ImmunoTools *special* Award 2015

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Tissue engineering and stem cell therapies

Our laboratory is working with cancer and stem cells and their interactions for several applications. We started our studies with the characterization of mesenchymal stem cells (MSC) from various sources. Now, we are using MSC from these sources for tissue engineering and cell therapy approaches. For example, we showed that priming MSC (pMSC) towards cardiomyogenic lineage enhances their beneficial effects in vivo as treatment option for acute phase myocardial infarction. Echocardiography analysis indicated that MSC-treated myocardium presented discrete improvement in function, but it also showed that pMSC treatment lead to superior beneficial results, compared with undifferentiated MSC. In another study, we demonstrated that human serum is a suitable supplement for the osteogenic differentiation of MSC seeded on poly-3-hydroxibutyrate-co-3-hydroxyvalerate (PHB-HV) scaffolds. The osteogenic capacity of MSC seeded on PHB-HV scaffolds indicates that this scaffold is adequate for cell growth and differentiation and that allogeneic human serum is a promising supplement for the in vitro expansion of MSC. We studied the endothelial differentiation of MSC seeded onto electrospun polyhydroxybutyrate/polyhydroxybutyrate-co-hydroxyvalerate (PHB/PHB-HV) fiber mesh. The in vitro evaluation demonstrated good adhesion and a normal morphology of the MSC. After 7, 14 and 21 days of seeding MSC onto electrospun PHB/PHB-HV fiber mesh, the cells remained viable and proliferative. Moreover, when cultured with endothelial differentiation medium (i.e., medium containing VEGF and bFGF), the MSC expressed endothelial markers such as VE-Cadherin and the vWF factor. Therefore, the electrospun PHB/PHB-HV fiber mesh appears to be a suitable material that can be used in combination with endothelial-differentiated cells to improve vascularization in engineered bone tissues. We also are using the MSC for

several toxicological and cell signalling studies. For example, we used an adenoviral (Ad) vector encoding the $Ca(^{2+})$ chelator protein parvalbumin (PV) fused to a nuclear exclusion signal (NES) and the Discosoma red fluorescent protein (DsRed) to investigate the function of cytoplasmic $Ca(^{2+})$ signals on MSC proliferation. Confocal microscopy was used to demonstrate that PV-NES-DsRed was expressed in the cytoplasm. $Ca(^{2+})$ signaling was monitored by using Fluo-4-AM. Fluorescence-activated cell sorting analysis of cells that were stained with propidium iodide was used as a quantitative measure of cell death. The mitotic index was assessed by immunofluorescence, and the expression of cyclins was examined with Western blot. Our results show that the Ad-PV-NES-DsRed fusion protein decreased serum-induced $Ca(^{2+})$ signaling and blocked the proliferation of rat adipose-derived MSCs (AT-MSCs) in prophase. Fluorescence-activated cell sorting analysis revealed that Ad-PV-NES-DsRed did not induce cell death in AT-MSCs. Furthermore, Western blot analysis demonstrated that Ad-PV-NES-DsRed reduced extracellular signal-regulated kinase (Erk1/2) phosphorylation and cyclin B1 expression. Buffering cytosolic $Ca(^{2+})$ did not alter the expression of cyclins A/D1/D2/D3/E and E2. We use the reagents from ImmunoTools for Immunophenotype characterization of MSC and for our studies of how cytokines and growth factors control migration and proliferation of these cell models in vitro and in vivo.