Investigation of microRNA-30a and selected target genes in prostate cancer

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INTRODUCTION
Prostate cancer (PCa) is the second most diagnosed cancer and the fifth cause of cancer mortality in men worldwide (1). It is now known that many genetic factors play a role in the development and progression of PCa. Among these factors are microRNAs (miRNAs) which are small non-coding RNAs that can silence target genes to regulate gene expression (2). In PCa, several miRNAs are aberrantly expressed, suggesting they could be useful biomarkers for the diagnosis and prognosis of this disease, as well as potentially acting as treatment targets (3,4,5). However, the expression and functionality of these miRNAs still requires more research to better understand how they contribute to disease.

Materials and Methods

IN VITRO ANALYSIS
Cell-lines: RWPE1 (Transformed normal prostate epithelial) & PCa (PCAs).

miRNA Transfections: 25 nM of pre-miR-30a-3p (mimic) transfected using Lipofectamine 2000 for 6 hours.

qRT-PCR: Expression of SOX4, ITGA2 and SOCS1 normalized to HPRT1. miR-30a-3p normalized to U6 snRNA. Fold change calculated using ΔΔCT method.

IN SILICO ANALYSIS
Database: The Cancer Genome Atlas (TCGA) prostate adenocarcinoma (PRAD) patient biopsy dataset

Bioinformatic Tools:
miRTarBase
https://miTarbase.cuhk.edu.cn/

UCSC Xena
CancerMiRNome
CancerMiRNome

RESULTS

Figure 1(a) qRT-PCR shows miR-30a expression is significantly higher in PCa cell line PC3 than normal prostate epithelial cell line RWPE1. (b) ITGA2, SOX4 and SOCS1 was identified as PCa-associated gene targets of miR-30a. (c) miR-30a was over-expressed by transient transfection in RWPE1 cells, resulting in (d) expression of SOX4 and ITGA2 being significantly reduced. SOCS1 expression was not significantly changed. Graphs show mean ± standard error (n = 4, paired t-test, "p < 0.01, ***p < 0.001, ns, non-significant).

Figure 2 UCSC Xena analysis of The Cancer Genome Atlas (TCGA) prostate adenocarcinoma (PRAD) patient biopsy dataset, including normal (n = 52) and tumour (n = 480) tissue samples, shows a significant negative correlation of miR-30a with (a) ITGA2 and (b) SOX4. (c) No significant correlation between miR-30a and SOCS1. (Spearman’s correlation, **p < 0.01, ***p < 0.001, ns, non-significant).

Figure 3 UCSC Xena analysis of TCGA PRAD dataset, including normal (n = 52) and tumour (n = 480) tissue samples from patient biopsies. (a) miR-30a expression is significantly higher in tumour tissue compared to normal. (b) Conversely, ITGA2 and SOCS1 expression is significantly lower in tumour tissue compared to normal, whereas SOX4 is higher. (c) Western blots would also demonstrate effect of miR-30a and ITGA2 in prostate cancer.

Figure 4 (a) CancerMiRNome ROC curve analysis demonstrating that miR-30a shows potential for distinguishing between tumour and normal tissue. (b) miR-30a is significantly elevated in the serum of prostate cancer patients compared to healthy, non-cancer control patients. Data from GEO dataset GSE112264 (n, Healthy = 41, PCa = 85) was used. (c) miR-30a is associated with improved survival of patients with high IGTA2 expression compared to those with low expression. (d) miR-30a is associated with a significant decrease in time to Disease-free interval compared to those with low expression. Data analysis for (a) to (c) was performed using UCSC Xena based on TCGA PRAD patient cohort.

SUMMARY AND FUTURE WORK

- These findings provide evidence that miR-30a over-expression is associated with PCas.
- Of the three targets genes examined, miR-30a appears to consistently show an inverse expression profile with the gene ITGA2, which has an important role in controlling prostate cell growth.
- However, similar experiments in other PCa cell lines are needed to definitively validate ITGA2 as a gene target.
- Western blots would also demonstrate effect of miR-30a upon ITGA2 protein levels.
- Effect of miR-30a on cells needs investigated by bioassays for migration, invasion, proliferation and clonogenicity.
- Both miR-30a and ITGA2 are potentially useful diagnostic and/or prognostic biomarkers for PCa, but this also needs to be robustly evaluated in combination with other miRNAs or gene markers.

REFERENCES & ACKNOWLEDGEMENTS


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Gleason Score

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