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Single-nuclei transcriptomics reveals TBX5-dependent targets in a patient with Holt-Oram syndrome

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COI: JFM is a cofounder of YapTx. No other COI exists.

To the Editor: Holt-Oram syndrome (HOS), characterized by heart and forelimb defects, is caused by mutations in the T-box transcription factor, *TBX5* (1). While much has been done to elucidate TBX5's transcriptional mechanisms in model systems (2–5), transcriptomics from HOS patient tissue is lacking. Here, we report a rare opportunity to interrogate the cardiac transcriptome of HOS using high-resolution, single-nuclei transcriptomics (snRNA-seq) of left ventricular (LV) tissue from a 10-year-old female HOS patient undergoing transplant surgery.

The patient presented with atrial and ventricular septal defects, right thenar hypoplasia, and sick sinus syndrome (**Supplemental Figure 1A**). The patient underwent genetic testing, revealing coding mutations in *TBX5* (p.P85R), *DNHD1*, and *ZNF469* (**Supplemental Table 1**). While phenotypically characteristic of HOS, *DNHD1* variants coincide with laterality defects and may contribute to the pathology (6). The *TBX5* mutation affects a conserved proline residue in a nuclear localization signal (**Figure 1A and Supplemental Figure 1B-C**). Structural predictions suggest that while nonpolar, cyclic proline-85 is interposed between nonpolar ring structures in adjacent helices, charged arginine-85 presents on the surface where it may disrupt surface interactions (**Supplemental Figure 1D**). In cell culture, TBX5-P85R was predominantly cytoplasmic compared to the reference allele (**Figure 1B**) and showed weaker expression (**Supplemental Figure 1E**), suggesting a mechanism for this loss-of-function allele (1, 7).

The HOS patient had a left coronary obstruction in the setting of pulmonary artery band removal and VSD closure requiring revascularization, leading to ventricular insufficiency, heart failure, and indication for transplant. Explanted LV tissue was collected and snRNA-seq was performed. As *TBX5* is predominantly expressed in cardiomyocytes (**Supplemental Figure 1F**), we performed differential expression testing comparing HOS and matched non-failing donor cardiomyocytes (8–10), identifying 338 down- and 262 up-regulated genes (**Figure 1C and Supplemental Table 2**). These genes are associated with contraction (11) and conduction (3, 12), known functions of *TBX5* (**Supplemental Figure 1G**). We compared these genes with reported TBX5 targets from induced pluripotent stem cell (iPSC)-derived cardiomyocytes and mouse models (2, 3, 12, 13). While there was an appreciable overlap among the datasets (122/600 with iPSC-derived cardiomyocytes and 229/600 with mouse atrial knockout), 143/338 down-regulated genes were newly identified (**Figure 1D and Supplemental Figure 1H**).

We used TBX5 ChIP-seq from iPSC-derived cardiomyocytes to identify direct targets (14). Two-thirds of the down-regulated and half of the up-regulated genes were associated with TBX5 binding (**Figure 1E**). Of the down-regulated genes, 80 of them were not in previous datasets, including *MTA1*, a gene found to genetically and physically interact with TBX5 in ventricular development (15). One-fifth of these direct TBX5 targets are associated with metabolism (**Figure**

1F), including glucose and glycogen metabolism (*HK1* and *GYS1*), TCA cycle (*IDH2* and *MDH1*), and amino acid synthesis (*GOT1* and *BCKDHB*). *TBX5* regulation of metabolism is underexplored and warrants further investigation.

To distinguish between the effects of TBX5-P85R and heart failure, we compared our gene lists with those of published pediatric and adult cardiomyopathies (8–10). We found that 28% of the down-regulated genes were not changed or up-regulated in cardiomyopathy with 78% of these being predicted direct targets, including several of the metabolic pathway genes (**Supplemental Figure 1I**). Conversely, 90% of the up-regulated genes were also up-regulated in cardiomyopathy.

Altogether, the HOS-patient transcriptome reveals undescribed TBX5 transcriptional targets, while sharing many features with HOS models and furthering our understanding of HOS pathophysiology. This exciting addition to the growing body of pediatric cardiovascular datasets benefits the wider community by offering a rare opportunity to study the HOS transcriptome firsthand.

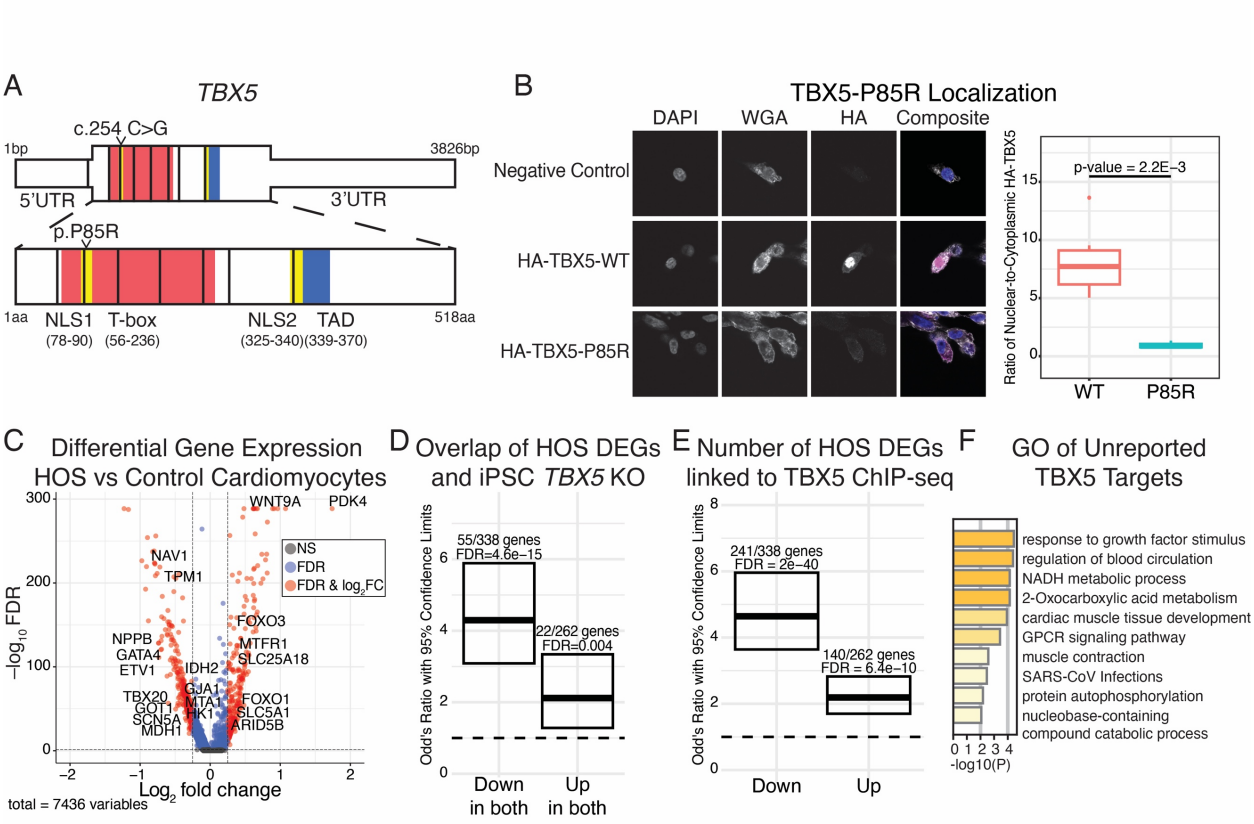

FIGURE 1. Identification of TBX5-dependent targets at single-cell resolution.

A. *TBX5* gene (top) and protein (bottom) with domains labeled. Patient mutation c.254C>G (p.P85R) is indicated by arrowhead.

B. Anti-HA and wheat germ agglutinin (WGA) immunofluorescence staining of FaDu cells transfected with HA-TBX5-wildtype (WT) or HA-TBX5-P85R. Ratiometric quantification of nucleus:cytoplasmic HA signal by boxplot (n=6); P-value by Welch Two Sample T-test.

C. Volcano plot showing the distribution of differentially expressed genes (FDR<0.05 & $|\log_2$Fold-Change$|>0.25$) comparing HOS and control cardiomyocytes.

D. Odds ratio by Fisher's exact test comparing the overlap of down- and up-regulated genes identified in both 1C and published TBX5 knockout (KO) iPSC-derived cardiomyocytes (2).

E. Odds ratio by Fisher's exact test comparing the overlap of down- and up-regulated genes identified in **Figure 1C** and published TBX5 ChIP-seq from iPSC-derived cardiomyocytes (14).

F. Gene ontology (GO) term analysis of *TBX5*-dependent genes associated with TBX5 ChIP-seq not previously reported in cardiomyocyte-derived iPSCs or mouse tissue.

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