing medium. 4.4×105 cells were seeded in each flask and were grown to 60–70% confluency. Different priming regimens were performed in five groups including, normoxia (Non-treated group), Interferon-gamma (IFN-γ-treated group) (50 ng/ml), Polyinosinic: polycytidylic acid (poly(I:C)-treated group) (42.22 μg/ml), Lipopolysaccharides (LPS-treated group) (1 μg/ml), and hypoxia (Hypoxia-treated group) (1% O2). After 48 hours of incubation, the supernatant was collected and frozen in a -80 °C freezer to use in further experiments. The cells were frozen in liquid nitrogen (-196 °C) as well. The immunomodulatory and regenerative properties of all primed human bone marrow conditioned MSCs (hBM-cMSCs) were evaluated by enzyme-linked immunosorbent assay (ELISA), western blotting, lymphocyte proliferation assay (LPA), and chorioallantoic membrane (CAM) assay.
Results: In this study, it has been shown that when BM-cMSCs were primed two days with hypoxia and LPS; a significant increase in the secretion of angiogenic factors (Vascular endothelial growth factor (VEGF), Hepatocyte growth factor (HGF), Insulin-like growth factor 1 (IGF-1), and basic fibroblast growth factor (bFGF)), also anti-inflammatory cytokines (IL-10, TGF-β1, PD-L1, IDO) were detected. On the other hand, LPS-treated MSCs have indicated an increase in the secretion of pro-inflammatory factors.
Conclusion: In conclusion, it was illustrated that the immunomodulatory and regenerative properties of Hypoxia-treated and LPS-treated MSCs were considerably higher than the other three groups. Hypoxia did not lead to apoptosis. It also did not alter the viability and confluency of the cultured cells. These findings confirmed the effectiveness, reproducibility, and capability of a pre-treatment developed for testing in clinical trials.
Keywords: Mesenchymal Stromal Cells, Immunomodulation, Regeneration, Priming, Paracrine Factors

Ps-51: NLRP3/ASC/NLRC4 Inflammasome Pathways Are Differentially Regulated by Adipogenic and Osteogenic Differentiation in Adipose Derived Mesenchymal Stem Cells

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Objective: Inflammasome is part of the innate immune recognition system, with its protein components activation (NLRP3/ASC/NLRC4) by inflammatory stimuli in immune-related disorders results in a release of inflammatory cytokines including IL-1β and IL-18. Hyperactivation of the inflammasome pathway affects the survival, proliferation, and function of many cells, including stem cells. Despite the confirmation of the expression of the inflammasome components in some stem cells, the impact of differentiation on the expression of inflammasome-associated genes has not been studied so far.
Materials and Methods: In this study, ADMSCs cells were isolated from adipose tissue of male C57BL/6 mice by collagenase digestion and serial passages and they were characterized phenotypically by the determination of expression surface protein markers (CD44, CD34, CD80, CD45, and CD138). The transcription levels of inflammasome-related genes (AIM2, ASC, NLRC4, NLRP1 and NLRP3, and IFF), the caspase1 activity, and the level of IL1β protein were measured in ATDMSc at days 7 and 14 after adipogenic and osteogenic differentiation.
Results: Our results showed that all inflammasome-related genes were expressed in ATDMSc. Despite the minimal gene expression of the IFF gene, induction of adipogenic differentiation resulted in the upregulation of all inflammasome-associated genes. Osteogenic differentiation caused a reduction in transcription of all studied genes. The expression pattern of the IFF gene showed different patterns to other inflammasome-related genes during ADMSCs differentiation. The activity of caspase1 and the protein level of IL1β were increased during the adipogenic differentiation, while those were reduced during the osteogenic differentiation.
Conclusion: Our results showed that gene components of the inflammasome pathway were differentially expressed in ATDMSC cells during differentiation. Changes in the expression of inflammasome-related genes during the osteogenic and adipogenic differentiation can be used for the improvement of adipose cell function in obesity or osteoblasts in osteogenic disorders.
Keywords: Adipose Derived Mesenchymal Stem Cells, Inflammasome, Differentiation

Ps-52: Anti-Aging Effects of Amniotic-Membrane Extract on H2O2-Induced Aging in Human Fibroblast

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Objective: In recent years, medical science and quality of life have got significantly improved; resulting in longevity elongation, and thus the average life expectancy at birth of the global population has risen. Skin is the largest organ of the body and plays an important role in human appearance health and beauty. Today people are surprisingly interested in rejuvenation and cosmetic procedures to ameliorate skin aging; hence, investigating new approaches is of great value. The human amniotic membrane is one of the extra-embryonic tissues and recently has had growing applications in regenerative medicine. It has anti-microbial, anti-fibrotic, and analgesic effects and contains different growth factors and cytokines. Besides, it is of less ethical concern and has reasonable processing procedure costs. Further, it showed anti-oxidative effects.
Materials and Methods: In this study, H2O2-induced aging human foreskin fibroblasts (HFFs) were pre and post-treated with amniotic membrane extract (AME) for 24 and 48 hours. Cellular senescence, viability, and anti-oxidant enzymes activity were evaluated.
Results: Results showed that AME significantly ($p<0.05$) increased cell proliferation and reduced cellular senescence. It also remarkably ($p<0.05$) decreased superoxide dismutase activity and raised reduced glutathione amounts after 48 hours as both pre and post-treatment regimens.
Conclusion: In this regard, due to AME proliferative and anti-oxidant-like effects; AME may consider as a potential natural-based anti-aging ingredient in skincare anti-aging products.
Keywords: Amniotic Membrane, Aging, Cellular Senescence, Anti-Oxidant, Fibroblast

Ps-53: Tissue Extract from Brittle Star Undergoing Arm Regeneration Promotes Wound Healing in Rat

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Objective: The important task of the skin is to create a defense barrier against external and infectious agents. When the wound is created, wound healing must be regulated to regains its normal function and shape of the skin. Marine organisms have been reported to have wound healing properties due to their effect on cell proliferation and collagen formation. Due to the high arm regeneration potential of the brittle star, Ophiocoma erinaceus, the present study aimed to evaluate the wound healing effect of hydroalcoholic extract of a brittle star undergoing arm regeneration in *in vitro* and *in vivo* studies.
Materials and Methods: The brittle star samples were collected from Nayband Bay, Bushehr, Iran. Two-thirds of one arm-tip of each brittle star was removed and they were kept in an aquarium for 3h, three days, five days, seven days, and fourteen days before extraction. Firstly, the macroscopical and histological evaluation of brittle star arms during regeneration were analyzed. Then, the hydroalcoholic extract of different times of arm regeneration was prepared. Then, *in vitro* MTT cell viability and cell wound healing tests were performed. Based on the *in vitro* findings, two brittle star extracts (BSEs) were chosen for the *in vivo* test. Rats were divided into 5 groups ($n = 9$), negative control (without treatment), alpha ointment, basal gel, 7d BSE in base gel, and 14d BSE in base gel. Two 2 cm circular wounds were created in the dorsal area of each rat. Once a day, the desired treatment was applied to the wound and after 7, 14, and 21 days, three rats from each group were randomly selected for macroscopic, histopathology, and biochemical evaluations.
Results: The brittle star arm regeneration was completed on day 14. Two different regenerative buds formed at early day 1 and day 7 for spine formation and arm regeneration, respectively. Forty-two compounds were detected in all groups of BSEs. All extracts had anti-inflammatory and anti-microbial compounds. The 0h BSE had muscle contraction and anti-proliferative compounds. The 3h BSE contained three different compounds as compared to the group without arm amputation, with anti-proliferative and neuro-regenerative effects. The 7d and 14d BSE had proliferation and migration compounds, respectively. The MTT assay showed that the 14d BSE had a higher significantly proliferation effect on human foreskin fibroblasts (HFFs) than 7d BSE. The image analysis of 24h cell wound healing analysis showed that the wound area in 7d and 14d BSEs were significantly lower than the control group. The *in vivo* analysis showed that evaluation of wound changes and the percentage of wound healing in the 7d BSE group was better in comparison with the other groups. Histopathological scores of the 7d BSE and 14d BSE groups were significantly higher than the other groups. The amount of collagen contents and hydroxyproline concentrations in the 7d BSE group was higher than the other groups.
Conclusion: The hydroalcoholic extract of Ophiocoma erinaceus undergoing arm regeneration after 7 and 14 days promoted the wound healing process in the rat skin due to proliferative and migration compounds.
Keywords: Wound Healing, Regeneration, Proliferation, Brittle Star, Rat

Ps-54: A Comparative Evaluation of Gene Expression of Interleukin Family in ADSCs With Green and IR Low-level Laser Therapy Using RNA-Seq Technique

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Objective: Adipose-derived mesenchymal stem cells (ADSCs) have anti-inflammatory and immunosuppressive effects, so recently they offer a great promising cell source for a variety of stem cell-based therapies, especially in inflammatory and autoimmune diseases. Photobiomodulation (PBM), also known as low-level laser therapy, is a therapeutic method that uses light to stimulate healing, relieve pain, and reduce inflammation. Among various types of inflammatory genes, interleukins belong to the cytokines superfamily and are key mediators of immunity and inflammation. They contribute to cell proliferation, differentiation, migration, maturation, and adhesion as well as systemic inflammation and immune system modulation. Therefore, their dysregulation is involved in many diseases, including onset, severity, and progression of cancer. This study investigates the effect of green and IR low-level laser therapy on the gene expression of the interleukin family in ADSCs.
Materials and Methods: Herein, we applied two different wavelengths of laser (830 nm and 532 nm) on human adipose-derived mesenchymal stem cells and RNA sequence analysis (RNA-seq) was used to evaluate the transcriptional expression level of interleukins.
Results: Sequencing data in both green and IR groups showed more than 250 genes whose expression was significantly different compared to the control group. Among them, the expression of IL1a, IL6, IL8, and IL11 decreased significantly, while the expression of other interleukins did not show significant upregulation or downregulation compared to the control group.
Conclusion: Our results suggest that using ADSCs with green and IR PBM may be a therapeutic method to reduce inflammation and decrease cancer progression.
Keywords: Mesenchymal Stem Cell, Low Level Laser Therapy, Inflammation, Interleukins

Ps-55: Proportions and Functional of Regulatory T Cells in MRL/lpr Mice Versus Normal Mice

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Objective: Naturally occurring CD4+, CD25+ Treg cells are central in the maintenance of peripheral tolerance. Impaired activity and/or a lower frequency of these cells are involved in the emergence of autoimmunity. This study aimed to analyze relative proportions and functional alterations of Treg cells in MRL/lpr mice.
Materials and Methods: The frequency of CD4+, CD25+ T cells in the peripheral blood of healthy and autoimmune mice was compared by flow cytometry. The capacity of CD4+, CD25+ T cells to inhibit the proliferation and cytokine secretion of CD4+, CD25- T cells was assessed after polyclonal activation.
Results: MRL/lpr mice exhibited a normal percentage of CD4+, CD25 high T cells, and forkhead box P3 messenger RNA and protein expression in Treg cells was not altered. However, MRL/lpr Treg cells displayed a reduced capacity to suppress proliferation and to inhibit interferon-gamma secretion by syngeneic effector CD4+, CD25- T cells, as compared with syngeneic cocultures of CBA/J T cells. Moreover, effector MRL/lpr CD4+, CD25- T cells were substantially less susceptible to suppression even when cultured with CBA/J or MRL/lpr Treg cells. Crossover experiments led us to conclude that in MRL/lpr mice, each partner engaged in T cell regulation displays altered functions. Molecules involved in suppressive mechanisms (CTLA-4 and CD80/CD86) are underexpressed, and antigen-presenting cells (APCs) produce raised levels of interleukin-6, which is known to abrogate suppression.
Conclusion: The results suggested that although the frequency and phenotype of Treg cells in MRL/lpr mice are similar to those in normal mice, Treg cells in MRL/lpr mice are not properly stimulated by APCs and are unable to suppress proinflammatory cytokine secretion from effector T cells.
Keywords: Treg Cells, Antigen-Presenting Cells, Effector T Cells, MRL/lpr Mice

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