Title: Cardiogenesis and congenital heart disease

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Introduction The cardiopharyngeal mesoderm (CPM) is a recently defined lineage that gives rise to part of the heart, branchiomeric muscle and other tissues of the lower face. It is a highly proliferative multipotent pools of the anterior mesoderm. Insights into the pathways that drive the formation, differentiation and regionalization of these cells into the heart and craniofacial structures is a central problem in developmental biology and it has important repercussions on our understanding of congenital heart disease and cardiac regeneration. TBX1 is a critical transcription factor for the development of the CPM and is the candidate gene for 22q11 deletion syndrome responsible of a wide range of defects ranging from mild learning disabilities and craniofacial malformation to lethal cardiovascular defects. We use mouse models and engineered mouse embryonic stem (ES) cells to study molecular, cellular and morphogenetic mechanisms regulated by TBX1 during development. Specifically, our focus is in three areas: 1) identification of critical pathways using phenotypic rescue of the \( Tbx1 \) mutant phenotype 2) identification of cell biology mechanisms regulating deployment of CPM cells during development 3) molecular mechanisms underlying enhancer activation or repression by TBX1.

Results We previously found that VitaminB12 (B12) is capable of enhancing \( Tbx1 \) gene expression in mouse embryonic fibroblasts (MEFs) and partially rescuing aortic arch patterning defects associated with \( Tbx1 \) haploinsufficiency (Lania et al. 2016). More recently, we extended the phenotypic anomalies that can be rescued by B12 treatment. Transcriptomic analyses of mouse embryos identified genes dysregulated by \( Tbx1 \) haploinsufficiency but rescued by B12 treatment in vivo. One of the rescued genes encodes the transcription factor SNAI2. We observed that SNAI2 identifies a population of mesodermal cells, partially overlapping with ISL1 and TBX1 expressing cells, that is mis-localized in \( Tbx1 \) heterozygous and homozygous mutant embryos. Suggesting that transcriptional properties of the CPM provide positional cues to lineages contributing to the development of the pharyngeal apparatus (Lania et al., manuscript in preparation). One of the key players of tissue morphogenesis and cell regionalization is the extracellular matrix (ECM) which can act to regulate signalling gradients at the cell surface, driving cellular processes and tissue patterning. Interestingly, we found that loss of TBX1 disrupts ECM-cell and cell-cell interactions, causing loss of cell polarity and tissue disorganization in the SHF cells of the splanchnic mesoderm of mouse embryos, and that loss of TBX1 has disruptive consequences on tissue architecture and heart development. In addition, the treatment of an inhibitor of alpha4 integrin-paxillin significantly reduces the length of cardiac out flow tract; \( Tbx1 \) null mice show a shorter cardiac out flow tract respect to wild type embryos (Alfano et al, 2019). Overall these findings reveal that TBX1 regulates ECM-integrin-focal adhesion signalling and that loss of function of TBX1 has major functional consequences for cell migration and spreading. Alteration in ECM could be responsible of alteration in positional cues for SNAI2 positive cell population. In order to identify TBX1 targets we preformed RNA-seq and chromatin accessibility assay (ATAC-seq) in differentiating mouse ES cells WT and \( Tbx1^{-/-} \) (Cirino et al., 2020). These studies confirmed that multiple genes related to the ECM are dysregulated in mutant cells. A set of these genes has been validated in the cellular model and are in the process of being tested in vivo. Overall, our results support the hypothesis that TBX1 function is critical for differentiation and regionalization of the CPM. Some of these functions are cell non-autonomous and may be mediated by regulation of the ECM and/or ECM-cellular interactions (Alfano et al, manuscript in preparation) while others are cell autonomous.

Prospective Future studies will address to specific cellular and molecular mechanisms by which \( Tbx1 \) regulates genes involved in the cell regionalization and in the ECM dynamics. We will also test whether targeting specific ECM components may modify the mutant phenotype.
References:


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