Ps-70: Experimental Validation of A Novel Alternative Polyadenylation Isoform of Human spp1 Gene in U87-MG Cell Line

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Objective: Osteopontin (OPN), also known as SPP1 (secreted phosphoprotein) is a secreted protein present in bodily fluids and tissues. It is also a tumour-associated protein, and elevated OPN levels are associated with tumour formation, progression and metastasis. In cancer patients expression of OPN has been associated with poor prognosis in several tumor types including breast, lung, and colorectal cancers. Research has revealed a promising role for OPN as a cancer biomarker. OPN is subject to alternative splicing, as well as post-translational modifications such as phosphorylation, glycosylation and proteolytic cleavage. Functional differences have been revealed for different isoforms and post-translational modifications. The pattern of isoform expression and post-translational modification is cell-type specific and may influence the potential role of OPN in malignancy and as a cancer biomarker. Tumor specific splice variants are being discovered at an increasing rate and their functions are also investigated in cancer progression.
Materials and Methods: After culturing U87-MG cell line in proper medium, RNA extraction following by single strand cDNA synthesis has been performed. Multiple products of RT-PCR by specific primers for spp1 mRNA was isolated and cloned in pTZ57R/T vector for sequencing.
Results: We found a novel transcription variant of spp1 gene in U87-MG cell line, experimentally. Our sequencing data revealed that this novel variant lacks a region that potentially is a binding site of some micro-RNAs.
Conclusion: Our study revealed that this novel variant has same function with other variants of this gene but we assume that post-transcriptional control mechanisms for this variant are different from the other variants. Computational analysis reveals that shorter variant of spp1 gene has so many regions that are complementary with some micro-RNAs. Then shorter variant is more stable than longer one and has a significant association with cancer.
Keywords: Spp1 Gene, ONP, Transcription Variant, U87-MG Cell Line

Ps-71: Evaluation of miR-210 Effect on Proliferation and Survival of Mouse Mesenchymal Stem Cells

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Objective: Bone marrow derived mesenchymal stem cells (MSCs) are a population of multipotent progenitors which have the capacity of proliferation and differentiation into mesenchymal lineage cells. Micro-RNAs are endogenous 22 nt RNAs that can play important roles in some processes such as proliferation and differentiation. We hypothesized that miR-210 could help for better prolifratation MSCs since this miRNA can activate HIF pathway. So MSCs could preserve their differentiation ability under normoxic conditions without any growth factors.
Materials and Methods: MSCs isolated from C57 BL/6 mice by flushing its femurs into cell culture media. After 72 hours MSCs which are plastic adherent cells were attached to the flask and nonadherent cells were removed. Subsequently MSCs differentiated into osteocytes and adipocytes with specific differentiation media to confirm their identity and multipotency. Also we were inserted miR-210 in Lentiviruse vectors and affected them on MSCs. The expressions of miR-210 and HIF-1α in each passage were evaluated by Real time PCR.
Results: Comparison between miR-210 infected MSCs and control cells showed that miR-210 has ability to increase proliferation of MSCs while maintained their ability to differentiate into adipocytes and osteocytes. The expression of miR-210 and HIF-1α were up regulated in each passage.
Conclusion: In order to important roles of MSCs, proliferation and maintenance of their ability are necessary. We showed that miR-210 has ability to proliferate MSCs without any effect on their differentiation abilities. Morther studies are needed for evaluation of probably miR-210 effects mechanism on MSCs.
Keywords: Mesenchymal Stem Cells, MiR-210,HIF-1α

Ps-72: Culture and Differentiation of Mouse Neural Stem and Progenitor Cells in A Polymeric Nano-Scaffold Poly-L Lactic Acid

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Objective: The selection of a good quality scaffold is an essential strategy for tissue engineering. Ideally, the scaffold should be a functional and structural biomimetic of the native extracellular matrix and support multiple tissue morphogenesis. Neural stem/progenitor cells (NSCs) are intrinsically capable of differentiating into different neural cell types within the nervous system, offering prospects for NSC-based cell therapies to treat neurodegenerative diseases. In this study, an attempt was made to develop porous polymeric Nano-fiber scaffold using a biodegradable Poly (l-lactic acid) (PLLA) for *in vitro* culture of neural stem and progenitor cells.
Materials and Methods: To fabricate Nano-fiber, we

used electrospinning techniques. The physico-chemical properties of the scaffold were fully characterized by using scanning electron microscopy. NSCs were isolated from the subventricular zone of the adult mouse brain and cultured in the scaffold and then the viability, proliferation and differentiation of these cells determined via both of Immunostaining and MTT assay. Scanning Electron Microscopy (SEM) was also used to observe the morphology of the cells cultured on PLLA fibrous scaffolds.
Results: DAPI and Immunostaining revealed the neuronal differentiation and adhering of cells on the surface of the electrospun scaffolds. Blue stained cells were found in Nano-fiber indicating that the scaffold had provided basic cell attachment. The MTT assay results showed that the absorption in 570 nm increased during 2 to 5 days. The morphology of differentiated cells was evaluated by SEM.
Conclusion: Nano-Scaffold PLLA due to the biocompatible properties is an appropriate structure for proliferation, differentiation and normal growth of the neural stem and progenitor cells.
Keywords: Tissue Engineering, Nano-Fiber Scaffold, Neural Stem Cells

Ps-73: Inside into ID Basic Helix-Loop-Helix Genes Expression during Retinoic Acid Induced Differentiation of Human Embryonic Carcinoma Cells

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Objective: The basic helix-loop-helix (bHLH) family of transcription factors are thought to affect the balance between cell proliferation and differentiation in coordinated regulation of gene expression and cell lineage commitment in most mammalian tissues. Helix-loop-helix ID (Inhibitor of DNA binding) proteins are distinct from bHLH transcription factors in that they do not possess basic domain necessary for DNA binding and function as dominant negative regulators of bHLH transcription factors . It is supposed that various members of the ID family have active roles in embryonic stem cell self-renewal and also in a range of human tumors. Since embryonal carcinoma (EC) cells are malignant but their terminally differentiated derivatives are not, understanding the expression profile of these embryonal cells may be of value for therapeutic purposes in embryology and development. These cells can be differentiate into neural linage upon exposure to retinoic acid (RA), an analogue of vitamin A, which is a robust inducer during normal mammalian development.
Materials and Methods: In the current work, differentiation was induced by retinoic acid in a human embryonal carcinoma cell line, named NT2/NTERA2. The cells were harvested at 0, 1, and 3 days of RA induction. The mRNA expression levels of all four ID genes were quantitatively evaluated before and after RA treatment by real time-PCR technique (qRT-PCR).
Results: The results declared significant expression levels of ID gene family in embryonal carcinoma cells. During development although onset of differentiation showed increase in the levels of ID genes, the levels of their mRNA decreased by day 3 of differentiation.
Conclusion: Elucidation of the detailed pattern of ID genes expression would reveal novel roles for promoting our understanding of how differentiation and proliferation are regulated.
Keywords: bHLH, ID, NTERA2, Differentiatin

Ps-74: Intra-Renal Arterial Injection of Autologous Bone Marrow-Mesenchymal Stem Cells Ameliorates Cisplatin-Induced Acute Kidney Injury in A Rhesus Macaque Mulatta Monkey Model

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Objective: Acute kidney injury (AKI) is a potentially devastating disease in clinical medicine. However, no specific therapy improves the rate or effectiveness of the repair process after AKI. Bone marrow-derived mesenchymal stem cells (BM-MSCs) have been proven to be benefit to the renal repair after AKI in different experimental models of rodent models, but the consequence of these results to large animals and eventually, to humans remains unknown. Thus, the aim of this study was to assessment the effect of autologous rhesus Macaque monkey BM-MSCs transplantation in cisplatin-induced AKI.
Materials and Methods: BM-MSCs were characterized for their growth characteristics, differentiation potential, immounophenotypic properties and chromosome content. According to design procedure, monkeys were divided into control, vehicle and cell transplantation (Cell Tx) groups and exposed to cisplatin 5mg/kg as intravenous for induce of AKI. Control animals were not treated with anything but cell Tx and vehicle animals were treated with intra-renal arterial injection of autologous BM-MSCs and normal saline, respectively. For cell tracking with magnetic resonance imaging (MRI), BM-MSCs labelled with nanoparticles superparamagnetic iron oxide (SPIO).
Results: Labelled BM-MSCs were found in both glomeruli and tubules. Transplantation of 5×10^6 cell/kg