Julia Jones
Bruce Aronow, PhD
James Lessard, PhD

Predicting androgen-dependent pathways for the development of male external genitalia via ChIP-seq data and analyses

Androgens, primarily testosterone and dihydrotestosterone (DHT), are responsible for the development of male genitalia during embryogensis. Malfunction of androgen receptors (AR), as seen in androgen insensitivity syndrome (AIS), can result in micropenis, hypospadias, and female external genitalia despite normal XY karyotype.

The aim of this study was to identify AR genomic targets that may be implicated in penile development. We hypothesized that genes which are syn-expressed with AR in male genitalia and also exhibit nearby AR binding sites (ChIP-seq analyses) can be implicated in the pathogenesis of anomolous external genitalia, such as those seen with AIS.

Using the Human Body Map (*GEO accession number:* GSE7307), we identified 74 genes exhibiting robust expression (3-fold upregulation in penis, prostate, or urethra) as well as syn-expression with AR in the male reproductive tract. These 74 probe sets were cross-referenced with AR ChIP-seq data from androgen-dependent prostate cancer cells (LNCaP; data obtained from Myles Brown laboratory) to identify genes with androgen response elements (ARE). Twelve AR-binding sequences were identified from this data set and were confirmed to contain ARE motifs by Multiple EM for Motif Elicitation (MEME). Through functional enrichment of this gene set (ToppGene Software Suite), several genes (TBX3, IGF1, DCN, APCDD1, and PTPN13) surfaced as strong candidates for AR interaction.

Due to pathogenic similarities between AIS and Ulnar-Mammary Syndrome (TBX3 mutation), we chose to focus on genetic interactions between AR and T-Box 3 (TBX3). To investigate TBX3 genomic targets, we obtained TBX3 ChIP-seq data from murine induced pluripotent stem cells (Bing Lim laboratory). The analysis outlined above for AR was repeated for TBX3 with minor modifications.

Our final analysis highlighted TBX3 regulatory binding sequences for several genes implicated in human genitourinary anomalies including SALL1 (Townes-Brocks Syndrome; Hand-Foot-Genital Syndrome), BMP4 (Microphthalmia, Syndromic 6), and GPC6 (Omodysplasia 1). From this analysis, we believe that AR may regulate the expression of TBX3 in the male reproductive tract, which may lead to downstream regulation of key genes responsible for the development of male external genitalia.