

Expression of Klf-4 and Zfp-161 during Mouse Gonadal Differentiation

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Background

Gonadal development in the mammalian fetus is an ideal model to study organogenesis because two completely different organs, the testis and ovary, arise from the same primordial structures, the genital ridges. This process involves the coordinated expression of different genes to divert the bipotential gonad toward the testicular or ovarian fate (1). In males, the expression of the Y-linked testis determining gene *Sry* acts as a switch that triggers a cascade of gene expression and molecular interactions to induce the morphological changes necessary for testis differentiation. In absence of the *Sry*, alternative molecular and cellular pathways induce the genital ridges to differentiate as an ovary (2). Although many genes related to testis development have been identified and their genetic pathways elucidated, there are many aspects of this complex developmental process that are still unknown. For example, the cause of more than 50% of Disorders of Sex Determination (DSD) cases in humans has not been identified (3).

Proposed Research

I have visited Dr. Erasmo Perera's Lab at the Department of Pediatrics at Leonard M. Miller School of Medicine several times and I have become very interested in their research. Dr. Perera's Lab is studying the molecular mechanisms involved in gonadal differentiation in mice. His laboratory is interested in testis differentiation, particularly in the role that two transcription factors, Klf-4 and Zfp-1, play in this process. They have recently found that Klf-4 and Zfp-161 are differentially expressed in the male gonad at 13.5 days post coitum (dpc), and that they are involved in the transcriptional regulation of *tescalctn*, a testicular gene identified in his laboratory (4,5 and unpublished data). This data suggests that these transcription factors are likely to be playing a role during the critical period of testis differentiation in mice. To investigate the role of Klf-4 and Zfp-161 in testis differentiation I propose:

1. Determination of the patten of gene expression of Klf-4 and Zfp-161 in male and female gonads during the period of gonadal differentiation (11.5 – 16.5 dpc). To accomplish this I will isolate total RNA from male and female embryonic gonads at different stages of development, prepare cDNA, and use Quantitative-PCR to measure their expression. This experiment will allow us to know the developmental stage at which Klf-4 and Zfp-161 are important for testis development.

2. Identification of the testicular cell lineage that expresses Klf-4 and Zfp-161. To accomplish this I will do immunohistochemical analysis of tissue sections prepared from mouse embryonic testis at the developmental stage in which the expression of the genes peaks. Specific antibodies against markers of Sertoli cells (Sox-9), Leydig cells (Cyp17), and Germ cells (PCAM-1) will be used. Antibody binding will be detected using fluorescence-conjugated secondary antibodies. The information will provided valuable information to determine the molecular pathway in which Klf-4 and Zfp-161 are implicated.

This research will be performed in Dr. Perera's Lab during this summer (May-August 2011) and I expect it will contribute to elucidate the role of Klf-4 and Zfp-161 transcription factors in testis differentiation.