Abstracts of

Royan International Twin Congress

6th Congress on Stem Cell Biology & Technology

15-17 September 2010

ROYAN INSTITUTE

Tehran, Islamic Republic of Iran

Guide for Authors

Aims and Scope: The "***Yakhteh Medical Journal (The Cell)***" is a publication of Cellular Sciences Research Centre, the Royan Institute. It is published both in Persian and English. The aim of the journal is to disseminate information through publishing the most recent scientific research studies on exclusively cellular, molecular and other related topics. Yakhteh Medical Journal (The Cell) has been certified as a quarterly publication by Ministry of Culture and Islamic Guidance in 1999 and was accredited as a scientific and research journal by HBI (Health and Biomedical Information) Journal Accreditation Commission in 2000.
This journal accepts (Original articles, Review articles, Case reports, Short communications, Editorial, Images in Biology papers and Letter to editors) in the field of cellular and molecular.

1. Types of articles

The articles published in *Yakhteh Medical Journal (The Cell)* fall into the following columns:
A. Original articles are scientific reports of the original research studies. The article consists of Abstract (English & Persian), Introduction, Materials and Methods, Results, Discussion, Conclusion, acknowledgements and References.
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Page 2**:** Running title; a maximum of 5 words (40 spaces and letters).
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Introduction

This should summarize the purpose and the rationale of the study. It should neither review the subject extensively nor should it have data or conclusions of the study.

Materials and Methods

This should include the exact method or observation or experiment. If an apparatus is used, its manufacturer's name and address should be given in parenthesis. If the method is established, give reference but if the method is new, give enough information so that another author is able to perform it. If a drug is used, its generic name, dose and route of administration must be given. For patients, age, sex with mean age ± standard deviation must be given. Statistical method must be mentioned and specify any general computer program used. The Info system used should be clearly mentioned.
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Discussion

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* Manicardi GC, Bianchi PG, Pantano S, Azzoni P, Bizzaro D, Bianchi U, et al. Presence of endogenous nicks in DNA of ejaculated human spermatozoa and its relationship to chromomycin A3 accessibility. Biol Reprod .1995; 52: 864-867.

Book:

* Name(s) of author(s).Book title. Edition. Publication place: publisher name; publication date (year); Page number.

* Edelman CL, Mandle CL. Health promotion throughout the life span. ST Louis: Mosby; 1998; 145-63.

* Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995; 465-78.

* Sigman M, Lipshultz LI, Howards SS. Evaluation of subfertile male. In infertility in the male. Lipshultz LI, Howards SS (eds).Philadelphia: Mosby Year Book; 1991; 179-210.

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Eftekhati Yazdi P, Comparison of Fragment Removal and Co-Culture with Vero Cell Monolayers on Development of Human Fragmented Embryos. Presented for the Ph.D., Tehran. Tarbiyat Modarres University. 2004.

Abstract book:

* Amini rad O. The Antioxidantal Effect of Pomegranate Juice on Sperm Parameters and Fertility Potential in Mice.Yakhteh.2008;10 Suppl 1:38.

Conferences:

* Harnden P,Joffe JK, Jones WG,editors.Germ cell tumors V.Proceedings of the 5th Germ cell tumors conference ;2001 Sep 13-15;Leeds,UK. New York:Springer;2002.

Internet References

Articles:

* Jahanshahi A, Mirnajafi-Zadeh J, Javan M, Mohammad-Zadeh M, Rohani M.Effect of low-frequency stimulation on adenosineA1 and A2A receptors gene expression in dentate gyrusof perforant path kindled rats. Yakhteh. 2008; 10(2): 87-92. Available from: http://www.yakhteh.org. (20 oct 2008).

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* Anderson SC, Poulsen KB. Anderson's electronic atlas of hematology. [CD-ROM]. Philadelphia: Lippincott Williams & Wilkins; 2002.

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9. Disclosure regarding source of funding and conflict of interest if any besides approval of the study from respective Ethics Committee/ Institution Review Board.

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IN THE NAME OF GOD

Gone But not Forgotten

In the memory of the late Director of Royan Institute, Founder of Stem Cells Research in Iran and Chairman Manager of Yakhteh Medical Journal. May he rest in peace.

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Abstracts of the 6th Royan International Congress on Stem Cell Biology & Technology

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Congress Chairman

Nasser Aghdami

Dear Friends and Colleagues;
On behalf of the organizing committee of Royan International Twin Congress, 11th Congress on Reproductive Biomedicine and 6th Congress on Stem Cell Biology & Technology, I would like to invite you to join us and attend this prominent event which will be held on September 15-17, 2010 in Tehran, Iran.
RITC-2010 will be held in conjunction with closing ceremony of the 11th Royan International Research Award, which contains five prizes for five best research projects in the field of Reproductive Biomedicine and Stem Cell Biology and Technology.
For the first time in 1999, when we had achieved the success of 1000 ART baby births, we decided to share this great experience with scientists and doctors from all around the world, and we held the first Royan International Research Award and Congress on Reproductive Biomedicine.
By September 2010, the time of 11th Royan International Congress, we will be reaching to the experience of more than 10,000 baby births resulting from the efforts of ART professionals and ART lab experts of the Royan Institute, and there would be too much to exchange with you throughout the scientific sessions of the congress.
Regenerative medicine and stem cells are of new hot scientific subjects which provide so many promising options for the future treatment of some major diseases such as cancers, nervous diseases, damaged organs and degenerative diseases.
In recent years the field of stem cell has witnessed enormous expansion in clinical and basic data. However the basic research is of vital interest to everybody working in this field and brings together scientists from all segments of basic and biomedical research and from various clinical disciplines, even if clinical applications to be developed finally require highly specialized knowledge and techniques.
Since 2006, we have been holding the Stem Cell Biology and Technology congress alongside the Reproductive Biomedicine part as a twin congress.
The scientific program of this twin congress will focus on breaking scientific developments and current issues from foremost opinion leaders. Each keynote speaker has been asked to not only review his/her particular area of expertise but also look ahead at possibilities for the next years.
The executive committee has also arranged social programs and pre/post congress tours to some historical cities of Iran like Isfahan, Shiraz, Kashan… for foreign guests to visit the cultural heritage and enormous tourism resources of our ancient country, Iran.
Hereby, I present my thanks to the participants of this conference, professors, young scientists and students! It is due to your keen interest that these fields are expanding and exploring new frontiers. We sincerely hope that each and every one of you has a wonderful time in Iran.
The organizers of Royan International Twin Congress are eagerly waiting to welcome you to this exciting extravaganza of knowledge and pleasure.

Best regards,
Nasser Aghdami M.D/PhD
Congress Chairman

Stem Cells

Is-1: ES Cell Lines after Double Nuclear Transfer into Mouse Oocytes

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Objective: In contrast to diploidy, tetraploidy is incompatible with full development in mammals; yet, tetraploid cells arise after normal diploid development and contribute to certain organs of the adult body. In this project we addressed the following question: Can a single oocyte confer pluripotency on two somatic nuclei and subsequently serve as a source of tetraploid pluripotent cell lines capable of participating in normal development and organ physiology?
Materials and Methods: We modified the classical nuclear cloning approach by simultaneously injecting two somatic nuclei (from cumulus cells) instead of one, into a single mouse ooplast of the B6C3F1 strain. Resultant tetraploid constructs were activated, cultured to blastocyst and derived into ES cells (ESCs) using the protocols routinely applied in our laboratory. The nature of these cells was assayed by *in vitro* differentiation, developmental chimerism and teratoma formation.
Results: Following activation of mouse ooplasts transplanted with two nuclei, blastocysts formed at comparable rates and with overlapping metabolic and gene expression profiles compared to diploid embryos. Tetraploid cloned embryos supported the derivation of tetraploid ESCs (tNT-ESCs), which maintained a stable karyotype (now passage 30+). tNT-ESCs populated the inner cell mass upon injection into normal blastocysts. Chimerism persisted after implantation of these blastocysts in utero; however, it was detected only in extraembryonic tissues. tNT-ESCs have the ability to form embryoid bodies and to differentiate into derivatives of the germ layers while maintaining their tetraploid nature. Interestingly, we did not observe teratoma formation upon transplantation of 1.106 early-passage tNT-ESCs subcutanously or into testis capsules of immunodeficient hosts (n=18). Notably, we obtain similar results from the ongoing characterization of tetraploid ESCs derived from fertilized embryos.
Conclusion: We showed that a single oocyte can reprogram more than one nucleus and that tNT-ESCs derived from developmentally incompetent tetraploid embryos have all desired pluripotency characteristics without being teratogenic, which is a long searched trait for the potential application of stem cells in tissue regeneration therapies.

Is-2: Functional Analyses of Gene-Corrected Liver Disease-Specific iPS Cells

Cantz T

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Objective: Direct reprogramming of somatic cells into induced pluripotent stem (iPS) cells by retrovirus-mediated expression of pluripotency-associated genes in mice and humans is a promising approach for the derivation of disease-specific iPS cells. In the clinical setting, patient-specific iPS cells must undergo repair prior to autologous cell transplantation if the underlying condition was caused by a genetic disorder. In our study, we focused on a mouse model of tyrosinemia type 1 (fumarylacetoacetate hydrolase deficiency; FAH-/- mice) as a paradigm for a genetic metabolic liver disorder that can be treated by an iPS cell-based appraoch, and analyzed the phenotype of disease specific– and gene-corrected iPS cells *in vitro* and *in vivo*.
Materials and Methods: Fetal fibroblasts (13.5 dpc.) were used for retroviral expression of Oct4, Sox2, Klf4, and c-Myc and embryonic stem cell–like colonies emerging 3 weeks after transduction were subcloned based on morphological selection and maintained as individual iPS cell lines. RT-PCR and immunofluorescence analyses were applied to investigate the expression of pluripotency markers (Oct4, Nanog, and Sox2) in iPS cells, and teratoma formation was performed to deomstrate the pluripotency of the iPS cell lines. Furthermore, we performed tetraploid embryo aggregation experiments, to evaluate the full pluripotency of the generated FAH-iPS cells,
Results: We were able to obtain viable mice, from FAH-/--iPS cells, which mimic the diseased phenotype of "normal" FAH-/--mice. In another experiment, we rescued the diseased phenotype by lentiviral transduction of an FAH transgene, which resulted in constitutive expression of FAH in the gene-corrected cells (FAHgc-iPSC). We then applied in-vitro differentiation protocols and embryo aggregation experiments to these FAHgc-iPS cells and analyzed their capability to form functional hepatic cells. Interestingly, we obtained healthy mice from FAHgc-iPS cells, which show FAH expression in the liver and did not exhibit any pathological abnormality.
Conclusion: We demonstrate that metabolic liver disease-specific iPS cells can be gene-corrected without loss of the pluripotent phenotype, and we provide strong evidence that these gene-corrected iPS cells do not acquire functional restrictions. Hence, derivatives of gene-corrected iPS cells may be obtained and applied toward cell-based therapeutic applications.

Is-3: Pluripotent Stem Cells as Source for Hepatic Cell Transplants

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Directed endodermal differentiation of various sources of pluripotent stem cells gives rise to a subset of cells with a hepatic phenotype. Such stem cell-derived hepatic progenitor cells (HPC) can acquire features of hepatocytes *in vitro*, but fail to form substantial hepatocyte clusters *in vivo*. In our recent work, we compared different sources of pluripotent stem cels and investigated whether the limited functional engraftment of stem cell-derived hepatic cells is due to inefficient engraftment or an immature phenotype of ES-HPC.
Cells arising from germ cells can be derived as alternative sources for pluripotent stem cells. In males, testis-derived pluripotent germline stem cells (gPS) can be derived from spermatogonial stem cells, whereas in females, parthenogenetic activation of oocytes can give rise to blastocysts, whose inner cell mass can be isolated and propagated as parthenogenetic embryonic stem cells (pES). Both germ-line-derived pluripotent cell lines (pES and gPS) can give rise to hepatic progenitor cells (HPC) using an embryoid body formation-based protocol. However, in our hands the generation of these EB-HPC was not efficient enough to generate considerable numbers of hepatic cells with an advanced hepatic phenotype and which were suitable for further transplantation experiments. Therefore, we evaluated a cytokine-based (Activin A, BMBP4, bFGF) differentiation protocol on adherent monolayers of ES and gPS to increase the efficiency of definitive endoderm formation with respect to higher numbers of cells gaining a hepatic phenotype [Loya et al., 2009]. However, further refinements of the differentiation protocols need to be evaluated to obtain more mature cell phenotypes and to get expandable hepatic precursor cells from these resources, which would be more suitable for transplantation experiments.
To elucidate varying repopulation efficiencies of given stem cell-derivatives we applied a competitive transplantation model for liver repopulation, which was established in the lab of our collaborator Michael Ott at Hannover Medical School. Heterozygous Alb-uPA transgenic mice express the uroplasminogen activator (uPA) under transcriptional control of the albumin promoter (Alb) in liver cells only from one allele. Spontaneous deletion or silencing of this transgene allows endogenous repopulation at low, but constant, frequencies. Transplanted cells need to compete with endogenous repopulating cells and, therefore, repopulation efficiencies closely reflect the repopulations capabilities of the transplanted cell population. Our results clearly demonstrate that adult hepatocytes of human and mouse origin generate liver tissue more efficiently than cells derived from fetal tissue or embryonic stem cells in the immunodeficient Alb-uPA transgenic mouse model [Haridass et al., 2009], which is in contrast to previous concepts hypothesizing that stem cell derived transplants exhibit an intrinsic proliferation potential.
Using another transplantation model, the FAH-/-- mice (see above) we were able to demonstrate that murine ES-derived hepatic cells engrafted throughout the liver at very low frequencies, without evidence for large hepatocyte cluster formation [Sharma et al., 2008]. Nevertheless, immunostaining for the FAH protein and quantitative RT-PCR indicated functional capabilities of the ES-derived hepatic cells, similar to fetal hepatoblasts of day 11.5 dpc.

Is-4: A Chemical Approach to Controlling Cell Fate

Ding Sh

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Recent advances in stem cell biology may make possible new approaches for the treatment of a number of diseases. A better understanding of molecular mechanisms that control stem cell fate as well as an improved ability to manipulate them are required. Toward these goals, we have developed and implemented high throughput cell-based phenotypic screens of arrayed chemical and gene libraries to identify and further characterize small molecules and genes that can control stem cell fate in various systems. This talk will provide latest examples of discovery efforts in my lab that have advanced our ability and understanding toward controlling stem cell fate, including self-renewal, survival, differentiation and reprogramming of pluripotent stem cells.

Is-5: Small Molecules Regulating Pluripotency and Reprogramming

Ding Sh

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The induced pluripotent stem cell (iPSC) technology, i.e. reprogramming somatic cells into pluripotent cells that closely resemble embryonic stem cells (ESCs) by introduction of defined factors, holds great potentials in biomedical research and regenerative medicin. Various strategies have been developed to generate iPSCs with less or no exogenous genetic manipulations, which represent a major hurdle for iPSC's applications. Toward an ultimate goal of generating iPSCs with a defined small molecule cocktail that would offer significant advantages over genetic manipulations or more difficult-to-manufacture/use biologics, substantial efforts and progresses have been made in identifying chemical compounds that can functionally replace exogenous reprogramming transcription factors (TFs) and/or enhance reprogramming efficiency and

kinetics. This presentation will focus on recent progress on identifications of small molecules that can regulate and/or induce pluripotency, as well as their underlying mechanisms.

Is-6: Stem Cells and Immunomodulation

Ferrara JLM

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Is-7: Stem Cell Transplantation: A Curative Treatment for Hematologic Malignancies

Ferrara JLM

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Is-8: Cell Therapy for Liver Disease

Forbes S

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Orthotopic liver transplantation is the accepted treatment for liver failure. Conditions currently requiring liver transplantation include inherited metabolic disorders resulting in functionally deficient hepatocytes, fulminant organ failure caused by acute insult to the previously healthy liver and cirrhosis due to chronic injury that ultimately leads to fibrosis and parenchymal insufficiency. Unfortunately, the applicability of liver transplantation is limited by donor organ availability. The mismatch between organ supply and demand requires the development of alternative strategies, such as regenerative therapies, to treat liver disease. Broadly, this could occur in two distinct ways: (1) Generation of hepatocyte-like cells for liver support or (2) cell therapy to remodel liver cirrhosis. End stage liver cirrhosis is by far the most frequent indication for transplantation. The characteristic disturbed vasculature, excessive fibrosis and reduced number of compromised hepatocytes is not an environment conducive to hepatocyte transplantation and clinical studies attempting to provide parenchymal support in this manner have been generally unsuccessful. This suggests that for the treatment of live cirrhosis, transplantation of stem cell derived hepatocyte like cells may also not be successful. Remodelling the cirrhotic liver to degrade excess scar tissue and promote endogenous regeneration may be a more realistic approach. The resolution of advanced liver scarring on cessation of injury has been well characterised in experimental models. Partial but significant cirrhosis remodelling has also been observed in patients following successful treatment of autoimmune liver disease and viral hepatitis.

Bone marrow (BM) derived cells influence hepatic scarring, scar resolution and liver regeneration. We have found that BM mesenchymal stem cells are a source of scar forming myofibroblasts in chronic liver injury and in patients with recurrent liver injury following liver transplantation. Furthermore BM-derived macrophages populate hepatic scars and are critical for co-ordinating the livers wound healing response. A technique that has been utilised in animal models and small clinical studies is the application of autologous BM stem cells. Autologous BM cell therapy for liver disease has shown promise in preliminary clinical studies however these studies have been non-randomised and the mechanisms of action are unclear. Further mechanistic studies are required as are randomised clinical trials to progress "regenerative medicine" for liver disease.

We have been developing cell therapy strategies to enhance liver regeneration. We have found that in rodent models that intra-portally infused BM derived naïve, unpolarised macrophages cultured in a low adherence environment promote liver regeneration and aid scar resolution. BM-derived macrophage (BMM) delivery resulted in early chemokine upregulation with hepatic recruitment of endogenous macrophages and neutrophils. These cells delivered matrix metalloproteinases-13 and -9 respectively, into the hepatic scar. The effector cell infiltrate was accompanied by increased levels of the anti-inflammatory cytokine IL-10. A reduction in hepatic myofibroblasts was followed by reduced fibrosis and elevation of serum albumin. Liver regeneration occurred through expansion of endogenous Liver Progenitor Cells. We hope that the use of a single cell type that promotes scar degradation and regeneration will aid the clinical development of cell therapy for liver cirrhosis.

Is-9: Stem Cells in Liver Regeneration

Forbes S

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The adult liver can regenerate from activation and division of normally quiescent hepatocytes. However during chronic damage this normal regenerative capacity is over whelmed and the liver progenitor (LPC) compartment becomes activated. LPCs are capable of regenerating both hepatocytes and biliary epithelium. The extent to which LPCs can fully regenerate the adult liver has not been well studies. To investigate

this specific and complete inhibition of hepatocyte proliferation is required. Mdm2 is a key inhibitor of p53, the tumour suppressor gene. p53 activation results in cell cycle arrest. Hepatocyte specific Mdm2 knockout and resulting p53 activation using Cre Lox technology therefore allows the examination of LPC mediated liver regeneration. We have used a novel model of LPCs activation in the $AhCre^{+}$ $Mdm2^{fl/-}$ mouse which demonstrates total parenchymal hepatocyte replacement from a non-differentiated LPC source. In this model marked p53 induced hepatocellular damage occurs with massive expansion of $p53^{low}$ LPCs. Isolated LPCs can be differentiated into hepatocytes *in vitro*. *In vivo*, expansion of $p53^{low}$ LPCs results in the production of phenotypically normal, Mdm2 competent, $p53^{low}$ hepatocytes. No cancers or significant fibrosis are observed following complete replacement of the hepatic parenchyma during long term follow up. We confirmed the requirement of LPCs for parenchymal restoration by crossing $Mdm2^{fl/fl}$ mice with $Fn14^{-/-}$ mice (the Tweak receptor on LPCs which controls their expansion). This resulted in loss of LPC activation, liver failure and death.

The mechanisms by which these lineages are delineated from LPCs is currently unknown. The role of Notch and Wnt signalling has been well described during hepatic ontogeny, however the role these signalling pathways play during regeneration of the adult organ from LPCs remains unclear. We have found that the cellular components of the niche act in a co-ordinated way to direct LPC proliferation and differentiation. During regeneration of the adult liver, myofibroblasts derived Notch signalling is required for biliary specification and LPC expansion. During hepatocyte regeneration macrophages secrete Wnt ligand which results in LPC NUMB upregulation and sequestration of Notch. Wnt signalling in macrophages is upregulated by the ingestion of hepatocyte debris suggesting a novel feedback role for macrophages in tissue repair.

Another feature of the LPC is laminin extracellular matrix. We have found that laminin matrix promotes expansion of undifferentiated LPCs *in vitro*. By utilising a murine model with a mutated collagen 1 resistant to metalloproteinase degradation we have found that extracellular remodelling and laminin formation is a requirement for LPC expansion. All of the signalling mechanisms and patterns of cellular regeneration we have studied in murine models have been correlated with the study of diseased human liver tissue. This data suggests that strategies that are aimed at promoting liver regeneration in chronic liver injury should also include the degradation of collagen matrix. By defining the signals that control LPC expansion and differentiation, there is the potential to modulate their behaviour *in situ* using pharmaceutical or other means.

Is-10: Endogenous Stem/Progenitor Cells in the Adult Pancreas

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Is-11: Stem Cell Therapy to Cure Diabetes in Mice

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Is-12: Stem Cells and the Molecular Control of Pluripotency

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The recent demonstration of *in vitro* reprogramming using transduction of 4 transcription factors by Yamanaka and colleagues represents a major advance in the field. However, major questions regarding the mechanism of *in vitro* reprogramming need to be understood and will be one focus of the talk.

Direct reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) can be achieved by over-expression of Oct4, Sox2, Klf4 and c-Myc transcription factors, but only a minority of donor somatic cells can be reprogrammed to pluripotency. We have demonstrated that reprogramming is a continuous stochastic process where almost all donor cells eventually give rise to iPSCs upon continued growth and transcription factor expression. Inhibition of the p53/p21 pathway or over expression of Lin28 increased the cell division rate and resulted in an accelerated kinetics of iPSC formation that was directly proportional to the increase in cell proliferation. These results suggest that the number of cell divisions is a key parameter driving epigenetic reprogramming to pluripotency. In contrast, Nanog over expression accelerated reprogramming in a predominantly cell division rate independent manner.

A major impediment in realizing the potential of ES and iPS cells to study human diseases is the inefficiency of gene targeting. Using Zn finger mediated genome editing we have established efficient protocols to target expressed and silent genes in human ES and iPS cells. Finally, our progress in using iPS cells for therapy and for the study of complex human diseases will be summarized.

Is-13: Stem Cells, Reprogramming and Personalized Medicine: *Promise, Problems, Reality*

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The recent demonstration of *in vitro* reprogramming using transduction of 4 transcription factors by Yamanaka has highlighted the concept that the state of differentiation is not fixed but can be changed experimentally. We are interested in studying the mechanism of direct reprogramming and to explore its potential to study human diseases in the Petri dish. Another goal of my laboratory is to define the state of pluripotency, how it is established and how its stability can be affected. For example, human ES or iPS cells are unlike their mouse counterparts and it has been realized that major differences in growth characteristics and in differentiation potential exist between different lines. To realize the medical potential of the ES and iPS cell technology we need to molecularly define the pluripotent state and what makes it distinct from the cell state of differentiated cells. The talk will focus on (i) molecular mechanism of direct reprogramming, on (ii) the analysis of newly isolated human ES cells (ESCs), which are epigenetically distinct from conventional hESCs and on (iii) novel approaches for the genetic manipulation of human ES and iPS cells. I will also discuss the current limitations of using iPS cells for studying human diseases and for their eventual use in transplantation therapy.

Is-14: Patient-Derived Stem Cell Models of Brain Diseases: New Tools for Drug Discovery

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There is a pressing need for patient-derived cell models of brain diseases that are relevant and robust enough to produce the large quantities of cells required for molecular and functional analyses, including drug screening. We have developed a new cell model based on patient-derived cells from the human olfactory mucosa, the organ of smell, which regenerates throughout life from a tissue-resident stem cell. Biopsies from olfactory mucosa were dissociated and grown as neurospheres in defined medium. Neurosphere-derived cell lines were grown in serum-containing medium as adherent monolayers and stored frozen for subsequent analysis. We now have an Adult Stem Cell Bank comprising 200 stem cell lines from healthy controls and from patients with neurological conditions including Parkinson's disease, schizophrenia, motor neuron disease, mitochondrial mutation disorders, hereditary spastic paraplegia and others. This list of diseases is growing as we develop strategic collaborations with neurologists and scientists investigating other nervous system diseases. The complete resource also includes the associated patient data (clinical and demographic) and cell biological data (genotype, gene expression, cell function).

In our first experiments we compared 42 stem cell lines obtained from healthy controls and patients with either schizophrenia, a neurodevelopmental disorder, or Parkinson's disease, a neurodegenerative disease. From each cell line we analyzed gene expression, protein expression and various metabolic cell functions. The aim was to identify genes, proteins, signaling pathways and cell functions that are altered in all the disease-derived cells compared to all healthy control cells. Expression profiling identified 1700 genes significantly altered in the schizophrenia stem cells and 514 altered in Parkinson's disease stem cells. Pathway analysis identified significantly dysregulated neurodevelopmental pathways in schizophrenia and significantly dysregulated mitochondrial function, oxidative stress and xenobiotic metabolism in Parkinson's disease. Fibroblasts from schizophrenia patients did not show these differences. Cell function assays confirmed these disease-specific differences: Caspase 3/7 activity was altered in schizophrenia whereas MTS metabolism and glutathione content were reduced in Parkinson's disease.

In subsequent experiments we discovered increased cell proliferation in schizophrenia due to dysregulation of the G0/S cell cycle transition. We also discovered decreased cell adhesion in schizophrenia with a doubled rate of cell migration due to disruptions in focal adhesion kinase signaling. In Parkinson's disease stem cells we discovered significant disruptions in NRF2 xenobiotic signaling. Upon stimulating this pathway we ameliorated disease-specific reductions in mitochondrial function and glutathione in the Parkinson's disease cells. These results confirm heuristic hypotheses for the aetiology of schizophrenia and Parkinson's disease and identify specific molecules and signaling pathways that may underlie these diseases. Significantly, we observed these disease-specific differences in diseases for which there are no obvious gene mutations. Schizophrenia is highly heritable but predicted to be caused by many genes of small effect. Sporadic Parkinson's disease is not heritable. Our results suggest that patients share a common complex "genetic background" of disrupted signaling pathways with functional consequences.

Patient-derived olfactory stem cells have many advantages over embryonic stem cells and induced pluripotent stem cells as brain disease models. They do not require genetic re-programming and they can be obtained from adults with complex genetic diseases. Our research demonstrates significant disease-specific differences in gene expression and cell function that provide novel insights into disease aetiologies as well as novel drug targets.

Is-15: Repair of Spinal Cord Injury with Olfactory Ensheathing Cells

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Traumatic spinal cord injury not only impairs or prevents movement but also leads to losses of sexual, bowel and bladder functions. Reduced movement leads to pressure sores and loss of lung function. Catheterization leads to increased risk of infection. Loss of autonomic controls can lead to bouts of dysreflexia when normally mild sensory stimuli, like a full bowel, cause an uncontrolled and life threatening increase in blood pressure. Traumatic spinal cord injury is a condition of the whole body, physical and mental.

Olfactory ensheathing cells are a specialized glial cell that surrounds the olfactory sensory axons coursing from the nose to the brain. Due to the neurogenesis occurring continually in the olfactory epithelium, newly born olfactory sensory neurons grow axons from the nose to the brain throughout adult life, assisted by the olfactory ensheathing cells. The cells exist in the periphery (in the olfactory mucosa) and in the brain (in the outer layer of the olfactory bulb). These properties have made olfactory ensheathing cells of intense interest for the transplantation repair of the spinal cord. In animal experiments olfactory ensheathing cells are obtained from the olfactory bulb and olfactory mucosa. Both sources of cells demonstrate positive outcomes when transplanted into the spinal cord injured by contusion, transection, electrolytic lesion or demyelinating lesion. Positive findings have included improved locomotion, improved skilled reaching, improved breathing, and reduced autonomic dysflexia. Anatomically, olfactory ensheathing cell transplants have resulted in growth of central axons across the injury site, reduction in gliosis and lesion cavity, and electrophysiological evidence for reconnection across the injury site. Interestingly, there is variability in these outcomes suggesting that unknown variables need to be optimized for consistent outcomes between laboratories. A major variable is in the culture of olfactory ensheathing cells and the location from which they are derived. Olfactory ensheathing cells from nose and brain differ in their behaviours *in vitro* and *in vivo*, and after transplantation into the spinal cord.

We have shown that olfactory ensheathing cells from the olfactory mucosa in the nose were therapeutic when transplanted into the injured spinal cord in rat models of spinal cord injury. When transplanted into a thoracic full transection injury they improved lower limb movement, stimulated growth of brainstem motor axons across the injury site and retrieved inhibition of lower motor neuron reflexes. In high lesions transplanted olfactory ensheathing cells improved autonomic dysreflexia after bowel distension. Human cells transplanted into thoracic contusion injury reduced lesion size and improved locomotion.

We recently completed a Phase I clinical trial of autologous transplantation of olfactory ensheathing cells into human paraplegics with complete, thoracic spinal cord injuries. This was a single-blinded controlled trial with 3 transplants and 3 non-surgical controls who were followed by the same blinded assessors for 3 years. This trial showed no adverse outcomes on MRI, no neuropathic pain, no respiratory changes, no altered hand functions or other functional changes. In one transplant there was an increase in light touch and pinprick sensation extending below the level of lesion suggesting a potential small improvement due to the procedure.

Spinal cord injuries pose special problems for translating promising pre-clinical studies into human clinical trials. Many aspects of human injury cannot be modeled in animals and there are important risks in human treatments that may mitigate the timing of an invasive therapy. Olfactory ensheathing cells present a potential autologous source for future human clinical trials if the risks associated with the timing and location of transplantation are appropriately balanced with the potential outcome in individual patients.

Is-16: Bone Marrow Cell Therapy for Treatment of Hemophilia A in Mice

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Objective: Bone marrow (BM) cells can directly differentiate into hepatocytes due to cellular plasticity. Regeneration of nonhematopoietic tissue by BM cells has been proposed as a promising novel class of therapies for the treatment of many diseases and injuries in mammals. Hemophilia A (HA) is an X-chromosome-linked recessive bleeding disorder. Since liver is the primary site of FVIII synthesis, we hypothesized that the partial replacement of mutated liver cells by healthy cells in HA mice could manage the severity of the bleeding disorder.

Materials and Methods: The phenotype of BM cells that can migrate to the damaged liver was characterized by *in vitro* migration assay. The migrated cells were analyzed by flowcytometry (FC) and immunocytochemistry (IC). To know the potential for hepatic differentiation, these cells were cultured on extracellular matrix protein-coated plate containing 10% damaged liver serum supplemented medium. The extent of differentiation of BM cells into hepatocytes was determined by analyzing hepatic genes (HNF-4a, albumin, TDO, TAT) and proteins (albumin and CK-

18) expression, and also by functional assay (CYP450 activity). Therapeutic potential of BM-derived cells was tested in HA mice model. The liver of male HA mice was perturbed with acetaminophen to facilitate the engraftment and differentiation of lineage-depleted (Lin-) enhanced green fluorescent protein (eGFP)-expressing female BMCs. The fusion, if any, between donor and recipient cells was identified by quantitative polymerase chain reaction (PCR) and fluorescent in situ hybridization (FISH) assays. The donor-derived hepatocytes expressing FVIII was determined by immunohistochemistry (IHC). This was confirmed by reverse transcriptase (RT)-PCR analysis of the light chain FVIII gene. The phenotypic correction in recipient HA mice was assessed by ELISA, Chromogenix and activated partial thromboplastin time (aPTT) based assays of plasma FVIII levels, and tail-clip challenge.
Results: *In vitro* studies showed that the damaged liver tissue is capable of inducing the migration of a distinct population of BM cells, phenotypically characterized as Lin-CXCR4+OSMRb+. The competent cells of the BM can differentiate into albumin and cytoketarin-18 expressing hepatocytes, which were functionally active as resorufin was produced from 5-pentoxyresorufin. In the recipient HA mice, BM-derived cells expressed the markers of both hepatocytes (albumin and cytokeratin-18) and endothelial cells (von Willbrand factor). The results of quantitative PCR and FISH followed by IC suggested that hepatocyte obtained from BM-derived cells was due to direct differentiation and not due to cell fusion. The recipient HA mice expressed FVIII light chain gene and protein. The coagulation assay confirmed that the plasma FVIII activity is maintained at 20.4 ± 3.6% of normal pooled plasma activity for more than a year without forming its inhibitor. Furthermore, 80% of the test mice survived in a tail-clip challenge during the study.
Conclusion: This report demonstrated that BM cells rescued the bleeding phenotype in HA mice, suggesting a potential therapy for this and other related disorders.
Keyword: Bone Marrow Cells, Hepatocytes, Hemophilia A, Transdifferentiation, Factor VIII

Is-17: Trans-Differentiation and Hepatogenesis

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Hepatogenesis means the formation of liver tissue. The development of mouse liver is induced by cardiac mesoderm at 7-8 somite stage when the hepatic diverticulum emerges from foregut endoderm. Following this induction, hepatic progenitors emerge from the foregut endodermal tissue, where they mature. Once the liver bud emerges from developing gut, hematopoietic cells migrate (E9-E10) there from aorta-gonad-mesonephros (AGM), proliferate and apparently produce liver morphogenic and growth factors till the end of the fetal liver hematopoiesis at about E16. Thus, a close relationship between endodermal and mesodermal tissue components in the fetal liver is expected. Experiments using explants and primary culture of hepatic precursors (hepatoblasts), isolated from the liver at later stages of development, support biopotential differentiation ability and their endodermal origin.
For the first time, Lipp show that about 80% of the liver parenchyma originates from mesoderm tissue. The argument in favor of possible involvement of hematopoietic cells in development of liver has become stronger after knowing the phenotypic similarities between hematopoietic stem cells (HSCs) and the liver stem cells, also known as oval cells. Moreover, recent reports showed that bone marrow-derived cells can directly differentiate into hepatocytes in mice liver injury model. Human HSCs are also shown to differentiate into hepatocytes in pre-immune fetal sheep by *in utero* transplantation. In recent past, we have shown that a sub-population of E10.5 fetal liver hematopoietic cells serves as progenitor of hepatocytes. This result probably suggests developmental plasticity in hematopoietic cells at fetal stage.

Is-18: Transposon-Mediated Reprogramming Allows Generation of Transgene-Free Induced Pluripotent Stem Cells

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Objective: Reprogramming of somatic cells with defined set of transcription factors to a pluripotent, embryonic stem cell equivalent state holds an enormous promise for future cell-based curative therapies of degenerative diseases. The original method for producing induced pluripotent stem cell (iPS cells) required viral transduction-mediated transgenic random integration into the cells, which poses a high risk of destroying genes essential for a healthy function of the human body. Furthermore, the reprogramming transcription factors are not "friendly" genes; the expression of most of them in the adult is associated with cancer. Their accidental reactivation in therapeutic cells could cause disease. Therefore, there is an urgent need for finding novel ways of generating iPS cells which do not leave transgenes and genetic changes in the genome.
Materials and Methods: We utilized the *piggyBac* transposon system to deliver the reprogramming transcription factors (N-myc, Klf4, Oct4 and Sox2) to embryonic and adult mouse and human fibroblast. We also combined this novel transgene delivery method with the tetracycline inducible system, which allowed

efficient temporal control of the reprogramming transgene expression. After reaching the bona fide stem cell state, the transcription factors could be turned off by simple withdrawal of the inducer doxycycline.
Results: The efficiency of generating iPS cell lines with the transposon system was comparable to that of the viral delivery system. We also put together all the four open reading frames defining the reprogramming factors in one polycistronic transgene with the 2A sequence links, which allowed the production of four proteins from a single transgene. This made possible the generation of single copy transgene reprogrammed iPS cell lines. With transient expression of transposase, it was possible to remove the reprogramming transgene for the genome in a seamless manner, where no trace was left behind at the transgene insertion site.
Conclusion: The transposon system offers an efficient generation of single polycistronic transgene reprogrammed iPS cell lines of both human and other species. The ability of seamless removal of the transgene allows for the production of clinically more acceptable cell lines for future regenerative medicine than by using the viral transgene delivery system.

Is-19: Transposon-Mediated Reprogramming as a Powerful Tool to Understand the Biology of Induction of Pluripotency

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Objective: The discovery of a defined set of transcription factors that can induce reprogramming of somatic cells to pluripotent stem cells (iPSc) had a massive impact on our view on future cell transplantation-based regenerative medicine. Somatic cell reprogramming is a several weeks long process through which the cells reach the developmental state similar to embryonic stem cells. This cascade of events and the driving forces behind the phenomenon are very poorly understood. It is however crucial to uncover the fine details of this process in order to comprehend the true property of iPS cells and so better tailor their future therapeutic use.
Materials and Methods: We have recently developed a reprogramming method utilizing a transposon-mediated delivery of the reprogramming transgenes. This system has several advantages over the viral delivery-based alternative. Most notably, it allows for a seamless removal of the transgenes once pluripotent stem cells have been generated and they are no longer needed for stem cell self renewal. We also combined the doxycycline inducible transgene expression system with the transposon delivery-based reprogramming and found that these transgenes are very efficiently regulatable by adding or withdrawing doxycycline. *In vivo* differentiated somatic cells derived from iPSc can be reprogrammed to “secondary” iPSc (2ºiPSc) by simply adding doxycycline to the culture medium. Somatic cell lines produced with this method frequently return to 2ºiPSc in a "population" manner, which allows us to study the cascade of molecular events during the entire process of reprogramming.
Results: We have recently revealed the importance of mesenchymal epithelial transition as a critical event at the early phase of the reprogramming process as well as the elasticity of the significant but early changes in cell properties. We are also investigating the genetic changes may be associated with the massive epigenetic change the cells undergo before they arrive to the pluripotent state.
Conclusion: We expect that utilizing genetic, epigenetic and proteomic profiling of the reprogramming process at a high definition level will give us answers to pivotal questions essential for the future potential and use of iPSc in human medicine.

Is-20: Molecular Signaling and the Transcription Factors of Buffalo Pluripotent Embryonic Stem Cells

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Objective: Present study was designed to investigate the role of transcription factors and signaling malecules in buffalo pluripotent embryonic stem cells.
Materials and Methods: A major advance in understanding the pluripotent state has come with the identification of a network of auto- and cross-regulatory control mediated by three key transcription factors—Oct4, Nanog and Sox2. Each of these factors is required for pluripotency both *in vivo* and *in vitro*. These transcription factors are expressed both in pluripotent ES cells and in the inner cell mass (ICM) of the blastocyst from which ES cells are derived. In our laboratory, the ICM separated from trophoectoderm of hatched buffalo blastocysts were developed to PSC colonies to study the expression pattern of Oct-4, SOX-2 and Nanog genes and immunolocalization of their protein product. Absolute quantification of the molecular markers was done by amplifying the transcripts through quantitative real time polymerase chain reaction (Q-PCR/qPCR).
Results: Our results showed an upregulation of Oct-4, SOX-2 and Nanog in late stage embryos which was maintained in PSCs, hence necessary for maintaining pluripotency. Cross regulation of Oct-4, SOX-2 and Nanog by their gene products confirm a regulatory network in the ICM required for pluripotency. We worked out physiological model of understanding the mitogenic factors during the PSCs development *in vitro* and found that pluripotency requires signaling from the members of fibroblast growth factor and transforming

growth factor families of growth factors. Oct-4 protein is localized both in ICM and trophoectoderm cells, whereas SOX-2 and Nanog proteins are restricted to ICM only.
Conclusion: Role of these functional molecules during embryogenesis and in the embryo derived PSCs shall be discussed. Probably expression of any of these genes solely may not act as reliable marker of pluripotency; rather their cumulative expression may help identify pluripotent buffalo embryonic stem cells.
Keyword: Buffalo, Pluripotent Stem Cells, Transcription Factors, Inner Cells Mass, Immunolocalization.

Is-21: Patient Specific Stem Cells

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Is-22: Stem Cells for Spinal Cord injury

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Is-23: The Role of miRNAs in Mouse ES Cells and Oocytes

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Objective: Maternal to-zygote transition resembles induction of pluripotency in stem cells. The same core pluripotency network found in ES cells is established upon zygotic genome activation. Post-transcriptional regulations play a key role in pluripotency. microRNAs, small RNAs selectively suppressing translation are essential for pluripotency in ES cells. At the same time, a large part of the maternal-to-zygote transition occurs in the absence of transcription, thus it depends on postransctiptiona control of the maternal mRNA pool synthesised during oocyte growth. Our objective is to understand how miRNAs control gene expression in mouse ES cells and early embryos
Materials and Methods: We analyzed phenotypes of ES cells and mouse oocytes depleted of miRNAs. We also used immunofluorescent staining of proteins and fluorescence in situ hybridization (FISH) to analyze dynamics of P-bodies, cytoplasmic foci enriched in mRNA-destabilizing proteins, translational repressors and other RNA binding proteins, and microRNAs (miRNAs)
Results: The same family of miRNAs, named the miR-290 family, becomes expressed during early mouse development and controls pluripotency in ES cells. On the other hand, differented ES cell and mouse oocytes express the Let-7 miRNA family, which is associated with the loss of pluripotency.
We also found that P-bodies disassemble early during oocyte growth and several P-body components, including RNA helicase DDX6 and polyadenylation regulator CPEB, form a novel type of mRNA storage granules in the cortex of fully-grown oocytes. As P-bodies form as a consequence of miRNA pathway activity, we analyzed activity of maternal miRNA. We found that P-body disappearance correlates with reduced ability of let-7 and miR-30c miRNAs to repress translation while they are present and loaded on AGO2 in the oocyte. Furthermore, transcriptome analysis of oocytes lacking miRNA processing enzyme Dicer did not reveal any miRNA specific footprint in the set of differentially expressed transcripts.
Conclusion: Our data suggest that miRNA function is suppressed in mouse oocytes, perhaps in order to support mRNA-stabilizing environment of the oocyte cytoplasm and reprogramming of differentiated oocytes into pluripotent cells of the early embryo. "Pluripotent" miRNAs are replacing the Let-7 family and other miRNAs during maternal-to-zygote transition in a "gear-shift" mechanism where translational repression by maternal miRNAs is suppressed and zygotic miRNAs acquire their ability to repress translation later during preimplantation development. Consistent with this, published data show that the loss of miRNAs has no strong phenotypic effect on oocyte development and early embryo development.

Is-24: Stem Cell Therapies for Autoimmune Diseases

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Since 1996 hematopoietic stem cell transplantation (HSCT) has been used clinically to treat patients with severe and refractory autoimmune diseases (AID) in Europe, North America and also in developing countries. About 1,500 patients have been treated using autologous HSC mobilized from the bone marrow to the peripheral blood with G-CSF +/- cyclophosphamide and about 1/2 to 2/3 patients achieve complete remission of their diseases. Depending on the nature of the disease, there was variable rate of mortality from transplant-related complications or disease progression and also the occurrence of disease relapse. Results of the phase I/II trials of autologous HSCT for multiple sclerosis,

systemic lupus erythematosus, systemic sclerosis, adult and juvenile rheumatoid arthritis will be presented and discussed as well the randomized phase III clinical trials that are running in several centers of the world. Finally, allogeneic HCST have been performed in small numbers of patients (<100) with severe AID, mostly in those who relapsed after autologous HSCT.

Is-25: Stem Cell Therapies for Diabetes Mellitus

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Type 1 and type 2 diabetes mellitus (DM) have been increasingly diagnosed in the last decades throughout the world, producing high morbidity/mortality and low quality of life of affected patients. Type I DM is a organ-specific autoimmune disease which has been treated with immonumodulatory agents in clinical trials, without sustained beneficial effect. We will present our own results using high dose immunosuppression and autologous hematopoietic stem cell transplantation in 25 patients with newly diagnosed DM1. For the first time, diabetes was reversed in most patients and some became long term insulin independent. Umbilical cord blood cells and mesenchymal stem cells have also been used to treat newly diagnosed DM1. Based on the inflammatory component present in DM2, some centers have been employing intraarterial infusion of bone marrow mononuclear cells in patients with DM1, showing beneficial results in the metabolic control of the disease.

Is-26: Direct Conversion of Fibroblasts to Functional Neurons by Defined Factors

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Cellular differentiation and lineage commitment are considered robust and irreversible processes during development. Recent work has shown that mouse and human fibroblasts can be reprogrammed to a pluripotent state with a combination of four transcription factors. This raised the question of whether transcription factors could directly induce other defined somatic cell fates, and not only an undifferentiated state. We hypothesized that combinatorial expression of neural lineage-specific transcription factors could directly convert fibroblasts into neurons. Starting from a pool of nineteen candidate genes, we identified a combination of only three factors, *Ascl1*, *Brn2*, and *Myt1l*, that suffice to rapidly and efficiently convert mouse embryonic and postnatal fibroblasts into functional neurons *in vitro*. These induced neuronal (iN) cells express multiple neuron-specific proteins, generate action potentials, and form functional synapses. Generation of iN cells from non-neural lineages could have important implications for studies of neural development, neurological disease modeling, and regenerative medicine.

Is-27: Potential Therapeutic Applications of Pluripotent Stem Cells

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Is-28: Genetic Modeling of Myeloproliferative Diseases

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Is-29: Treatment of Jak2-Mediated Myeloid Leukemia

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Stem Cells

Os-1: Inhibitory Effects of Estradiol on Differentiation of Germ Cells, Leydig or Sertoli Cell in Male Reproductive System

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Objective: Estrogens play important roles in differentiation of male and female reproductive system. In particular, environmental estrogens have potentially adverse effects in the male. This study investigated whether exposure of rats to estradiol valerate altered cellular differentiation in male reproductive system.
Materials and Methods: Adult Wistar male rats were purchased and raised in our colony from an original stock. Estradiol valerate (200 microg /kg /day) was applied subcutaneously for 4 weeks. After death, testes were removed and testes weight and size measured and compared with control group (ANOVA). Slides prepared for testis tissue and compared with those of untreated to detect changes or marked histological trends associated with treatment. In this concern, the general integrity of tissue structure, luminal complexity and size and the presence or absence of luminal products were studied in testes.
Results: Estradiol valerate treatment caused to decreasing of testis size and weight ($p<0.01$), and semeniferous tubules were markedly different and deformed in appearance from those in control rats. The number of germ cells, Leydig cells and Sertoli cells was significantly less than of control rats ($p<0.05$). The number of spermatozoa (luminal products) was also reduced compared with control group ($p<0.01$).
Conclusion: Our results indicate that estradiol valerate has inhibitory effects on testis weight, size and cellular structure and density in males. Estradiol valerat administration, as environmental estrogen, prevents the normal differentiation of most testis cells, particularly germ cells, to mature cells or results in cell death.

Keywords: Estradiol Valerate, Testis, Differentiation

Os-2: Establishing a Uniform Environment of Olfactory Ensheathing Cells Enhances Axon Extension during Regeneration

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Olfactory ensheathing cells (OECs) support the regeneration of primary olfactory neurons throughout life and, unlike other glial cells, can cross the boundary between the peripheral nervous system and the central nervous system. OECs have been shown to have a remarkable ability to promote neural regeneration after spinal cord injury, and are thus a promising candidate for transplant-mediated repair. While OECs are able to migrate in the lesioned area their migration is often limited. While it has been proposed that increasing OEC migration will lead to improved axon regeneration, this has not yet been tested.
We have developed a regeneration model to examine whether (1) the migration of OECs ahead of olfactory axons affects axonal growth and (2) the migration rate of OECs is altered by the presence of olfactory axons. We performed unilateral bulbectomy to ablate olfactory axons followed 4 days later by methimazole administration to further delay neuronal
We found that delaying axon growth increased the migration rate of OECs and that subsequently the axons grew over a significantly larger region and formed more distinct fascicles and glomeruli in comparison with growth in animals that had undergone bulbectomy alone. *In vitro*, we confirmed that (i) olfactory axon growth was more rapid in the presence of pre-existing OECs and (ii) OECs migrated more rapidly in the absence of axons and also (iii) the outgrowth rate of olfactory axon was highest over OECs compared to the other glial cells.
These results demonstrate that olfactory axon growth is significantly enhanced if a permissive OEC environment is present prior to axon growth and that the distribution of OECs is repressed by the presence of olfactory axons.
Key words: Glia, Olfactory Bulb, Neuron, Fascicle

Os-3: Fetal Maternal Stem Cell Trafficking and Cardiac Repair

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Objective: Fetal cells enter the maternal circulation during all pregnancies. They may persist in maternal blood and tissues for decades, creating a state of physiologic microchimerism. We hypothesize that fetal stem cells may be associated with a maternal response to cardiac injury. This may even represent a mechanism that contributes to cardiac recovery in Peripartum Cardiomyopathy.
Materials and Methods: In order to examine whether

fetal stem cells cross the placenta and participate in cardiac repair in the maternal heart, we created a mouse model of cardiac injury during pregnancy. We crossed wild-type B6CBA female mice (age 3-6 months) with male GFP transgenic mice. The female mice then underwent ligation of the left-anterior descending (LAD) artery in order to induce an anterolateral myocardial infarction (MI) at gestation day 12. Approximately 50% of the embryos were GFP-positive. 13 experimental females underwent infarction; 9 female pregnant controls did not undergo surgery. Post-partum females were sacrificed at 1 week post-MI (8 experimental, 4 control) or 2 weeks post-MI (5 experimental, 5 control). Genomic DNA was extracted from each total heart. Using primers designed to amplify GFP and an internal control gene, ApoB, real time PCR was done in triplicate. The ΔΔCt method was used to calculate the relative quantity of GFP in experimental hearts compared to controls. Unpaired t-test was used to evaluate the ΔCt values generated from the PCR reactions.

Results: Experimental hearts harvested at 1 week post-MI contained 4.1 times more GFP than controls ($p=0.0477$). Experimental hearts harvested 2 weeks post-MI contained 3.9 times more GFP than controls ($p=0.0312$). In a separate group of experimental and control mice, immunofluorescence analysis with confocal microscopy was utilized to detect GFP-positive cells in ventricular tissue sections of maternal hearts. GFP-positive cells were noted only in infarct zones of experimental maternal hearts at 1, 2, and 3 weeks post-MI. Further analysis of ventricular sections at 2 weeks and 3 weeks post-MI indicate co-expression of GFP and the cardiac markers alpha-sarcomeric actin and troponin, the vascular markers alpha smooth muscle actin and alpha smooth muscle MHC, and the endothelial marker PECAM. GFP positive fetal cells were isolated from the injured maternal hearts at various time points post-injury. When plated on mesenchymal feeder layers, the GFP positive cells form vascular structures and the expression of PECAM and Smooth muscle cell actin can be detected. Isolated GFP positive fetal cells differentiate into beating cardiomyocytes when co-cultured with neonatal murine cardiomyocytes and express the cardiac markers alpha-sarcomeric actin and troponin.

Conclusion: These data indicate that fetal cells home to the injured maternal heart and differentiate into cardiomyocytes, smooth muscle cells and endothelial cells. Furthermore, these differentiation pathways are recapitulated *in vitro* thus implicating fetal maternal stem cell transfer as an important mechanism in the maternal response to myocardial injury.

Keywords: Fetal Cells, Stem Cells, Fetal Maternal Michrochimerism, Fetal Maternal Stem Cell Transfer, Cardiomyocyte Differentiation, Endothelial Cells, Smooth Muscle Cells, Vascular Differentiation

Os-4: Effect of Adipose Tissue Derived Mesenchymal Stem Cell Injection on Wound Healing in Diabetic Rats

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Objective: Diabetic ulcer is a major morbidity for diabetic patients. Stem cells are a new hope to ameliorate delayed diabetic wound healing. We designed this study to analyze the effect of adipose tissue derived mesenchymal stem cells (ADMSC) on wound healing in diabetic rats.

Materials and Methods: Twenty six white male rats weighing 180-250 grams were injected intraperitoneally with streptozotocin. All the rats became diabetic (blood sugar>280) and were divided into two similar groups. After 6 weeks, wound healing rate was studied by creating dorsal full-thickness round excisional wounds (diameter=1cm). Stem cells were derived from abdominal subcutaneous fat of a single white male rat. After the third passage, surface markers were tested using flowcytometry. 10^6 ADMSCs, stained with PKH26 and dissolved in phosphate buffer saline (PBS) were injected around the wound area subcutaneously in case group rats. PBS was injected in control group. Wound surface area was calculated using a computerized algorithm every other day. When the wounds were completely healed, the wound tissue was excised for histological study. Stereomicroscopy was used to measure the volume density of collagen, vessels, fibroblasts and also vessel length. All the rats' blood glucose level was measured at the end of the study, again.

Results: Flowcytometric analysis showed that the cells are positive for CD90 and negative for CD 31, 45 and 11b. Wound closure rate in case group had no significant difference comparing with control group ($p>0.05$).There was no significant difference between the case and the control group blood glucose level changes. PKH26 stained cells existed in epidermis, dermis and hypodermis of case group wound tissue. Stereomicroscopy results are not ready yet.

Conclusion: Adipose tissue is a possible source of adult

stem cell. ADMSCs had no effect on wound healing rate and no effect on blood glucose levels in diabetic rat model.

Keywords: Diabetic Ulcer, Adipose Tissue Derived Mesenchymal Stem Cells, Stereomicroscopy

Os-5: Propagation of Human Spermatogonial Stem Cells *in Vitro*

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Objective: Recently developed, highly effective, cancer therapy for children allows the majority of them to survive their cancer. One of the major side effects of cancer therapy in male patients is sterility. There are currently no means to preserve reproductive potential in prepubertal boys, which contrasts with adolescents and adults, for whom cryopreservation of semen prior to chemotherapy or radiotherapy is available and widely used. Therefore, establishing a human spermatogonial stem cell (hSSC) culture system to allow successful autotransplantation for young boys diagnosed with cancer is of utmost importance. As the final number of transplantable cells will influence the success rate of this technique, we first focus on the propagation of hSSC in culture.

Materials and Methods: We used testicular tissue from six men undergoing bilateral castration as part of prostate cancer treatment. Testicular cells were isolated with a two steps enzymatic digestion and overnight differential plating. Testicular cells were cultured in supplemented StemPro medium. Some formed germline stem cell (GSC) clusters were taken out of the culture and subcultured on human placental laminin coated dishes.The presence of spermatogonia in the cultures was determined by immunohistochemistry and RT-PCR for spermatogonial markers (integrin-α6, integrin-β1, PLZF). The spermatogonial stem cell transplantation assay was performed using busulphan treated nude mice, as the functional test of stem cell capability. Human cells in recipient mouse testis were detected by Fluorescent In situ hybridization (FISH) using the most common human specific repetitive DNA sequence (COT) as a probe.

Results: Germline stem cell (GSC) cluster formation was observed in the testicular cell cultures of all six men in testicular cell cultures and in subcultures GSCs. Testicular cells and subcultured GSCs could be cultured for at least 15 and 28 weeks respectively, while expression of spermatogonial cell surface markers integrin-α6 and integrin-β1 (on RNA level) and spermatogonial nuclear marker PLZF (on RNA and protein levels) was maintained.The mouse transplantation assay showed successful colonization of cultured testicular cells in 4 out of 6 patients and from the subcultured GSCs in 1 out of 2 patients, indicating the presence of functional spermatogonial stem cells. By determining the number of colonies of transplanted cultured cells from early and later passages of the same culture, we found a more than 50 fold increase of hSSC in 19 days in our testicularcell culture when cultured from day 28 to 47 (passage 2 to 5) and a more than 18000 fold increase in number of hSSC in 64 days in our subcultured GSCs when cultured from day 77 to 141 (passage 7 to 12).

Conclusion: This report outlines the first successful long term culture and proliferation of hSSC *in vitro*. This is an important step forward to future clinical application of SSC autotransplantation in prepubertal boys diagnosed with cancer to preserve their fertility

Keyword: Cancer Survivors, Infertility, Stem Cells, Spermatogonial Stem Cells, Autotransplantation

Os-6: Therapeutic Potential of Genetically Modified Neural Stem Cells (NSCs) in a Mouse Model of Globoid Cell Leukodystrophy (GLD)

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Objective: To evaluate the potential of Neural Stem Cell (NSC)-based approach to correct the metabolic defect and to ameliorate pathology in Twitcher (Twi) mice, a true model of Globoid Cell Leukodystrophy (GLD).

Materials and Methods: Twi mice display severe demyelination and neurodegeneration, with a median survival of 46 days. We derived NSC lines from the subventricular zone of neonatal Twi mice and of wt littermates. Using bidirectional lentiviral vectors (bdLV) encoding for galactocerebrosidase (GALC) and GFP, we achieved high efficiency of transduction (>80%) and supraphysiological GALC levels (2-3 fold the wt levels) in mutant NSC, with no toxicity or functional impairment due to transgene over-expression. Similar supraphysiological GALC levels were obtained following bdLV. GALC transduction of wt NSC. Of importance, gene-corrected cells allowed more efficient metabolic cross-correction of GALC-deficient NSC then wt cells *in vitro*, due to more efficient enzyme secretion in the extracellular milieau. In order to obtain a stable source of GALC-secreting cells in the brain we transplanted wt or GALC over-expressing NSCs into the telencephalic lateral ventricles of neonatal (post-natal day 2) Twi mice (1x10^6 total cells, bilateral injection). Forty days

after transplant we evaluated on NSC-treated mice and on untreated controls: i) NSC engraftment, distribution and fate (by immunocytochemistry); ii) the presence of a functional GALC protein in tissues and cerebrospinal fluid (by western blot and enzymatic assays); iii) intracellular storage (by lectin staining); iv) improvement of pathology (by immunocytochemistry and qPCR). A group of treated mice was monitored for body weight and motor skills until a fixed human endpoint, in order to assess the impact of NSC therapy in delaying the onset of symptoms and prolonging lifespan.

Results: Forty days after transplant we found NSC (identified by GFP expression) widely distributed into the brain parenchyma, from the olfactory bulb to the hippocampus. Many cells were found lining the ventricles, along the corpus callosum and in the external capsula. Engrafted NSC (1-3% of the total injected cells) either expressed glial cell markers or retained antigenic features of immature neural cells and did not show proliferative activity. Of note, they robustly produced and secreted the GALC protein, as demonstrated by immunohistochemistry and by western blot using anti-GALC antibody. Most important, GALC activity was restored to 50% of wt levels in brain and spinal cord tissues of NSC-transplanted Twi mice, indicating widespread and efficient transport of the bioactive enzyme in CNS tissues (mainly through CSF flow) coupled to active cross-correction. The metabolic correction correlated with amelioration of pathology, clearance of tissue storage and partial rescue of the phenotype. We are currently evaluating the potential therapeutic advantage of GALC-overexpressing NSC as compared to the wt counterpart.

Conclusion: These results, together with our preliminary data indicating the feasibility of safe GALC-overexpression in human fetal NSC (a therapeutically relevant cell type), warrant further consideration of NSC gene therapy for the treatment of GLD, likely in combination with other approaches (i.e. bone marrow transplant, currently under evaluation in our laboratory) ensuring enzymatic reconstitution in visceral organs and in the PNS.

Keywords: Neural Stem Cells, Transplant, Genetic Diseases, Neurodegenerative Diseases

Os-7: Small Molecule Induction of Neural Crest-like Cells derived from Human Neural Progenitors

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Objective: Neural crest cells are stem/progenitor cells that are specified within the embryonic neuroectodermal epithelium, and migrate to stereotyped peripheral sites for differentiation into many cell types important for the formation of the craniofacial tissues, cardiac outflow tract and sensory and autonomic nervous systems. Several neurocristopathies involve a deficit of neural crest-derived cells, raising the possibility of stem cell therapy. In Hirschsprung's Disease the distal bowel lacks an enteric nervous system due to a failure of colonisation by neural crest-derived cells. Our aim was to develop a robust method of producing migrating neural crest-like cells from human embryonic stem (ES) cells, and to evaluate their developmental potential *in vitro* and *in vivo*.

Materials and Methods: We used human ES cells to derive neural progenitors as neurospheres via exposure to Noggin, then co-cultured the neurospheres with mouse embryonic fibroblasts. Following this we exposed the neurospheres to Y27632, a small molecule inhibitor of the Rho effectors ROCKI/II.

Results: This sequential treatment dramatically increased the efficiency of differentiation of human ES cells into neural crest-like cells, identified by cell behavior and molecular marker expression *in vitro*. Neural crest-like cells derived by this method were able to migrate along neural crest pathways in avian embryos in ovo and within organotypic explants of murine bowel. In both these normal tissue contexts, the human ES-derived cells differentiated into cells with neural crest-like neuronal and glial markers.

Conclusion: This is the first study to report the use of a small molecule to efficiently induce cells with neural crest characteristics from ES cells, that can migrate and generate neurons and support cells in complex tissue. This study demonstrates that small molecule regulators of ROCKI/II signaling may be valuable tools for stem cell research aimed at treatment of neurocristopathies

Keywords: Neural Crest, Human Embryonic Stem Cells, Small Molecule, Hirschsprung's Disease, Stem Cell Therapy

Os-8: Cancer Stem Cells in Lung Cancer: Distinct Differences between Small Cell and Non-Small Cell Lung Carcinomas with Special Reference to Expression and Activity of Aldehyde Dehydrogenase

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Objective: Of the many markers for cancer stem cells (CSCs) in lung cancer reported to date, the cell surface antigen CD133, nuclear β-catenin accumulation, the side population phenotype, and high aldehyde dehydrogenase (ALDH) activity seem to be most reliable. In this presentation, we review the results of studies on lung CSCs and discuss the significance of these markers from a biological, pathological, and clinical viewpoint. In addition, we present our own data, focusing primarily upon ALDH1A1.
Materials and Methods: Twenty-seven lung cancer cell lines (nine small cell lung carcinoma (SCLC) cell lines and eighteen non-small cell lung carcinoma (NSCLC) cell lines) were examined for mRNA and protein expression and fractions of cells with activity.
Results: ALDH1A1 mRNA was strongly expressed in five cell lines, of which three were SCLC cell lines and two were NSCLC cell lines. Two of the SCLC cell lines consistently expressed the protein and had a large fraction of cells with ALDH activity, but the third did not. Both the NSCLC cell lines expressed the protein, but only one had a large fraction of cells with strong ALDH activity. In brief, the level of ALDH1A1 mRNA did not always parallel that of the protein in SCLC cell lines, while the level of ALDH1A1 protein did not necessarily parallel that of ALDH activity in NSCLC cell lines. The ALDH1A1 protein level or ALDH activity level was well associated with the mRNA level of CD133, which is the most commonly used marker for CSCs, in SCLC cell lines, but not in NSCLC cell lines, suggesting an abundance of CSC populations in SCLC compared to in NSCLC. From the current findings, the mechanism and pathway that regulate the expression of ALDH1A1 mRNA and its protein, and its enzymatic activity as well differ greatly between SCLC and NSCLC cells. We speculate that ALDH (its expression and activity) is only one of the factors determining the stemness of CSCs in lung cancers.
Conclusion: In conclusion, the CSCs in SCLC and NSCLC differ distinctly in terms not only of their abundance but also of the regulatory mechanism of ALDH1A1 expression and activity, as well as its role in the maintenance/activation of stemness. Exploring the mechanism of ALDH's activation and its role in the maintenance of the stemness not only of CSCs but also of normal stem cells would provide a novel paradigm for stem cell biology.
Keywords: Cancer Stem Cell, Small Cell Lung Carcinoma, Non-Small Cell Lung Carcinoma, Aldehyde Dehydrogenase

Os-9: Cell Adhesion and Spreading Affect Adipogenesis from ES Cells: the Role of Calreticulin

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Objective: To we characterize the adhesive properties of ES cells lacking calreticulin
Materials and Methods: ES cells
Results: In the present study we characterized the adhesive properties of ES cells lacking calreticulin and shown that adipogenesis from ES cells is directly and reciprocally controlled by the adhesive status of a cell, which in turn is modulated by calreticulin. Calreticulin-deficient ES cells are not only highly adipogenic, but also show elevated calmodulin/CaMKII signalling and poor adhesiveness compared to the wild type ES cells. Calreticulin deficiency leads to a disorganized cytoskeleton and low levels of focal adhesion-related proteins, such as vinculin, paxillin, and phosphorylated focal adhesion kinase, which cause limited focal adhesion formation and limited fibronectin deposition. Moreover, differentiation on non-adhesive substrata, which hinder cell spreading, promoted adipogenesis in the wild type ES cells that normally have low adipogenic potential, causing a decrease in focal adhesion protein expression as well as an increase in calmodulin/CaMKII signalling. In contrast, inhibition of CaMKII effectively increased focal adhesion protein levels and inhibited adipogenesis in calreticulin-deficient ES cells, causing them to behave like the low adipogenic, wild type ES cells.
Conclusion: The adipogenic potential of ES cells is proportional to their calmodulin/CaMKII activity, but is inversely related to their focal adhesion protein levels and degree of adhesiveness/spreading.
Keywords: Adhesion, ES Cells, Calreticulin

Os-10: Evidence for Calreticulin Attenuation of Cardiac Hypertrophy Induced by Pressure Overload and Soluble Agonists

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Objective: To investigate here the role of calreticulin in cardiac hypertrophy.
Materials and Methods: ES cells
Results: While calreticulin has been shown to be critical for cardiac development, its role in cardiac pathology is unclear. Previous studies have shown the detrimental effects on the heart of sustained germline calreticulin overexpression, yet without calreticulin, the heart does not develop normally. Thus, carefully balanced calreticulin levels are required for the heart to develop and to function properly into adulthood. But what happens to calreticulin levels, and how is this regulated, during cardiac hypertrophy, during which the fetal gene program is reactivated, at least partially?

Our working hypothesis was that c-Src, a kinase whose activity we previously found to be correlated with calreticulin expression, was involved with calreticulin in regulating the response to hypertrophic signals. Thus, we subjected adult mice to transverse aortic constriction to induce left ventricular hypertrophy. We found that aortic constriction caused calreticulin levels to increase, while those of c-Src fell with longer constriction time. We also examined the ability of ES cell-derived cardiomyocytes to respond to soluble hypertrophic agonists. Endothelin-1 treatment caused a significantly greater cell area increase of calreticulin-null cardiomyocytes, which had higher c-Src activity, compared to wild type cells. c-Src inhibition abolished this difference. Greater c-Src activity may explain the efficacy with which calreticulin-null cells are able to induce the hypertrophic program, while cells containing calreticulin may be able to attenuate the hypertrophic response as a result of decreased c-Src activity.
Conclusion: Calreticulin may have a protective effect on the heart in the face of cardiac hypertrophy.
Keywords: Cardiomyocyte, ES Cell, Calreticulin

Os-11: Differentiation Capability of Mouse Bone Marrow-Derived Mesenchymal Stem Cells into Hepatocyte-Like Cells on Artificial Basement Membrane Containing Ultraweb Nanofibers and their Transplantation into Carbon Tetrachloride Injured Liver Model

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Objective: Liver fibrosis, the wound-healing response of the liver to chronic injury, is one of the most problems in health care. At present, liver transplantation is the only curative therapy available for the patients; however, there are various problems with this approach, including donor shortages, surgery related complications, immunological rejection, and high cost. Recent studies showed that regeneration therapy has the potential to become a useful minimally invasive technique with minimal complications. Bone marrow mesenchymal stem cells (BM-MSC) have been shown to be capable of differentiating into a liver cell lineage *in vitro* and *in vivo*. However, MSC differentiation to hepatocyte is poor, and the cell characteristics and their role in liver repair are poorly understood.
Materials and Methods: we differentiate mouse BM-MSCs into early (day 18) and late (day 36) hepatocyte-like cells (HLCs) *in vitro* in presence or absence of ultraweb nanofibers (nano+ and nano-). Then we evaluated their hepatocyte specific genes and proteins by Real-time PCR and immunofluorescence. We also evaluated ultrastructure of the differentiated cells by electron microscope and examined their functionality by PAS and PROD techniques and measurement of hepatocyte specific secretions in culture medium. Then we investigated potential of the untreated MSCs and the early and late HLCs of nano+ and nano- groups for recovery of mice with CCl4 induced hepatic fibrosis.
Results: We have demonstrated that, the markers of hepatocytes-albumin (ALB), HNF4α, CX32 and CYP1A1- were upregulated consistently in a time-dependent manner in the nano+ group, but not changed or decreased in nano- group. Moreover, the urea production, secretion of ALB and α-fetoprotein, and metabolic activity of the CYP450 enzymes were significantly more within differentiated HLCs on nanofibers *in vitro* at day 36. MSCs and early and late HLCs in nano+ and nano- culture conditions, transplanted by intravenous route, decreased liver fibrosis, engrafted in recipient liver, and differentiated into functional hepatocytes (ALB+), except late HLCs in nano- group.
Conclusion: These results showed that transplantation of late HLCs of nano+ group was more effective in rescuing liver failure, enhancement of serum ALB, homing of transplanted cells, and functional engraftment, than other groups. It seems that topographic properties of nanofibers enhanced hepatogenic differentiation profile and maintain the function of MSC-derived hepatocytes in long-term culture, which has implications for cell therapies.
Keyword: Mesenchymal Stem Cells, Hepatocyte-Like Cells, Nanofibers, Liver Fibrosis, Differentiation

Os-12: Human Neural Stem Cells Ameliorate Autoimmune Encephalomyelitis in Non-human Primates

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Objective: Transplanted neural stem/precursor cells (NPCs) display peculiar therapeutic plasticity *in vivo*. Although the replacement of cells was first expected

as the prime therapeutic mechanism of stem cells in regenerative medicine, it is now clear that transplanted NPCs simultaneously instruct several therapeutic mechanisms, among which replacement of cells might not necessarily prevail. A comprehensive understanding of the mechanism(s) by which NPCs exert their therapeutic plasticity is lacking. This study was designed as a preclinical approach to test the feasibility of human NPC transplantation in an outbreed nonhuman primate experimental autoimmune encephalomyelitis (EAE) model approximating the clinical and complex neuropathological situation of human multiple sclerosis (MS) more closely than EAE in the standard laboratory rodent.

Materials and Methods: We examined the safety and efficacy of the intravenous (IV) and intrathecal (IT) administration of human NPCs in common marmosets affected by human myelin oligodendrocyte glycoprotein 1-125–induced EAE. Treatment commenced upon the occurrence of detectable brain lesions on a 4.7T spectrometer.

Results: EAE marmosets injected IV or IT with NPCs accumulated lower disability and displayed increased survival, as compared with sham-treated controls. Transplanted NPCs persisted within the host central nervous system (CNS), but were also found in draining lymph nodes, for up to 3 months after transplantation and exhibited remarkable immune regulatory capacity *in vitro*.

Conclusion: Herein, we provide the first evidence that human CNS stem cells ameliorate EAE in nonhuman primates without overt side effects. Immune regulation (rather than neural differentiation) is suggested as the major putative mechanism by which NPCs ameliorate EAE *in vivo*. Our findings represent a critical step toward the clinical use of human NPCs in MS.

Keywords: Neural Stem Cells, Transplantation, Multiple Sclerosis

Os-13: VEGF, IL-8 and MMP2 Expression Profile in Adipose-Derived Stem Cells (ASCs) of Breast Cancer Patients

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Objective: Angiogenesis is an important process for progression and metastasis of most types of tumors such as breast cancer. Vascular endothelial growth factor (VEGF), CXCL8 (IL-8) and matrix metalloproteinases (MMPs) are reported to play crucial roles in cancer development and metastasis. It has been shown that MSCs recruit to the tumor microenvironment and may contribute to the production of proangiogenic factors. Herein, we investigated the expressions of VEGF, IL-8 and MMP2 mRNA levels in adipose-derived stem cells (ASCs) of twenty one women with breast cancer.

Materials and Methods: ASCs were isolated from fragments of breast adipose tissue after mincing and incubating with chollagenase. They were characterized as mesenchymal stem cells by flow cytometry method for the expressions of MSC related markers and the hepatogenic and chondrogenic differentiation capabilities. The expression of extracted mRNAs was determined using real-time quantitative PCR (Q-PCR). Results were compared to those of a sex and age matched control group.

Results: ASCs were appeared with a spindle shape in the culture and flow cytometry analysis for stem cell specific markers revealed that ASCs from both patients and controls expressed CD44, CD105 and CD166. They were negative for the expressions of CD14, CD34 and CD45. ASCs were changed morphologically through hepatogenic and chondrogenic differentiation to the polygonal-flattened and cobuidal shapes, respectively. Relative Quantitation (RQ) of VEGF and IL-8 were about 2.4 and 2 folds higher in patients than controls, respectively. The expression of both molecules were higher in patients with stage 3 compared to stage 1 and 2. No difference was found in the expression of MMP2 between patients and controls. There were a statistically significant correlation between the expression of MMP2 and esterogen and progesteron receptors (ER/PR) expression in breast cancer patients (P value<0.05).

Conclusion: These data suggest that the higher expression of VEGF and IL-8 by ASCs of breast cancer patients can probably change the prognosis and susceptibility of women to breast cancer. Thus, these molecules might be introduced as potential therapeutic targets for human breast cancer.

Keywords: ASCs, Angiogenesis, VEGF, IL-8, Breast Cancer

Os-14: Substance P and Calcitonin-Gene-Related Neuropeptides as Novel Growth Factors for *Ex vivo* Expansion of Cord Blood CD34+ Hematopoietic Stem Cells

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Objective: There is little evidence on roles of growth factors other than cytokines in expansion of cord blood (CB) stem cells. We aimed to explore a novel approach for expansion, using Substance P (SP) and Calcitonin Gene-Related Peptide (CGRP) neuropeptides.
Materials and Methods: CB CD34+ cells were cultured in different concentrations of SP and/or CGRP in combination with a cytokine cocktail. Phenotypic and functional analysis was performed by flowcytometry and colonogenic assay.
Results: Our results show a significant improvement of total expansion of neuropeptide treated cells. There was a selective effect of CGRP on CD34+ CD133+ cells, SP on CD34+ CD45dim cells and 10-9 M SP and/or CGRP on expansion of CD34+ CD38- cells. There was also a tendency for erythroid and granulocyte-myeloid colony formation in SP and CGRP treated cultures, respectively.
Conclusion: Supplementation of cytokines with other growth factors, such as neuropeptides, might enable us to overcome the difficulties of *ex vivo* expansion of CB cells.
Keywords: CGRP, Cord Blood, Expansion, Stem Cell, SP

Os-15: Human Mesenchymal Stem Cells Transplantation in the Sensory Cortex Are Able to Decrease Pain Perception in Neuropathic Rodents

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Objective: Neuropathic pain is a very complex disease, involving several molecular pathways. Current available drugs are usually not acting on the several mechanisms underlying the generation and propagation of pain. Nowadays, pain research is focusing on newer molecular ways, such as stem cell therapy. These methods could provide a new therapeutic approach to neuropathic pain relief. Stem cells have been used in a variety of nervous system injury models. As neurodegenerative disease, also neuropathic pain could undergo to stem cell therapy.
Materials and Methods: We used spared nerve injury (SNI) model of neuropathic pain to assess the possible use of human mesenchymal stem cells (hMSCs) as neuroprotective tool in the regenerative medicine. Human MSCs were isolated from a small aspirate of bone marrow and *in vitro* expanded in FGF-containing medium. hMSCs (50,000 cells) were transplanted in several brain areas involved in neuropathic pain controlling and processing. Stem cells injection was performed 4 days after sciatic nerve surgery. Neuropathic mice were monitored 7, 10, 14, 17, and 21 days after surgery.
Results: hMSCs were able to reduce pain like behaviors, such as mechanical allodynia once transplanted in the somatosensory cortex, in the rostral agranular insular cortex, in the striatum, and in the ventricle. Best result was achieved in the striatum area. Anti-nociceptive effect was detectable from day 10 after sugery. hMSCs did not affect thermal hyperalgesia. Stem cell transplantation did not affect motor coordination of the neuropathic mice.
Conclusion: Stem cell transplantation could be an useful therapeutic tool in the future of regenerative medicine.
Keywords: Stem Cell Therapy, Neuropathic Pain

Os-16: Immortalized Human Skin Fibroblast Feeder Cells Support Growth and Maintenance of Both Human Embryonic and Induced Pluripotent Stem Cells

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Objective: Feeder cells are frequently used for the early-stage of derivation and culture of human embryonic stem cell (hESC) lines.
Materials and Methods: We established a conditionally immortalized human foreskin fibroblast line that secreted basic fibroblast growth factor (bFGF). These cells were used as feeder cells for hESC culture and induced pluripotent stem (iPS) cell derivation and expansion. This conditional immortalization was performed using lentiviral vector (LV) mediated transduction of Bmi-1 and human telomerase reverse transcriptase genes and the resulting cell line was further modified by LV-mediated transduction of a secreted form of bFGF gene product. Three different laboratories have tested whether this feeder cell line could support the maintenance of four different hESC lines.
Results: Immortalized fibroblasts secreting stable amounts of bFGF supported the growth of all hESC lines, which remained pluripotent and had a normal karyotype for at least 10 passages. Even at high passage (p56), these modified cells, when used as feeders, could support iPS derivation and propagation. Derived iPS cells expressed pluripotency markers, had hESC morphology and produced tissue components of the three germ layers when differentiated *in vitro*.
Conclusion: These modified fibroblasts are useful as a genetically-defined feeder cell line for reproducible and cost-effective culture of both hESC and iPS cells.

Keywords: Feeder Cells, Human Embryonic Stem Cells, Induced Pluripotent Stem Cells, Human Fibroblasts, Immortalization

Os-17: Abrogation of E-Cadherin Mediated Cell-Cell Contact in Mouse Embryonic Stem Cells Results in Reversible LIF-Independent Self-Renewal

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We have previously demonstrated that differentiation of embryonic stem (ES) cells is associated with down-regulation of cell surface E-cadherin (Spencer et al, 2007 - paper attached). In this study we have assessed the function of E-cadherin in mouse ES cell pluripotency and differentiation.
We show that inhibition of E-cadherin mediated cell-cell contact in ES cells using gene knockout (Ecad-/-), RNA interference (EcadRNAi) or a trans homodimerisation inhibiting peptide (CHAVC) results in cellular proliferation and maintenance of an undifferentiated phenotype in FBS-supplemented medium in the absence of LIF. Re-expression of E-cadherin in Ecad-/-, EcadRNAi and CHAVC-treated ES cells restores cellular dependence to LIF supplementation. Whilst reversal of the LIF-independent phenotype in Ecad-/- ES cells is dependent on the β-catenin binding domain of E-cadherin, we show that β-catenin null (βcat-/-) ES cells also remain undifferentiated in the absence of LIF. This suggests that LIF-independent self-renewal of Ecad-/- ES cells is unlikely to be via β-catenin signalling. Exposure of Ecad-/-, EcadRNAi and CHAVC-treated ES cells to the activin receptor-like kinase inhibitor SB431542 led to differentiation of the cells, which could be prevented by re-expression of E-cadherin. To confirm the role of TGFβ-family signalling in the self-renewal of Ecad-/- ES cells we show that these cells maintain an undifferentiated phenotype when cultured in serum-free medium supplemented with Activin A and Nodal, with FGF-2 required for cellular proliferation.
We conclude that E-cadherin protein is required for LIF-dependent ES cell self-renewal and that multiple self-renewal signalling networks subsist in ES cells, with activity dependent upon cellular context.
Keywords: E-Cadherin, Embryonic Stem Cells, β-Catenin, Activin, FGF, Nodal

Stem Cells

Ps-1: Efficacy of Human Adipose Tissue-Derived Stem Cells in Cardiac Muscle Repair in An Experimental Acute Myocardial Infarction Model Using Nude Rats Strain Crl: NIH-Fox1

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Objective: Left ventricular remodeling is a major cause of progressive heart failure after acute myocardial infarction (AMI) in human beings.1-4 Heart failure following AMI remains a leading cause of morbidity and death in industrialized countries.1 Until recently, it has been believed that cardiomyocytes lack the ability to regenerate after AMI and that the extent of damage is closely related to the loss of cardiac functions and ultimately survival of the patient.5,6,14 Cell therapy has been used in the treatment of various conditions including cardiac muscle diseases. Transplanted cells transdifferentiate to replace the irreversibly damaged tissues.7-12,15-19 Challenges in the field of cardiac, stem cell-based therapy (cardiomyoplasty) are fading.22-25 In recent literature, there is a growing evidence that application of skeletal myoblasts, bone marrow-derived stem cells (BMSCs) and human adipose tissue-derived stem cells (hADSCs) are effective in the treatment of AMI.1,11-13,23-28 This technology holds a great promise in the medical management of AMI leading to a major improvement in the outcome of patients with cardiac disease.7-9 In this study, we hypothesized that human ADSCs injected into infracted myocardium are capable of differentiation into functional myocardial muscle following AMI in rat model and reduce the extent of post-AMI remodeling effects leading to significant improvement of cardiac performance.
Materials and Methods: Acute myocardial infarction was induced in 99 adult nude rats (strain Crl:NIH-Fox1, Charles Rivers Labs). Rats were randomly allocated into 6 groups and received either no cells (control, group I) or 2 million, freshly prepared hADSCs as follows: group II (n = 19) received intraventricular injection only immediately after AMI; group III (n = 18) received echocardiography-guided intraventricular injection only 5 days after AMI; group IV (n = 15) received echocardiography-guided intrventricular injection 5 days after AMI and intramyocardial injection directly into the infarct and peri-infarct regions through a second thoracotomy 10 days after AMI; group V (n = 17) received intraventricular injection immediately after AMI and intramyocardial injection 10 days after AMI and group VI (n = 15) received intramyocardial injection only 10 days after AMI. Five rats from each group were euthanized and hearts were collected at 10, 30 and 60 days after hADSCs injection. Hearts were studied using H & E stained sections and immunohistochemistry. Immunohistochemical staining was performed using antibodies against ?- smooth muscle actin, ?- actinin, heavy chain cardiac myosin, cardiac tropinin I, VEGF, von willebrand factor, and phospholamban.
Results: Upon histological analysis, the control group showed a tendency toward granular tissue formation (scar formation), active phagocytosis, variable angiogenesis when evaluated at 10 days, early fibrosis (fibrous connective tissue formation) when evaluated at 30 days and established fibrosis when evaluated at 60 days. However, hADSCs treated groups showed a tendency toward cardiomyocyte regeneration, prominent angiogenesis (growth of new blood vessels) when evaluated at 10 days, and lower infarction size when evaluated at 60 days. In some of the treated animals, minimal scarring area was observed when compared to the control group.
Conclusion: Injection of hADSCs decreased the amount of scar tissue formation following myocardial infarction and enhanced the regenerative capacity of myocardial cells. Significant evidence of regeneration was evident following a single, ventricular injection of 2 million hADSCs immediately after AMI.
Keywords: Stem Cells, Adipose Tissue, Myocardial Infarction, Heart Failure, Cellular Cardiomyoplaty

Ps-2: MicroRNAs Involved in Lymphoid Lineage Differentiation

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Objective: MicroRNAs are small noncoding molecules in genome of plants and animals. These molecules have 19-25 nucleotides and regulate gen expression in post transcriptional level (epigenetic mechanism). The number of these molecules is different, however, between 200-100 in primates up to more than 1000 in human.

Materials and Methods: MicroRNAs are involved in several processes such as development, cell proliferation, cell death, apoptosis, fat metabolism and cell differentiation. These molecules are responsible for the regulation 30% of expressed genes.
Results: MiRNA function done through a similar siRNA complex (RISC) but distinction is that full identity between miRNA and its target genes is not necessary. Like mRNA profile, miRNA expression varies from tissue to tissue, but is similar among individuals of a unique species.
Conclusion: The main involved miRNA in lymphocyte differentiation is miR-181 that has increased in all lymphoid lineages. This Mir targets Bcl-2, CD69 and TCR-α genes. In B-cell differentiation mir-150 is the prominent decreased microRNA at Pro-B stage but mir-17-92 cluster and mir-181 increased in pre-B and B cell stage respectively. Both miR-223 and miR-142 that are present in bone marrow increased in primary steps of T cell differentiation.
Keywords: MicroRNAs, Lymphoid, Differentiation

Ps-3: Production and Transduction of a Recombinant Lentiviral Vector Carrying EGFP Gene

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Objective: Lentivector mediated production of EGFP carrying murine spermatogonial stem cells.
Materials and Methods: EGFP gene was subloned into plenti6v5/dest vector. The transgene and/or the packaging vectors were cloned and purified using JM109 bacterial cells. Calcium phosphate transfection using HEK293FT cell line was performed to produce the EGFP carrying lentivectors. Spermatogonial stem cells were then transduced with the EGFP carrying lentivectors.
Results: PCR and Sequencing confirmed the integration of EGFP in to the lentiviral vector. Transduction with the 24 hour supernatant demonstrated a better result than 48 hour supernatant. Green color in spermatogonial cells under the UV light after cell passage illustrated the maintenance and expression of EGFP which indicates the entrance of EGFP gene into the cell genome.
Conclusion: By means of recombinant lentiviral vectors it is possible to integrate the gene of interest into the cell genome. This can lead to the final production of transgenic animals
Keywords: Lentivirus, EGFP Reporter Gene, HEK293FT Cell Line, Transfection, Transduction

Ps-4: Dose Dependent Effects of the Herb Arnebia euchroma on Epidermal Stem Cell Proliferation

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Objective: Studies of skin graft behaviour in rodent excisional wound models are limited by the dominance of wound contracture and graft sloughing as primary healing responses. In this study we used Arnebia euchroma hydro alcoholic extract on epidermal stem cells *in vivo* to prove the growth inducing effect of this herb.
Materials and Methods: 50 Wistar rats of albino portion were divided into 3 groups and each got a special treatment; AE10%, AE 20% and Control-group with no treament. Treatments was done on grafts and wounds were analyzed by stereologist and pathologist.
Results: We conclude that Arnebia euchroma herb induces proliferation of epidermal stem cells. Arnebia euchroma in both 20% and 10% doses had inducing effect on stemm cell growth ($p<0.05$)and there was no hepatotoxicity. There was no significant difference between 10% and 20% doses. ($p>0.05$).
Conclusion: Herbal medicine can be helpful in treatments using stem cells and Arnebia euchroma is one of the herbs that has inducing effect specially on proliferation of stem cells.
Keywords: Epidermal Stem Cell, Arnebia euchroma, Proliferation

Ps-5: G-CSF Mobilized Peripheral Blood Does Not Support High Yield Isolation of Endothelial Progenitor Colony Forming Units

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Objective: Endothelial progenitor colony forming units (CFU-ECs) were first believed to be the progenitors of endothelial cells and were named as endothelial progenitor cells (EPCs). But further it was discovered that they are monocytes regulating vasculogenesis. CFU-derived cells have been shown to improve blood flow recovery and capillary density in animal models of hind-limb or myocardial ischemia. The problem of working with these cells for therapeutic purposes is their low frequency and limited replicative potentials. The aim of this study was to evaluate G-CSF mobilized peripheral blood as a potential candidate for high yeild isolation of CFU-ECs.

Materials and Methods: EPCs were isolated using a standard cell culture assay developed by Hill et al. G-CSF mobilized and normal peripheral blood mononuclear cells were isolated on ficoll gradient and transferred into 6-well fibronectin coated plates. After 2 days nonadherent cells were transferred into 24-well fibronectin coated plates and after 5 days formation of colonies were investigated.
Results: Cells from normal peripheral blood showed excellent colonies during culture improving proper isolation of EPCs but no colonies and only few cell aggregates were reached in G-CSF mobilized group.
Conclusion: G-CSF mobilized peripheral blood can not be used as a good source fro isolation of CFU-ECs for laboratory or clinical purposes.
Keywords: Endothelial Progenitor Cell, Colony Forming Unit Endothelial Cell, G-CSF Mobilized Peripheral Blood

Ps-6: Assess the Pluripotency of Caprine Umbilical Cord Wharton's Jelly Mesenchymal Cells by RT-PCR Analysis of Early Transcription Factor Nanog

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Objective: In the present study we investigated the isolation protocol, population doubling time (PDT) and the expression of a pluripotential gene by RT-PCR analysis of early transcription factor Nanog in caprine umbilical cord (CUC) Wharton's jelly mesenchymal cells (WJMCs).
Materials and Methods: CUCs were collected from abattoir pregnant uteri and their Wharton's jellies (WJs) were cut into 2 × 2 mm2 segments for explanting. 8-10 segments were explanted into each 35 mm culture dish. WJ explants were removed 5 days after plating and the remaining adherent cells were cultured for another 5 days. Isolated cells were histochemically assayed for the presence of alkaline phosphatase (AP) activity. RT-PCR was used to assess the presence of Nanog mRNA. Besides, in this study the growth kinetic was evaluated for the isolated cells.
Results: CUC isolated cells displayed spindle-form and small round-shape with high nucleus. Confluent cells formed colonies that indicated AP activity. Initial seeding concentration of 2 × 104 CUC-WJMCs resulted in shorter PDT compared to fetal fibroblasts (46.57 vs. 54.29 hours, respectively). Expression of Nanog was undetectable in 9th passage of CUC-WJMCs.
Conclusion: CUC contains an easily obtainable source of mesenchymal cells which exhibit stem cells properties but probably these cells are not pluripotent.
Keywords: Wharton's Jelly, Mesenchymal Cells, Alkaline Phosphatase, Growth Kinetic, Nanog

Ps-7: Assessment of Morphological and Biological Characteristics of Mesenchymal Stem Cells and Hepatocytes Derived from Rat Mesenchymal Stem Cells *In Vitro*

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Objective: It has been demonstrated that mesenchymal stem cells (MSCs) can differentiate into hepatocytes in the presence of induction by fibroblast growth factor-4 and hepatocyte growth factor. The degree of cells differentiation in-vitro has been distinguished by measurements of the cell surface antigens. Unfortunately, these measurements of mRNA expression are destructive and not applicable to cells used for autologous transplantation. Images of cell morphology can be obtained nondestructively.
Materials and Methods: To using a CCD camera (DP12, Olympus, Tokyo) and a microscope (IX71, Olympus, Tokyo) recorded the cultured cell images. This study attempted to investigate several morphological parameters of MSCs and hepatocytes derived from rat MSCs, in-vitro, and to examine apoptosis rate of MSCs examined by TUNEL assay
Results: Our results showed that MSCs are clonogenic and self-renewal. These cells showed low percentage of apoptotic property. Approximately 80-90% of MSCs differentiated into hepatocyte, and cell morphology changed from fibroblast-like to polygonal and the percentage of the large polygonal cells increased during the differentiation culture of MSCs into hepatocytes. There were not significant difference in confluence and doubling time rate among serial passages. However, low rate of apoptotic cells was distinguished with usage of Tunel assay.
Conclusion: Morphological parameters such as polygonal index, cell adhesion area, morphologic changes and double nucleoli rate might be use as an indication of differentiation of BM-MSCs to hepatocytes.
Keywords: Mesenchymal Stem Cell, Hepatocyte, Morphological Parameter, Polygonal Index, Differentiation

Ps-8: Human Neural Stem Cell Line as an Alternative Model for *in Vitro* Developmental Neurotoxicity Testing

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Objective: Systematic testing for neurotoxicity and developmental neurotoxicity (DNT) is not foreseen in most regulatory schemes. The toxic effects of organic and inorganic chemicals in the developing human brain are not known, and chemicals are not regulated to protect children. Our aim was to investigate whether a human neural stem cell line, derived from umbilical cord blood, can serve as a reliable test model for DNT
Materials and Methods: Cells of the human umbilical cord blood-derived neural stem cell line (HUCB-NSC) were cultured at different developmental stages, ranging from non-differentiated floating stem cell aggregates, to attached and committed neural progenitors, and differentiating neuronal, astroglial and oligodendroglial cells. Conventional methods (culture in 96-well plates) and emerging nano/micro technologies (piezoelectric spotting and microcontact printing of different biomolecules to create protein microarrays) were used for toxicological screening. The selected compounds, acetaminophen, theophylline, methylmercury chloride (MeHgCl), chlorpyrifos, tellurite, cadmium chloride, L-glutamate (L-Glu) and D-glutamate (D-Glu), were applied at different concentrations, and dose range cytotoxicity curves established, using colorimetric and immunofluorescent-based cytotoxicity assays. The primary endpoints, representing processes essential for neurodevelopment, were cell proliferation, apoptotic cell death and neuronal and glial differentiation. The effect of test compounds on these features at different stages of HUCB-NSC commitment into neurons and glia was monitored in terms of immunocytochemical expression of cell type specific markers.
Results: HUCB-NSCs were differentiated into neural lineages by defined exposure to selected growth factors and neuromorphogenes. Dibutyryl cyclic AMP (dBcAMP), thyroid hormone (T3) and platelet derived neurotrophic factor (PDGF-BB) in combination with retinoic acid (RA) were the most potent in directing the differentiation of HUCB-NSCs towards neurons, astrocytes and oligodendrocytes respectively. Dose range finding for tested compounds allowed windows of exposure at non-cytotoxic concentrations to be established. Sensitivity of HUCB-NSC to MeHgCl, chlorpyrifos, tellurite, cadmium chloride at early stages of development was higher than at the late, differentiated stage. A low dose of MeHgCl (0•05μM) inhibited cell proliferation and induced apoptosis significantly, while morphological changes, detectable in phase-contrast, required concentrations of 1μM or more. At the differentiated stage, 1μM MeHgCl induced selective break down of S100μ expressing astrocytic cells. L-Glu at a concentration of 1mM did not influence early stages of HUCB-NSC development, but did affect cells directed into neurons at their late stage of differentiation. Acetaminophen, theophylline and D-Glu, did not affect HUCB-NSC proliferation, apoptosis or neural differentiation at any stage of development. Vulnerability of HUCB-NSC to MeHgCl, when tested on biomolecule microarrays, were dependent upon the type of the biomolecule (extra-cellular matrix proteins versus polyamino-acid arrays) and reflected connection of the stem cell reaction to the specific (receptor mediated) or non-specific type of cell/surface interactions.
Conclusion: The susceptibility of HUCB-NSCs to the selected neurotoxins was developmental stage and cell-type dependent. HUCB-NSC-derived cells accordingly fulfill one important criterion for serving as a human stem cell-based *in vitro* model for DNT priority setting. Sensitivity of HUCB-NSCs to low doses of neurotoxins may hence allow detection of pandemic neurotoxic effects, which are not clinically recognized.
Keywords: Human Cord Blood, Neural Stem Cells, Developmental Neurotoxicity

Ps-9: Inhibition of CSC Specific miRNAs Result in Differentiation of CSC into Cardiomyocytes

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Objective: Myocardial infarction is leading killer disease in the world. There are many different treatment options but the most recent one is cellular therapy after MI. Several studies suggest that miRNA have important roles in the development of the heart and cardiac function. Cardiomyocytes can be derived from hESC and human cardiomyocyte progenitor cells by treatment with activin A (Acv), bone morphogenic protein (BMP) or transforming growth factor, beta receptor III (TGF-βIII). We hypothesized that inhibition of specific miRNA in mouse cardiac stem cells (CSC) and CSC with GATA-4 (CSCG) could induce differentiation in cardiomyocytes by activation of their target genes.
Materials and Methods: We studied 569 unique miRNAs probes in mouse heart cells (MHtC), CSC and CSCG and identified high expression of miR762, 21 and 31 in CSC and CSCG compared to MHtC. CSC and CSCG were cultured in matrigel and anti-mRNAs

were transfected with oligofectamine reagent twice (3 and 15 days) after plating cells. We inhibited miR-762; 762+21; 762+31 and 762+21+31, which present as target genes activin A, BMP and TGF-β receptors. The target genes as TGF-βIII, activin A (receptors type-IB, II-like-1, 2a) BMP (receptors type 2), as well as Smad-4 and Dicer were analyzed by RT-PCR, 30 days after the last transfection, also cardiac sarcomeric proteins (α and β-MHC, MLC-2a, MLC-2v and cTNI). Beating cells were recorded on Confocal microscopy. As a positive control the cells were treated with activin A and BMP-4 cytokines and also compared to MHtC exrpession. The anti-sense was tested at 1, 5, 10, 50 and 100nM concentration. After 72hs of the treatment the target genes were analyzed by RT-PCR

Results: In CSC and CSCG the TGF-βIII, activin AR, BMPR and Smad4 expression increased up 50nM and beginning inhibition with 100nM concentration. The maximum miRNA expression inhibition was observed 6hs after the treatment, and 24hs the miRNA expression was similar of the control cells, however the target genes expression was increased. As expected cytokines treatment increased the target genes and signaling pathway increasing Smad-4. In CSC the miR-762, 762+21 and 762+21+31 anti-sense increased the target genes TGFβIII, AcvR (AcvR 2a and like-1), but not BMPR. Furthermore, increase the transcription factor Smad-4 and MLC-2a. α-MHC expression increased with 762+21 anti-sense association. In CSCG the 762+31 and 762+21+31 inhibition induced the genes, including TGFβIII, AcvR (2a and BI), but not BMPR. Also, increase Smad4 and MLC-2a and α-MHC (with 762+31 association).

Conclusion: The results show that CSC and CSCG differentiate in cardiomyocytes by activation of TGFβIII and AcvR signaling pathway, but not BMPR. Also, CSC differentiation was most activated by inhibition miR-762+21, while in CSCG by inhibition miR-763+31.

Keywords: Stem/Progenitor Cells, Cardiac Regeneration, Stem Cell Therapy, Cardiomyocytes, Gene Expression

Ps-10: Isolation of CD133 Positive Stem Cells from Meningioma Brain Tumor Tissues

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Objective: Meningiomas form one-fifth of all primary brain tumors and account for approximately 15% of all brain tumors with a slow pattern of grow and development. This type of tumor originates from cells of arachnoids granulations. Recently several studies reported CD133 as a specific marker for isolation and characterization of brain tumor stem cells. . Aim: To investigate the presence of CD133+ stem cells in meningioma tissues by flow cytometry analysis.

Materials and Methods: Surgically removed tumors were received in sterile conditions. Single cells were prepared and cultured in DMEM, 10% FBS. Tumor cells were propagated for 3 weeks. Cells from passage 1 and 4 were assessed for expressions of CD166, CD105, CD44, CD133, CD14, CD45 and CD34.

Results: Isolated tumor cells were highly positive for CD166, CD105, CD44 and specially for CD133, but they were negative for the expressions of CD34, CD45 and CD14.

Conclusion: Results of our investigation clearly indicate that a high portion of tumor cells derived from meningioma tissues express most specific markers of stem cells. To understand the significance of this finding, more investigations are required to study the functional properties of CD133+ isolated cells and their role in the process of tumorgenesis of manangioma.

Keywords: Brain Tumor, Stem Cell, CD133+ Cells

Ps-11: Effect of p-Nonylphenol on Viability and Induction of Apoptosis in Rat Bone Marrow Mesenchymal Stem Cell

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Objective: para-Nonylphenol (p-NP) which is used in detergents and preparation of cosmetics as well as plastic food containers is an einvironmental pollutant with toxic effects on living animals. In this study the effects of p-NP on viability and induction of apoptosis in rat bone marrow mesenchymal stem cells (BMCs) was investigated.

Materials and Methods: With the help of flash out techniqe and under sterile conditon, BMCs were extracted in the DMEM contaning 15% of FBS and pen/strep which were expanded till the 3rd passage. The cells were treated with 0, 0.5, 2.5 and 5 μM of p-NP for 21 days (as pilot study) and at the end of treatment period, viability of the cells was investigated using MTT test and trypan blue staining. Then the cells were treated for 21 days with the selected dose (2.5 μM) of p-NP and colony forming ability (CFA) of the cells were determined using colony forming assay, furthermore, foloroscent dyes were used to study the morphology of cells. Comet assay and agarose gel electrophoresis for DNA study, and TUNEL as well as caspase were applied for understanding the cell death. Data were analyzed using one-way ANOVA and the $p<0.05$ was considerd as the level of signifiacans.

Results: Treatment with different doses of p-NP showed

that the significant reduction ($p<0.05$) of viability was dose dependent. In addition significant reduction of CFA ($p<0.05$), neuclei diameter ($p<0.001$), chromatin condensation and cytoplasem shrinkage after treatment with selected dose of 2.5 µM were observed. Comet assay and agarose gel electrophoresis revealed the DNA breakage and positive TUNEL as well as caspase were also seen in the cells treated with selected dose.
Conclusion: Based on the results of this study, low concentration of p-NP caused reduction of viability due to induction of apoptosis. Therefore, long term exposure to this environmental pollutant may have unwanted effects on the health of the BMCs.
Keywords: para-Nonylphenol, Mesenchymal Stem Cell, Cell Viability, Apoptosis, Rat

Ps-12: Low Dose of para-Nonylphenol Causes Impairment of *in vitro* Differentiation of Rat Bone Marrow Mesenchymal Stem Cell to Osteoblasts

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Objective: Previous investigations have indicated the presence of 0.26 and 1.2 µM of para- Nonylphenol (p-NP) in human milk and plasma respectively. The aim of this study was to investigate the effect of 0.5 and 2.5 µM of p-NP on osteogenic differentiation of rat bone marrow mesenchymal stem cells (BMCs).
Materials and Methods: Using flash out techniqe under sterile condition, BMCs were extracted in the DMEM contaning 15% of FBS and pen/strep, and the cells were expanded till the 3rd passage. Then the cells were divided into three groups including negative control (without the ostogenic media), positive control (with the osteogenic media having dexametasone, ascorbic acid and β-glycerol phosphate) and treated group (with the osteogenic media and 0.5, 2.5 µM of p-NP) which were cultured for 21 days. Using MTT assay the viability of the cells was determined and also the cell morphology was studied with the help of pripidium iodide, Hoechst and acridin orang dyes. BMCs calcium concentration as well as activity of alkaline phosphatase were determined using comertial kits after 21 days of osteogenic incubation. In addition quantitative alizarine red measurment was carried out to follow the level of minirilization of BMCs to osteoblasts for 5, 10, 15 and 20 days. Data were analyzed using one-way ANOVA and the means difference was considered significant at the level of $p<0.05$.
Results: Following treatment of the BMCs with 0.5 µM of p-NP, no morphological as well as viability changes were observed whereas treatment with 2.5µM of p-NP casued significant reduction ($p<0.05$) of cell viability and also condensed the chromatin. In addition calcium concentration and activity of alkaline phosphatase were significantly reduced ($p<0.05$) when compared to control and 0.5µM groups. The level of miniralization in term of quantitative alizarin red estimation reduced significantly ($p<0.05$) from the day 10th in the 2.5 µM group as compared to the control and 0.5 µM groups.
Conclusion: Our results showed that the exposure of BMCs during osteogenic differentiation period to low dose of p-NP reduced viability and caused impairment of differentiation of these cells to osteoblasts.
Keywords: Mesenchymal Stem Cell, para-Nonylphenol, Differentiation, Alizarin Red

Ps-13: Can Mesenchymal Stem Cell Ameliorate Motor Dysfunction in Cerebellar Degeneration?

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Objective: The cerebellum has been considered a key structure for the processes involved in sensori-motor integration ultimately leading to motor planning and execution of coordinated movement. Thus, motor deficits and behavioral changes can be associated with cerebellar degeneration.
Materials and Methods: Here, the neurotoxin Pyridine-2,3-dicarboxylic Acid (Quinolinic Acid,QA) used to create partially cerebellum degeneration in adult wistar rats suitable for use in mesenchymal stem cell transplantation studies. Stereotaxicaly administration of QA (0.2 mmol) in the right cerebellar hemisphere (folia VI) caused noticeable motor disturbance in all treated animals. 48 h after causing lesion, rat bone marrow derived mesenchymal stem cells (MSCs) transplanted into damage cerebellar hemisphere. The efficiency of cellular transplantation for improvement of motor disorder was assessed by several motor tests, during seven weeks after engraftment.
Results: These results indicate that transplantation of MSCs can significantly reduce the behavioral abnormalities of these animals.
Conclusion: According to results of this assay, cell therapy by means of bone marrow derived adult stem cells promises for treatment of cerebellar diseases.
Keywords: Cerebellar Degeneration, Mesenchymal Stem Cell, Quinolinic Acid

Ps-14: Effect of PPARγ Agonists and Antagonist on Mouse Embryonic Stem Cells Pluripotency

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Objective: Mouse embryonic stem (mESC) cells are pluripotent cells, can differentiate *in vitro* and *in vivo* into cells representative of the three embryonic germ layers. Proliferation of mESCs is affected by leukemia inhibitory factor (LIF) which is a pleiotropic cytokine that maintains pluripotency of mESCs. Peroxisome proliferator-activated receptor γ (PPARγ) is member of the nuclear receptors, involved in growth and differentiation of many cell types, can be activated in presence of ligands. In this study, we have investigated the effects of agonists and antagonist ligands of PPARγ, on pluripotency of mESCs in the presence and absence of LIF.
Materials and Methods: Stable transformant of mouse embryonic stem cells expression EGFP under regulation of mouse OCT4 promoter , were cultured in the presence and absence of LIF (leukemia inhibitor factor) for 3 days. PPARγ agonists (Rosiglitazone and Ciglitazone) and antagonist (GW9662) were added to media at the final concentration of 1μM. Three day post treating, the expression of EGFP was measured by flow cytometry analysis.
Results: Flow cytometry data were indicated that that expression of EGFP was increased to 60 percent in the presence of LIF . However, PPARγ agonists and antagonist didn't have significant effect on the EGFP expression level. Expression of the marker genes of three embryonic layers did not change significantly upon treating with PPARγ agonists and antagonist.
Conclusion: our data revealed that PPARγ did not exert any effects on pluripotency of mESCs.

Ps-15: Polymorphism of Interleukin-23 Receptor Gene But Not of NOD2/CARD15 Is Associated with Graft-versus-Host Disease after Hematopoietic Stem Cell Transplantation in Children

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Objective: Graft-versus-host disease (GVHD) is a major cause of morbidity and mortality after hematopoietic stem cell transplantation (HSCT). The selection of a suitable donor is the most critical issue in preventing severe GVHD. Recent data suggest that the risk of GVHD does not only depend on human leukocyte antigens (HLA) but also on polymorphisms of genes that influence immune responses.
Materials and Methods: We analyzed the 1142G>A single-nucleotide polymorphism (SNP) in the interleukin-23 receptor gene (IL23R) and three SNPs in the NOD2/CARD15 gene in a cohort of 231 children who underwent allogeneic stem cell transplantation and their respective donors.
Results: No association was observed between any of the NOD2/CARD15 polymorphisms and GVHD in either donor or recipient. Likewise, the IL23R polymorphism in the recipient was not significantly associated with GVHD. We found a significantly reduced incidence of GVHD grade II-IV in patients who were transplanted from a donor with the IL23R polymorphism (5.0% versus 33.3%; p=0.009). There was no case of GVHD grade III-IV if this polymorphism occurred in the donor.
Conclusion: These findings could be particularly relevant for children with inborn metabolic or immunologic disorders who do not benefit from graft-versus-tumor effect, and therefore, selection of a donor with the IL23R polymorphism might be beneficial.
Keywords: Hematopoietic Stem Cell Transplantation, Graft-versus-Host Disease, Children, IL-23R, NOD2/CARD15

Ps-16: Transdifferentiation of Mouse Embryonic Fibroblasts into Cardiomyocytes

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Objective: Various cell populations, such as embryonic stem cells, cord blood cells, and mesenchymal stem cells, have been suggested as a source for cell therapy in curing heart diseases. Transdifferentiation of fully differentiated cells can be use as a potential source of cell therapy. In this study, we showed that heart extract could induce transdifferentiation of mouse embryonic fibroblasts (MEFs) into cardiomyocytes.
Materials and Methods: Mouse embryonic fibroblasts (MEFs) were used as primary source of cells.

Cardiomyocyte extract was prepared from adult mouse cardiomyocytes. MEFs were treated with 5- Azacytidin and Trichostatin A. Some cells were also treated with DMSO that was the dilution solvents of the Azacytidin and Trichstatin A. The treated cells were permeabilized with streptolysin O, and exposed to the mouse cardiomyocyte extract. The cells were cultured for 10 days. Immunocytochemistry technique was performed for α-actinin and cardiac troponin T antibodies as cardiomyocyte markers. The results were compared with control group.

Results: The results of immunocytochemistry for α-actinin antibody revealed that 75% of the cells were treated with azacytidin and trichostatin A were expressed α-actinin 10 days after exposure to the extract. However, 13.3% of the cells were treated with DMSO expressed α-actinin. The results of immunocytochemistry for cardiac troponin T antibody showed no expression of this antibody in all groups.

Conclusion: Mouse embryonic fibroblasts expressed α-actinin, so that, it seems that the cardiomyocyte extract can induce tarnsdifferentiation of mouse embryonic fibroblasts into cardiomyocyte. No expression of cardiac troponin T after 10 days may arise of deficient maturity in reprogrammed-mouse embryonic fibroblasts. This study shows that mouse embryonic fibroblasts have the capability to use in the future research as a source of cells for reprogramming procedures.

Keywords: Reprogramming, Trichostatin A, 5-deoxyazacytidin, Transdifferentiation, Cell Extract

Ps-17: Activation of Adenosine A3 Receptors Potentiates Stimulatory Effects of IL-3, SCF, and GM-CSF on Mouse Granulocyte-Macrophage Hematopoietic Progenitor Cells

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Objective: Adenosine A3 receptor agonist N6-(3-iodobenzyl)adenosine-5'-N-methyluronamide (IB-MECA) was tested from the point of view of potentiating the effects of hematopoietic growth factors interleukin-3 (IL-3), stem cell factor (SCF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and granulocyte colony-stimulating factor (G-CSF) on the growth of hematopoietic progenitor cells for granulocytes and macrophages (GM-CFC) in suspension of normal mouse bone marrow cells *in vitro*.

Materials and Methods: Tested drugs: IB-MECA, granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), stem cell factor (SCF), and interleukin-3 (IL-3). Normal mouse bone marrow for use in *in vitro* testing was obtained from male B10CBAF1 mice. For determination of granulocyte-macrophage colony-forming cells (GM-CFC), a standard *in vitro* cultivation technique was used.

Results: IB-MECA alone induced no GM-CFC growth. Significant elevation of numbers of GM-CFC evoked by the combinations of IB-MECA with IL-3, SCF, or GM-CSF as compared with these growth factors alone was noted. Combination of IB-MECA with G-CSF did not induce significantly higher numbers of GM-CFC in comparison with G-CSF alone. Joint action of three drugs, namely of IB-MECA + IL-3 + GM-CSF, produced significantly higher numbers of GM-CFC in comparison with the combinations of IB-MECA + IL-3, IB-MECA + GM-CSF, or IL-3 + GM-CSF.

Conclusion: These findings, together with those on the ability of IB-MECA to stimulate proliferation of hematopoietic progenitor cells *in vivo* in normal and irradiated mice, give evidence of a significant role of adenosine A3 receptors in regulation of early hematopoietic cells.

Keywords: Adenosine A3 Receptors, Hematopoietic Progenitor Cells, IB-MECA

Ps-18: Age-Dependent Depletion of Human Skin-Derived Precursor Cells

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Objective: A major unanswered question in autologous cell therapy is the appropriate timing for cell isolation. Many of the putative target diseases arise with old age and previous evidence, mainly from animal models, suggests that the stem/progenitor cell pool decreases steadily with age. Studies with human cells have been generally hampered to date by poor sample availability. In recent years, sev- eral laboratories have reported on the existence, both in rodents and humans, of skin-derived precursor (SKP) cells with the capacity to generate neural and mesodermal progenies. This easily obtainable multipotent cell population has raised expectations for their potential use in cell therapy of neurodegeneration. However, we still lack a clear understanding of the spatiotemporal abundance and phenotype of human SKPs.

Materials and Methods: We show an analysis of human SKP abundance and *in vitro* differentiation potential, by

using SKPs isolated from four distinct anatomic sites (abdomen, breast, foreskin, and scalp) from 102 healthy subjects aged 8 months to 85 years.
Results: Human SKP abundance and differentiation potential decrease sharply with age, being extremely difficult to isolate, expand, and differentiate when obtained from the elderly.
Conclusion: Our data suggest preserving human SKP cell banks early in life would be desirable for use in clinical protocols in the aging population
Keywords: Stem Cells, Aging, Human Skin Derived-precursors

Ps-19: Sodium Arsenite Impairs Mineralization by Affecting Calcium Level and Alkaline Phosphatase Activity in Differentiated Rat Bone Marrow Mesenchymal Stem Cells

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Objective: Sodium arsenite is a well known toxic which is presented in drinking water and foods in polluted areas. The mutagenic property of this chemical has been determined and it is categorized in the class I carcinogen. Studies have shown that sodium arsenite causes DNA breakage and hypomethylation as well as hyper acetylation of histones. In addition, this environmental pollutant causes inactivation of enzymes specially those with SH group in their active site. To the best of our knowledge there is no data available to indicate the effect of sodium arsenite on differentiation of mesenchymal stem cells to osteoblasts. As the bone marrow mesenchymal stem cells are a very important source of osteoblasts generation thus the aim of this study was to investigate the effect of 25 nM of sodium arsenite on differentiation of rat bone marrow mesenchymal stem cells (BMCs) to osteoblasts.
Materials and Methods: Using flash out technique under sterile condition, BMCs were extracted in the DMEM containing 15% of FBS and pen/strep then expanded till the 3rd passage and treated with osteogenic media(having dexametasone, ascorbic acid and β-glycerol phosphate) and 25 nM of sodium arsenite for 5, 10, 15 and 21 days. At the end of treatment periods, the cells were washed with tris buffere saline TBS (pH 7.4) then the calcium concentration, and alkaline phosphatase acitivity of the cells were determined at 5, 10, 15 and 21 days of treatments. In addition, using qualitative alizarin red S estimation the level of mineralization of the differentiated cells was determined in treatment days. Furthermore, the morphology of the cells was studied using Hoechst, Propidium Iodide and Acridine Orange after 21 days of the treatment. Data was analyzed using one-way ANOVA and the $p<0.05$ was considered as the level of significance.
Results: Calcium concentration reduced significantly ($p<0.05$) from the day 5 when cells treated with 25 nM of sodium arsenite. Significant reduction of alkaline phosphatase activity was observed in the days 5, 10, 15 and 21 when cells treated with sodium arsenite. Result of this study showed that the mineralization in control group starts from the day 10 and its maximum was observed at day 21, while it significantly reduced ($p<0.05$) when the cells were treated with 25 nM of sodium arsenite.
Conclusion: As the results of this study showed, sodium arsenite impairs the mineralization process by reducing the calcium level and alkaline phosphatase activity in differentiated BMCs. Thus exposure to this chemical might cause the reduction of mineralization which can be considered as the main reason of osteoporosis.
Keywords: Sodium Arsenite, Rat Bone Marrow Mesenchymal Stem Cells, Mineralization, Calcium, Alkaline Phosphatase

Ps-20: Variation of Morphological Character and Induction of Caspase Dependent Apoptosis in Rat Bone Marrow Mesenchymal Stem Cells Following Exposure to Low Dose of Sodium Arsenite

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Objective: Based on the United State FDA recommendation, arsenite trioxide is used in the treatment of promyelocytic leukemia, where the presence of ≤5μM of this chemical has been reported in the blood serum of these patients. Different studies have been conducted on toxicity of sodium arsenite, but no report has given attention to the effect of very low concentration in long term with respect to stem cells. With this respect, the aim of this study was to investigate the probable effects of sodium arsenite on viability, morphology and cell death of rat bone marrow mesenchymal stem cells (BMCs), as a model of stem cells response to the toxicity of arsenite trioxide.
Materials and Methods: Using flash out technique under sterile conditon, BMCs were extracted in the DMEM contaning 15% of FBS and pen/strep which were expanded till the 3rd passage. The cells were treated with 0, 0.4, 0.8, 25 and 75 nM of sodium arsenite for 21 days (as pilot study) and at the end of treatment period, viability of the cells was investigated using MTT test and trypan blue staining. Then the cells were treated with the selected dose (0.8 nM) for determination of colony forming ability (CFA) in 7, 14 and 21 days where as for population doubling number (PDN) cell counting was carreid out on 5, 10,

15 and 21 days of treatment, furthermore, fluorescent dyes were used to study the morphology of the cells. Comet assay and agarose gel electrophoresis for DNA study, and TUNEL as well as caspase were applied for understanding the cell death. Data were analyzed using one-way ANOVA and the $p<0.05$ was considerd as the level of significance.
Results: Treatment of the cells with sodium arsenite for 21 days showed a significant reduction in viability ($p<0.05$) and nuclei diameter ($p<0.05$) as well as chromatin condensation and cytoplasm shrinkage as compared to control. In addition sodium arsenite caused significant reduction ($p<0.05$) of colony diameter and PDN in day 21th, where as number of the colony reduced significantly ($p<0.05$) in 7, 14 and 21 days of treatment. Comet assay and agarose gel electrophoresis revealed the breakage of DNA and positive TUNEL as well as caspase were also seen in the cells treated with selected dose.
Conclusion: Our results showed that the sodium arsenite much below the concentration found in the blood serum of the patients treated for malignancy , affect the vital factors of BMCs and induced caspase dependent apoptosis.Therefore, long term exposure to this medication may have unwanted effects on the health of the BMCs or other adult stem cells.
Keywords: Bone Marrow Mesenchymal Stem Cells, Sodium Arsenite, Cell Viability, Apoptosis, Rat

Ps-21: Differentiation Of Human Menstrual Blood Derived Stem Cells into Chondrocyte-Like Cells on A Nanofibrous Scaffold

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Objective: Recently, menstrual blood derived stem cells (MenSCs) have been identified as unique population of stem cells created enormous promise for the cell based therapies. However, much more studies are needed to introduce these cells suitable for tissue engineering and regenerative medicine. In this study, chondrogenic differentiation of MenSCs has been evaluated on a nanofibrous scaffold with high infiltration capacity.
Materials and Methods: MenSCs were isolated of menstrual blood by discontinuous density gradient centrifugation and plastic adherence. After characterization of isolated cells, cells differentiation into chondrocytes was investigated on a nanofibrous scaffold with specific growth and differentiation factors. The scaffold was prepared from polyethersulfoune and surface modified by plasma treatment and collagen grafting.
Results: The isolated cells were positive for stem cells markers such as CD9 (95%), CD29 (99%), CD44 (99%) and CD73 (98%) as judged by flow cytometric analysis. Based on scanning electron microscope images, the scaffold had a highly porous scaffold that cells adhered, penetrated and proliferated properly. The scaffold contained an extensive cartilage-like extracellular matrix with more than 50% greater glycoseaminoglycan content than control MenSCs differentiated in standard cell pellet culture. Proteoglycan production by cell differentiated on the scaffold was more demonstrated by alcian blue staining. Unlike non differentiated MenSCs, imunoreactivity of cells with monoclonal antibodies against for collagen type II was strongly positive in cells differentiated on the scaffold.
Conclusion: The evidences presented in this study introduce MenSCs as an applicable stem cell population suitable for cartilage tissue engineering.
Keywords: Menstrual Blood Derived Stem Cells, Chondrocyte, Differentiation, Nanofiber

Ps-22: Expression of Integrins α2β1 and α5β1 in Dedifferentiated NIH3T3 Cells by Mouse Embryonic Stem Cell Extract

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Objective: Generation of patient specific stem cell is among the ultimate goals in regenerative medicine. On the other hand contact between matrix proteins and integrin receptors adjust many cell's function such as migration during embryogenesis, immune responses and tumour invasion. In this study we de-differentiated NIH3T3 cells by mouse Embryonic Stem Cell (mESC) extract in order to determine the expression level of pluripotency markers as well as α2β1 and α5β1 integrins.
Materials and Methods: Mouse embryonic stem cell extracts were prepared and NIH3T3 cells were reprogrammed by mESC extract. Generated iPS (induced Pluripotent Stem cells) colonies were picked up in the sterile conditions and checked for the pluripotency by alkaline phosphatase kit, Reverse Transcriptase PCR (RT-PCR) for oct3/4 and immunocytochemistry for oct3/4, sox2 and nanog. Then the expression level of oct3/4, α2β1 and α5β1 integrins was studied by RT-PCR.
Results: NIH3T3 cells treated with mESC extracts

showed noticeable changes in cell morphology as early as day 3 post-treatment forming colonies similar to typical mESC morphology by day 10, after three passages. Alkaline phosphatase test and immunocytochemistry staining were performed for the iPS colonies. The results indicated that these colonies not only showed the alkaline phosphatase activity, but also the expression of sox2, oct3/4 and nanog by their specific antibodies in immunocytochemistry. Also RT-PCR results revealed the expression of oct3/4 α2β1 and α5β1 integrins in iPS cells.

Conclusion: These data provide evidence for the generation of iPS cells from differentiated somatic cells by mESC extract also the expression of α2β1 and α5β1 integrins in iPS cells.

Keywords: Somatic Cell de-differentiation, NIH3T3 Cells, Integrins, mESC Extract

Ps-23: CXCR1 and CXCR2 Receptors Participate in Regulation of Expansion and Differentiation of Umbilical Cord Blood CD133+ Stem Cells

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Objective: Umbilical cord blood (UCB) has been recognized as an alternative source of hematopoietic stem cells (HSCs) for transplantation. However, low number of HSCs in UCB, is a major problem that limits wide use of UCB transplantation. Furthermore, Patients transplanted with cord blood have higher risk of graft failure and delay hematopoietic cells recovery compared with those transplanted with bone marrow. Two overcome on these limitations, many studies have performed with the aim of achieving optimal expansion and differentiation of UCB stem cells in the presence of different combination of grows factors. Some studies have been shown that chemokines regulate hematopoiesis via different effects. In this study, we aimed to survey the regulatory role of chemokine receptors –CXCR1 and CXCR2 – which are expressing on umbilical cord blood CD133+ cells, via inhibition of function of these receptors.

Materials and Methods: UCB CD133+ cells were purified using magnetic cell sorting method and were cultured in serum free liquid medium, in the presence or absence of neutralizing anti-CXCR1 and/or anti-CXCR2 in combination with a cytokine cocktail including IL3, IL6, SCF, and TPO. Flow cytometric analysis was performed for determination of CD133, CXCR1 and CXCR2 receptors expression on days 0, 7, and 12. Total number of grown cells was counted and absolute number of CD133+ cells was calculated on days 7 and 12. Cell viability was evaluated using dye exclusion assay and 7-AAD. Furthermore, expression of CD41 and CD61 evaluated as megakaryocytic differentiation markers.

Results: Our results showed that CXCR1 and CXCR2 receptors express on UCB CD133+ cells and their expression, significantly increase in period of culture on days 7 and 12($p<0.05$). The absolute number of CD133+ cells decreased after inhibition of CXCR1 or CXCR2, but this decrees was not significant ($p>0.05$). Interestingly, inhibition of CXCR1 and CXCR2 receptors together, induced significant decrees in absolute number of CD133+ cells compared with control group both on days 7 and 12($p<0.05$). Based on these results, simultaneous inhibition of CXCR1 and CXCR2 receptors inhibit significantly the stimulatory effect of IL3, IL 6, TPO and SCF on *ex vivo* expansion of CD133+ cells while enhance differentiation to megakaryocyte progenitor cells as an increase in the expression of megakaryocytic markers; CD41 and CD61($p<0.05$).

Conclusion: Expression of CXCR1 and CXCR2 receptors on hematopoietic stem cells is crucial for regulation of hematopoisis. Considering with delay in platelet recovery after UCB transplantation, attention to this regulatory effect could be novel approach for overcoming on limitations of use of umbilical cord blood for transplantation.

Keywords: Umbilical Cord Blood, CD133+ Cells, CXCR1, CXCR2

Ps-24: Induction of Intestinal Stem Cells in Mouse Embrionic Stem Cell

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Objective: Embryonic stem (ES) cells have been proposed as candidates for cell replacement therapy in patients with intestinal failure because these cells can be expanded indefinitely without losing their pluripotent phenotype. We investigated the differentiation capacity of mouse ES cells into gut-like structures (ES-guts), including intestinal stem cells, and defined culture conditions for efficient induction of formation of these structures.

Materials and Methods: We first examined whether mouse ES cells can differentiate into gut-like structures. In the absence of LIF, ES cells differentiate spontaneously and aggregate into EBs in hanging-drop cultures. When EBs are allowed to attach to tissue culture slides, visceral endoderm develops and spreads

around a core of primitive ectoderm cells. To characterize these ES-guts, immunohistochemical analysis of EBs was performed on day 24 of differentiation. We next examined gene expression in ES-guts. ES-guts were isolated from day 28 EBs with a scalpel under phase-contrast microscopy. After extraction of total RNA from ES-guts and other areas of the same EBs, expression of several endodermal markers was analyzed by reverse transcription (RT)-PCR. Because it is difficult to culture ES-guts for more than 4 weeks *in vitro*, we attempted to grow these structures *in vivo* to evaluate their developmental potential. ES-guts were isolated under a phase-contrast microscope from EBs on day 21 of differentiation, then transplanted under the kidney capsules of SCID mice. Three weeks after transplantation, kidneys were harvested and analyzed histologically. We next examined the effect of SR culture conditions on expression of endoderm-associated genes in differentiating EBs by RT-PCR. To confirm that SR conditions promote generation of intestinal stem cells in differentiating EBs, Western blot analysis of Msi-1 was performed.

Results: ES cell-derived gut-like structures (ES-guts) were reproducibly induced in developing embryoid bodies (EBs) by day 21 of differentiation culture. ES-guts contained an endodermal epithelium, a smooth muscle layer, interstitial cells of Cajal, and enteric neurons and showed spontaneous contraction. Transplantation of ES-guts under the kidney capsules of immunodeficient mice induced formation of highly differentiated epithelium composed of absorptive cells and goblet cells in the grafts. Immunoreactivity for Musashi-1, a marker of intestinal stem cells, was detected in 1.9% of the columnar epithelial cells in the graft. Culture with 0.1% dimethyl sulfoxide increased the numbers of ES-guts in EBs, and serum-replacement (SR) culture, in comparison to standard ES culture containing 15% serum, increased the area ratio of ES-guts to EBs. SR culture also promoted maturation of epithelium to form a single layer of columnar epithelial cells, including absorptive cells and goblet cells. Expression of Musashi-1 mRNA and protein was significantly enhanced when EBs were cultured under SR conditions.

Conclusion: SR conditions efficiently induce formation of ES-guts and promote differentiation of epithelium, including intestinal stem cells. These results suggest the feasibility of cell-based therapy for intestinal failure based on ES cell culture systems

Keywords: Mouse Embryonic Stem Cell, Embryoid Body, Gut, Serum Replacement, Musashi-1

Ps-25: Optimization Of CD133+ Stem Cell Isolation From Human Cord Blood

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Objective: Hematopoiesis is a highly regulated and hierarchical process wherein hematopoietic stem cell (HSCs) differentiates into mature hematopoietic cells. It is a process controlled by complex interactions between numerous genetic processes in blood cells and their environment. HSCs can be collected from mobilized peripheral blood (PB), bone marrow (BM) and cord blood (CB). Lately, CB has been increasingly utilized because it is readily available, HLA mismatches are better tolerated and there is a decreased risk of graft-versus-host disease when using CB-derived HSCs when compared to the other sources. Even though the cell content of CB is limited, it has a higher frequency of progenitor cells compared to PB or BM. However, enrichment of stem cells is challenging due to the lack of stem cell-specific markers and gentle protocols for the isolation of highly pure stem cell fractions

Materials and Methods: Umbilical CB was obtained from informed and consenting donors and CD133+ stem cell were isolated according to optimized method

Results: In this study, immunomagnetic cell sorting protocols to purify CD133+ from fresh cord blood was optimized. The selected cells were highly viable having substantial colony-forming potential.

Conclusion: The optimized protocols enable rapid enrichment of highly pure hematopoietic stem cells from fresh cord blood

Keywords: Stem Cell, MACS, CD133+

Ps-26: Improvement in Cardiac Function Following Transplantation of Human Umbilical Cord Matrix-Derived Mesenchymal Cells

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Objective: Loss of cardiomyocytes after myocardial infarction is a causative factor in progression to heart failure because adult cardiomyocytes have essentially no regenerative capacity. Pathologic ventricular remodeling ensues as damaged myocardium is replaced by fibrous scar resulting in Ventricular function loss. In an effort to replace cardiomyocytes lost after infarction, cellular transplantation has been introduced as a potential therapy. The aim of present study was to investigate the potency of human Mysenchymal stem cells isolated from the human umbilical cord (hUCM).

Materials and Methods: Isolated hUCMs were treated with 5-azacytidine for cardiomyocyte differentiation.

Thirty five adult Female New Zealand rabbits were divided into 5 groups: 1, intact; 2, control; 3, PBS; 4, undifferentiated human Umbilical Cord cells (hUCM) and 5, differentiated human Umbilical Cord cells (dhUCM). After anesthesia, rabbits were mechanically ventilated and, their hearts were exposed by means of a left thoracotomy through the fourth and fifth intercostals space. The proximal end of the left anterior descending coronary artery was ligated. After ligation for 1 h, 5×106 human Umbilical Cord Matrix cells in 50 µl PBS, 5×106 cardiomyocyte-like cells in 50 µl PBS and 50 µl PBS was carefully injected at the border area of the ischemic myocardium respectively in hUCM, dhUCM and PBS groups. Echocardiography, general pathology and immunohistochemistry were performed in order to detect any myocardial regeneration and improvement of cardiac function.
Results: 5-azacytidine Treated cells in comparison to non-treated group were positive for F-actin and negative for troponin I. The rate of ejection fraction, diameter of left ventricular wall at the end of diastole and systole was significantly different in cell transplanted groups when compared to PBS (p=0/001) and control (p=0/002) groups. A significant reduction in the fibrous tissue and fibroblasts occurred in cell-transplanted groups versus PBS and control groups. Surviving hUCM and dhUCM were identified by BrdU positive spots in infarcted region and few transdifferentiated cells into cardiomyocytes were characterized with a positive cardiac phenotype: troponin I and human F actin.
Conclusion: Our data show that mesenchymal stem cells from Wharton's jelly may improve heart function and undergo differentiation to cardiomyocyte – like cells following transplantation. These cells may provide an appropriate source of cells for cell therapy procedures.
Keywords: Human Umbilical Cord Matrix Cells, Cardiomyocytes, 5-Azacytidine, Heart Function

Ps-27: Treatment of Type 1 Diabetes with Adipose Tissue-Derived Stem Cells Expressing Pancratic Duodenal Homeobox 1

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Objective: To treat a type 1 diabetic rat model with Adipose Tissue-Derived Stem Cells (ADSC) Expressing Pancratic Duodenal Homeobox 1 (Pdx1)
Materials and Methods: Human or rat ADSC were transduced with Pdx1, examined for pancreatic cell characteristics, and transplanted into type 1 diabetic rats.
Results: ADSC tranduced with Pdx1 exhibited beta cell characteristics. Transplantation of Pdx1-expressing ADSC partially reverted the progress of type 1diabetes
Conclusion: ADSC-expressing Pdx1 is promising for treating type1 diabetes
Keywords: ADSC, Diabetes, Pdx1

Ps-28: Effects of Ectopic Nanog and Oct4 Overexpression on Mesenchymal Stem Cells

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Objective: Mesenchymal stem cells (MSCs) represent a source of pluripotent cells that are already in various phases of clinical application. However, the use of MSCs in tissue engineering has been hampered largely due to their limitations including low proliferation, finite life span and gradual loss of their stem cell properties during *ex vivo* expansion. Nanog and Oct4 are key transcription factors essential to the pluripotent and self-renewing phenotypes of undifferentiated embryonic stem cells. To determine whether Nanog and Oct4 improve human bone marrow-MSC quality,
Materials and Methods: We therefore established stable Nanog and Oct4 overexpressing MSCs using a lentiviral system.
Results: Our results showed that overexpression of Oct4 and Nanog promoted cell proliferation and enhanced colony formation of MSCs. In differentiating MSCs, Nanog and Oct4 overexpression had converse effects on adipogenesis of MSCs, Nanog overexpression slowed down adipogenesis whereas Oct4 overexpression improved adipogenesis. Nanog and Oct4 overexpression both improved chondrogenesis. Microarray data showed many differences in transcriptional targets in undifferentiated MSCs overexpressing Nanog and Oct4.
Conclusion: These results provide insight into the improvement of the stemness of MSCs by genetic modification with stemness-related genes.
Keywords: Mesenchymal Stem Cells, Nanog, Oct4, Overexpression

Ps-29: Evidence for Transcriptional Regulation of the Glucose-6-Phosphate Transporter by HIF-1 alpha: Targeting G6PT with Mumbaistatin Analogs in Hypoxic Mesenchymal Stromal Cells

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Objective: Mesenchymal stromal cell (MSC) markers are expressed on brain tumor-initiating cells involved in the development of hypoxic glioblastoma. Given that MSCs can survive hypoxia and that the glucose-6-phosphate transporter (G6PT) provides metabolic control that contributes to MSC mobilization and survival, we investigated the effects of low oxygen (1.2% O2) exposure on G6PT gene expression.
Materials and Methods: Bone marrow-derived MSCs were isolated from the whole femur and tibia bone marrow. Nuclear extracts from MSCs or MSC-HIF-1a cells were isolated using the NE-PER Nuclear and cytoplasmic extraction kit (Pierce) and proteins were separated by SDS-PAGE. Boyden chambers were used to assess cell migration. Total RNA was extracted and cDNA syynthetized. qPCR was used to monitor gene expression profiles.
Results: We found that MSCs significantly expressed G6PT and the glucose-6-phosphatase catalytic subunit beta, whereas expression of the glucose-6-phosphatase catalytic subunit alpha and the islet-specific glucose-6-phosphatase catalytic subunit- related protein was low to undetectable. Analysis of the G6PT promoter sequence revealed potential binding sites for hypoxia inducible factor (HIF)-1alpha and for the aryl hydrocarbon receptor (AhR) and its dimerization partner, the AhR nuclear translocator (ARNT), AhR:ARNT. In agreement with this, hypoxia and the hypoxia mimetic cobalt chloride induced the expression of G6PT, vascular endothelial growth factor (VEGF), and HIF-1alpha. Gene silencing of HIF-1alpha prevented G6PT and VEGF induction in hypoxic MSCs whereas generation of cells stably expressing HIF-1alpha resulted in increased endogenous G6PT gene expression. A semisynthetic analog of the polyketide mumbaistatin, a potent G6PT inhibitor, specifically reduced MSC-HIF-1alpha cell survival.
Conclusion: Collectively, our data suggest that G6PT may account for the metabolic flexibility that enables MSCs to survive under conditions characterized by hypoxia and could be specifically targeted within developing tumors.
Keywords: Mesenchymal Stem Cells, Metabolic Adaptation, Brain Tumors, Hypoxia, Anticancer Therapy

Ps-30: Decellularization of Rat Heart for Preparing a Biologic Scaffold to Organ Engineering

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Objective: Biologic scaffolds composed of extracellular matrix (ECM) or components of ECM have been successfully used in regenerative medicine for the reconstruction of functional tissues. The tissues and organs which ECM is harvested include skin, lower urinary tract, small intestine and musculoskeletal tissues, among others. Recent studies have shown that tissue/organ specific ECMs can support site appropriate cell phenotype and lineage-directed differentiation. It is intuitive that the native 3-dimentional architecture and microenviromental niche provided by the extracellular matrix are important for cell attachment and differentiation. By decellularizing the entire organ, most of the 3D structure of the ECM can be retained to provide a native framework for organ reconstruction. The objective of this study was to prepare a native scaffold from rat heart in order to seeding of stem cells in it for make of bioartificial heart in future.
Materials and Methods: After killing of rat and its systemic heparinization through the left femoral vein, a median sternotomy allowed us to open the pericardium. We then removed the retrosternal fat body, dissected the ascending thoracic aorta and ligated its branches. After transecting the caval and pulmonary veins, the pulmonary artery and the thoracic aorta, we removed the heart from the chest. A prefilled 1.8-mm aortic cannula inserted into the ascending aorta allowed retrograde coronary perfusion. Heparinized PBS at a coronary perfusion pressure of 77.4 mm Hg for 15 min followed by 1% SDS (Invitrogen) in deionized water for 8 h served as the perfusate. This was followed by 15 min of deionized water perfusion and 30 min of perfusion with 1% Triton-X100 (Sigma) in deionized water. We used antibiotic-containing PBS (100 U/ml penicillin-G; Gibco), 100 U/ml streptomycin (Gibco) to perfuse the heart for 124 h. For histology, we fixed, paraffin-embedded and sectioned heart following standard protocols. We cut hearts into 5-mm sections, stained them with Masson's trichrome and photographed the sections on a microscope. We stained the specimen with Hoechst for nuclear staining.
Results: To create a whole-organ scaffold with intact three-dimensional geometry and vasculature, we attempted to decellularize rat heart by coronary perfusion with detergents, which have been shown to generate acellular scaffolds for less complex tissues, by direct immersion. The use of SDS for full removal of cellular constituents was best treatment. Antegrade coronary SDS perfusion over 8 h yielded a fully decellularized construct. Histological evaluation revealed no remaining nuclei or contractile elements.
Conclusion: Immersion decellularization has been used previously to create scaffolds in various thin (nonperfused) cardiovascular tissues including vessel wall, pericardium and valve leaflets. Decellularization of whole heart, first time was reported by Ott et al. in 2008. Using perfusion decellularization, we produced a complex, biocompatible cardiac ECM scaffold with a perfusable vascular tree, patent valves and a four-chamber-geometry template for biomimetic tissue

engineering. Reseeding of decellularized heart is possible both by intramural injection of adult stem cells and by perfusion of endothelial cells into the vascular conduits. These researches are studying in our laboratory.
Keywords: Decellularization, Scaffold, Rat, Heart, Recellularization

Ps-31: Effect of Bone Morphogenetic Protein-4 on the Gene Expression of β1 Integrin of Mouse Embryonic Stem Cell

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Objective: To determine the effect of bone morphogenetic protein 4 (BMP4) on the differentiation of ESCs into germ cells, β1 integrin expression that express during germ cell development was calculated.
Materials and Methods: ESCs were cultured in DMED containing 20% fetal bovine serum (FBS) for 1 day in order to embryoid body (EB) formation (the first step of germ cell induction) and then cultured for 4 days in the presence or absence of 5 ng/ml BMP4 (the second step of germ cell induction). The expression of β1 integrin was calculated in the first and second steps of germ cell induction by quantitative RT-PCR.
Results: Quantitative RT-PCR results showed that β1 integrin was expressed in a higher significant rate in BMP4-treated cells compared to BMP4-free group.
Conclusion: Quantitative RT-PCR was used to estimate the level of germ cell gene expression. The results confirmed that the addition of 5ng/ml BMP4 improve the differentiation germ cells from mouse ESCs.
Keywords: Embryonic Stem Cell, BMP4, Germ Cell, β1 Integrin

Ps-32: Assessment of Expression Stra-8 Gene in Primordial Germ Cells Derived Bone Marrow Mesenchymal Stem Cell after Treatment with Different Doses of Bone Morphogenetic Protein 4

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Objective: In this study, we investigated the expression of Stra-8 as one of the specific genes in primordial germ cells after treatment with different doses of bone morphogenetic protein 4 (BMP4) on primordial germ cells which have been derived from Bone Mesenchymal Stem Cells(BMSCs).
Materials and Methods: Following isolation of BMSCs from male mouse femur and tibia, cells were cultured in medium for 72 hours. After 4 passages, spindle shaped cells were prepared for characterization of mesenchymal cell surface markers, CD44 and CD45 which were analyzed using flowcytometery and immunocytochemistry. Then cells were differentiated to osteogenic and adipogenic leanages by defined medium. 30000 cells of BMSCs were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing different doses of BMP4 (0, 0.5, 1, 5, 25 and 50 ng/mL) for 4 days. Stra8 gene expression, proliferation rate and viability rates in BMP4-treated cells were evaluated. The mean number of whole cells and living cells were considered as proliferation and survival rates respectively; Stra8 gene expression in the different doses of Bmp4 was also examined. Data analysis was done with ANOVA test.
Results: The results revealed that Stra-8 expression in mediums containing 25 and 50ng/mL of BMP4 increased significantly ($p \leq 0.05$). Proliferation rate and viability percent were raised significantly by adding 5 ng/ml of BMP4 and there were decreased to the lowest rate after adding 0.5 and 50 ng/ml BMP4 ($p \leq 0.05$).
Conclusion: The results suggest that addition of 25ng/ml BMP4 had the best effects on the expression of Stra-8 gene in primordial germ cells derived from bone marrow mesenchymal stem cells.
Keywords: Mesenchymal Stem Cells, BMP4, Stra 8 Gene

Ps-33: Gene Expression Changes during Chondrogenic Differentiation of Human Mesenchymal Stem Cells Induced by Synovial Fluid

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Objective: Human bone marrow mesenchymal stem cells (hMSCs) can differentiate into several types of mesenchymal cells, inducing osteocytes, adipocytes, and chondrocytes. Until now, many protocol for inducing chondrocyte-differentiation in hMSCs *in vitro* have been reported. In this study, we induced differentiation into chondrocyte phenotype in the MSCs population by new protocol. Our objective was to study the effects of

synovial fluid on chondrogenic differentiation of hMSCs in culture. synovial fluid could promote expression of chondrogenic markers, Sox9 and collagen II mRNA. Chondrogenesis was induced by synovial fluid, which encourage tissue engineering applications of MSC in chondral defects, as the natural environment in the joint is favorable for chodrogenic differentiation.

Materials and Methods: hMSCs (passage2) were plated in 6-well plates in DMEM containing 1% FBS .To induce chondrogenic differentiation, the cells were treated with Synovial fluid. Differentiation along the chondrogenic lineage was documented by Sox9 and type II collagen expression for 21 days. The expression of the identified genes was confirmed by RT-PCR and cells staining.

Results: Human MSCs cultured in monolayer and treated with SF exhibited a more rounded shape than the cells from the control group. The expression of chondrocyte-specific genes that occurred following SF treatment was found to be dose-dependent and time-dependent. We checked the expression of well-known cartilage-specific genes. An expression analysis for the known chondrogenic markers Sox9 and Col2a1 using RT-PCR in differentiated hMSCs was performed. In this manner, we showed that Sox9 and Col2a1 were induced during the course of hMSCs differentiaton into chondrogenic lineage .

Conclusion: Our results showed that hMSCs treatment with 200µl/ml Synovial Fluid differentiated to chondrocytes . Phenotype of cells and gene expression changed during this differentiation. These data confirmed that MSCs can exhibit chondrocyte differentiation potential *in vitro*, depending on the protocols of inducement.

Keywords: Mesenchymal Stem Cells, Synovial Fluid, Differentiation, RT-PCR

Ps-34: A novel Pax6 Negative Neural Precursor Cell Line Derived Human Embryonic Stem Cell

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Objective: Embryonic stem (ES) cells can differentiate into all cell types and have been used extensively to study factors affecting neuronal differentiation. Neural precursor cells derived ES cells lines have the potential to provide useful *in vitro* models for the study of gene function during neuronal differentiation. Recently, mouse ES cell lines lacking the neurogenic transcription factor Pax6 were reported; neurons derived from these Pax6-/- ES cells died rapidly after neuronal differentiation *in vitro*.

Materials and Methods: NPCs were derived from hESCs (hESCs induced by bFGF, Noggin and Retinoic Acid during 2weeks).NPCs were analyzed by flowcytometry, immunocytochemitry, Real time RT-PCR, Karyotype and patch clamp analysis. These cells differentiated *in vitro* for 35days and after analyzed.

Results: Here we report the derivation of new lines of Pax6-/- NPC- ES cells and the assessment of their ability to survive and differentiate *in vitro*. Neurons derived from our new Pax6-/- line were viable and continued to elaborate processes in culture under conditions that resulted in the death of neurons derived from previously reported Pax6-/- NPC- ES cell lines. The new lines of Pax6-/- NPC- ES cells showed reduced neurogenic potential, mimicking the effects of loss of Pax6 *in vivo*

Conclusion: We suggest that loss of Pax6 from ES cells reduces their neurogenic capacity but does not necessarily result in the death of derived neurons. We offer these new line as additional tools for those interested *in vitro* NPC-ES cell models of Pax6 function during neuronal differentiation, embryonic and postnatal development.

Keywords: Neural Precursor, Embryonic Stem Cell, Pax6, Differentiation

Ps-35: Effect of Mesenchymal Stem Cells on Gene Expression of Breast Cancer Cells

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Objective: Stem cells may have inhibitory effects on tumor cell growth and apoptosis. However, it is unknown whether Stem cells regulate the biological functions of breast cancer cells, especially gene expression.

Materials and Methods: We co-cultured mesenchymal stem cells and breast cancer cells in serum-free conditioned medium and detect gene expression by RT-PCR.

Results: Down-regulation of bone morphogenetic protein 4 (BMP4), Bcl-2, and c-Myc and upregulation of PTEN occurred.

Conclusion: Mesenchymal stem cells could inhibit the tumorigenicity of breast cancer cells, by upregulation of tumor suppressor genes and down-regulation of oncogenes.

Keywords: Oncogenes, Tumor Suppressor Genes, Breast Cancer, Stem Cell

Ps-36: Induction of Apoptosis in Cancer Cells by Mesenchymal Stem Cells

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Objective: Several studies have demonstrated the suppresive effects of mesenchymal stem cells. Cell-cell contact inhibition and secretion of suppressive soluble factors have been suggested in this regard. We aimed to investigate if mesenchymal stem cells could induce apoptosis in breast and colon cancer cells during co-culture.
Materials and Methods: We used mesenchymal stem cells enriched in CD44 and CD133 cells for our studies to induce apoptosis in breast and colon cancer cells co-cultured with them. TUNEL assay was carried out to determine the degree of apoptotic induction.
Results: We observed that breast and colon cancer cells co-cultured with mesenchymal stem cells had a higher number of TUNEL-positive characteristics compared to the controls.
Conclusion: These results demonstrate that mesenchymal stem cells have therapeutic potential with possible clinical implications.
Keywords: Colorectal Cancer, Breast Cancer, Stem Cell, Apoptosis

Ps-37: Upregulation of Tumor Suppressor Gene Expression in Colorectal Cancer Cells By Mesenchymal Stem Cells

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Objective: PTEN (phosphatase and tensin homologue deleted on chromosome ten) is a tumor suppressor gene implicated in a wide variety of human cancers, including colorectal cancer. PTEN is a major negative regulator of the PI3K/Akt signaling pathway. Most human colorectal cancer show high levels of activated Akt, and decreased expression of PTEN protein. The unique ability of mesenchymal stem cells to track down tumor cells makes them as potential therapeutic agents. Based on this capability, new therapeutic approaches have been developed using mesenchymal stem cells to cure colorectal cancer.
Materials and Methods: In order to study the mechanisms by which migration of colorectal cells can be inhibited by the upregulation of the PTEN gene, we studied colorectal cell line alone and in co-culture with mesenchymal stem cells .
Results: Co-cultures of colorectal cells showed increased expression of PTEN as evaluated by immunofluorescence assay. Upregulation of PTEN gene is correlated with the downregulation of Akt. These results have been confirmed by reverse-transcription based PCR analysis of PTEN and Akt genes. Upregulation of PTEN resulted in the inhibition of migration capability of colorectal cells under *in vitro* conditions.
Conclusion: Our study indicated that upregulation of PTEN by stem cells in colorectal cancer cells downregulated Akt signaling pathway molecule. This resulted in the inhibition of migration of the colorectal cells. Taken together, our results suggest stem cells as a therapeutic agent in treating colorectal cancer.
Keywords: PTEN, Tumor Suppressor Gene, Colorectal Cancer, Stem Cell

Ps-38: Calreticulin Inhibits Commitment to Adipocyte Differentiation

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Objective: To show whether or not calreticulin modulates adipogenesis
Materials and Methods: ES cells
Results: Calreticulin, an endoplasmic reticulum-resident protein, affects many critical cellular functions including protein folding and calcium-homeostasis. Using embryonic stem cells and 3T3-L1 preadipocytes, we show that calreticulin modulates adipogenesis. We find that calreticulin-deficient cells show increased potency for adipogenesis when compared to wild-type or calreticulin overexpressing cells. In the highly adipogenic crt-/- cells, the endoplasmic reticulum lumenal calcium concentration was reduced. Increasing the endoplasmic reticulum lumenal calcium concentration lead to a decrease in adipogenesis. In calreticulin-deficient cells, the calmodulin/CaMKII pathway was upregulated and inhibition of CaMKII reduced adipogenesis. Calreticulin inhibits adipogenesis via a negative-feedback mechanism whereby the expression of calreticulin is initially upregulated by PPARγ. This abundance of calreticulin subsequently negatively regulates the expression of PPARγ, LPL, C/EBPα and aP2.
Conclusion: Calreticulin appears to function as a Ca2+-dependent molecular switch that regulates commitment to adipocyte differentiation by preventing the expression and transcriptional activation of critical pro-adipogenic transcription factors.
Keywords: Calreticulin, Adipogenesis, ES Cells

Ps-39: Stimulation of Osteogenesis in "Mesenchymal Stem Cells" by Combined Bone Morphogenetic Protein-2, Dexamethasone and Dihydroxy-vitamin D

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Objective: *In vitro* experiments with Mesenchymal Stem

Cells (MSC) are beneficial for better understanding of bone biology and bone formation. In this investigation, we carried out experiments on human MSC obtained from bone marrow of a healthy 44 year old male. Our aim is to examine and stimulate hMSC differentiation into osteoblasts by different inducers either in serum or serum free medium.

Materials and Methods: HMSC were treated with either 10−8M 1, 25-dihydroxyvitamin D (vitamin D), 10−7M Dexamethasone, 100 ng/ml BMP-2 or in combinations in 2D cultures and as a new work in 3D polyurethyane scaffolds. The osteoblast induction was determined by alkaline phosphatase (Alp) activity, calcium and collagen production, osteopontin, collagen type 1 and Msx2 expressions.

Results: BMP-2, Dex and Vit.D treatment increased Alp activity, Msx2 and osteopontin expressions but BMP-2 alone had less effect on Alp activity in 3D cultures in serum medium however Alp activity was increased with BMP-2 alone in serum free conditions in 2D and 3D cultures. According to our results Alp Activity was highest with combination of all three treatments either in serum or serum withdrawl in both 3D and 2D cultures of hMSC. BMP-2 alone or in combination with Dex and Vit.D enhanced calcium and collagen production in both serum and serum free medium. In the end, *in vitro* mineralization was significantly increased in hMSC cultures treated with these inducers. Cell proliferation was greater increased by BMP-2 than Dex treatment either in serum free or serum medium.

Conclusion: To recapture briefly, hMSC can be differentiated into osteoblast with Dex, BMP-2 and Vit.D inducers *in vitro*. However, these three stimulators produce different characteristics osteoblastic phenotypes with different levels of early genes expressions in serum and serum free medium in either 2D or 3D hMSC cultures which indicates the interactions with other signalling pathways during this induction.

Keywords: Mesenchymal Stem Cells, 1, 25 Dihydroxy-vitamin D, Dexamethasone, Bone Morphogenetic Protein-2, Alkaline Phosphatase

Ps-40: Effect of Inhibitors of Signalling Pathways on Derivation of Embryonic Stem Cell Lines from Bovine IVP Embryos and Parthenotes

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Objective: Efficient isolation and maintenance of pluripotent embryonic stem cell (ESC) lines are important for the application of ESCs in research and biotechnology. The aim of this research was to assess the efficiency of 3i medium containing 3 small-molecule inhibitors (3i), SU5402, PD184352 and CHIR99021, which affect ERK and Wnt-β-catenin signalling pathways, on isolation of ESCs from bovine IVP embryos and parthenotes.

Materials and Methods: Bovine embryos were produced by *in vitro* fertilisation and bovine parthenotes were created by exposure to 6-dimethylaminopurine (6-DMAP). Embryos or parthenotes were pressed to feeder layers (Mouse Embryonic Fibroblasts) and cultured with 3i medium. Embryonic outgrowths of presumptive ESCs were continually passaged to fresh feeder layers to create presumptive bovine ESC lines, which were subsequently characterized. Expression of pluripotency related genes (oct4, rex1, sox2, ssea1, alp and nanog) and proteins were assessed by RT-PCR and immuno-cytochemistry. Differentiation ability was analyzed by embryoid body (EB) formation and assessment of gene expression of all three embryonic germ layers. The cell lines were vitrified for further investigation.

Results: Efficient isolation of bovine parthenogenetic ESC lines (8/15, 53%) and IVP cell lines (11/12, ~ 92%) was achieved from *in vitro* produced parthenotes and IVP embryos cultured in 3i medium. Five parthenote cell lines and 8 IVP-cell lines were cultured for 15 and 30 passages, respectively. All cell lines expressed markers of pluripotency. The cells formed EBs containing cells of the three embryonic germ layers. Following vitrification and warming, cell lines survived and proliferated, with good morphology, in 3i medium.

Conclusion: The results indicate that 3i medium can improve isolation and increase the pluripotent component of putative bovine ES cells and parthenote ES cells. This research demonstrates that altering the ERK and Wnt-β-catenin signalling pathways improves the efficiency of bovine ESC isolation.

Keywords: Inhibitors, Signalling Pathways, IVP Embryos, Parthenotes, Embryonic Stem Cells

Ps-41: LIF Induced Mouse Embryonic Stem Cells Proliferation Is Increased upon Treating the Cells with Peroxisome Proliferator-Activated Receptor Gamma Agonists

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Objective: Mouse embryonic stem (ES) cells are specialized cell types , capable of self renewal and pluripotency. To maintain mouse embryonic stem cells in undifferentiated state, leukemia inhibitory factor (LIF) is used in the culture medium. Furthermore, Peroxisome proliferator-activated receptor gamma (PPARγ) is a member of a class of nuclear hormone receptors which regulates growth and differentiation of several cell types. The activation of this receptor is dependent to its ligands. In the present study we have investigated the effects of PPARγ ligands on the proliferation of mESCs in the presence and absence of LIF.
Materials and Methods: Mouse embryonic stem (ES) cells were cultured in 96 wells in the absence and presence of LIF. Various concentrations of agonists (Rosiglitazone and Ciglitazone) and antagonist (GW9662) were added to the cell culture media for 72 hours. Then, MTS was done to assess the absorbance of the proliferation rate of the cells. Moreover, the expression of PPARγ was assayed by Real time PCR in the presence and absence of LIF.
Results: Data indicated that PPARγ agonists decreased the proliferation of mouse ES cells in the absence of LIF and increased the proliferation these cells in the presence of LIF. The antagonist reversed the above phenomenon. On the other hand, results were indicated that LIF induced the expression of PPAR γ too.
Conclusion: Our data suggest that there is an increase in LIF induced cell proliferation in the presence of PPARγ agonists which its details should be clarified in further studies.

Ps-42: DNA Damage Mediated S and G2 Checkpoints in Human Embryonal Carcinoma Cells

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For mouse embryonic stem (ES) cells, the importance of the S and G2 cell cycle checkpoints for genomic integrity is increased by the absence of the G1 checkpoint. We have investigated ionizing radiation (IR)-mediated cell cycle checkpoints in undifferentiated and retinoic acid-differentiated human embryonal carcinoma (EC) cells. Like mouse ES cells, human EC cells did not undergo G1 arrest after IR but displayed a prominent S-phase delay followed by a G2-phase delay. In contrast, although differentiated EC cells also failed to arrest at G1-phase after IR, they quickly exited S-phase and arrested in G2-phase. In differentiated EC cells, the G2-M-phase cyclin B1/CDC2 complex was upregulated after IR, but the G1-S-phase cyclin E and the cyclin E/ CDK2 complex were expressed at constitutively low levels, which could be an important factor distinguishing DNA damage responses between undifferentiated and differentiated EC cells. S-phase arrest and expression of p21 could be inhibited by 7-hydroxystaurosporine, suggesting that the ataxia-telangiectasia and Rad-3-related-checkpoint kinase 1 (ATR-CHK1), and p21 pathways might play a role in the IR-mediated S-phase checkpoint in EC cells. IR-mediated phosphorylation of ataxia-telangiectasia mutated, (CHK1), and checkpoint kinase 2 were distinctly higher in undifferentiated EC cells compared with differentiated EC cells. Combined with the prominent S and G2 checkpoints and a more efficient DNA damage repair system, these mechanisms operate together in the maintenance of genome stability for EC cells.
Keywords: Embryonal Carcinoma Cell, Cell Cycle, Checkpoint, Ionizing Radiation

Ps-43: Serum-Free Isolation of Adipose Tissue Derived Multipotent Mesenchymal Stromal Cells

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Objective: Mesenchymal stromal cells (MSCs) are multipotent cells with the capacity to differentiate into several cell lineages such as bone, adipose, cardiac and even neural cells *in vitro*. They are a promising source for cell therapy and tissue engineering nowadays. Today's most culturing media are supplemented with fetal bovine serum (FBS) as the resource of growth factors. But FBS containing culturing media may raise the possibility of zonotic infections and immunological reactions in cell therapy conditions, therefore usage of FBS is considered hazardous for the patients. Numerous researches have been performed to assess the use of FBS-free culturing systems for bone marrow derived mesenchymal stromal cell isolation. Since MSCs can be isolated from several tissues including bone marrow, adipose tissue and umbilical cord blood, we aimed the present investigation to assess the effect of serum free media on growth and differentiate capacity of adipose tissue derived MSCs.
Materials and Methods: Approximately 1cm3 surgically waste sterile adipose tissue was digested with collagenase-I leading to a single cell suspension. The isolated cells were cultured in Ultra Culture media

supplemented with 2% Ultroser G. MSCs isolation was confirmed with respect to morphology, flowcytometry, adipogenic and osteogenic differentiation potentials.
Results: The isolated cells showed adherent spindle-shaped morphology, expanded rapidly and showed expected MSC flowcytometric characteristics; they were positive for CD73, CD90, CD105, CD44, CD166, CD44 and were negative for hematopoietic antigen such as CD45, CD34 and CD14. They could also differentiate successfully into osteoblast and adipocyte which was confirmed with Alizarin Red and Oil red O staining respectively.
Conclusion: According to present study, we come to the conclusion that adipose derived MSCs can be cultured in serum-free media with no change in their differentiation capacity. This finding gives us a hope for future cell therapy studies and trials with fewer worries about zonotic infections or immunological reaction.
Keywords: Serum-Free, Adipose Tissue Derived MSCs, Ultra Culture

Ps-44: A Rapid Protocol for Isolation and Expansion of Neural Stem Cells from Human Embroyid Bodies by New Approach

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Objective: Human embryonic stem cells (hESCs) can be differentiated into Neural stem cells (NSCs) and functional neurons. These cells have a great potential to be used in basic research, in the pharmaceutical industry and finally in regenerative medicine. Different varieties of protocols were used for induction of hESCs into NSCs. Amount all of them, using embryoid body (EB) protocol is a cheapest way for this differentiation. EB is aggregate of cells derived from embryonic stem cells and made up of a large variety of differentiated cell types. Neural stem cells (in this case neuroectoderm) composed only few percent of the differentiated cells. Previous protocols based on EBs were time consuming (around 21 days), but in this experiment, we succeeded in isolation of these cells from EBs by new approaches, just in 11 days.
Materials and Methods: Human embryonic stem cells (Royan H6) were used as a source of cells for differentiation into neural stem. The differentiation was induced by removing of self renewal factors from medium in suspension culture and the purification was preformed by combination of adherent culture and mechanical passage of colonies center. Then Expression levels of transcripts characteristic for undifferentiated stem cells and neural stem cells were analyzed by Reverse Transcription PCR (RT-PCR) for and in the translation level was confirmed by fluorescence immune staining for Pax6 and Sox1. Also the produced neural stem cells were passaged in NSCs expansion medium and purity of the cells was confirmed by Flow Cytometry technique for Nestin (83%±5). The differentiation ability of the NSCs was checked by spontaneous differentiation and by directed differentiation into Dopaminergic neurons.
Results: NA
Conclusion: We demonstrated a novel efficient method for isolation and expansion of NSCs from human EBs. This differentiation technology would be extended to iPSC, and broadly contribute to central envious system regeneration by providing cell sources for transplantation strategies, or drug discovery.
Keywords: Human Embryonic Stem Cells, Neural Stem Cells, Differentiation

Ps-45: Human Placenta Is a Potent Hematopoietic Niche Containing Hematopoietic Stem and Progenitor Cells throughout Development

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Objective: To determine if the human placenta contains hematopoietic stem cells and if so when in development are they found
Materials and Methods: Human placentas from week 6 to term were obtained made into single cell suspensions for flow cytometric analysis, assessment of *in vitro* hematopoietic colony-forming ability, and hematopietic stem cell potential by *in vivo* xenotransplantation into immundeficient adult mice.
Results: The human placenta contains hematopoietic progenitors and HSCs from week 6 in gestation until term. Stromal cell lines generated from human placenta at several developmental time points are pericyte-like cells and support human hematopoiesis. Immunostaining of placenta sections during development localizes hematopoietic cells in close contact with pericytes/ perivascular cells.
Conclusion: The human placenta is a potent hematopoietic niche containg hematopoietic stem and progenitor cells throughout development.
Keywords: Human Placenta, Hematopoietic Stem Cells, Development, Pericytes

Ps-46: *In vitro* Differentiation of Blastema Cells Derived from New Zealand White Rabbit Pinna into Insulin Producing Cells

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After injury, in some animals, blastema tissue is formed which helps in restoration and healing of the injured tissue. One probability for production of these cells is dedifferentiation of mesenchymal cells to undifferentiated cells that can divide and differentiate to various cell types. In this study, New Zealand white rabbit pinnas were punched and after the second punch, a thin ring containing blastema cells was transferred into culture medium. Cells derived from this tissue were grown and then induced to differentiate into insulin producing cells by culturing in serum free medium containing high glucose concentration, nicotinamide and β-mercaptoethanol.
Dithizone staining and measuring the concentration of insulin in culture medium showed that these cells were able to produce insulin from two days after induction. Also the defferentiated cells produced some clusters of cells similar to islets of langerhans within one week to one month of treatment. Finally, RT-PCR analyseis showed that expression of insulin mRNA was induced in these cells.
These results demonstrate high capacity of blastema cells to differentiate into insulin producing cells and therefore they might be useful in finding novel approaches or new cell source with the potential for cell therapy.
Keywords: Blastema Cells, Differentiation, Insulin Producing Cells, Cell Therapy

Ps-47: Repair of Spinal Cord Injury by Co-Transplantation of Embryonic Stem Cell-Derived Motor Neuron and Olfactory Ensheathing Cell

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Objective: The failure of regeneration after spinal cord injury (SCI) has been attributed to axonal demyelination and neuronal death. Cellular replacement and white matter regeneration are both necessary for SCI repair. In this study, we evaluated the co-transplantation of olfactory ensheathing cells (OEC) and embryonic stem (ES) cell-derived motor neurons (ESMN) on contused SCI.
Materials and Methods: OEC cultured from olfactory nerve rootlets and olfactory bulbs. ESMN was generated by exposing mouse ES cells to retinoic acid and sonic hedgehog. Thirty female rats were used to prepare SCI models in five groups. Control and medium-injected groups was subjected to induce lesion without cell transplantation. OEC or ESMN or both were transplanted into the site of the lesion in other groups.
Results: The purity of OEC culture was 95%. Motor neuron progenitor markers (Olig2, Nkx6.1 and Pax6) and motor neuron markers (Isl1, Isl2 and Hb9) were expressed. Histological analysis showed that significantly more ($p<0.001$) spinal tissue was spared in OEC, ESMN and OEC+ ESMN groups but the OEC+ ESMN group had a significantly greater percentage of spared tissue and myelination than other groups ($p< 0.05$). The numbers of ESMN in co-transplanted group were significantly higher than ESMN group ($p<0.05$). A significant ($p<0.05$) recovery of hindlimb function was observed in rats in the transplanted groups.
Conclusion: We found that the co-transplantation of ESMN and OEC into an injured spinal cord has a synergistic effect, promoting neural regeneration, ESMN survival and partial functional recovery.
Keywords: Spinal Cord Injury, Embryonic Stem Cell, Motor Neuron, Olfactory Ensheathing Cell

Ps-48: PPARγ Expression Profile upon Ectodermal and Mesodermal Differentiation of Mouse Embryonic Stem Cells

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Objective: Mouse embryonic stem (ES) cells are pluripotent cell lines derived from the inner cell mass of the blastocyst. Their properties include self renewal and capacity to differentiate into different cell types. Neural differentiation of ES cells has been achieved by several different protocols such as treating with retinoic acid and serum decreasing in culture media. The PPARs (peroxisome proliferator-activated receptors) are members of the nuclear hormone receptor superfamily. Three major isoforms of PPARs (α, β and γ) have been identified. PPARγ has been demonstrated to play an important role in the regulation of cell differentiation. Mouse PPARγ has 2 isoforms termed: γ1, γ2. PPARγ2 primarily expresses in adipocytes but PPARγ1 expresses in various cell types such as neuron and astroglial cells. In order to understand the molecular mechanisms of PPARγ during the neurogenesis we set

serial experiments to evaluate the relative expression patterns of these genes.
Materials and Methods: Embryonic stem cells were cultured to form multicellular aggregates, called embryoid bodies (EBs). EBs were separated into two groups. In order to ectodermal differentiation, one group was cultured as a suspension form with KDMEM supplemented ES-FCS 10% media in the presence of retinoic acid for four days. The other group was cultured on the same media except Retinoic acid for endodermal differentiation. Retinoic acid treated and untreated EBs were plated in Neurobasal ES-FCS 5% media for 7 days to form mature neuron and cardiomyocytes.
Results and Discussion: Real Time quantitative PCR data were shown that PPARγ1 expression was increased in the early satge of ectodermal differentiation and the late stage of mesodermal differentiation. However, There was no expression of PPARγ2.
Keywords: Peroxisome Proliferator-Activated Receptors, Mouse Embryonic Stem Cells, Ectodermal Differentiation, Mesodermal Differentiation

Ps-49: Expression of Neural Progenitor Cell in Mouse Dental Pulp Stem Cells Cultured in Low Serum Media

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Objective: Stem cells are the source of cells that has ability to differentiate to another cells. Among these cells, dental pulp, entrapped with the pulp chamber, is rich site for stem cell collection. These stem cells are called dental pulp stem cells. These cells exhibited a differentiation potential for neural cytotypes. The aim of this study was to isolate and proliferation dental pulp Stem cells and survey effect on serum on differentiation to nerve progenitor. In the present study, the *in vitro* neurogenic differentiating potential of mouse dental pulp stem cell was examined. The induction was carried out under the same inducting system as used for bone marrow cells.
Materials and Methods: Teeth were collected from mouse. The pulp tissue gently separated from the crown and root, and then digested in a solution of 4 mg/ml collagenase type I for 1 h at 37◦C. The dental pulp sample from each individual was pooled, and cultured with alpha-modified Eagle's medium (A-MEM) supplemented with 20% fetal bovine serum (FBS) then harvested pellete cells by centrifugation at 1200 g for 10 minutes. Dental pulp cells are seeded into T25 culture dishes with alpha-modified Eagle's medium supplemented with 20% (v/v) fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin at 37° C and 5% CO2. After 48 hours, the cells were washed with PBS for removal of another cell. Dental pulp stem cells were treated with A-MEM containing 5% (v/v) FBS and 1% (v/v) penicillin-streptomycin, so that the cells could proliferate sufficiently. After passage 4, we found the existence of progenitor nerve cell in culture cells.
Results: When mouse dental pulp cell were cultured in 5% of fetal bovine serum (FBS), the ratio of nerve cells and oligodenrocyte was significantly higher in comparison with incubation in 20% FBS.
Conclusion: We demonstrate the characterization and distinctiveness of the dental pulp and showed that, when cultured with the medium containing serum, they were highly proliferative. However, when cultured in low concentration of FBS, cells were differentiate into nerve cells. Lower concentration of FBS may provide the chance for the cells to differentiate into nerve cells and it useful for cell-based therapies to treat dental diseases.
Keywords: Dental Pulp Stem Cells, Differentiation, Nerve Cell, Fetal Bovine Serum

Ps-50: Study Effects of the 3 Different Feeder Layers on the Culture of Embryonic Stem Cells

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Objective: Using of embryonic & adult stem cells (Sc) in medical research and treatment has made a new horizon to progress in many disorders. So finding methods for better isolation & culture and maintenance of Sc has a specific importance. Aim of this reach was to evaluate of protective effects of 3 different layers of Mesenchymal stem cells (MSC), mouse embryonic fibroblast (MEF) & 3T3 on Sc for finding the best feeder layer for increasing the amounts of sells for transplantation, & differentiation. Purpose: evaluation of protective effect of MEF, MSC and 3T3 feeder layers in isolation and culture of mouse embryonic stem cells.
Materials and Methods: Blastocysts were obtained from Balb/c pregnant mice. Collected embryos were put on 3 different feeder layers of MEF, MSC & 3T3 cell lines. Two or 3 days later, the zona pellucida was removed and after 5 days the inner cell mass (ICM) growth, was removed mechanically and changed to small multi-cellular clumps through trypsination. Colonies of

stem cells were formed after 2 to 3 days. This repeated to two other passages and each time, the amount of colonies formation was increased.Then formed colonies were cultured on feeder layers. Finally the colonies were identified through staining with alkaline phosphates for their numbers and morphological characteristics.
Results: From 29 embryos put on MEF layer all t of them (100%) was attached. From 32 embryos put on the MSC layer (64.5%) was attached. While the amount of attaching of 20 blastocysts to the3T3 feeder layer was law and none of them reach to the growth stage of the inner cell mass. The amount of colonies formation during three passages on MEF feeder layer was more than MSC feeder layer. Data analysis demonstrated a significant difference ($p<0.05$) in percentage and time of hatching and time for living of blastocysts.
Conclusion: Considering these three feeder layers, MEF was better than MSC and both of them had priority to 3T3.
Keywords: Embryonic Stem Cells, Feeder Layer, 3T3, MEF, MSC, Alkaline Phosphates

Ps-51: Renal Tubule Function in Beta-Thalassemia after Hematopoietic Stem Cell Transplantation

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Objective: Advances in hematopoietic stem cell transplantation (HSCT) for beta-thalassemia major make the longterm outcome of these patients very important. Few data on long-term renal function of thalassemia patients are available.
Materials and Methods: We evaluated the renal function in children after successful allogeneic HSCT for beta-thalassemia.
Results: Twenty-nine patients were included; the mean age at HSCT was 4.9 years. Mean follow-up time was 7.6 years. After HSCT,two patients eveloped acute renal failure and two had graft versus host disease. At last follow up, height standard deviation score (SDS) remained the same, but weight SDS had improved. Mean hemoglobin was 12.5 g/dl, and serum ferritin level was 545 ng/ml. All children had normal estimated glomerular filtration rate (GFR). One patient had hypertension and proteinuria, 10 years after HSCT. When comparing 39 children of the same age with beta-thalassemia of similar disease severity but who had not experienced HSCT, we found that the parameters of renal tubule function were better in patients that had undergone HSCT, as demonstrated by urine protein level (0.36 mg/mg creatinine vs 3.03 mg/mg creatinine, $p<0.001$), osmolality (712 mosmol/kg vs 573 mosmol/kg, $p=0.006$), N-acetyl-beta-D-glucosaminidase (17.7 U/g creatinine vs 42.9 U/g creatinine, $p=0.045$), and beta 2 microglobulin (0.09 µg/mg creatinine vs 0.13 µg/mg creatinine, $p=0.029$).
Conclusion: This study showed a low incidence of long-term renal impairment after HSCT and indicated that renal tubule function may be better in beta-thalassemia patients after HSCT.
Keywords: Beta-Thalassemia, Hematopoietic Stem Cell Transplantation, Renal Function

Ps-52: Comparison of Manual and Automated Closed System in Umbilical Cord Blood Stem Cells Separation

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Objective: According to advanced human diseases and its interest in longevity , the sciences of stem cell biology and repair tissues and damaged organs have been used routinely to develop the cell therapies and regenerative medicine for more than three decades. Therefore, the aim of stem cells utilization as a source of appropriate differentiated cells is to replace those lost through physical, chemical or ischemic injury, or as a result of degenerative disease. For this purpose, there are two main kinds of stem cells according to their origin containing embryonic and adult stem cells. Essentially, embryonic stem cells are extracted fetal blood, amnion fluid and membrane or umbilical cord blood (UCB), however adult stem cells are commonly isolated from bone marrow, peripheral blood or skin. Whereas UCB is a biological waste product post childbirth, it is mostly preferred in comparison to other sources. Also, it is a source of hematopoietic progenitors cells coupled to the immaturity of the immune system at birth that is one of the advantages of using these cells for transplantation. In 1993, the first three UCB programs were set up to cryopreserve UCB from healthy newborn infants in New York, Milan, and Dusseldorf. The first placental blood banking program sponsored by the National Heart, Lung, and Blood Institute (NHLBI) was initiated by Pablo Rubenstein in 1993. So the separation of these useful cells requires a standard manual technique or some important protocols and technological innovations. Nowadays, an accurate and efficient system is the automated closed system "Sepax®" that it is employed largely in the routine processing of cord blood banks worldwide.
Materials and Methods: In this consideration the results of many stem cells separation processes by both of manual and automated closed system are evaluated.
Results: Many results showed TNC recovery were significantly higher after Sepax processing ($P<0.0001$-0.05) whereas no significance was found for red blood

cell depletion.
Conclusion: Nowadays, it is proved that one of the best methods is using the automated closed system "Sepax®", whereas this system is a simple, safe, user-friendly and a time-saving method which provides automatic clinical-grade stem cells, the more standardized and user independent processing procedure, releases high amount of stem cells as a final product ready for cryo-preservation, decreases the risk of contamination, reduces final sample volume efficiently that causes to solve the storage space problem and decreasing the cost of cryogenic storage in cord blood banks, providing optimal red blood cell depletion for the storage of the final cellular product.
Keywords: Stem Cells, Sepax®, Cell Therapy, Umbilical Cord Blood

Ps-53: Effects of Red Blood Cells on the Final Product of Automated Closed System in Stem Cells Isolation and its Elimination Method

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Objective: Nowadays, the use of umbilical cord blood (UCB) stem cells is promoting for related and unrelated donor transplantation. A successful transplant happens when quality of isolated cells will be high. Therefore, in addition to yield of quantitative production, to provide safe and effective products is very important in cord blood banks, because these products have to create the conditions and results allowing a therapeutic exploitation. So these principles are the main aims of cord blood banks to achieve an accurate transplantation from stem cells (originating from: bone marrow, peripheral blood, cord blood). For this purpose, it requires some important technological innovations. This goal remains to be achieved for cord blood cells too. In many researches, quality of fresh samples and final products after thawing are evaluated by staining. For assessing the quality and recovery of UCB cells following laboratory manipulation in a study, for example, mononuclear cells (MNC) from fresh (<48 h old) and thawed UCB units were stained with 7-amino-actinomycin D (7-AAD) and illustrated revealing 2-3% dead cells. The frequencies of apoptotic cells in fresh and thawed sample were similar. However, UCB held for 72 h showed higher levels of cell deterioration. Finally, the utilization of an automated closed system "Sepax®" with washing after thawing is one of the best solution methods before transplantation.
Materials and Methods: In this consideration the effect of red blood cells on final products and their quality is evaluated. The automatic method is compared with the manual washing procedure. For this purpose, viability and mean recovery of nucleated cells (TNC) post-thaw are evaluated.
Results: Umbilical cord blood (UCB) products are thawed using an automatic washing method intended to reduce DMSO toxicity and remove RBC. The results show that the automatic washing method has no significant differences on final products. So mean TNC recovery depends on thawing method.
Conclusion: It is proved that the automatic washing method is as effective as the manual method on viability, but this procedure is faster and easier for the operators. Safety of final products is very important that the automatic method is safe and suitable for washing of thawed final products. So an accurate washing method after thawing is necessary.
Keywords: Washing, Umbilical Cord Blood, Transplantation, Sepax®, Automated Closed System

Ps-54: *In vitro* Embryo Production in Camel (Camelus dromedarius) from *In vitro* Matured Oocytes Fertilized with Epididymal Spermatozoa Stored at 4°C

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Objective: The objectives of this study were to determine the effect of storing epididymal spermatozoa, collected from slaughtered animals, in tris–tes- and tris–lactose egg yolk extenders, on their fertilizing ability and subsequent embryo development from *in vitro* matured oocytes.
Materials and Methods: Ovaries and testes were collected from a local slaughterhouse in normal saline solution (NSS) at 37 ◦C and on ice (0-1 ◦C), respectively. Cumulus oocyte complexes (COCs) aspirated from the follicles were randomly distributed to 4-well culture plates (20–25 COCs/well) containing 500 L of maturation medium and cultured at 38.5 ◦C in an atmosphere of 5% CO2 in air for 36 h. Spermatozoa were collected from the cauda epididymides in syringes containing 2–3mL of either tris–tes- or tris–lactose egg yolk extender. They were cooled down slowly and stored at refrigeration (4 ◦C) temperature. The spermatozoa were evaluated for motility and used for IVF of IVM oocytes on the day of collection and after 2, 4, 6 and 8 days of storage. On the day of IVF, spermatozoa were prepared by the swim up technique and both spermatozoa and oocytes were co-incubated at 38.5 ◦C in a humidified atmosphere of 5% CO2 in air for 15-16 h. Presumptive zygotes were either fixed and stained with Hoechst 33342 for evaluation of fertilization or were cultured in 500L of the culture medium at 38.5 ◦C in an atmosphere of 5% CO2, 5% O2 and 90% N2 in air
Results: There was no significant difference ($p > 0.05$) in the proportion of oocytes fertilized with spermatozoa

stored in either of the two extenders for up to 8 days. The proportion of oocytes that cleaved (43-60%) and those that developed to blastocysts (14-21%) did not show any difference ($p > 0.05$) either, when spermatozoa from different days of storage were used. First cleavage was observed as early as 16 h after IVF, early blastocysts had developed by day 4, expanded blastocysts after day 5 and hatching of blastocysts started after day 6 of culture.

Conclusion: It may be concluded that dromedary epididymal spermatozoa survive in storage for at least 8 days in tris-lactose- and tris-tes egg yolk diluents at 4 ◦C. These spermatozoa maintain their fertilizing ability and may be suitable for use in IVF and other assisted reproductive procedures. Further, *in vitro* matured oocytes develop to blastocysts and hatch in *in vitro* culture, but at a rate lower than in other domestic animal species like cattle. Therefore, a need exists to investigate the requirements of dromedary embryos in *in vitro* culture to develop an efficient embryo production system for this species.

Keywords: Dromedary Camel, Epididymal Spermatozoa, Semen Extenders, *In vitro* Fertilization, Embryo Development

Ps-55: Production of the First Cloned Camel by Somatic Cell Nuclear Transfer

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Objective: In this study, we demonstrate the use of somatic cell nuclear transfer to produce the first cloned camelid, a dromedary camel (Camelus dromedarius) belonging to the family Camelidae.

Materials and Methods: Donor karyoplasts were obtained from adult skin fibroblasts, cumulus cells, or fetal fibroblasts, and *in vivo*-matured oocytes, obtained from preovulatory follicles of superstimulated female camels by transvaginal ultrasound guided ovum pick-up, were used as cytoplasts. Reconstructed embryos were cultured *in vitro* for 7 days up to the hatching/hatched blastocyst stage before they were transferred to synchronized recipients on Day 6 after ovulation.

Results: Pregnancies were achieved from the embryos reconstructed from all cell types, and a healthy calf, named Injaz, was born from the pregnancy by an embryo reconstructed with cumulus cells. Genotype analyses, using 25 dromedary camel microsatellite markers, confirmed that the cloned calf was derived from the donor cell line and the ovarian tissue.

Conclusion: The present study reports, for the first time, establishment of pregnancies and birth of the first cloned camelid, a dromedary camel (C. dromedarius), by use of somatic cell nuclear transfer. This has opened doors for the amelioration and preservation of genetically valuable animals like high milk producers, racing champions, and males of high genetic merit in camelids. We also demonstrated, for the first time, that adult and fetal fibroblasts can be cultured, expanded, and frozen without losing their ability to support the development of nuclear transfer embryos, a technology that may potentially be used to modify fibroblast genome by homologous recombination so as to generate genetically altered cloned animals.

Keywords: Assisted Reproductive Technology, Camel, Cloning, Ovum Pick-Up/Transport, Somatic Cell Nuclear Transfer

Ps-56: Meta-Analysis of HLA Matching and the Outcome of Unrelated Umbilical Cord Blood Transplantation (CBT)

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Objective: The aim of this meta-analysis is to compare the HLA disparities and the outcome of UCBT, i.e. the disease free survival (DFS), engraftment, graft-versus-host disease, (GVHD), and transplantation related mortality (TRM).

Materials and Methods: We electronically searched the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, Pubmed, IBMTR and critically appraised all relevant articles (1989.01–2008.12). Comparative studies are carried on HLA typing and cord blood transplantation with research on stem cells engraftment, GVHD, TRM, and DFS. A meta-analysis is performed using Review Manager 5.0 software and adopted funnel plot regression assessed the publication bias.

Results: We got 882 records, and 10 trials totaling 1589 patients assessed. Pooled comparisons of studies of outcomes found that the incidence of neutrophil and platelet engraftment failure increased with HLA mismatched antigen increased, ≥2-Ag mismatched group had a higher risk of ≥II degree GVHD and a lower DFS rate than the HLA matched group.

Conclusion: Our meta-analysis confirmed that with the HLA-mismatched antigen increased, the rate of graft failure, severe GVHD and TRMi ncreased, and the DFS decreased.We cannot fully exclude the possibility of center biases in treatment and selection of patients and well-designed trials need to carry out.

Keywords: HLA Typing, Transplant, Meta-Analysis, Cord Blood, Outcome

Ps-57: Interaction Analysis of Recombinant Mouse Peroxisomal Protein (PEP) in Mouse Embryonic Stem Cells Derived Neural Differentiated Cells

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Objective: Nowadays proteomics techniques are widely used for better understanding of genes functions. As PEP (Peroxisomal Protein) which was the target of our research project showed that might have an essential role/s in neural and muscular evolution in embryonic stage, we have under taken this project to analyze its interaction with proteins involved in neural differentiated of mouse embryonic stem cells.
Materialss and Methods: At the first step GST-PEP fused protein produced using bacterial BL21 competent cells. The purification of recombinant protein was achieved by GSH-Sepharose. At the next step, neural differentiated mouse embryonic cells were harvested for further analyses. The lysate cells were subjected to the Glutathione sepharose beads conjugated with GST-PEP in a pull down assay. The pull-down products were analysed to identify the possible interacting partners of PEP.
Results: Using the above mentioned approach, several proteins were detected to be the candidate of interacting partners for the PEP. One of the identified proteins is [MKIAA- CTTNBP2NL] which potentially is involved in apoptosis and has proline rich domains which interact with actin fibers of cytoskeleton. The other candidate was a kind of serine protein kinase precursor which is involved in cell cycle arrest and apoptosis and might be one of the triggers for differentiation. The third one was K5 keratin acted in accompanying with K14 keratin for neonatal cells differentiation. The last identified protein was vomeronasal sensory neurons receptor, a member MHC1b, plays an important role in progress of neural connectivity and function.
Conclusion: In this experiment several proteins were identified for as candidates for interaction with PEP in neural differentiated cells which guide us unravel the molecular mechanism of PEP in neural differentiation process. However, more documents are needed to elucidate the exact role of PEP in the process of neurogenesis.
Keywords: Proteomics, Peroxisomal Protein (PEP), Tandem Mass Spectrometry, Glutathione Sepharose

Pre-Congress Educational Course on Stem Cells

Beyond the 11th Congress on Reproductive Biomedicine and 6th Congress on Stem Cell Biology & Technology, Royan Institute is going to hold the second pre-congress educational course on Stem Cells Biology and Technology on September 14th, 2010.
The topics to be covered include an overview of the different aspects of stem cell biology and application of stem cells in regenerative medicine. The target audience for the pre-congress educational courses includes all of biological and medical students and all of interested persons to the stem cell subjects.

Chairman:
Hossein Baharvand, Ph.D.

Scientific and Executive Manager:
Sahar Kiani, Ph.D.

Workshop on Isolation and Culture of Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are defined as non-hematopoietic cells that are able to replicate for a long time while maintaining their multilineage differentiation potential.
MSCs are considered as a suitable candidate for cell therapy strategies owing to their ability to undergo extensive proliferation and their potential to undergo differentiation into different cell lineages. Their efficacy has been indicated in curing osteogenesis imperfecta, regenerating bone and cardiac muscle and resurfacing articular cartilage as well as restoring hematopoiesis in patients receiving chemotherapy. The potential of MSC in differentiating into cells other than those of skeletal lineages, such as neurons and keratinocytes as well as liver, intestine and kidney epithelial cells has also been demonstrated by several experiments.
In this workshop, the main components that are involved in cell preparation for therapy including cell isolation and culture, evaluation of differentiation potential of extracted cells and quality control of cells of aspects such as growth rate and viability will be trained.

Chairman:
M. R. Baghban Eslaminejad

Executive Manager:
Hamid Nazarian, M. Sc.

Authors Index

In memorial of
Dr.Saeid Kazemi Ashtiani

Symposium of :

Application of Pluripotent Stem Cell in Gastroenterology and Hepatology

Organiser : Royan Institute of Reproductive Medicine and Stem Cell in Cooperation with Digestive Disease Research Center (DDRC)

January 6, 2010 ,Royan Congress Hall, Tehran,Iran

The 2nd Royan Institute International Summer School:

Developmental Biology and Stem Cells

Time: July 11-14, 2011
Place:Royan Institute

The first symposium on stem cell differentiation

- Time: Feb.10, 2011
- Place:Royan Institute
- Duration: 1 day
- Price (Rials):250/000
- Capacity:10 Person

List of stem cell group workshops 2010-2011

NO	Titles	Scientific Secretary / Executive Secretary	Duration (days)	Suggested time	Price (Rials)
1	Seventh Workshop theory and practice cultivation and maintenance of mouse embryonic stem cells	Dr. H. Baharvand / S.N. Hassani	1	11 Oct	2/000/000
2	The second workshop theory and practice cultivation and maintenance of ploripotent human stem cells	Dr. H. Baharvand / S.N. Hassani	2	23-24 Feb	3/500/000
3	The second workshop of theory and practical two-dimensional electrophoresis and Western blotting	Dr.Gh. Hosseini / F. Shekari	4	7-10 Jan	5/000/000
4	The first workshop in cell Sorting methods and Flow cytometry (Theoretical and practical course)	Dr. M. Ebrahimi / E. Janzamin	3	21-23 Oct	4/000/000
5	Theoretical and practical workshops and RNA extraction and cDNA synthesis PCR method to study gene expression Real-Time PCR	Dr. H. Baharvand / A. Farrokhi	2	26-27 Jan	2/000/000
6	The first workshop of theory and practical electrophysiology	Dr. S. Kiani / Dr. S. Kiani	2	1-2 Nov	2/500/000
7	the second workshop theory and practice mesenchymal stem cells	Dr. R. Eslami / H. Nazarian	1	13 Aug	2/000/000
8	The second workshop of bone and cartilage tissue engineering	Dr. R. Eslami / H. Nazarian	1	27 Jan	2/000/000
9	Bioinformatics workshop theory and practice	Dr.Gh. Hosseini / F. Shekari	1	Dec	1/000/000

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Stem Cell Technology
Research Center

مرکز تحقیقات فناوری بن یاخته
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Technology Research Center

Molecular Biology & Genetic Engineering Department

Molecular biology and genetic engineering department to provid escientific support for the Stem Cell Research group of the stem cell technology research center studies. Our research team exploits extensive nation-wide intellectual collaboration toward the development of cutting-edge technologies for miRNAs profiling and design/construction of tissue specific vectors for gene therapy and stem cell differentiation studies.



Nano Technology & Tissue Engineering Department

The main objectives and tasks of this department include various and worldwide research projects in Tissue engineering and nanotechnology field. This research department attempts to produce scaffolds with defined and special physical, chemical, biological and morphological properties making use of various materials, equipments, processing methods, and high technologies especially nanotechnology. Having different well-equipped laboratories and workshops, this research department performs various activities in designing, processing, modification, and characterization of biomaterials for medical applications.

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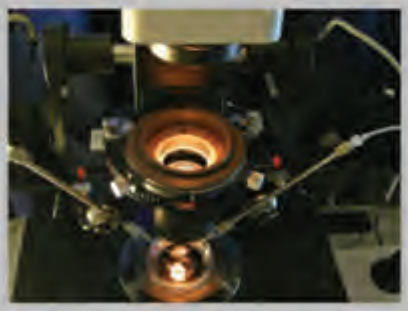
Stem Cell Biology Department

Stem cells biology department is committed to applied and basic researches in the field of stem cell biology and tissue engineering. We are evaluating the therpeutic potential of different embryonic, cord blood and adult stem cells to be used in conjunction of some disease models.



The most important research topics of this group are as follows:

1. Study of biology of stem cell types including embryonic stem cells, mesenchymal stem cells etc
2. Regenerative medicine of spinal cord, heart, kidney by autologous transplantation of human mesenchymal stem cell
3. Bone, liver and skin tissue engineering using use of stem cells and appropriate nanoscaffolds
4. Design specific bioreactors for three dimensional culture, proliferation and differentiation of stem cells
5. Nuclear transfer in mouse model as primary model to achieve cloning therapy
6. Production of transgenic mice with the ultimate goal of producing transgenic products

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1. Commercializing R & D ideas.
2. Quality improvement and customer satisfaction.
3. Improving bioscientific products.

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