RESEARCH GROUP: Genomic Medicine

Project Title:
Regulation of microRNA by DNA methylation in prostate cancer

Supervisors:
Declan McKenna, Colum Walsh

Contact Details:
Declan McKenna (dj.mckenna@ulster.ac.uk)
or Colum Walsh (cp.walsh@ulster.ac.uk)
School of Biomedical Sciences
University of Ulster, Coleraine

Level: MRes/ PhD
PhD

Background to the project:
The role of epigenetic regulation in the onset of cancer is now well established (1). In prostate cancer (PCa), the methylation status of several candidate genes has been studied (2), such as hypermethylation of the GSTP1 gene promoter (3), which has recently been shown to be a more sensitive and specific marker than other PCa markers (4). Hence, the use of epigenetics-based markers is believed to hold great potential for PCa diagnosis and prognosis, although continued research on different genes is required if this approach is to gain widespread acceptance. A recently discovered class of non-coding genes called microRNAs (miRNAs) may help prove this potential as there is increasing evidence that many of them are also epigenetically regulated. In PCa, several miRNAs express abnormally, suggesting they may be useful in the diagnosis, prognosis, and potential therapeutic intervention of this disease (5). It has also become apparent that many miRNAs are controlled by epigenetic mechanisms and several tumour suppressor miRNAs have now been shown to be silenced by hypermethylation in a range of tumour types, including PCa (6). Thus, reactivating the expression of these miRNAs may prove successful as a novel therapeutic strategy in advanced prostate cancers.

In our laboratory, we have identified a number of miRNAs which are downregulated in PCa cell lines and which show changed expression response to depleted levels of DNA Methyltransferase 1 (DNMT1). We have also profiled miRNA expression in a panel of biopsy specimens in collaboration with colleagues at Altnagelvin Area Hospital (7,8). We now want to further investigate selected miRNAs from these lead candidates to confirm which are under epigenetic regulation and which have potential value as biomarkers for monitoring PCa progression.

Methods to be used:
The methods outlined below are routinely used in the laboratories of both supervisors and papers featuring similar experimental approaches have been published by them (7-10). The equipment and expertise available in the labs means there should be minimum difficulty in commencing experimental work.

Cell culture
A panel of PCa cells will be cultured and treated to generate in vitro samples. Treatments will involve altering methylation levels by knockdown technology (e.g. of DNMT1) and by use of demethylation agents (e.g. decitabine (Aza))

Expression Analysis of miRNA
Quantitative Real-Time PCR for specific miRNAs

Epigenetic Changes
Analysis of DNA methylation in miRNA regions by MS-PCR, bisulfite sequencing, COBRA analysis

Functional Analysis of selected miRNA
miRNA inhibition (transfection using miRNA-specific antisense molecules)
Over-expression (transfection using miRNA-mimic molecules)
Subsequent effect on putative protein target(s) (assessed by Western Blotting)
Validation of target(s) (Assessed by luciferase reporter assay)
Effect on cell behaviour (proliferation / apoptosis / invasion )
Bioinformatic analysis & Data-mining

Data-mining of online data repositories will be performed to identify interesting and clinically relevant patterns of methylation and miRNA expression. This data includes datasets from prostate cell lines and from patient biopsies and this analysis will be carried out in parallel with in vitro lab work to help inform choice of miRNA/target to focus on.

Objectives of the research:

HYPOTHESIS
Methylation-related changes impacts upon miRNA expression in PCa cells.

AIMS & OBJECTIVES
1. To confirm that expression changes of key miRNAs is related to methylation in PCa cells
2. To characterize the response of prostate cancer cell lines to re-expression of miRNA following Aza treatment or other interventions
3. To evaluate the potential of miRNA and methylation profiling as diagnostic/prognostic biomarkers in PCa using bioinformatic analysis

Skills required of applicant:
- Good Laboratory skills
- Good oral and written presentation skills
- Good critical thinking and analytical skills
- Good IT skills
- Good work ethic and ability to work independently
- Experience of biostatistics and using statistical packages (e.g. SPSS)
- Interest in bioinformatic analysis

References: