Regulation of miR-200c and miR-141 by Methylation in Prostate Cancer

Seodhna M. Lynch1, Karla M. O’Neill1,2, Michael M. McKenna3, Colum P. Walsh1, Declan J. McKenna1

1Biomedical Sciences Research Institute, Ulster University, Cromore Road, Coleraine, BT52 1SA
2School of Medicine, Dentistry and Biomedical Sciences, Queen’s University Belfast, BT9 7BL
3Dept of Cellular Pathology, Western Health & Social Care Trust, Altnagelvin Area Hospital, Co. Derry, BT47 6SB

INTRODUCTION

In prostate cancer, many miRNAs are aberrantly expressed. This abnormal expression suggests that miRNAs are potentially promising in terms of diagnosis, prognosis and subsequent therapeutic intervention in this disease. miR-200c and miR-141 are co-expressed members of the miR-200 family, which play a key role in epithelial-to-mesenchymal transition (EMT) in cancer. miR-200c and miR-141 are known to be aberrantly expressed in several cancers, including prostate cancer. Epigenetic regulation of miR-200c via DNA methylation has also been reported in various cancers, but no studies to date have investigated this in prostate cancer.

We therefore proceeded to profile and correlate miR-200c/miR-141 expression and methylation status in prostate cell lines and clinical prostate samples.

This work was accepted for publication in the Prostate Journal in May 2016 doi: 10.1002/pros.23201 [Epub ahead of print]

METHODS AND MATERIALS

Cell Lines: Non-malignant prostate epithelial cell-line RWPE1 and human prostate cancer cell-lines PC3, 22Rv1 and LNCaP. Transfections performed at final concentration of 25nM.

Clinical Prostate Samples: Five 10µM sections were prepared from FFPE needle core biopsies (n=14) and FFPE prostatectomy biopsy samples (n=22) for RNA and DNA extraction using RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE (Life Technologies). ORENCNI Ref: 10/NIR02/13.

PCR: miRCURY LNA™ microRNA PCR system (Exiqon, Denmark) used to measure miR-200c and miR-141 expression from 50ng (clinical samples) or 20ng (cell-line samples) template RNA, mRNA and gene expression performed on Roche LC480 Lightcycler.

Flow Cytometry: performed on Beckman-Coulter Gallios™ instrument using PI staining (cell cycle) or PI and AlexaFlour 488 Annexin V Kit (apoptosis) (Life Technologies).

Aza and Genisteen Treatment: PC3 cells were treated with decitabine (5-aza-2'-deoxycytidine) (1µM) for 72hours or genisteen (40µM) for 120 hours. RNA and DNA extracted for analysis.

Pyrosequencing/COBRA: Bisulfite converted DNA was produced using the EpiTect Bisulfite Kit (Qiagen). The PyroMark™ Q2 pyrosequencer (Qiagen) was used to measure methylation within the miR-200c/miR-141 promoter region across CpG sites. Restriction enzymes BstU1 and HinfI was used in COBRA analysis.

Other Assays: XTT proliferation assay (Roche) and colony assay performed on transfected and control cells.

RESULTS

SUMMARY AND CONCLUSION

- This is the first study to show a correlation between DNA methylation and expression of miR-200c and miR-141 in clinical prostate specimens.
- Our findings provide evidence that expression of miR-200c and miR-141 is regulated by methylation of CpG sites in their promoter in PCa cells.
- Profiling their expression and methylation status may therefore have potential as a novel biomarker in the diagnosis and prognosis of PCa.
- Our data also suggests that aberrant miR-200c/miR-141 expression will affect DNM3A and TET genes, key components of the methylation machinery which will influence global cellular methylation levels.
- Furthermore, we propose that manipulation of miR-200c and miR-141 expression by epigenetic alterations, either through chemical or dietary means, may be the basis for a possible therapeutic intervention for this disease.