P19

Utilization of Fibronectin for the Isolation of Specific Valve Cell Subpopulations: Application for the Study of Mitral Valve Diseases

Elizabeth Stephens[1], Trevor Huynh[2], Jennifer Cieluch[1], Jane Grande-Allen[1]
[1]Rice University, Houston, TX, United States, [2]University of Minnesota, Minneapolis, MN, United States

Objectives:
Diseased valves have been shown to contain unique cell populations, but it has been difficult to isolate such cells for further study. In this study fibronectin (FN), a protein known to be up-regulated in diseased tissues, was used to isolate subpopulations of cells from the mitral valve (MV).

Method:
Cells from the posterior leaflet of porcine MVs were separated based on time-dependent adhesion to either tissue culture plastic (TCP) flasks or FN-coated flasks. The resultant "FAST" adhering and "SLOW" adhering subpopulations from each technique were phenotyped using flow cytometry (FC) and immunocytochemistry (ICC). Markers evaluated included prolyl 4-hydroxylase (P4H), heat shock protein-47 (HSP47), smooth muscle alpha-actin (SMαA), non-muscle myosin (Smem), smooth muscle myosin (SMM), extracellular-related signaling kinase (ERK) 1, ERK2, and phosphorylated-ERK (PERK).

Results:
By FC, FN FAST showed higher expression of P4H, HSP47, SMαA, Smem, ERK1, ERK2, and PERK. In contrast, there was only a significant difference between the two TCP subpopulations in ERK1, which was decreased in the TCP FAST group compared to the TCP SLOW group. Similarly in ICC FN FAST showed higher expression of ERK2, PERK, HSP47, SMaA, and Smem compared to FN SLOW, while TCP subpopulations were not as markedly different and only showed higher expression of HSP47, SMaA, and Smem compared to TCP slow.

Conclusions:
Differential adhesion to FN successfully separated a myofibroblast-like subpopulation from the posterior MV. This subpopulation may be useful in studying myxomatous MV disease, although additional studies remain to verify that this myofibroblast-like population is that seen in myxomatous MV disease.