Properties of human cardiac fibroblasts in Hypertrophic Cardiomyopathy

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Fibroblast overgrowth and secretion of collagen (fibrosis) represent major determinants in structural (cardiac hypertrophy, myocyte misalignment) and functional abnormalities (arrhythmias) associated with Hypertrophic Cardiomyopathy (HCM). 5-HT2 receptors play a key role in the modulation of cardiomyocyte and non-cardiomyocyte cells cycle. Recent data obtained by our laboratory, suggested that 5HT receptors are overexpressed in human HCM tissue.
The aim of this study was to set up primary cultures of human fibroblasts from affected and non-affected hearts and assess the effect of 5-HT2 receptor stimulation on morphological, secretory, and proliferative response indexes of human fibroblasts.
Human cardiac fibroblasts (hCF) were isolated from fibrotic tissue derived from endocardial tissue from HCM patients undergoing surgical myectomy. The tissue was cut into small pieces and dissociated by enzymatic digestion with Collagenase II (1 mg/mL) and Albumin (1mg/mL). The medium was changed after 24 h and fibroblast cells began to proliferate from fibrotic tissue. The settled cells were maintained in culture, fixed for immunocytochemistry and harvested to extract RNA for real-time PCR analysis.
The fibroblast phenotype of isolated cells, and during the different passages in culture, was verified by the staining with the Fibroblast Surface Protein 11333 antibody. hCF isolated from HCM patients showed a highly variable shape and dimension among different cells. Immunocytochemistry staining showed that the phenotype did not change after treatment with angiotensin II (100 nM) and α-methyl-serotonin (1 μM). Cell viability and proliferation of hCFs HCM culture were performed by MTT test in control conditions and after stimulation with endothelin-1 (10 nM), angiotensin II (100 nM) and α-methyl-serotonin (1 μM) and basic fibroblast growth factor (bFGF; 100 ng/ml) as positive control. Cell viability and proliferation were increased by AngiotensinII and α-methyl serotonin.
5HT2B receptors were expressed in both control and HCM samples. The expression level, evaluated by Real time PCR, was significantly increased in ventricular and septal tissue from HCM with respect to control; in isolated fibroblasts, the expression being similar in the two groups. Stimulation of fibroblasts with α-methyl-5HT, a selective 5-HT2 agonist, elicited a marked response as documented by appearance of calcium transients due to release from intracellular stores, as suggested by the occurrence of transients also in the absence of extracellular calcium in the medium. 5HT2 receptor does not modify HCM fibroblast proliferation. The mRNA level for type-1 alpha collagen (Col1A1), an index of fibroblast activation, was significantly higher in hCF from HCM respect to control. It was reverted by angiotensin II (100 nM) and α-methyl-serotonin (1 μM). In summary, we have tested and validated a novel method to isolate viable fibroblasts from fresh samples from HCM patients. AngiotensinII and α-methyl- serotonin exerted a clear stimulating effect on fibroblast growth, suggesting a modulatory role of the pathway downstream AT1 and 5HT2. In HCM fibroblasts stimulation of 5HT2 receptor elicits a transient calcium release from intracellular stores and a reduction of the expression of Col1A1 which is over-expressed in fibroblasts from HCM patients. This signal may represent a limiting event in the development of cardiac hypertrophy in HCM.