Mitral valve endothelial cells secrete osteoprotegerin during endothelial mesenchymal transition

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Background: Mitral valve prolapse (MVP) affects more than 176 million people worldwide. Despite this, little is known about the molecular and cellular mechanisms involved in the progression of MVP and surgical intervention is the only available option. MVP is a disorder characterized by extracellular matrix (ECM) remodelling. The disruption of ECM tight regulation and the constitutive activation of valve interstitial cells (VIC) have been linked to MVP, as well as valve endothelial cell (VEC) mesenchymal transition (EndMT). Osteoprotegerin (OPG) is involved in a myriad of physiological and pathological processes. In the vascular environment, endothelial cells and smooth muscle cells constitutively secrete OPG. Here, we investigated the role of OPG during EndMT in MVP.
Methods: Human VECs and VICs were isolated from posterior mitral valve leaflets of patients who underwent mitral valve repair (n=25). Plasma was collected from 57 subjects (29 controls and 28 MVP patients).
Results: Osteoprotegerin was significantly elevated in prolapsed tissues when compared to healthy tissues (100±49.1% vs. 309±49.8%, respectively; $p<0.05$). During EndMT, VECs showed a significant up-regulation of OPG mRNA levels (4.1±1.3 fold vs. untreated cells; $p<0.05$). In addition, VECs undergoing EndMT secreted more OPG when compared to untreated cells (2369±564.7 pg/μg and 923.5±261.1 pg/μg of total protein, respectively; $p<0.05$). Moreover, the OPG treatments triggered autocrine effects characterised by increased mRNA levels of Col1A1 (+4.0±0.6; $p<0.001$), Col3A1 (+3.0±0.6; $p<0.01$), BMP4 (+2.4±0.7; $p<0.05$), and accelerated migration (43.3±3.9% for OPG treated cells vs. 21.8±2.6% area closed for untreated cells; $p<0.001$). OPG promoted also VICs proliferation (22.4% increment; $p <0.05$) and significantly up-regulated mRNA levels of Col1A1 (+2.24±0.29; $p<0.001$), Col3A1 (+1.58±0.29; $p<0.05$) and BMP4 (+1.6±0.1; $p<0.05$). Finally, OPG plasma levels were significantly higher in MVP patients compared to control subjects (1953±127.5 pg/mL and 1109±45.3 pg/mL, respectively; $p<0.0001$), with an area under the receiving operator characteristic (ROC) curve of 0.92.
Conclusion: We reported, for the first time, the involvement of OPG in cellular and molecular changes in MVP isolated cells. In addition, we detected elevated circulating OPG levels in MVP patients when compared to controls, which supports the hypothesis that OPG could be involved in MVP development and/or progression.