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Identification of Mechanisms Underlying Sex Determination and Differentiation Using Single-cell Multiome Analysis

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Embryonic gonadal development toward male or female fate is a sequential and intricate process beginning with the formation of a bipotential gonad. The initiation and maintenance of sex-specific pathways are regulated by transcription factors, which repress the opposite pathway to ensure proper sex development. Disruptions in these regulatory networks can lead to disorders of sex development (DSDs). While significant progress has been made in identifying genes involved in gonad development, particularly from the perspectives of non-coding RNAs and epigenetics, a comprehensive understanding of the regulatory networks governing the transition from gonad to testis or ovary remains limited.

The zebrafish model offers distinct advantages for studying these mechanisms. Zebrafish share conserved cell types and functions with mammals, and key genes involved in sex determination and differentiation are well preserved across species. Additionally, the large number of fertilized eggs makes it easier to obtain samples across developmental stages, a challenge with other models.

To uncover the genes and transcriptional regulatory mechanisms involved in gonadal development into ovaries or testes, I conducted a single-cell multiome analysis on zebrafish samples collected at 20 days post-fertilization (dpf), 30 dpf, adult ovaries, and adult testes. Through downstream analysis, I identified distinct cell types and markers within zebrafish gonads and sex organs. I also identified key transcription factors specific to each fate, displaying coordinated patterns between chromatin accessibility and gene expression. Notable testis-specific genes included foxj1a, cpeb1b, hmga1a, setdb1b, and dmrt1. Ovary-specific genes included bach1b, irf8, nr1h3, and mef2cb.

This study enhances our understanding of sex determination and differentiation while addressing limitations of prior analyses focusing solely on transcriptomics or epigenomics. By integrating multiple molecular datasets, this research advances knowledge of complex and previously unexplored mechanisms underlying sex determination and differentiation.