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Selective inhibition of the Mek1/2-Erk1/2 signalling pathway induces the differentiation of human cardiac pericyte-like cells into contractile vascular smooth muscle cells

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Background: Arteriogenesis is key for tissue repair but whether myocardial stromal cells contribute to this phenomenon remains unknown.
Purpose: Investigate if cardiac pericytes are a druggable target for therapeutic arteriogenesis.
Methods and results: The localization of pericyte-like cells (PCs) was assessed in the human and murine heart by immunohistochemistry of typical antigenic markers. CD34+ PCs co-expressing NG2 and PDGFRβ but not endothelial cell (EC) or vascular smooth muscle cell (VSMC) antigens were identified in peri-arterial position in normal hearts. Interestingly, we also found rare PCs co-expressing αSMA in the peri-infarct myocardium, suggesting these cells could represent a transitory phenotype between PCs and VSMCs. Next, we isolated human cardiac PCs by immunosorting for CD31 and CD34 and established the purity of the isolated CD34+CD31- fraction by flow cytometry. Following culture expansion, cardiac PCs maintain the typical antigenic profile except for CD34. Moreover, we confirmed the PCs' ability to promote angiogenesis in-vitro. The withdrawal of EGF and bFGF from the culture media for 10 days induced the differentiation of PCs into mature VSMCs, as documented by a massive upregulation of contractile genes MYH11, CNN1 and ACTA2 (200-, 35- and 15-folds increase versus naïve PCs, $p<0.01$), which was followed by the induction of SM-MHC, Smoothelin B, αSMA, Calponin and SM22α proteins ($p<0.05$ versus PCs). In addition, PC-derived cells lost migratory capacity, secreted elastin, and responded to endothelin-1 in a contraction assay, thus phenocopying the behaviour of control coronary artery-derived VSMCs. We excluded contamination of the PC preparation by verifying similar phenomena occur in PCs expanded from single cell clonogeneic assays. Moreover, the process is partially reversible, with PC-derived VSMCs being able to reacquire some intermediate markers following EGF/bFGF re-challenge. ECs secrete EGF and bFGF, with this GF signalling being enhanced by hypoxia, suggesting ECs may control the PC phenotype in a paracrine fashion. Mechanistic studies revealed the Mek1/2-Erk1/2-Elk1 signalling is accountable for the transcriptional repression of VSMC genes in PCs. Accordingly, a selective Mek1/2 inhibitor (PD0325901) was able to switch the definitive VSMC phenotype of PCs maintained in full media. The drug prevented the phosphorylation of Erk1/2 and its downstream target Elk1. This likely relieves the complex SRF/MyocD and abolishes the transcriptional repression at the gene promoter.
Conclusions: Cardiac PCs have a VSMC potential which is under the inhibitory control of the Mek1/2-Erk1/2-Elk1 signalling. Mek1/2 inhibitors showed promises for the treatment of melanoma and solid tumours. A novel application of this class of compounds to improve arteriogenesis in myocardial ischemia is fascinating. The caveat about their potential cardiotoxicity could be less relevant with short duration treatments of myocardial ischemia.