miR-24 Regulates p27 Expression in Prostate Cancer

Seodhna M. Lynch 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

Faculty of Life & Health Sciences

INTRODUCTION

In prostate cancer, several miRNAs express abnormally, raising the possibility that miRNAs will be useful for diagnosis, prognosis, and potential therapeutic intervention in this disease (1,2).

Aberrant expression of miR-24 has been associated with several disorders, including cancer, but very few studies have specifically investigated the expression and targets of miR-24 in relation to prostate cancer (3).

We were interested in its relationship between miR-24 and the cyclin dependent kinase inhibitors p27 (CDKN1B) and p16 (CDKN2A), which are both known to be deregulated in prostate cancer (4), and which we had previously demonstrated as targets of miR-24 in human keratinocytes (5).

We therefore proceeded to investigate miR-24 expression in prostate cell lines and clinical prostate samples, as well as examining how manipulation of miR-24 levels affects cell behaviour and p27/p16 expression in prostate cancer cells.

MATERIALS AND METHODS

Cell Lines: Non-malignant prostate epithelial cell-lines RWPE1 and PWR-1E and human prostate cancer cell-lines PC3, 22Rv1, LNCaP and DU145. 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 extraction using RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE (Life Technologies).

PCR: miRCURY LNA™ microRNA PCR system (Exiqon, Denmark) used to measure miR-24 expression from 50ng (clinical samples) or 20ng (cell-line samples) template RNA. miRNA and gene expression performed on Roche LC480 Lightcycler.

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

Luciferase Reporter Assay: Co-transfection with 300ng wild-type or mutant p27-3’UTR reporters (Origene Technologies) with 25nM pre-miR-24 or control, using 30ng Renilla luciferase vector for transfection efficiency. Luciferase activity measured using the Dual-Glo® Luciferase Assay Kit (Promega).

Antibodies: Monoclonal Anti-human p16 Antibody (Roche), Anti-human p27Kip1 (DAKO), anti-B-actin (Sigma). IHC performed on Benchmark ULTRA IHC/ISH Staining instrument (Ventana/Roche).

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

RESULTS

1. REFERENCES

Antibodies transfection Annexin instrument used biopsy Clinical and PWR behaviour We cancer in raising prostate (2014) (cell 24 expression is significantly lower in prostate cancer cell lines compared to normal prostate epithelial cells (PC3)). FFPE miR(24) expression in prostate cancer is regulated by miR-24 targeting of p27.

2. MATERIALS AND METHODS

Cell Lines: Non-malignant prostate epithelial cell-lines RWPE1 and PWR-1E and human prostate cancer cell-lines PC3, 22Rv1, LNCaP and DU145. 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 extraction using RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE (Life Technologies).

PCR: miRCURY LNA™ microRNA PCR system (Exiqon, Denmark) used to measure miR-24 expression from 50ng (clinical samples) or 20ng (cell-line samples) template RNA. miRNA and gene expression performed on Roche LC480 Lightcycler.

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

Luciferase Reporter Assay: Co-transfection with 300ng wild-type or mutant p27-3’UTR reporters (Origene Technologies) with 25nM pre-miR-24 or control, using 30ng Renilla luciferase vector for transfection efficiency. Luciferase activity measured using the Dual-Glo® Luciferase Assay Kit (Promega).

Antibodies: Monoclonal Anti-human p16 Antibody (Roche), Anti-human p27Kip1 (DAKO), anti-B-actin (Sigma). IHC performed on Benchmark ULTRA IHC/ISH Staining instrument (Ventana/Roche).

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

3. RESULTS

Correlation of miR-24 and p27 in clinical prostatectomy biopsy cases. Representative staining of (A) p16 and (B) p27 in normal and tumour tissues from 2 separate prostatectomy cases. (Original mag40X).

Correlation of miR-24 expression with (B) p27 and (C) p16 protein levels, as measured by immunostaining, revealed an inverse relationship (p-values generated using Spearman’s rank test). (F) Correlation miR-24 with prostate cancer progression (A4) and (B) using clinical prostatectomy samples.

4. DISCUSSION

We show that miR-24 expression is significantly lower in prostate cancer cell lines compared to a normal prostate epithelial cell line. Decreased expression of miR-24 is also more frequently observed in both needle core and prostatectomy tumour tissue relative to matched normal tissue. Low miR-24 expression correlates with high PSA serum levels, suggesting that stratifying patients into groups which show high or low miR-24 levels could be a useful prognostic tool. Restoration of miR-24 expression inhibited cell cycle, proliferation, migration and clonogenic potential of prostate cancer cells, as well as inducing apoptosis. p27 and p16 were confirmed as targets of miR-24 in prostate cancer cells and a significant inverse correlation between miR-24 and p27 was revealed in clinical prostatectomy specimens. We conclude that miR-24 likely acts in a tumour suppressor role in prostate cancer and helps regulate cell growth by targeting p27 and p16 in the prostate cancer cells. We propose that miR-24 may be a useful progression biomarker in prostate cancer and is an attractive target for a possible therapeutic intervention for this disease.

REFERENCES

5. McKenna et al. Urology. 2014 84(2):210-6

SUMMARY AND CONCLUSION

We show that miR-24 expression is significantly lower in prostate cancer cell lines compared to a normal prostate epithelial cell line. Decreased expression of miR-24 is also more frequently observed in both needle core and prostatectomy tumour tissue relative to matched normal tissue. Low miR-24 expression correlates with high PSA serum levels, suggesting that stratifying patients into groups which show high or low miR-24 levels could be a useful prognostic tool. Restoration of miR-24 expression inhibited cell cycle, proliferation, migration and clonogenic potential of prostate cancer cells, as well as inducing apoptosis. p27 and p16 were confirmed as targets of miR-24 in prostate cancer cells and a significant inverse correlation between miR-24 and p27 was revealed in clinical prostatectomy specimens. We conclude that miR-24 likely acts in a tumour suppressor role in prostate cancer and helps regulate cell growth by targeting p27 and p16 in the prostate cancer cells. We propose that miR-24 may be a useful progression biomarker in prostate cancer and is an attractive target for a possible therapeutic intervention for this disease.