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

Chloe McCully & Declan J. McKenna*

Genomic Medicine Research Group, School of Biomedical Sciences, Ulster University, Coleraine, Northern Ireland

*** Correspondence e-mail: dj.mckenna@ulster.ac.uk**

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 abnormally 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.

Hsa-miR-30a-5p (**miR-30a**) is a microRNA that has been linked with different cancers, where it targets various genes to help regulate cell proliferation, apoptosis, invasion and metastasis (6).

However, it has not been extensively explored in PCa and many targets in prostate cells have not been validated.

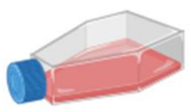
Therefore, this study aims to investigate the role of miR-30a in PCa by investigating its expression and the expression of three selected target genes in prostate samples.

This includes *in vitro* analysis of cell lines and *in silico* analysis of patient data repositories.

Hypothesis: Upregulation of miR-30a expression will result in a downregulation expression of target genes believed to play a role in prostate cancer progression

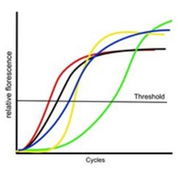
MATERIALS AND METHODS

IN VITRO ANALYSIS



Cell-lines: RWPE1 (Transformed normal prostate epithelial) & PC3 (PCa).

miRNA Transfections: 25 nM of pre-miR-30a-5p (mimic) transfected using Lipofectamine 2000 for 6 hours.



qRT-PCR: Expression of *SOX4*, *ITGA2* and *SOCS1* normalized to *HPRT1*. miR-30a-5p 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
http://portal.gdc.cancer.gov/projects

Bioinformatic Tools:

miRTarBase
https://mirtarbase.cuhk.edu.cn/

UCSC Xena
https://xenabrowser.net/

CancerMIRNome
http://bioinfo.jialab-ucr.org/CancerMIRNome/

RESULTS

FIGURE 1. Effect of miR-30a expression on selected target genes *in vitro*

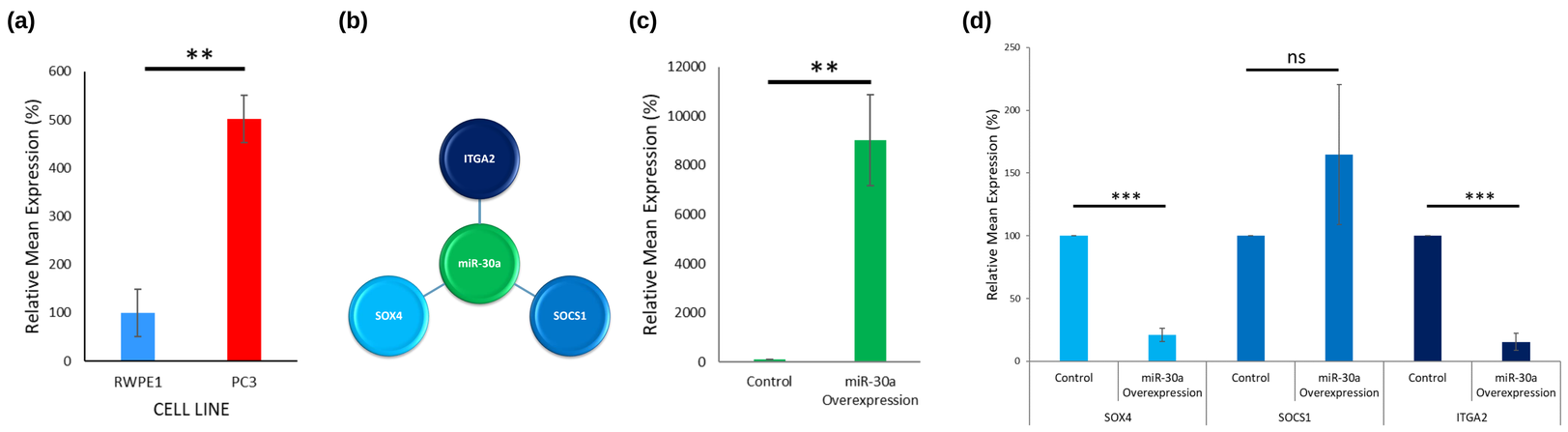


Figure 1 (a) qRT-PCR shows miR-30a expression is significantly higher in PCa cell line PC3 than normal prostate epithelial cell line RWPE1. **(b)** *ITGA2*, *SOX4* and *SOCS1* were identified as PCa-associated gene targets of miR-30a. **(c)** miR-30a was over-expressed by transient transfection in RWPE1 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. Correlation of miR-30a and target genes in TCGA PRAD biopsy dataset

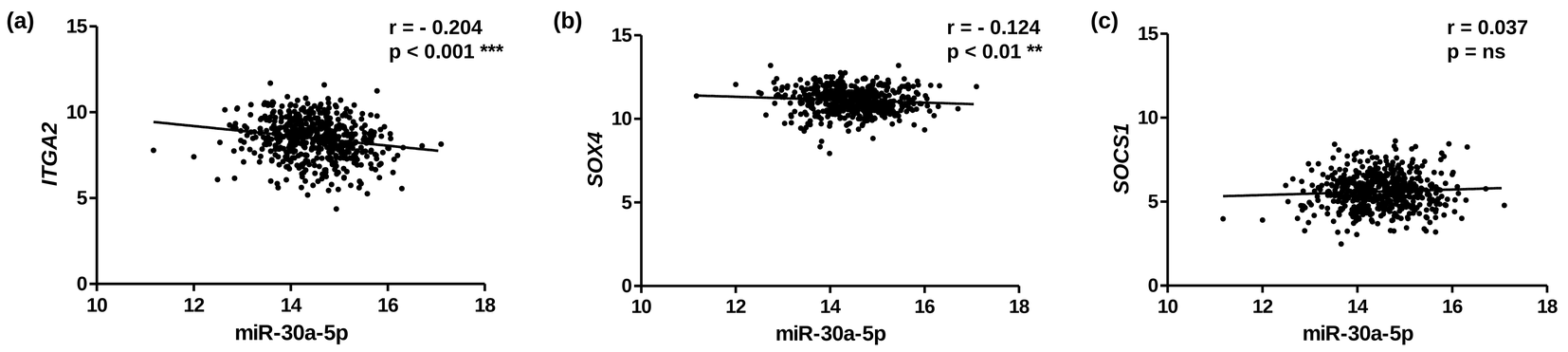


Figure 2 UCSC Xena analysis of The Cancer Genome Atlas (TCGA) prostate adenocarcinoma (PRAD) patient biopsy dataset, including normal (n = 52) and tumour (n = 490) tissue samples, shows a significant negative correlation of miR-30a with **(a)** *ITGA2* and **(b)** *SOX4.* **(c)** No significant correlation between miR-30a and *SOCS1*. (Spearman's correlation, ** p < 0.01, *** p < 0.001, ns; non-significant).

FIGURE 3. Expression of miR-30a-5p and target genes in normal and tumour prostate tissue

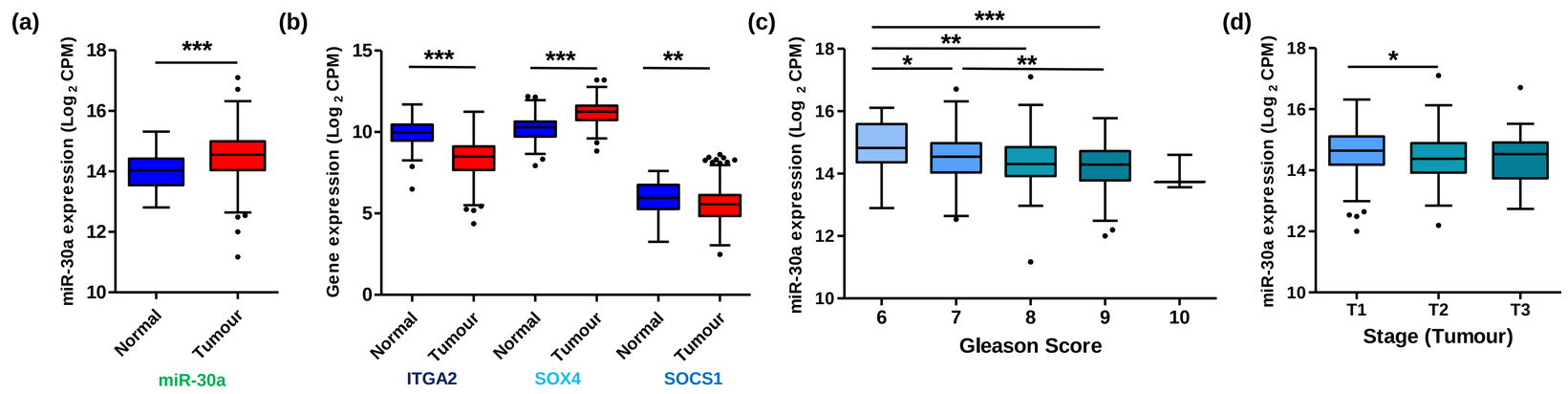


Figure 3 UCSC Xena analysis of TCGA PRAD dataset, including normal (n = 52) and tumour (n = 490) tissue samples from patient biopsies. **(a)** miR-30a expression is significantly higher in tumour tissue compared to normal. **(b)** Conversely, *ITGA2* and *SOCS1* expression is significantly lower in tumour tissue compared to normal, whereas *SOX4* is higher. (Welsh's unpaired t-test, ** p < 0.01, *** p < 0.001). In prostate tumour samples, miR-30a expression significantly decreases with advanced disease, as measured by **(c)** Gleason score and **(d)** clinical T staging. (Kruksal-Wallis ANOVA with Dunn's multiple comparison test, * p < 0.05, ** p < 0.01, *** p < 0.001).

FIGURE 4. Biomarker potential of miR-30a and ITGA2 in prostate cancer

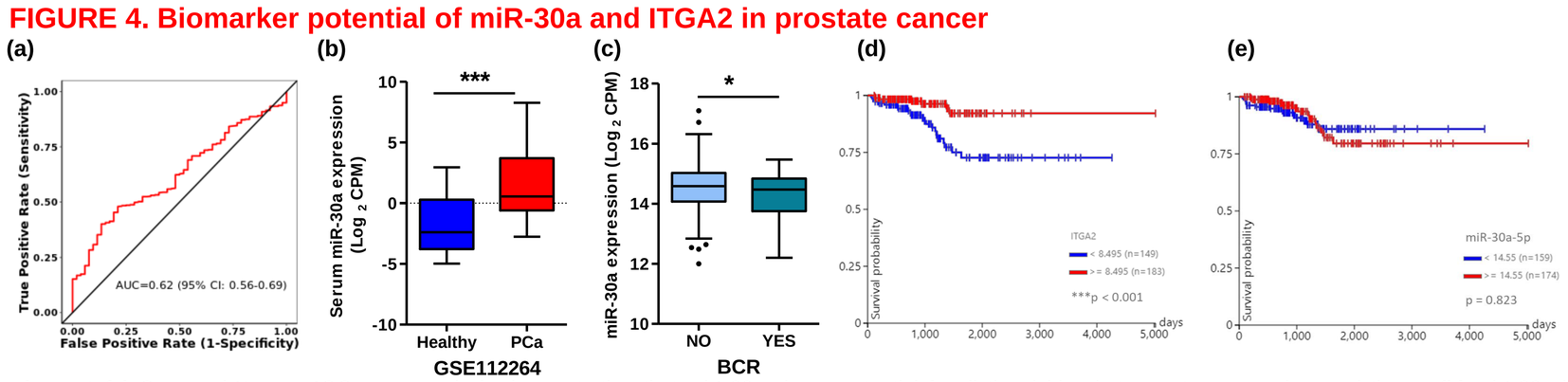


Figure 4 (a) CancermiRNome ROC curve analysis demonstrating that miR-30a shows potential for distinguishing between tumour and normal tissue. **(b)** miR-30a is significantly elevated in the serum of prostate cancer patients compared to healthy, non-cancer control patients. Data from GEO dataset GSE112264 (n, Healthy = 41, PCa = 809)(Welch's t-test, * p > 0.001). **(c)** Significantly higher miR-30a levels associated with patients showing no biochemical recurrence (BCR) compared to those who do (n, no recurrence = 407, recurrence = 61)(Welch's t-test, * p > 0.05). **(d)** For survival analysis, patients with high *ITGA2* expression show significantly reduced time for Disease-free interval, compared to those with low expression. (Log-rank (Mantel–Cox) test, *** p < 0.001). **(e)** No significant difference in time for Disease-free interval between patients with high and low miR-30a expression. (Log-rank (Mantel–Cox) test). Data analysis for (b) to (e) was performed using UCSC Xena based on TCGA PRAD patient cohort.

REFERENCES & ACKNOWLEDGEMENTS

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SUMMARY AND FUTURE WORK

- These findings provide evidence that miR-30a over-expression is associated with PCa.
- 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.