Abnormal Cardiac Differentiation Underlies Cardiac Hypertrophy in Noonan Syndrome With RAF1 Mutation

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Noonan Syndrome (NS), an autosomal dominant RASopathy disorder, is caused by germ-line mutations that affect the canonical RAS-MAPK pathway. >95% of NS patients with an S257L mutation in *Raf1* exhibit cardiac hypertrophy (CH) at a very young age. However, the molecular and developmental mechanisms that elicit CH in these patients remain unknown. Hence, we aimed at modeling CH by differentiating human NS *Raf1* induced-pluripotent stem cells (iPSCs) towards the cardiomyocyte fate. We generated iPSCs from an NS pediatric patient with the S257L/+ mutation in the *Raf1* gene and corrected the mutation using CRISPR-Cas9 double nickase system. Differentiation of the mutant NS line led to a significantly increased number of cardiomyocytes (CMs) as measured by FACS sorting at day 20, as compared to isogenic corrected cells (5.71 million ± 0.38 of cTNT+ cells vs 3.77±0.54, n=6, $p<0.01$). Differentiation of mutant iPSCs into mesodermal cells was normal; however, differentiation into cardiac progenitor cells was enhanced by the mutation, as demonstrated by the increased number of NKX2.5+ cells in the mutant culture at this stage of development. In addition, differentiated S257L/+ CMs had a significantly increased cell surface area compared to the control CMs (3,331 $\mu m^2 \pm 325$ vs 1,638 $\mu m^2 \pm 97$, n=6, $p<0.01$). To uncover the aberrant signaling pathways underlying these phenotypes, we generated RAF1 S257L/+ knockin and RAF1 null (KO) Hela cell lines using CRISPR-Cas9n technology. Interestingly, we found that ERK1/2 activity was enhanced in KO cells, both at baseline and in response to growth factor (GF) stimulation. At the opposite, ERK1/2 activity was reduced in mutant cells, suggesting that the RAF1 S257L/+ mutant actually suppresses ERK1/2 activation. Finally, we found that AKT activation was upregulated in S257L/+ cells under GF stimulation, due to decreased ERK1/2-dependent p70-S6 kinase activity and subsequent increase in mTORC2 activity (n=6). Similar experiments are underway in S257L/+, corrected and KO RAF1 iPSC-derived CMs. In conclusion, we propose that the S257L/+ mutation modulates development of CH in NS RAF1 patients through a reduction in ERK1/2 activity and overactivation of the AKT/mTOR signaling pathway, leading to both an increased number and size of CMs.

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