Project proposal

Title: Identification of a potential biomarker for PCOS

Alignment with the CRE New Knowledge program research activities

This project aligns well with the aims of CRE in the following areas:

- Improve PCOS diagnosis: This research project will investigate the potential of the use of novel biomarkers to allow improved PCOS diagnosis and thus allow a precise diagnosis of the PCOS pathophysiology and subsequently improved clinical management of PCOS patients.
- Advance fundamental understandings of biological origin, aetiology and pathology of PCOS: If the findings from this research project identify a clear link in altered androgen receptor (AR)-mediated mechanisms and PCOS, then this will support AR signalling as a therapeutic target.

Background

The most prominent effect of androgens on women's health is hyperandrogenism in women with polycystic ovary syndrome (PCOS). Worldwide, PCOS is the most frequent endocrine disorder of reproductive-aged women, causing infertility due to arrested follicular maturation and ovulation, hyperandrogenism causing acne and hirsutism, and metabolic abnormalities including obesity, insulin resistance, dyslipidemia, cardiovascular disease and type 2 diabetes (1;2). AR signalling pathways are implicated as key factors in the pathogenesis of PCOS with androgen excess being the most consistent feature (3). The source of androgen excess is most likely the ovary, although the adrenal glands cannot yet be fully discounted. Notably, whether hyperandrogenism is cause or effect remains to be determined. Recently two papers have identified mechanisms of altered AR-mediated regulation that may lead to dysfunctional ovarian function. In the 1st paper by Sen et al (4), it is reported that AR regulates follicular atresia by enhancing the expression of the anti-apoptotic microRNA (miR) miR-125b, and thus miR-125b plays a key role in androgen-induced follicular survival. Recent findings indicate that miRNAs are involved in the regulation of key processes involved in follicular development, and in addition altered expression of miRNAs have been proposed as a potential as diagnostic/prognostic biomarkers for PCOS (5;6). In the 2nd paper, Wang et al. (7) reported two alternative AR spice variants present in the granulosa cells of most (~62%) women with PCOS but none in non-PCOS controls. The AR variants comprise of in-frame modifications, one being an insertion (69bp insertion into intron 2), and the other a deletion (exon 3). The authors proposed that the presence of these variants may be an important mechanism causing ovarian hyperandrogenism and aberrant follicle development characteristics of PCOS. However, there is evidence to support that these variants are a consequence rather than a cause of PCOS (8). The exon 3 deletion is a well-known mutation causing complete androgen insensitivity in humans (9) and mice (10). Furthermore, in conjunction with the mouse PCOS model, the deletion of exon 3 protects against rather than causing typical PCOS features (11). Whether the insertion mutation is a cause or consequence of PCOS is more speculative. However, the reduced functionally of cells expressing this mutation argues in favour of this variant also being an adaption of follicles in a PCOS ovary to an aberrant hyperandrogenic environment rather than a cause of ovarian hyperandrogenism. Thus, while it appears that the AR variants are a consequence rather than cause of PCOS, these findings strengthen the link between AR signalling and PCOS. Taken together, these findings support that miRNAs and AR variants may serve as new biomarkers of an aberrant follicular androgen environment and/or targets for novel therapies directly targeting AR signalling.

Rationale/Project Plan

At present there is no diagnostic test for PCOS and as a result diagnosing PCOS is dependent on the specific criteria used (Rotterdam criteria, NIH criteria and Androgen Excess and PCOS Society). The identification of a valid diagnostic marker(s) for PCOS has the potential to facilitate the development of clinical diagnostic and classification tests for PCOS, allowing customised treatment for the various features of the disease.

In this project we aim to identify if miR-125b and the AR variants identified by Wang et al. (7) are present in follicular fluid samples collected from control and PCOS patients undergoing IVF treatment. The presence and level of expression of miR-125b and AR variants will be measured and correlated with a range of endpoints including steroid profiles and IVF outcomes (number of eggs retrieved, fertilisation rates, embryo quality, pregnancy and live birth rates). The presence of miR-125b and the AR variants in our samples would add support to the use of miR-125b and these AR variants as potential biomarkers associated with ovarian PCOS phenotypes.

Methods

Follicular fluid (FF) is being collected from women undergoing IVF treatment at Royal Prince Alfred Hospital, Sydney, NSW. PCOS is diagnosed according to the Rotterdam Consensus. Follicular fluid is obtained by follicular aspiration from women undergoing oocyte retrieval for IVF. Ethical approval has already been obtained from Sydney Local Health District Ethics Review committee for the collection of FF samples, and ~80% of target number of samples is already available for LC-MS steroid profiles in FF. An amendment to the approved protocol will be submitted for the same samples to be used to assess the presence of miR-125b and the two AR variants in FF.

RNA will be isolated and reverse transcribed from follicular fluid as previously described (6). Quantitative real-time PCR will then be carried out on the samples. Quantitative real-time PCR analysis will be performed on a Corbett Rotorgene 6000. A standard curve will be generated for miR-125b and each AR variant from five serial dilutions of purified PCR product from the same primers. Standards (dilutions used for each gene will be 10^{-2} to 10^{-6}) will be assigned an arbitrary value, and mean relative mRNA expression of samples determined in duplicate and standardized to the internal housekeeping gene. GAPDH and PPIA will be used as an internal housekeeping genes, as previously described (4;6). No template controls, substituting water for cDNA, and a negative reverse transcription will be included in each run.

Significance

Currently there is no diagnostic test for PCOS and, despite substantial research, the origins of PCOS have remained elusive. As a result, diagnosing PCOS is dependent on the criteria used and mechanism-based interventions are not feasible leaving management to rely on empirical, symptomatic treatment. The study of miRNAs and androgen receptor splice variants is a new area in PCOS, but has important implications for developing novel biomarkers for more specific diagnosis of the PCOS pathophysiology, and ultimately towards personalized treatment oriented towards the specific mechanisms of the disease. The findings from this study will provide an insight into potential biomarkers associated with ovarian PCOS phenotypes and IVF outcomes.

Expected outcomes

We expect that our results will increase our understanding of molecular milieu of human follicular fluid, especially in regard to the hyperandrogenism of PCOS, and potentially be predictive for embryo and pregnancy outcomes. This may lay the foundation for the future refinement of biomarkers in follicular fluid as indicators of PCOS, aberrant follicular androgen environment and potentially embryo quality and other IVF-related outcomes.

Proposed statistical analysis

Statistical analysis will performed using NCSS software (NCSS Statistical Software, Kaysville, UT). Data that is not normally distributed will be transformed prior to analysis. Statistical difference will be tested by ANOVA with post hoc test using Fishers's least significant difference multiple comparison test. P vales <0.05 will be considered statistically significant.

Summary Budget

I am seeking $15,000 funding for this research project.

Item	**Cost**
RNA and miRNA isolation reagents and kits	$5000
Reverse transcription kits	$5000
Primers and MicroRNA assays	$1500
PCR plates and tubes	$2000
Agarose gel electrophoresis reagents, DNA ladders	$500
Disposable plastics, gloves, tissues	$1000
Total	**$15,000**

References

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