Integrin linked kinase expression prevents anoikis in human mesenchymal stem cells

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Statement of Purpose:

Tissue engineering is the regeneration of new tissue to replace that which has been damaged. One method of tissue engineering is to encapsulate mesenchymal stem cells (MSCs) in a poly (ethylene glycol) (PEG) based hydrogel, which acts as a scaffold to support cell growth. However, since PEG hydrogels are resistant to protein adhesion, and MSCs are adhesion-dependent cells, viability has been a major issue (1). MSCs rely on adhesion to the extracellular matrix (ECM) for survival. Cell adhesion is regulated by signaling effects of various intracellular proteins, including integrin-linked kinase (ILK) (2). This experiment tests a possible method to increase viability of PEG-encapsulated MSCs by infecting them with a virus that will increase the expression of ILK in the cells without adhesion to the ECM.

Methods:

Infecting MSCs with ILK-producing virus

MSCs were seeded onto 12-well plates and cultured in control media. The cells were then infected with a green fluorescent protein (GFP) producing virus at multiplicities of infection (MOI) of 1, 10, 1000, and 10000 to determine the necessary viral concentration for infection. The cells were then infected with a virus that produces ILK, as well as a cyclization recombinase producing virus for use as a control. The cells were lysed and analyzed using western blot analysis to verify an increase of ILK production. The immunoblots were incubated in rabbit anti-ILK antibody overnight and then in peroxidase-coupled goat anti-rabbit IgG for 1 h and developed using an Opti 4CN kit.

Examining effects of ILK

MSCs were infected with the ILK-producing virus and encapsulated in PEG-based gels. Using confocal microscopy and a live/dead assay, the survivability of the infected cells was monitored.

Viculin Quantification

Western blot analysis was used to verify the production of viculin in the cells. Immunoblots were incubated in mouse anti-viculin antibody overnight and peroxidase-coupled goat anti-mouse IgG for 1h and developed using an Opti 4CN kit.

Results:

Viral Infection/ILK production

The western blot analysis of the ILK infected cells not only verified the production of ILK, but also showed that ILK production in MSCs increases with increased concentration of virus in a dose-dependent manner.

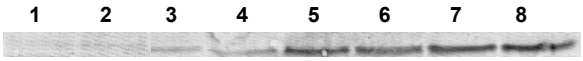

Figure 1. Western blot analysis of MSCs infected with ILK-producing virus. Lanes 1,2 – MOI = 1; lanes 3,4 – MOI = 10; lanes 5,6 – MOI = 100; lanes 7,8 – MOI = 10000.

Effects of ILK increase on cell viability

The results of the live/dead assay showed that an increase in the production of ILK increases the viability of encapsulated MSCs.

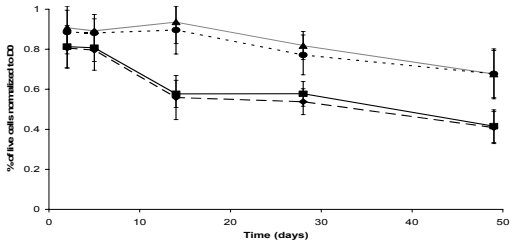

Figure 2. 10% PEGDA + uninfected hMSCs (--♦--), 10% PEGDA + CRE-infected hMSCs (—■—), 10% PEGDA + 5 mM RGD + uninfected hMSCs (—▲—), and 10% PEGDA + ILK-infected hMSCs (··●··). Uninfected hMSCs were encapsulated in unmodified PEGDA or PEGDA containing 5 mM Ac-PEG-RGD.

Viculin Quantification

The results of the immunoblot staining showed similar levels of viculin for cells infected with ILK and encapsulated in PEGDA and for uninfected cells encapsulated in RGD-modified PEG. Viculin is a protein that is associated with focal adhesions that occur from interaction with the ECM, so this result is expected.

Conclusions:

The results of the western blot analysis verified that the production of ILK in MSCs can be increased by infecting the cells with an ILK-producing virus. The results of the live/dead study showed that viability is increased with increased production of ILK and is unchanged by the presence of CRE, which verifies that the increase in viability of ILK infected cells is due to ILK production and not the infection process. The increase of viability when ILK levels are increased verifies that the pathway to prevent anoikis is triggered by ILK.

References:

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