Introduction

We previously showed 16p11.2 microdeletion to be a major cause of congenital anomalies of the kidney and urinary tract (CAKUT), and identified Tbx6 as the most likely driver. Whether Tbx6 dose reduction causes the full spectrum of CAKUT seen in 16p11.2 microdeletion has yet to be demonstrated, and consequent mechanisms remain elusive. Here we show the full phenotypic spectrum of CAKUT in Tbx6 mouse models and identify, in silico, downstream signaling pathways and targets that impact development of the genitourinary (GU) tract.

Methods

We studied a Tbx6 allelic series using two independent alleles: a null allele (Tbx6<sup>-/-</sup>, hereafter Tbx6<sup>-</sup>) and a hypomorphic allele (Tbx6<sup>rv/rv</sup>). We conducted detailed phenotypic analysis on histological sections of these models across developmental stages. Gene expression analysis was performed on dissected models, and coexpression from scRNA-seq data identifies 28 potential targets of Tbx6. Of these genes, 4 have been previously identified in the literature as causes of CAKUT, including Sufu, Chd7, Arid1b, and Kif11. This analysis also identifies Fgfr7 as a promising new cause of CAKUT and target of Tbx6, as the two are significantly coexpressed in the intermediate mesoderm. Fgfr7 is important for patterning of the intermediate mesoderm from embryonic stem cells in vitro, and has been shown in vivo to maintain stemness of nephron progenitors along with Fgfr2.

Results

Pleiotropy of CAKUT in mice with reduced Tbx6 gene dosage

Gene set enrichment analysis and deconvolution of E9.5 tail gene expression identify disrupted cell types and pathways in Tbx6 mutants

Graphical Abstract

Tbx6<sup>rv/rv</sup>

Methods

We conducted a Tbx6 allelic series using two independent alleles: a null allele (Tbx6<sup>-/-</sup>, hereafter Tbx6<sup>-</sup>) and a hypomorphic allele (Tbx6<sup>rv/rv</sup>). We conducted deconvolution of Tbx6<sup>-/-</sup> and Tbx6<sup>rv/rv</sup> scRNA-seq clusters from Orchard et al. (PMID: 30380057). TFBS predictions were subsequently prioritized by intersection with ATAC-seq of E9.5 tail and intersection with our expression data. TFBS predictions were then determined using the scRNA-seq data used for deconvolution.

Conclusions

This study strongly supports a role of Tbx6 in pleiotropic CAKUT, uncovering causal mechanisms, and identifies genes and pathways regulated by Tbx6. Involvement of Notch signaling is of particular relevance as mutations of NOTCH2 and related genes cause Aplagge syndrome and spondyloostotic dysostosis, characterized by CAKUT and spine defects similar to those observed both in our Tbx6 models and in patients with 16p11.2 microdeletion. These data implicate loss of Tbx6-mediated regulation of Notch signaling as critical to the development of CAKUT and spine defects. The integration of large scale TFBS analysis with single-cell transcriptomics and epigenomics allowed for the identification of downstream mechanisms, pathways, and target genes. Of these targets, Fgfr7 is attractive as a novel candidate Tbx6 gene, given its importance in specification and maintenance of nephron progenitors.

Future Directions

We are conducting confirmatory studies to validate the differential expression of genes from our E9.5 tail dataset. Protein-DNA interactions will be investigated to confirm newly predicted targets of Tbx6. We will also examine the utility of Tbx6 dose reduction as a model of low nephron number at birth and subsequent risk for kidney diseases across the lifespan.

References

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