The Correlation Between Substrate Stiffness and TGF-β1-Induced Hepatic Stellate Cell Activation

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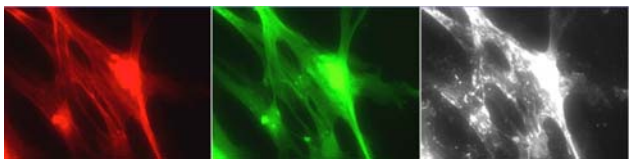
Statement of purpose: Fibrosis in the liver is caused by inflammation triggered by alcoholism or other types of injury. Hepatic stellate cells (HSCs) in the liver become activated when the liver is inflamed and can cause fibrosis, which consists of excessive extracellular matrix production. Inactive HSCs appear round and express PPAR-γ (Hazra et al. 2004). Activated HSCs are characterized by α-smooth muscle actin (α-SMA) expression, and loss of PPAR-γ expression. Published work has shown that TGF-β1 and substrate stiffness induce activation in HSCs (Olsen et al. 2011). TGF-β1 signaling stimulates collagen-rich ECM production, which increases tissue stiffness. Gao et al. have shown that BMP-2 can inhibit activation and even has the potential to induce inactivation; other sources have suggested that BMP-7 may act similarly. The aim of this project is to investigate the effect of substrate stiffness, TGF- β1, BMP-2 and BMP-7 on HSC activation.

TGF-β1, BMP-2 and BMP-7 are members of the TGF-β superfamily (Hayashi et al. 2012). TGF-β1 and fibronectin (FN) are up regulated during inflammation. FN is assembled by cellular contractile forces and is a key component of ECM remodeling. When unraveled, growth factor binding sites on FN are exposed, allowing TGF-β1 to bind to it (Olsen et al. 2011).

The objective was to vary TGF-β1, BMP-2, and BMP-7 concentrations in addition to varying the stiffness so that the effects of these signals on activation can be observed. HSCs were plated on 0.4, 1, 2, 3, 5, 8, 10, and 20kPa gels. Activation was observed in cells plated on gels with stiffness above 5 kPa.

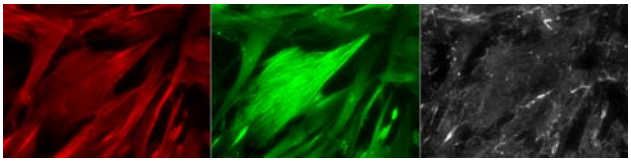
Methods: HSCs were maintained in collagen-coated flasks, and then transferred to polyacrylamide gels of various stiffness. Gels were made by varying the acrylamide and bis-acrylamide, concentrations. Gels were then coated with FN for 30 minutes. HSCs were allowed to plate for 24 hours before adding TGF-β1 (2 ng/ml) for 48 hours. Cells were fixed and immunofluorescently labeled for actin, α-SMA (marker of activation), and either FN (marker of activation) or PPAR-γ (marker of inactivation).

Results:



a b c

Figure 1: HSCs plated on 20kPa gels with no added TGF- β: (a) actin (b) α SMA (c) FN.



a b c

Figure 2: HSCs plated on 20kPa gels with 2ng/mL of TGF- β: (a) actin (b) α SMA (c) FN.

As a control experiment, HSCs were plated onto glass coverslips and TGF-β1 was added to them every 48 hours for 10 days in order to observe the long-term effects of TGF-β. On glass coverslips, there was not a clear distinction in activation levels of + and - TGF-β1 coverslips because the stiffness of glass (~ 3 Gpa) causes cells to become activated without the addition of growth factors (Olsen et al. 2011). The presence of TGF-β1 for a longer period resulted in more activation and less presence of PPAR-γ as compared to the control samples. The cells were activated on all of the coverslips.

HSCs generally showed activation on gels stiffer than 5 kPa. These cells were α-SMA positive, displaying strong stress fibers, and lost PPAR-γ expression. They also exhibited higher FN production and assembly. The plating on gels with stiffness lower than 5kPa was sparse, presumably due to poor attachment. Cells on softer gels were round and the addition of TGF-β1 appeared to induce apoptosis, which is consistent with TGF- β1 activity in other cell types (Leight et al. 2012).

Conclusion:

HSC activation was enhanced on gels with stiffness higher than 5kPa and in cells that were exposed to TGF-β1. Addition of TGF-β1 to gels with lower stiffness induced apoptosis. Activation on glass did not require TGF- β1.

References:

Gao, X., Cao, Y., Yang, W. et al. "BMP2 inhibits TGF-β-induced pancreatic stellate cell activation and extracellular matrix formation." American Journal of Physiology- Gastrointestinal Liver Physiology. 304. (2013); 804-13. Web. 25 Apr. 2014.

Hayashi, H., and Sakai,T. "Biological Significance of Local TGF-β activation in liver diseases." Frontiers in Physiology. 3. (2012); 1-11. Web. 25 Apr. 2014.

Hazra,S., Miyahara., T., Rippe, R., Tsukamoto, H,. "PPAR Gamma and Hepatic Stellate Cells." Comparative Hepatology.(2004); 3. Web. 24 Oct. 2014.

Leight, J., Wozniak, M., Chen, S., Lynch, M., Chen,C., "Matrix Rigidity regulates a switch between TGF-β1-induced apoptosis and epithelial-mesenchymal transition." Molecular Biology of the Cell. (2012); 13781-91. Web. 25.Oct.2014.

Olsen AL, Bloomer SA, Chan EP, Gaca MD, Georges PC, Sackey B, Uemura M, Janmey PA, Wells, RG. "Hepatic stellate cells require a stiff environment for myofibroblastic differentiation. American Journal of Physiology." Gastrointestinal and liver physiology. (2011); 301(1): G110-8. Web. 25 Apr 2014.

Wells RG. "The role of matrix stiffness in hepatic stellate cell activation and liver fibrosis." Journal of Clinical Gastroenterology. (2005); 39(4 Suppl 2):S158-61. PubMed. Web. 15 Oct. 2014.