SRF ACADEMIC SCHOLARSHIP RECIPIENT 2016

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PROJECT SUMMARY

The role of DAZL in chromosomal structure in early meiosis

In women, the primordial follicles which contain the lifetime supply of oocytes are formed during fetal life, from 17 weeks gestation. Oocytes within primordial follicles must then maintain a state of meiotic arrest (with inherent chromosomal instability) until meiosis resumes at the time of ovulation: this will be not months but years or decades later. It is now recognized that most aneuploidies results from errors arising in meiosis I, and understanding of the germ cell master regulators involved at the time of initiation and subsequent arrest of meiosis and their role in determining oocyte quality and quantity is critical to our understanding of reproductive potential in women. Specifically found in germ cells, Dazl is a key determinant of germ cell maturation and entry into meiosis in mice. Although the $Dazl^{-/-}$ fetal mouse ovary was shown to have reduced expression of numerous meiotic genes, there has been little work establishing the direct dependency on Dazl for translation of these key mRNAs, and thus limited understanding of the specific functional consequences of Dazl regulation on these targets. Consequently, the role of DAZL in oocyte maturation, survival and meiotic stability remain unclear.

We have investigated novel DAZL targets in the human fetal ovary using RNA-immunoprecipitation and RNA-Seq, and identified targets involved in synaptonemal complex formation (*SYCP3*, *SYCP1, TRIP13*), and cohesin establishment (*SMC1B, RAD21L*), This work therefore indicates novel potential roles for DAZL function in chromosome cohesion regulation during early meiosis in the fetal oocyte, key pathways that underpin lifelong oocyte quality, in addition to targets involved in entry into meiosis.

The work to be supported by this funding will investigate the consequences of depletion of Dazl for chromosomal structure in the newly formed oocytes. To investigate Dazl's involvement in cohesin formation and DNA repair enzyme regulation, we will use chromosome spreads from oocytes at different substages of meiotic prophase I using a hypomorph model we have developed of RNAi to knock-down Dazl expression in mouse fetal ovaries in vitro from the onset of meiosis to primordial follicle formation. Measurements of the number and chromosomal distribution of late recombination foci will be performed on control and Dazl-depleted oocytes. Incorporation of cohesin into chromosome axes will also be assessed by immunostaining these fetal oocyte chromosome spreads for specific cohesion subunits. These techniques are well established in the laboratory of our collaborator Professor Ian Adams with whom we have obtained preliminary data from our model to show that this approach is technically achievable. The outcome of this experimental plan will be to demonstrate whether Dazl deficiency in fetal oocytes is sufficient to perturb meiotic recombination and progression in meiosis I in a manner that would be detrimental to oocyte quality and reproductive success in adult life. This will provide a basis for future work demonstrating the impact of this on adult oocyte aneuploidy, and its relation to age.