Epigenetic regulation of miR-200c in prostate cancer

Seodhna M. Lynch 1, Karla M. O’Neill 1, Michael M. McKenna 2, Colum P. Walsh 1, Declan J. McKenna 1
1 Biomedical Sciences Research Institute, Ulster University, Coleraine, Northern Ireland, UK
2 Department of Cellular Pathology, Altnagelvin Area Hospital, Derry, Northern Ireland, UK

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 1-2.

miR-200c is believed to play a key role in epithelial-to-mesenchymal transition (EMT) and is known to be aberrantly expressed in several cancers, including prostate cancer 3-4.

Epigenetic regulation of miR-200c via DNA methylation has also been reported in various cancers 5-6, but no studies to date have investigated this in prostate cancer.

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

We also investigated how manipulation of miR-200c levels affected cell behaviour and impacted upon target genes.

MATERIALS AND METHODS

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=12) and FFPE prostatectomy biopsy samples (n = 24) for RNA and DNA extraction using RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE (Life Technologies).

PCR: miRCURY LNA™ microRNA PCR system (Exiqon, Denmark) used to measure miR-200c 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-Couler Gallios™ instrument using PI staining (cell cycle) or PI and AlexaFlour 488-Annexin V Kit (apoptosis) (Life Technologies).

AzaTreatment: PC3 cells were treated with decitabine (5-aza-2’-deoxycytidine) for 72hours. Final concentration of decitabine was 1µM. RNA and DNA extracted for analysis.

Pyrosequencing/COBRA: The PyroMark™ Q24 pyrosequencer (Qiagen) was used to measure methylation levels within the miR-200c promoter region across CpG sites. Restriction enzyme BstU1 was used in COBRA analysis.

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

SUMMARY AND CONCLUSION

We show that miR-200c expression is highly elevated in LNCaP and 22Rv1 cells, but much lower in PC3 cells.

Expression in LNCaP and PC3 cells correlates inversely with the methylation status of its promoter region, which is unmethylated in LNCaP and hypermethylated in PC3.

In PC3 cells, miR-200c expression is elevated by treatment with decitabine and by knockdown of DNA Methyltransferase 1 (DNMT1), suggesting its expression is regulated by methylation.

Altered expression and methylation of miR-200c is frequently observed in clinical biopsy tissue relative to matched normal tissue, which may be useful measurements to help stratify patient cohorts.

Restoration of miR-200c expression in PC3 cells inhibited cell proliferation, migration and clonogenic potential of prostate cancer cells, as well as inducing apoptosis.

Over-expression of miR-200c in PC3 cells down-regulated expression of DNA Methyltransferase 3A (DNMT3A), suggesting miR-200c may itself have a role in epigenetic regulation within the cell.

We conclude that DNA methylation contributes to the expression of miR-200c in prostate cancer cells, which impacts upon its role in controlling cell growth and apoptosis.

We propose that miR-200c may be a useful prognostic biomarker in prostate cancer and is an attractive target for a possible therapeutic intervention for this disease.

REFERENCES
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ADDRESS FOR CORRESPONDENCE
Dr Declan McKenna, Ulster University, Coleraine, N. Ireland, UK, BT52 1SA
E-mail: d.mckenna@ulster.ac.uk Tel: +4428 7012 4256

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