HDAC1 depletion in human cardiac mesenchymal stromal cells facilitates paracrine-mediated endothelial cell growth and tube formation through a mechanism involving enhanced bFGF production and secretion

David Hagan
University of Louisville, USA

Introduction: Cardiac mesenchymal stromal cell (CMC) administration has been documented to improve cardiac function in pre-clinical animal models of heart failure. While the precise mechanism(s) underlying their therapeutic benefits remain unclear, both transdifferentiation (contributing to formation of new cardiac parenchyma) and secretion of paracrine signaling molecules (promoting neovascularization, cell survival, etc.) have been implicated as major modes by which transplanted cells exert their cardiac reparative effects. Thus, many laboratories have focused on novel methods to improve donor cell cardiogenic differentiation and/or cytokine secretion to enhance their therapeutic potential. We have previously shown either pharmacologic inhibition or genetic depletion of *HDAC1* to promote CMC lineage commitment towards a cardiomyogenic/endothelial cell-like fate. Further, in a pilot study, human CMCs pre-treated with the benzamide *HDAC1* inhibitor, entinostat (MS-275), exhibited superior ability to attenuate adverse left ventricular remodeling and yielded greater improvement in ventricular function relative to untreated CMCs when transplanted into a rat infarct model. While cardiogenic differentiation of *HDAC1*-inhibited CMCs may account for these functional improvements, we have previously shown inhibition of *HDAC* activity to alter CMC cytokine secretion – an effect that may have profound consequences on endogenous repair mechanisms (including cell proliferation and neovascularization). To this end, in the current study, we sought to investigate the influence of *HDAC1*-depletion on CMC cytokine secretion and associated paracrine-mediated activities on endothelial cell function *in vitro*.

Methods: Patient-derived CMCs were transduced with shRNA constructs targeting human *HDAC1* (sh*HDAC1*) or non-target (shNT) controls. Conditioned media (CM) was collected from shHDAC1 or shNT transduced CMCs cultured in F12 media in the absence of FBS for 24 h. Cytokine protein arrays were employed to comprehensively assess and compare/contrast the expression of >100 secreted proteins in CM from sh*HDAC1* or shNT-transduced CMCs. *In vitro* functional assays for cell proliferation, protection from oxidative stress, cell migration, and tube formation were performed on human endothelial cells incubated with CM from untransduced, shNT, or sh*HDAC1* human CMCs to compare/contrast paracrine signaling activity.

Results: Cytokine protein arrays revealed a pronounced increase in the secretion of a number of cytokines involved in cell growth, migration, and differentiation in CM from sh*HDAC1*-transduced CMCs. Consistent with these observations, sh*HDAC1* CM more efficiently promoted endothelial cell proliferation and tube formation compared to that of CM from shNT or untransduced CMCs. In an effort to narrow down which secreted factors may be responsible for these affects, key cytokines previously implicated in cell therapy-mediated cardiac repair were interrogated in shHDAC1, shNT, and untransduced CMCs. We revealed bFGF to be significantly upregulated at both the mRNA and protein levels in sh*HDAC1*-transduced CMCs vis-à-vis shNT and untransduced CMCs. Furthermore, shRNA-mediated depletion of *bFGF* in *HDAC1*-depleted CMCs was able to inhibit the effects of sh*HDAC1* CM in promoting both endothelial proliferation and tube formation. Thus, our results demonstrate that *HDAC1* depletion activates CMC proangiogenic paracrine signaling through a mechanism involving the enhanced secretion of bFGF. Conclusion: These results reveal a hitherto unknown role for *HDAC1* in the modulation of CMC cytokine secretion and implicate the targeted inhibition of *HDAC1* in CMCs as a means to enhance paracrine-mediated neovascularization in cardiac cell therapy applications.

dphaga01@louisville.edu