*Wnt9b/*β-catenin signaling controls Wolffian duct maintenance.

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The maintenance and differentiation of the Wolffian duct (WD), the precursor of the male reproductive tract, is predominantly controlled by the androgen action. During sexual differentiation, under the control of testicular androgens, the Wolffian duct differentiates into the epididymis, vas deferens and seminal vesicle in the male embryos. By contrast, the Wolffian duct degenerates in the absence of testicular androgens in the female embryo. Although disruptions of androgen signaling by environmental and genetic factors during male sex differentiation can cause male reproductive disorders, the etiology of such birth defects remains unknown in many cases. Here, we uncovered the critical role of *Wnt9b/*β-catenin pathway in Wolffian duct maintenance and morphogenesis in mice. *Wnt9b* belongs to the Wnt family of secreted glycoproteins, which are known to be involved in every aspect of embryonic development. The loss of *Wnt9b* caused Wolffian duct degeneration in male embryos that had normal testicular androgen production. We observed comparable expression of two critical steroidogenic enzymes (*Hsd3β* and *Cyp17a1)* and comparable testosterone level between $Wnt9b^{+/+}$ and $Wnt9b^{-/-}$ testes. Since the androgen action is mediated by its receptor AR in the mesenchyme, we next investigated whether mesenchymal AR expression in the Wolffian duct was impaired in the absence of *Wnt9b* by immunohistochemistry. In the normal condition, AR was normally expressed in most of mesenchymal cells at the initiation of Wolffian duct maintenance (E14.5), which promoted Wolffian duct epithelial proliferation. However, in the absence of *Wnt9b*, the percentage of mesenchymal cell expressing AR was significantly decreased. Consistent with reduced AR+ mesenchymal cells, epithelial and mesenchymal proliferation was also significantly decreased in the absence of *Wnt9b*. These observations demonstrate that *Wnt9b* promotes the number of AR+ mesenchymal cells in Wolffian duct maintenance. The epithelium-derived WNT9B can activate two intracellular pathways in the mesenchyme: β-catenin dependent and independent pathways. We detected nuclear localization of β-catenin in the Wolffian duct mesenchyme, which indicated the activation of β-catenin-mediated WNT pathway. We therefore investigated whether β-catenin operated downstream of WNT9B in the Wolffian duct mesenchyme. When β-catenin was deleted in the Wolffian duct mesenchyme, the caudal region of the Wolffian duct degenerated while the WD at the cranial region was still maintained. These observations indicate that mesenchymal β-catenin is required for promoting Wolffian duct maintenance at the caudal region. Taken together, our study demonstrates that WNT9B/β-catenin pathway facilitates epithelial-mesenchymal crosstalk in promoting Wolffian duct survival.