Mechanotransduction in cardiovascular physiology and pathology

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Background: Age and pathology exert a negative impact on cardiac progenitor cells (CPCs), increasing their senescence rates and altering their functions. Importantly, mechanotransduction plays a relevant role in stem cell biology, modulating cell proliferation and differentiation. Last, cardiac remodeling is characterized by alterations of the mechanical properties of the myocardium (e.g. stiffening and increased wall stress).

Aim: to evaluate if cardiac remodeling is coupled with alteration of the mechanotransduction pathways of CPC.

Methods and Results

To gain insights into the mechanisms associated with CPC dysfunction, we studied cells obtained from normal donors (D-CPC) and from explanted, failing hearts (E-CPC). KEGG pathway analysis of genes differentially expressed by D- vs E-CPC (as assessed by cDNA microarray) revealed a significant enrichment of themes associated with cytoskeletal dynamics and mechanotransduction. To assess functional differences, we cultured D-and E-CPC in a specifically designed microfluidic chip that imposes them a cyclic equiaxial mechanical loading (15%, 1Hz) or a commercial platform (FlexCell) designed to impose cyclic strain to large cell cultures. D- and E-CPC were analyzed at 24 and 48 hours after stretching, by immunofluorescence, to assess both the cell density and the subcellular localization of YAP (a transcriptional coactivator that transduces mechanical forces into cell proliferation). Cyclic stretch was significantly associated with both increased proliferation of D- ($n=6$, $p<0.0001$) and E-CPC ($n=4$, $p=0.003$), and with a significant reduction of nuclear localized YAP (N-YAP) as a function of time ($p<0.05$). However, with respect to un-stretched cells, mechanical load increased the fraction of CPC with N-YAP at high confluence ($p<0.017$). While E-CPCs showed, even in static conditions, a significant correlation between cell density and decreased N-YAP ($p=0.003$, $r2= 0.37$), this was not evident for D-CPC, suggesting a less stringent control of YAP nuclear localization and contact inhibition in D-CPC.

To directly verify this latter feature, we grew CPC at 50, 100, and 500 cells/mm2. Intriguingly, while D-CPC did not reduce N-YAP positivity as a function of cell density, E-CPC did. These data were corroborated by realtime PCR analysis of YAP-regulated genes (ANKRD1, CTGF, CYR61) and immediate early genes (cFOS and IER3).

Finally, we assessed the effect of substrate elasticity on N-YAP and cell proliferation by culturing D- and E-CPC on polyacrylamide gels with mechanical compliance ranging from 1kPa to 231kPa. We could observe only in E-CPC a significant positive correlation among proliferation, N-YAP activation and substrate stiffness. Importantly, the expression of YAP-regulated targets (ANKRD1, CTGF, CYR61) paralleled N-YAP localization, while only D-CPC upregulated Stem Cell markers (Oct4 and cKit) on stiff substrates.

Conclusions

D- and E-CPC differ in their ability to respond to mechanical stimuli such as: cyclic mechanical loading, cell to cell contact, and cyclic stretching. The ability to modulate the nucleo/cytoplasmic shuttling of YAP seems to be involved in these differences, however the molecular details of this different behaviour are under scrutiny.