Antagonism between retinoic acid and estrogen signaling maintains uterine cytodifferentiation

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ABSTRACT

Classical tissue recombination experiments demonstrate that cell-fate determination along the anterior-posterior axis of the Mullerian duct epithelium occurs between postnatal days 5-7 in mice. However, little is known about how these cell types are maintained in adults and whether cellular plasticity can mediate transdifferentiation between uterine simple columnar and cervicovaginal stratified epithelia. In this study, we provide genetic evidence that a balance between antagonistic estrogen and retinoic acid signaling activity is required to maintain simple columnar cell fate in postnatal uterine epithelium. Transformation of simple columnar uterine epithelium into stratified cervicovaginal epithelium was observed in two mouse genetic models, one expressing a dominant-negative RARα and the other missing all three retinoic acid receptors in the postnatal uterus. Single cell RNA sequencing (scRNA-seq) analysis identified the transformed epithelial cell populations and revealed extensive immune cell infiltration as a result of loss of retinoic acid signaling, which was confirmed by immunostaining. Surprisingly, differential gene expression analysis revealed a group of strongly upregulated estrogen targets in the transformed cell clusters, suggesting that these two pathways may functionally oppose each other during uterine epithelial cell-fate determination. Consistent with this model, exposure to diethylstilbestrol, a strong synthetic estrogen downregulated a group of retinoic acid target genes, led to epithelial stratification and immune cell infiltration later in life. Fulvestrant, an estrogen antagonist, -treated RAR triple conditional knockout pups reestablished the balance between the two signaling pathways, resulting in the astonishing complete rescue of the metaplastic stratified epithelia back to the normal simple columnar morphology. Together, these results implicate an essential role for retinoic acid signaling in maintaining uterine cytodifferentiation by antagonizing estrogen signaling in the postnatal uterus.