Abstract

Infertility is a prevalent issue that often has underlying genetic causes, so this project aims to construct an in vitro platform to study meiotic mechanisms of gametogenesis and the factors that affect it in order to model infertility causing SNPs. Following previously published experiments, embryonic stem cells (ESCs) will be induced into epiblast-like cells (EpiLCs) and then into primordial germ cell-like cells (PGCLCs). The PGCLCs will then be cocultured with somatic testicular cells from a mouse model, inducing the entry of the PGCLCs into meiosis and ultimately forming spermatid-like cells. Key stages of germ cell development will be visualized using cellular markers. CRISPR/Cas9 modified cell lines with SNPs lacking expression of one or more of these cellular markers will point to infertility-causing alleles, and preliminary experiments using cell lines with a Cdk2 allele mutation experimentally shown to disrupt spermatogenesis in vivo will be used to test the potential of the in vitro platform. Ultimately, the development and use of vitro gamete cell lines will allow the efficient analysis of thousands of SNPs suspected of causing infertility.

Biographical Sketch

I am a junior from Buffalo Grove, Illinois, majoring in Biological Sciences with a concentration in Genetics, Genomics, and Development in the College of Arts and Sciences.

One of my first experiences with research was at the Eric Weiss Lab at Northwestern University, where I studied the genes regulating the RAM network in budding yeast. There, I was able to use what I had learned in AP Biology in real-life applications, discovered the ups and downs of designing and conducting experiments, and developed a strange fondness for pipetting for hours on end. At Cornell, I am currently working in the John Schimenti lab. In conjunction with Xinbao Ding, a postdoc in the lab, I am working on developing an in vitro platform to study infertility, and plan on continuing this project this summer with the Tanner Dean's Scholars Grant. After graduation, I plan to pursue a PhD in the biological sciences.

Outside of research, I am the treasurer for Splash!, an organization with the motto "Teach Anything, Learn Anything" that plans a biannual event inviting local middle and high school students to Cornell for a day so Cornell students can teach them classes in a wide variety of subjects (topics have ranged from epigenetics to memes to interactions with live reptiles!). I am also a member of Asian American Intervarsity, a Christian fellowship that explores faith and seeks to love the campus, which has provided a wonderful community here these last few years. In my free time, I enjoy playing badminton at Helen Newman, fermenting a variety of foods, and growing my succulent collection.

Shu Zhang, Class of 2020

Biological Sciences

Developing an In Vitro Platform to Model Infertility-Causing SNPs

Statement of Purpose

Infertility, a prevalent issue affecting up to 15% of couples, is often linked to genetic causes (Zhou et al, 2016). Thus far, traditional methods for studying infertility include genome-wide association studies and linkage analyses, while recent methods in our laboratory of identifying infertility-causing alleles have included using CRISPR/Cas9 gene editing technology to model humanized SNPs in mice in vivo (Singh and Schimenti, 2015). These earlier studies are often associated with a variety of confounding variables and often cannot provide experimentally confirmed results, and this current method, while effective in the determination of infertile alleles and the evaluation of their physiological consequences, is costly and more time-consuming, and thus not well-suited for the analysis of the numerous alleles suspected of causing infertility.

This project thus looks to create an in vitro platform to study meiotic mechanisms of gametogenesis and the factors that affect it, which may eventually lead to the development of methods of treating infertility resulting from gametogenesis irregularities. In mice, specification of PGCs in embryonic testes allows differentiations into pro-spermatogonia that, in postnatal testes, differentiate into spermatogonia (Ishikura et al, 2016). Prior studies have differentiated mouse ESCs into EpiLCs and then PGCLCs in vitro. Co-culture of the PGCLCs with testicular somatic cells from a Kit^{W}/Kit^{W-V} mouse model, after exposure to specific morphogens and hormones, have reproduced key markers of meiosis and the formation of haploid spermatid-like cells. Furthermore, these spermatozoa have contributed to fertile offspring after intracytoplasmic sperm injection (Zhou et al, 2016). This project seeks to differentiate stem cells into spermatid-like cells in a similar fashion in vitro, and then use these methods to model suspected infertility-causing alleles.

This project is a continuation of my work in the lab these last two semesters. Previous semesters' work has included the culture of somatic testicular cells, for later co-culture with PGCs. ID4-GFP mouse tail fibroblasts have been infected with lentiviruses containing five different transcription factors and induced with doxycycline to create induced pluripotent stem cells (iPSCs) (Welstead et al., 2008). Furthermore, plasmids such as pStra8-mVenus and pPrm1-dsRed2 have been constructed, which will eventually serve as markers of early meiotic development and haploid cell formation, respectively. Many other relevant experiments have been performed by Xinbao Ding, a postdoc in the lab, and all these experiments will provide the basis for my work this summer and guide the future directions for the project.

An additional aspect of this project will involve modeling the mutation of a Cdk2 allele through this in vitro platform. Previously, the $Cdk2^{Y15S}$ allele, which alters a tyrosine phosphorylation site and mimicks SNP rs3087335, in homozygous mutant mice has been identified to be deleterious, disrupting spermatogenesis and playing a role in spermatogonial stem cell (SSC) maintenance (Singh and Schimenti, 2015). Current work in the lab further suggests that the absence of this allele leads to the lack of differentiation of SSC-like cells, which ultimately become lost. Specifically, GFRA1 (glial cell line derived neurotrophic factor family receptor alpha 1), a cell surface receptor, is a marker of spermatogonia differentiation that is overexpressed in SSCs. In $Cdk2^{Y15S}$ mutant cells, there was more GFRA+ expression in both adolescent and adult cells when compared to WT cells, suggesting that the $Cdk2^{Y15S}$ allele creates an imbalance between SSC differentiation and maintenance.

We would like to use the constructed in vitro platform to study the effects of the $Cdk2^{Y15S}$ allele on spermatogenesis and see if our platform can detect expected defects in spermatogonia differentiation. Although the in vitro platform is still under construction, spermatogenesis occurs after the formation of PGCs and before the undergoing of meiosis. While

a bulk of the project remains in inducing cells to undergo meiosis, we have successfully induced the formation of PGCs. Thus the $Cdk2^{Y15S}$ allele is ideal to study, since it disrupts normal differentiation of the cells prior to the onset of meiosis, and has been confirmed to be deleterious in vivo. Therefore, in vitro cell lines lacking the $Cdk2^{Y15S}$ allele theoretically would demonstrate spermatogenesis defects which can be measured by GFRA1 expression and histological analysis. If this holds to be true, this would further the support for development of the in vitro platform and increase the potential that this novel method for studying infertility-causing SNPs holds.

Thus, we would ultimately like to construct an in vitro platform in order to study infertility and subfertility causing SNPs. Eventually, CRISPR/Cas9 modified ESCs/iPSCs containing SNPs can be induced to differentiate into PGCs, and then into gonad-like structures containing the SNPs, such that cell lines constructed with mutations in the coding or regulatory regions of the genome. These SNP modified cell lines lacking expression of one or more of the previously mentioned cellular markers will point to infertility-causing alleles. We are further able to test the preliminary effectiveness of our constructed in vitro platform by studying alleles like $Cdk2^{Y15S}$, which has already been experimentally proven in vivo to affect fertility. Furthermore, performing these experiments in an in vitro platform allows more controlled observation and measurement of results when compared to an in vivo study. Ideally, the development and use of in vitro gamete cell lines will allow the efficient analysis of thousands of SNPs suspected of causing infertility.

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