to-mesenchymal transition (EMT) is the main cause of death in cancer patients. The stem cell protein, PIWIL2, is a member of the Argonaute family, plays an important role in the EMT process by controlling and enhancing the invasive properties of cells. In this study, we aimed to examine the effect of overexpression of piwil2 gene and its effects on EMT process in the prostate cancer cell line, DU145.

Methods: DU145 cell line was cultured in the RPMI media containing 10% fetal bovine serum (FBS) with 1% penicillin-streptomycin (Pen-Strep) suggested by ATCC. In order to provide DU145-PIWIL2 cell line, these cells were transfected by PCDNA3 vector expressing human piwil2 gene driven by CMV promoter via electroporation method. To provide DU145-mock cells, PCDNA3 empty vector was transferred to the cells via electroporation. G418 was then applied to the transfected cells for 4 weeks to establish stable cell lines before further cell and molecular analysis. To investigate the effect of *Piwil2* gene overexpression on cell growth, the doubling time method was performed to compare the cells growth rate between DU145-PIWIL2 and DU145-mock cells. Moreover, the expression of EMT biomarkers was investigated in the two cell lines using RT-PCR and Real-Time PCR.

Results: The doubling time analysis profiled a significant increase in DU145-PIWIL2 cells growth rate compared to DU145-mock cells, exhibiting 2.31 times higher growth in the cells overexpressing PIWIL2. Quantitative real-time PCR indicated changes in some canonical EMT biomarkers including E-cadherin, Vimentin, and Snail, with 0.77 decreases in E-cadherin expression, as well as 1.36 and 2.01 increase in Vimentin and Snail expressions respectively.

Conclusion: In this study our data showed that overexpression of *Piwil2* gene promoted the growth rate of DU145 cells and induced the EMT process in these cells, hence enhancing the invasive potential of cancer cells. Therefore, these data profiled piwil2 as an important biomarker in assessing the malignant state of prostate cancer with application in procedures related to cancer diagnostics and targeted therapies.

Keywords: Piwil2; DU145; Prostate cancer; Epithelial-to-mesenchymal transition

PS-085. Neuroprotective and Neurodifferentiative Properties of Ferulic Acid

Farshad Homayouni Moghadam[1*], Mehrnaz Mesbah Ardakani[2], Mohammad Hossein Nasr-Esfahani[1]

1. Department of Cellular Biotechnology at Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
2. Imam Hossein Hospital of Sepidan, Shiraz University of Medical Sciences, Isfahan, Iran

Email: f_homayounim@yahoo.com

Background and Aim: Ferulic acid (FA) is a phenolic compound with known anti-apoptotic and anti-oxidative properties. Effect of FA on the induction of neurogenesis and neuro-differentiation beside its effect on neuroprotection makes this compound very interesting for stem cell researches. We designed this set of experiments to evaluate the effect of FA on neuronal differentiation and neuroprotection.

Methods: ROS-mediated apoptosis was induced on PC12 cells by using hydrogen peroxide then with the treatment of different concentrations of FA was started. To evaluate the differentiation-inducing the effect of FA, PC12 cells and mouse neural stem cells (mNSCs) were treated with different concentrations of FA. MTT, quantitative real-time RT-PCR and immunostaining assays were performed on cells.

Results: FA treatment at low concentrations significantly reduced the apoptosis rate in treated PC12 cells. Real-time RT-PCR and western blot assays confirmed that FA revealed this effect through stabilization and degradation of P53 by increasing the expression rate of SIRT1, SIRT7 and MDM2 and down-regulation of USP7. Beside this anti-apoptotic effect, FA treatments on PC12 cells and mNSCs at higher concentrations on PC12 cells and mNSCs increased their differentiation toward mature neurons. Immunocytochemical staining against beta-tubulin III and Map2 verified the presence of mature neurons, and western blot assay on FA treated PC12 cells showed a stepwise rise of phosphorylated-ERK1/2 as the concentration of FA was increased.

Conclusion: our findings showed that FA at low concentrations has a neuroprotective effect through up-regulation of SIRT1, SIRT7, and MDM2, and in higher concentrations can promote neural differentiation and neurite outgrowth.

Keywords: Ferulic Acid, PC12 Cells, Apoptosis, Survival, Neuronal differentiation

PS-086. Investigating Cytotoxic and Anticancer Properties of Coumarin Derivatives Contain Geranyl Groups at Different Position on Cervical Cancer

Ebrahim H. Maleki[1], Elahe Merajifar[1], Ahmad Reza Bahrami[1], Hamid Sadeghian[2], Maryam M. Matin[1*]

1. Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran
2. Neurogenic Inflammation Research Center, Department of Laboratory Sciences, School of Paramedical Sciences, Mashhad University of Medical Science, Mashhad, Iran

Email: matin@um.ac.ir

Background and Aim: Coumarin compounds have been known to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antimicrobial, anti-arrhythmic, anti-osteoporosis, antiviral, and anticarcinogenic activities. Due to this wide variety of pharmacological values, coumarins and its derivatives have received more attention in synthesis and production.

Methods: In the present study, we aimed to investigate the effect of geranyl position on coumarin backbone on the cytotoxicity of these synthetic compounds. To do so, first, the cytotoxic effect of 3-geranyloxycoumarin (3-GC), 4-geranyloxycoumarin (4-GC), 5-geranyloxycoumarin (5-GC), 6-geranyloxycoumarin (6-GC), 7-geranyloxycoumarin (7-GC) and 8-geranyloxycoumarin (8-GC) were assessed by MTT assay on HeLa (cervical cancer) and HDF (human dermal fibroblast) cells. Furthermore, the apoptosis-inducing potential of these coumarin compounds was determined by flow cytometry.

Results: The results of the MTT assay revealed that 3-GC, 4-GC, 5-GC, 6-GC, and 8-GC had significant cytotoxic effects on HeLa cells. While they did not show any significant cytotoxicity on normal HDF cells. Moreover, the flow cytometric analysis, using PI/ FITC-Annexin V, showed that 3-GC, 4-GC, 5-GC, 6-GC and 8-GC induced apoptosis in HeLa cancer cells. Thus, they can be used as potent anticancer compounds.

Conclusion: Altogether, our results indicate that changes in the level of cellular toxicity on HeLa cells are associated with the position of the geranyl group; which puts an emphasis on the importance of the relationship between structure and activity in coumarin compounds. Our results showed that position 6 was the best site for the geranyl group. Further studies on various cancer cell lines are required to confirm these findings and future studies need to be focused on the molecular mechanisms of toxicity inducing potential of these compounds both in vitro and in vivo.

Keywords: Cervical cancer; Anticancer; Geranyloxycoumarin

PS-087. Quantitative Efficiency Comparison of Mouse Embryonic Fibroblast Isolation Methods

Vahid Hosseini[1], Ashkan Kalantary-Charvadeh[1], Mahdi Totunchi[2,3], Amir Mahdizadeh[4], Reza Rahbarghazi[1], Masoud Darabi[1*]

1. Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
4. Endocrine Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Email: darabim@tbzmed.ac.ir