addition, expression levels of telomerase reverse transcriptase (TERT), histone deacetylase 1 (HDAC1), DNA (cytosine-5)-methyltransferase 1 (DNMT1), dyskerin pseudouridine synthase 1 (DKC1), B-cell lymphoma 2 (BCL2), vascular endothelial growth factor (VEGF), Ki-67 and proliferating cell nuclear antigen (PCNA) were analyzed using quantitative real-time polymerase chain reaction (qPCR).

Results: The viability and S phase (the phase of DNA synthesis) of eAD-MSCs were increased significantly after cells were treated with vegetable-based resources (VR #9) under serum-free condition. Also, expression levels of anti-senescence and cell survival-related genes such as TERT, HDAC1, DNMT1, BCL2, VEGF, Ki-67, and PCNA were significantly increased after cells were treated with vegetable-based resources (VR #9) under serum-free condition.

Conclusions: Our findings revealed that the vegetable-based resources could promote proliferation of eAD-MSCs under serum-free condition. In addition, results of this study suggest that induction of stem cell proliferation by vegetable-based resources is likely to be related to its expression of anti-senescence and cell survival-related genes such as TERT, HDAC1, DNMT1, BCL2, VEGF, Ki-67, and PCNA under serum-free condition.

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P-178

Osteogenic Potential and Proliferating Effect of Three-Dimensional Hydrogel Scaffolds on Equine Mesenchymal Stem Cells

Mi Jeong Park, Na-Yeon Gu, Da-Un Jeong, Jeong Su Byeon, Jienny Lee, In-Soo Cho, Sang-Ho Cha*

Viral Disease Research Division, Animal and Plant Quarantine Agency, 177, Hyeoksin 8-ro, Gimcheon-si, Gyeongsangbuk-do, 39660 Republic of Korea

Introduction: Physiological cell environment not only connects cells to each other, but also connects cells to the extracellular matrix (ECM) that provide mechanical support, thus exposing the entire cell surface and activating signaling pathways. All cells in tissues consist of a complex three-dimensional (3D) structure and are connected with neighboring cells with ECM [1]. Two-dimensional (2D) culture system is typically used for cell growth, but the method reduces the characteristics of cells. Hydrogel is a polymeric material that swells in water. It can maintain a distinct 3D network structure by cross linking. Its soft and fibrous nature together with high water content and the possibility for diffusion of small components make this class of materials an ideal mimic of ECM. Hydrogel is regarded as excellent materials for encapsulating cells. In this study, to investigate the enhancing properties of 3D culture system in equine adipose tissue-derived mesenchymal stem cells (eAD-MSCs), we performed encapsulating cells and determined changes in gene expression levels of eAD-MSCs.

Materials and Methods: eAD-MSCs were seeded at normal plate (1×10^5 cells/well, condition 1), normal plate with hydrogel (1×10^5 cells/well, condition 2), transwell membrane (0.4×10^5 cells/well, condition 3) and transwell membrane with hydrogel (0.4×10^5 cells/well, condition 4) of 6-well culture plate. After culturing for 5 days, cells from these four different conditions were collected and gene expression levels of Ki-67, proliferating cell nuclear antigen (PCNA), OCT4, and SOX2 were determined by quantitative real-time PCR (qPCR) and normalized against the expression level of GAPDH. Also, differentiation into mesodermal cell lineages was performed by using osteogenic differentiation medium. Then, the mRNA expression level of specific differentiation marker such as osteocalcin (OC) was also examined by qPCR.

Results: We observed that the expression levels of Ki-67, PCNA, OCT4, and SOX2 were significantly ($p < 0.05$) increased in conditions including hydrogel (condition 2 and 4) compared to those in conditions 1 and 3. In addition, expression levels of Ki-67, PCNA, and SOX2 were significantly ($p < 0.05$) higher in condition 4 compared to those in condition 2. Also, eAD-MSCs under all conditions were well differentiated into osteocytes in osteognic differentiation medium. Among them, expression level of OC was significantly ($p < 0.05$) increased in conditions including hydrogel (conditions 2 and 4) compared to those in conditions 1 and 3.

Conclusions: These results suggest that 3D culture of eAD-MSCs through hydrogel scaffolds method can enhance the expression of proliferating and osteogenic factors. Also, surrounding culture condition is found to be better than plating culture condition including hydrogel. Therefore, these 3D culture systems can be used to enhance the efficiency of tissue engineering in eAD-MSCs compared to the traditional cell culture system.

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P-179

Transforming growth factor-beta induced epithelial-mesenchymal transition abolished without TGF-beta condition: Cancer stem cell properties and cisplatin resistance in lungcancer cell lines

Seong-Kwan Kim, Jin-A Park, Sang-Hyun Cho, Weijia Zheing, Jeong-Min Choi, Ho-Chul Shin*

Department of Veterinary Pharmacology and Toxicology, College

of Veterinary Medicine, Konkuk University, Seoul 143-701, Republic of Korea

Introduction: Epithelial-Mesenchymal transition (EMT) hypothesis have been developedto explain metastasis, chemotherapy resistance, even though cancer stem cell (CSC) theory. Also Mesenchymal-Epithelial transition (MET), reverse process of EMT, have been connected with EMT for explainmetastasis theoretically. However, it is not clear if MET is essential phenomenonfor cancer metastasis, especially in lung cancer. In lung cancer cells,there were few reports regarding the relationship between cancer metastasis and MET, compared to breast cancer. In this study, we examined lungcancer cell line, A549, H23, H358, andH1299 cell inducing EMT and METusing transforming growth factor beta (TGF-beta) and investigated some propertiesconcerned with cancer metastasis andchemo-resistance.

Materials and Methods: TGF-beta (10 ng/mL) was added everyother day to normal RPMI 10% FBSmedia for 6 days and the EMT properties was monitored using Western Blot. After treatment of TGF-beta, the media waswashed with PBS and the cells were incubated for 6 extra day to make MET group.Epithelial cell marker, E-cadherin, and mesenchymal cell marker Vimentinwere used to check the portion of cell protein. Furthermore, a chemotherapy resistance property was measuredusing MTT assay in 100 ng/mL cisplatin exposure for 24 h.

Results: InTGF-beta induced EMT group, while E-cadherin was decreased, the Vimentin was increased; in MET group, E-cadherin and Vimentinwas approximately similar to negative control on day 12 in resting group without TGF-beta. Concerning the signaling mechanism, active caspase 3 was decreased in EMT group and MET group. Moreover TGF-beta induced EMT and MET groupwas susceptible to Cisplatin compared to negative group.

Conclusions: In conclusion, lung cancer celllines needs spontaneous TGF-beta supplement to maintain mesenchymal states, butonce TGF-beta triggered EMT, it induces become more susceptible to cisplatinmedication and decreased caspase 3 activation.

P-180

Upregulation of death receptor 5 mediated by telmisartan enhances TRAIL-mediated apoptosis via inhibition of autophagy flux in lung cancer

Mohammad Rasheduzzaman, Jeong-Min Hong, Uddin MD Nazim, Hong-hua Yin, You-Jin Lee, Jae-Won Seol, Sang-Youel Park*

Biosafety Research Institute, College of Veterinary Medicine, Chonbuk National University, Iksan, Jeonbuk 54596, South Korea

Introduction: Telmisartan is an angiotensin II type 1 (AT1) receptor blocker (ARB) broadly used for the treatment of hypertension also familiar for its anti-cancerous properties. However, Anti -cancerous effects of telmisartan and its underlying mechanism still remain elusive. TRAIL has the potential to kill tumor cell beyond minimal toxicity in the normal cell, despite this resistant of TRAIL is the growing concern in lung cancer. Autophagy is a highly conserved self-degrading lysosomal pathway that is crucial for maintaining cellular death, survival in stress condition and finally maintaining cellular homeostasis. Role of autophagy is the double-edged sword in the cancer cell.

Materials and Methods: A549 cells were seeded at a density of 1.0×10^4 cells onto 12-well plates and incubated at 37°C for 24 h. The A549 cells were pretreated with telmisartan at concentrations of (0, 10, 20, 40μM) following 12 h then recombinant TRAIL protein (100 ng/ml)was treated and co-incubated for additional 2.3 h. Additionally, cells were pretreated with chloroquine (20 μM) for 1 h, followed by telmisartan treatment. Cell morphology was analyzed by taking photographs under inverted microscopy (Nikon, Japan). Cell viability was determined by using the crystal violet staining method, MTT and LDH assay. Activation of DR5, apoptotic cascade and autophagy function was evaluated by ROS assay, Immunoblotting, Immunocytochemistry analysis.

Results: In the current research, we figured for the first time telmisartan combined with TRAIL enhance the apoptosis in TRAIL-resistant non-small-cell lung carcinoma (NSCLC) cells. The mechanism includes the inhibition of autophagy flux by telmisartan resulting ROS generation leading to death receptor (DR5)activation and subsequent activation of caspase cascade by TRAIL treatment. Furthermore, application of ROS scavenger N-acetyl-cysteine (NAC) rescue the cells undergo apoptosis by abrogating the activation of DR5 and finally the caspase cascade.

Conclusions: Therefore, application of telmisartan attenuates TRAIL resistance and enhances TRAIL-mediated tumor cells death that would be the novel therapeutic approaches to fight against lung cancer.

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