Müllerian duct maintenance at the cranial region is dependent upon GATA2

Shuai Jia[1], Fei Zhao[1]

[1]Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI 53706, USA

Abstract

Congenital anomalies of the female reproductive tract (FRT), also known as Müllerian anomalies, are a major cause of female infertility. However, our understanding of normal FRT development remains incomplete. The FRT organs including the oviduct, uterus, cervix, and the upper vagina derive from the same embryonic progenitor, the Müllerian duct (MD). The MD is surrounded by multiple layers of undifferentiated connective tissues known as the mesenchyme, which governs the maintenance and differentiation of the MD. During sexual differentiation, in male embryos, the fetal testis produces AMH (Anti-Müllerian hormone), which acts upon its receptor AMHR2 expressed in the mesenchyme to induce MD regression. On the other hand, ovaries in female embryos do not produce AMH; as a result, MDs are maintained. Therefore, it has been believed that MD maintenance in the female embryo is a passive outcome arising by default in the absence of testicular AMH. In contrast to this prevailing view, we here provided genetic evidence that the maintenance of MD at the cranial region is actively promoted by GATA2 in the mesenchyme. *Gata2* belongs to the GATA family of transcription factors that bind to the characteristic "GATA" DNA sequence. Among the six members of the GATA family, GATA2 is the only member that is expressed in MD mesenchyme. GATA2 has been known to play crucial roles in morphogenesis and differentiation of other tubular organs. We therefore hypothesize that mesenchymal *Gata2* plays critical roles in MD development. To test this hypothesis, we established a conditional *Gata2* knockout mice (Osr2-Cre; Gata2-flox, *Gata2*^{cKO}), where *Gata2* was deleted specifically in MD mesenchyme. At birth, *Gata2*^{cKO} females had a shorter MD compared to that of the control. More strikingly, *Gata2*^{cKO} females completely lost the cranial (head) MD, which is the precursor of the oviductal infundibulum essential for oocyte pickup. These observations indicate that *Gata2* is essential for promoting the survival of cranial MD. To identify the potential molecular mechanism, we performed bulk RNA-seq on collected cranial MD region from control and *Gata2*^{cKO} female embryos at the initiation stage of ductal degeneration (E16.5). We obtained 296 differentially expressed genes (DEGs), which did not significantly overlap with the published list of AMH-induced genes. This observation indicated that the regression of the cranial MD in *Gata2*^{cKO} female embryos was not caused by ectopic AMH signaling. It is established that mesenchyme-derived secreted ligands mediate the instructive role of the mesenchyme in epithelial fate and differentiation. In our list of 296 DEGs, we identified 13 secreted ligands, among which we were particularly interested in the downregulated growth factor *Nrg1* (Neuregulin 1). *Nrg1* has been known to play critical roles in growth of other organs including heart and testis. Its receptors (Erbb2 and Erbb3) are expressed in MD epithelium. We are now in process of confirming the decreased *Nrg1* signaling and investigating whether NRG1 supplementation can rescue the phenotype of cranial MD degeneration. Overall, our study challenges the notion that MD maintenance is a passive

outcome, and demonstrates that the maintenance of cranial MD is actively promoted by mesenchymal GATA2 .