

**Notch signaling is abnormally activated in cardiac progenitor cells of tetralogy of Fallot patients**

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Tetralogy of Fallot (TOF) is a congenital heart defect that is characterized by four anatomical abnormalities: ventricular septal defect, biventricular connection of the aorta, subpulmonary stenosis, and right-ventricular hypertrophy. TOF is the most common cyanotic heart defect and the most common cause of blue baby syndrome. The cellular and genetic mechanisms of this defect are so far unclear. Recently fine-tuned sequential activation of Notch genes have been shown to be responsible for the proper heart chamber development. In addition mutations in several genes of the Notch pathway have been shown to be associated with TOF. The aim of this study was to analyze the activity of Notch pathway in the cardiac stem cells (CSC) derived from myocardial tissue of TOF patients.

We isolated CPC from 8 TOF patients (saturation 70-80%, EF 60-80%) and from 4 patients with ventricular septal defects (saturation 100%, EF 70-80%). We analyzed the activity of Notch pathway by estimating the expression of Notch genes and receptors as well as the main Notch target gene HEY1 by qPCR. To test the differentiation capacity of CPC we differentiated CPC to cardiogenic, adipogenic and osteogenic lineages. To verify that the activation of Notch signaling influences the differentiation potential of CPC we infected the CPC with lentiviruses bearing activated Notch intracellular domain (NICD) and also estimated differentiation capacity of CPC. The CPC derived from TOF patients had significantly higher NOTCH1 and HEY1 gene expression. This elevated Notch activity corresponded to higher differentiation rate to cardiac lineage estimated by Nkx2.5, MEF2C and Troponin T expression by qPCR as well as alpha-actinin and Troponin I levels by ICH staining of differentiated cells. Activation of Notch by lentiviral NICD expression also caused elevation of cardiogenic markers in the CPC confirming the role of Notch signaling in determining cardiogenic potential of CPC.

Our data on CPC derived from TOF patients suggest direct involvement of Notch pathway dysregulation in the pathology of TOF and further confirms that fine-tuned Notch signaling is one of the key factors responsible for the appropriate heart development.