

Exploring Sex Differences in Prenatal Human Fetal Mammary Bud Development

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The early stages of mammary development are independent of sex steroid hormones but at 15 weeks of fetal development, breast tissue is transiently sensitive to testosterone. Milk ducts are formed by weeks 20–32 unless significant testosterone exposure prevents alveolar ductal system development. Mammary ducts and lobules are present in both sexes meaning that like women, men can develop breast cancer although accounting for less than 1% of all diagnosed cases. Despite tissue similarities, female mammary gland development is far more dynamic, starting during fetal life, pausing after birth, then resuming at puberty in response to hormonal cues and culminating at the end of pregnancy with functional lactogenesis. Little is known about sex-dimorphism in the human prenatal mammary bud; indeed, the nipple is not visible in 1st and early-mid 2nd trimesters. With this study, we have begun analysis of region-specific transcriptomes to provide novel data on the regulation of sex-specific development of the prenatal mammary bud.

Collection of human fetuses from normally progressing pregnancies was approved by the National Health Service (NHS) Grampian Research Ethics Committees (REC 15/NS/0123). Dissected tissues were fixed overnight in 10% Neutral Buffered Formalin prior to dehydration and paraffin embedding processes. Fetal sex was further confirmed by PCR. Three male and 3 female mammary bud (15-16 gestational weeks) sections were mounted as recommended by Nanostring[®]. Adipose tissue was identified using FABP4 immunolabelling while vimentin and PanCK immunolabelling highlighted stroma and epithelium, respectively. We compared differential gene expression (DEG) in similar mammary bud regions (regions of interest) between sexes using an unpaired t-test ($p\text{-value} \leq 0.05$ and $|FC| \geq 2$). As a follow-up, proliferating and dying epithelial cells within male and female mammary buds were immunolabelled using KI67 and cleaved-Caspase3 as respective markers. Counting is currently ongoing.

Visualisation of the dissimilarities in gene expression between regions of interest, by PCA biplot, showed clear separation of samples based on region of interest type but no separation based on fetal sex. Indeed, PC1 and PC2 accounted for 32% and 22% of expression variability, respectively. The first dimension separated stroma from other region of interest types while the second dimension separated epithelial regions (central and basal) from the nipple area. More gene expression changes were sex-related in the central epithelium compared to other regions of interest (301 *versus* 192 in stroma and 135 in basal epithelial layer). Gene set enrichment analysis based on sex related DEGs in stroma, central epithelium and basal epithelial layer showed significant enrichment of the “DNA replication” pathway ($p\text{-value}=0.0002$, $p\text{-value}=0.005$ and, $p\text{-value}=0.0007$, respectively). Sex related DEGs in the basal epithelial layer were significantly enriched in pathways such as “DNA methylation” ($p\text{-value}=0.0002$) and “estrogen-dependent gene expression” ($p\text{-value}=0.0009$). Genes encoding for proteins involved in lipid metabolic process such as MVK (mevalonate kinase, $p\text{-value}=0.00734$) and CH25H (cholesterol 25-hydroxylase, $p\text{-value}=0.00312$) were upregulated in female central epithelium compared to male. Surprisingly, prolactin transcript was almost 3-fold more highly expressed in male compared to female central epithelium ($p\text{-value}=0.00919$). No sex differences in prolactin receptor transcript level were observed in basal or central epithelium.

These data represent early but important steps towards better understanding of sex-dependent human prenatal development of the mammary bud and may contribute to better understanding of sex differences in breast cancer risks.

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