ABSTRACT

DETERMINING THE ROLE OF IRF6 IN OOGENESIS AND EXTRA EMBRYONIC DEVELOPMENT

By

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Interferon Regulatory Factor 6 (IRF6) is a member for the IRF family of transcription factors. Mutations in IRF6 cause two autosomal dominant Mendelian disorders characterized by cleft lip and palate. In addition, DNA variation in IRF6 contributes risk for non-syndromic cleft lip and palate. Mouse models developed to study Irf6 function indicate a critical role in regulation of proliferation and differentiation of keratinocytes during embryogenesis. Irf6 has also been implicated in adult developmental processes and adult diseases. These include mammary development, breast cancer, squamous cell carcinoma, and wound healing. In addition, Irf6 has been implicated in a number of processes surrounding reproduction. Studies using ovine models indicate a role for Irf6 in trophoblast cell types, the cell lineage that composes the placenta. Irf6 was also found to be expressed in bovine oocytes, indicating that it is a maternally expressed gene. Maternal expression of irf6 is conserved in zebrafish and frog. Inhibition of maternally deposited Irf6 in zebrafish results in early embryonic lethality. The aim of this work was to elucidate the role of maternally expressed Irf6 in early embryonic development and to study the function of Irf6 in placental development.

To study the function of Irf6 in a tissue specifc manner, a novel conditional allele of Irf6, carrying LoxP sites in introns two and four, was generated. We validated the functionality of this allele of Irf6 using three Cre transgenic lines: Gdf9-Cre, CAG–Cre and Ella-Cre. Cre-mediated recombination of the conditional allele was sufficient to produce a null allele of Irf6. However, not all Cre transgenic lines were able to facilitate recombination with the same efficiency. We conclude that the Irf6 conditional allele is a novel tool for analysis of Irf6 function in a tissues specific manner.

The conditional allele of Irf6, in combination with the Gdf9-Cre transgenic line,

was utilized to generate mice with oocyte specific deletion of Irf6. Genetic analysis of progeny indicated that Gdf9-Cre efficiently recombined the Irf6 conditional allele in oocytes prior to meiosis I despite persistence of gene products. Female mice with this oocyte specific excision of Irf6 displayed an increase in litter size when compared to control counterparts. This increase in litter size was accompanied by an increase in ovulation. Females with oocyte specific excision of Irf6 also displayed an increase in multiple oocyte follicles (MOFs). These MOFs did not appear to contribute to the observed increase in ovulation. MOFs are caused by impaired breakdown of germ cell nest. Irf6 expression was observed at critical time points in germ cell nest breakdown. This expression pattern suggests that Irf6 plays a role in germ cell nest breakdown. From this work, a novel role for Irf6 in regulating female fertility and folliculogenesis was identified. The mechanisms underlying these phenotypes have not yet been elucidated. Lastly, a conventional knockout mouse model was used to study the role of Irf6 in placental development. Irf6 expression was observed in the mouse placenta during embryogenesis. Analysis of Irf6-deficient and wildtype placenta was conducted. We observed no morphological differences in Irf6-deficient placenta. Along with this, there was no difference in wet weights between Irf6-deficient and wildtype embryos, suggesting normal placental function. We conclude that there is a non-essential role for Irf6 in placental development.