Regulation of the transition from gonocytes to spermatogonia

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Declaration

This thesis is submitted in the form of a series of published papers of which I am a co-author. I have included within this work a written statement signed by each author attesting to my major contribution to each published work. This statement is has been endorsed by the Faculty Assistant Dean (Research Training).

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(Signed)
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Publications included

Literature Review:

Chapter 1:

Chapter 2:
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Chapter 3:

Final Discussion:

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Statements of Contribution

The research higher degree candidate Skye Courtney McIver as contributed upwards of 50% towards the data collection and analysis as well as the manuscript preparation for all the publications for which I am a co-author that are included for consideration in this thesis.

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Abstract

In the western world there has been a significant decline in male reproductive health over the last 100 years, e.g. decreased sperm production and function and increased male infertility. Along with these more subtle changes the incidence of defects of reproductive development, including undescended testis and testicular germ cell cancer (seminoma and non-seminoma), have increased. Seminoma and non-seminoma are now the most common malignancy observed in young men. Both seminoma and non-seminoma develop from the pre-invasive lesion Carcinoma in Situ (CIS). The origin of CIS cells is still under debate however CIS cells are believed to result from improperly differentiated primordial germ cells (PGCs) or gonocytes. In general CIS cells express remarkably similar transcriptomes to gonocytes. Therefore it is essential to understand the cellular transition between gonocytes to spermatogonia to gain a better understanding of the aetiology of testicular germ cell tumours.

MicroRNA (miRNA) are important regulators of posttranscriptional gene expression and have been identified as essential for embryogenesis, primordial germ cell differentiation and spermatogenesis. The aberrant expression of miRNA molecules is associated with male infertility and tumourigenesis including that of testicular germ cell tumours. We therefore examined the change in miRNA expression between postnatal gonocytes and spermatogonia for insights into how miRNA expression could influence this essential developmental process.

We identified seven differentially expressed miRNA molecules between gonocytes and spermatogonia. The down regulated miRNA (miR-290.5p, 291a-5p, 293 and 294*) all belong to the miR-290 family, which is a key regulator of pluripotency as well as primordial germ cell survival and proliferation. The up-regulated miRNA (miR-136, 463* and 743a) are not as well known however miR-136 is thought to be a tumour suppressor and miR-743a is involved in oxidative stress. Target prediction software identified an abundance of targets in both the PTEN and Wnt signalling pathways. Wnt signalling is known to help maintain the undifferentiated spermatogonial stem cell population promote
their proliferation. The ablation of PTEN expression is associated with the transformation of CIS into overt testicular germ cell cancer.

The most highly expressed miRNAs, which were differentially expressed between gonocytes and spermatogonia, were selected for target identification using RNA/RNA pull-down as well as knockdown technology. The array analysis of the pull-down assay identified the presence of multiple targets from each of the miRNAs assayed (miR291a-5p, 293 and 743a) in the PDGF and RAC signalling pathways. PDGF has been documented to be involved in both the migration and proliferation of gonocytes. RAC signalling is required to transverse the blood testis barrier, which develops at postnatal day 8, and therefore is essential for later stages of spermatogenesis. Knockdown of miR291a-5p, 293 and 743a resulted in a significant reduction of Igfbp7 (Insulin-like growth factor binding protein 7), which is a known regulator of cell proliferation and migration in tumours. Therefore this cohort of differentially expressed miRNA molecules (miR290-5p, 291a-5p, 293, 294*, 136, 463* and 743a) is likely to impact on gonocyte proliferation and migration, both of which are disrupted to result in CIS development.

Another feature of the differentiation of gonocytes into spermatogonia is migration into the stem cell niche. The cytokine CXCL12 and its receptor CXCR4 are known to control this migration and are involved in the maintenance of the undifferentiated spermatogonial stem cell pool. Additionally the majority of the differentially expressed miRNA between gonocytes and spermatogonia were predicted to modulate this pathway. In particular they targeted members of the MAP kinase signalling pathway, which has previously been identified to be activated in response to CXCL12 in the seminoma cell line TCam-2. Therefore we examined the role of CXCL12 in seminomas and non-seminomas. CXCR4 mRNA expression was elevated in seminoma tumours compared to normal testis. Treatment of seminoma (TCam-2) and non-seminoma (833ke and Ntera2/D1) cell lines with CXCL12 did not result in increased proliferation or survival. CXCL12 stimulated invasion of TCam-2 but a similar response was not observed in 833ke or Ntera2/D1 cells. Therefore we propose that CXCL12 is likely to play a role in seminoma metastasis rather than non-seminoma metastasis.
The differentiation of gonocytes to spermatogonia is a key developmental step and is essential for continued reproductive productivity and health of men. By concentrating on this narrow developmental window we were able to identify seven miRNA molecules with the potential to regulate this process. These miRNA molecules are predicted to control stem cell development and the control several key signalling pathways i.e. WNT, PENT, PDGF, RAC as well as CXCR4. These pathways have previously been implicated in the differentiation of germ cells as well as the development of cancer.
Aims and Hypothesises

Aim 1: To elucidate the changes in miRNA expression between murine postnatal gonocytes and spermatogonia.

Aim 2: To infer the function of the differentially expressed miRNA molecules between gonocytes and spermatogonia by identifying their targets.

Aim 3: To determine the role of CXCL12 and CXCR4 signalling in seminoma and non-seminoma testicular tumours.

Hypothesis 1: There will be subtle changes in the miRNA expression profile between gonocytes and spermatogonia. These miRNA molecules will target genes, which control key pathways including proliferation, migration and apoptosis within these cells. If these miRNA molecules are dysregulated improper differentiation of gonocytes, which is a key step in the development of seminoma and non-seminoma testicular germ cell tumours could result.

Hypothesis 2: miRNA molecules differentially expressed between gonocytes and spermatogonia will target members of the CXCL12/CXCR4 signalling pathway. Additionally CXCR4 and/or CXCL12 will be up-regulated in seminoma and non-seminoma tumours. Treatment of cell lines with CXCL12 will increase cell proliferation survival or invasion indicating that CXCR4 signalling is central to the progression of seminoma and non-seminoma tumours