

REVIEW

A novel microRNA-1207-3p/FNDC1/FN1/AR regulatory pathway in prostate cancer

Dibash K. Das^{1,2,3}, Olorunseun O. Ogunwobi^{1,2,3}

¹Department of Biological Sciences, Hunter College of The City University of New York, New York, NY, 10065, USA

²The Graduate Center Departments of Biology and Biochemistry, The City University of New York, New York, NY, 10016, USA

³Department of Medicine, Weill Cornell Medicine, Cornell University, New York, NY, 10065, USA

Correspondence: Olorunseun O. Ogunwobi

E-mail: ogunwobi@genectr.hunter.cuny.edu

Received: December 29, 2017

Published: August 06, 2018

Prostate cancer (PCa) is the second most common cause of cancer-specific deaths in the U.S. Unfortunately, the underlying molecular mechanisms for its development and progression remain unclear. Studies have established that microRNAs (miRNAs) are dysregulated in PCa. The intron-derived microRNA-1207-3p (miR-1207-3p) is encoded at the non-protein coding gene locus *PVT1* on the 8q24 human chromosomal region, an established PCa susceptibility locus. However, miR-1207-3p in PCa had not previously been investigated. Therefore, we explored if miR-1207-3p plays any regulatory role in PCa. We discovered that miR-1207-3p is significantly underexpressed in PCa cell lines in comparison to normal prostate epithelial cells, and that increased expression of microRNA-1207-3p in PCa cells significantly inhibits proliferation, migration, and induces apoptosis via direct molecular targeting of fibronectin type III domain containing 1 (FNDC1). Our studies also revealed significant overexpression of FNDC1, fibronectin (FN1) and the androgen receptor (AR) in human PCa cell lines as well as tissues, and FNDC1, FN1, and AR positively correlate with aggressive PCa. These findings, recently published in *Experimental Cell Research*, are the first to describe a novel miR-1207-3p/FNDC1/FN1/AR novel regulatory pathway in PCa.

Keywords: miR-1207-3p; prostate cancer; FNDC1; FN1; AR

To cite this article: Dibash K. Das, et al. A novel microRNA-1207-3p/FNDC1/FN1/AR regulatory pathway in prostate cancer. *RNA Dis* 2018; 5: e1503. doi: 10.14800/rd.1503.

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Prostate cancer (PCa) is the most commonly diagnosed male cancer in the western world^[1, 2]. With an estimated 22,800 newly diagnosed men in the US in 2015, PCa is a major cause of cancer morbidity and mortality^[3]. Although there has been progress made in the field, several areas of critical unmet needs remain in PCa, such as the molecular mechanisms underlying its development and progression^[1, 2, 4-6]. The complexity of the disease lies in its heterogeneity^[1, 2, 7]. The current tool for screening for PCa, prostate specific

antigen (PSA), is highly controversial as it is not PCa-specific and has a high false positive rate^[8, 9]. This poor correlation leads to adverse consequences associated with unnecessary diagnosis and overtreatment of non-aggressive PCa^[8-10]. Currently, there are no biomarkers available that are effective for the early detection of PCa and sensitive enough to discriminate between indolent and aggressive PCa^[1, 11, 12]. Therefore, the search continues for improved and reliable biomarkers which offer specificity, and improve risk

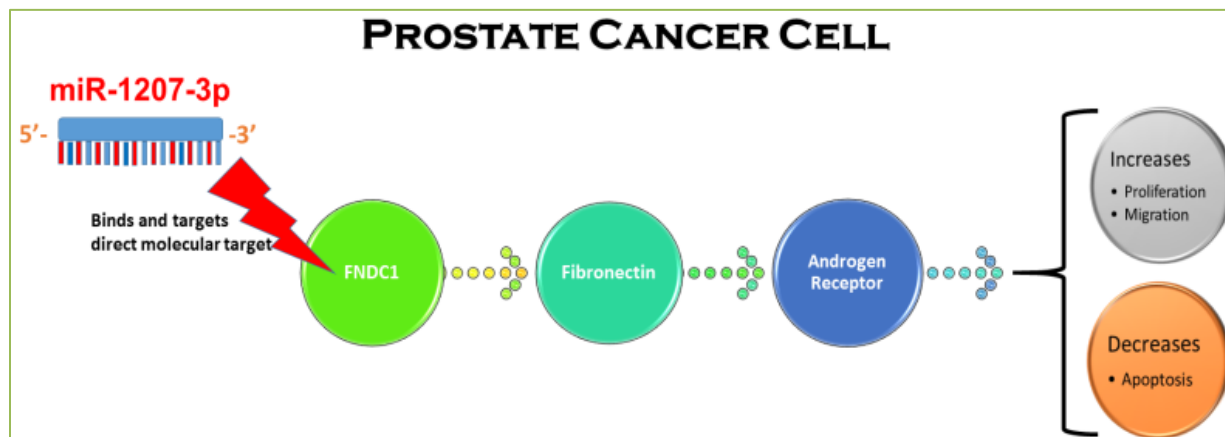


Figure 1. A novel miR-1207-3p/FNDC1/FN1/AR regulatory pathway in prostate cancer. MicroRNA-1207-3p inhibits proliferation and migration and induces apoptosis of prostate cancer cells by the direct molecular targeting of the 3'UTR of FNDC1. miR-1207-3p induced loss of FNDC1 expression leads to the sequential loss of FN1 and AR expression.

stratification and clinical outcomes for patients.

Progressively increasing data have established an important role for microRNAs (miRNAs) in human cancers [13-16]. miRNAs are small single-stranded non-coding RNAs, which play a vital role in post-transcriptional gene regulation [16]. With their locations typically in regions of the genome that are overexpressed, deleted or epigenetically modified, their regulatory effects are extensive [17]. Understanding their roles in the pathogenesis of cancer such as their differential expression in the different stages of cancer may inform their potential use as prognostic biomarkers or as possible therapeutic targets. Studies have implicated various roles of miRNAs in PCa [13, 18-20]. However, much work is still required to elucidate which specific miRNAs directly promote or suppress PCa and what their mechanisms of regulation and action are.

It is now well known that the 8q24 human chromosomal locus has profound genomic instability in PCa [2, 21-24]. Several studies, including one published recently from our lab, "PVT1 exon 9: a potential biomarker of aggressive prostate cancer?" Int. J. Environ. Res. Public Health 2016, 13(1)12, have shown that the PVT1 gene locus, located on the 8q24 chromosomal locus, is dysregulated in PCa [21]. PVT1 has at least 12 different exons and encodes six microRNAs, including microRNA-1207-3p (miR-1207-3p) [1, 5, 21, 24]. However, to the best of our knowledge, no study had been previously performed to determine the role and biological functions for miR-1207-3p in PCa. Our study, recently published in Experimental Cell Research Volume 348, Issue 2, Pages 190–200, is the first to determine the role of miR-1207-3p in PCa.

To investigate the expression profile, the functional role, and the molecular mechanisms of action of miR-1207-3p in

PCa, a panel of 8 prostate cell lines modeling different clinical characteristics of PCa was used because of the widely known heterogeneity of PCa [1]. The panel of cell lines included the RWPE-1 (non-tumorigenic prostate epithelial cell line, Caucasian male (CM)), WPE1-NA22 (derived from RWPE-1, indolent, androgen-dependent, CM), MDA PCa 2b (aggressive, androgen dependent, from a male of African ancestry (moAA)), PC-3 (aggressive, androgen-independent, CM), E006AA (indolent, androgen-independent, moAA), E006AA-hT (derived from E006AA, aggressive, androgen-independent, moAA), LNCaP (aggressive, androgen-dependent, CM) and C4-2b (derived from LNCaP, aggressive, androgen-independent, CM)[1]. In this study, we discovered that miR-1207-3p was significantly underexpressed in all the human PCa cell lines in comparison to RWPE-1, by real-time quantitative polymerase chain reaction analysis [1]. We observed approximately a 50% decrease in miR-1207-3p expression in androgen-dependent PCa cell lines, and a further reduction, nearly 80%, in androgen-independent cell lines [1]. The data suggest that miR-1207-3p underexpression may be associated with the onset and progression of PCa and could be used as a new predictive biomarker for development of androgen-independent PCa.

To explore the molecular mechanisms through which miR-1207-3p exerts its PCa-inhibitory effects, the molecular targets of miR-1207-3p have to be identified. No previous studies had ever shed light on the direct molecular targets of miR-1207-3p in any system. Using two miRNA molecular target prediction algorithms, we identified fibronectin type III domain containing 1 (FNDC1) as a putative molecular target of miR-1207-3p. FNDC1 contains the conserved 'fibronectin type III domain' of fibronectin (FN1) [1, 25, 26]. FN1 is a known regulator of tumorigenesis with roles in cellular proliferation, migration and apoptosis [1, 27-31]. Many

of the FN1 domains (type I, type II and type III) have been found in many other molecules due to exon shuffling^[1, 25, 26]. The biological function of FNDC1 has not been well studied. The FNDC1 gene is located on the 6q25.3 human chromosomal region^[26]. The limited data available on FNDC1 show that FNDC1 was formally known as Activator of G protein signaling 8 (AGS8) and it has been previously observed to be expressed in different tissue types including fetal cartilage, thyroid gland, heart, kidney and adipose tissue^[26, 32-34]. In addition, FNDC1 may have a role in inflammation and hypoxia-induced apoptosis of cardiomyocytes^[32, 33]. However, there had been no reported functional role for FNDC1 in PCa. We investigated the association between miR-1207-3p and its potential target, FNDC1. We observed that FNDC1 protein expression was consistently higher in all the PCa cell lines compared to RWPE1, and overexpression of miR-1207-3p significantly inhibits FNDC1 protein expression^[1]. In addition, dual-luciferase reporter assay and subsequent RNA pull-down assay confirmed, for the first time, that FNDC1 is a direct molecular target of miR-1207-3p^[1]. Furthermore, our custom-designed FNDC1 siRNA confirmed that the effects of loss of FNDC1 expression positively correlates to the effects of overexpression of miR-1207-3p on PCa cellular functions^[1]. Consequently, our study is the first to demonstrate that FNDC1 is directly targeted by miR-1207-3p and associated with PCa.

To determine if a relationship exists between miR-1207-3p and FN1, we also analyzed mRNA and protein expression of FN1. Our data revealed that compared to the non-tumorigenic RWPE-1 cell line, FN1 mRNA and protein expression was significantly higher in all the PCa cell lines^[1]. Moreover, overexpression of miR-1207-3p downregulates FN1. As expected, knockdown of FN1 by our custom-designed FN1 siRNA demonstrated similar effects on PCa cellular functions to that observed by the overexpression of miR-1207-3p^[1]. These findings demonstrate that FN1 is a component of the molecular pathway regulated by miR-1207-3p in PCa.

It is well established that aberrant AR expression and activity play a critical role in the development and progression of PCa^[6, 35-37]. And given our observation that miR-1207-3p expression was less in androgen-independent PCa cell lines than androgen-dependent PCa cell lines, we wanted to investigate if miR-1207-3p regulates AR in PCa. Inverse trends between AR protein expression and miR-1207-3p were observed. AR protein expression, similar to FN1 and FNDC1 protein expression, is overexpressed in PCa cell lines^[1]. In addition, we demonstrated that the molecular effects of miR-1207-3p: loss of FNDC1 expression and consequent loss of FN1 expression leads to

loss of AR expression^[1]. Therefore, we demonstrated that miR-1207-3p regulates AR expression via FNDC1 and FN1. Thus, our work published in *Experimental Cell Research* Volume 348, Issue 2, Pages 190–200, is the first description of the miR-1207-3p/FNDC1/FN1/AR regulatory pathway in PCa^[1].

To investigate the role of miR-1207-3p in proliferation, migration and apoptosis of PCa cells, dose response experiments were initially performed which determined that a 50nM concentration of a miR-1207-3p inhibitor and a miR-1207-3p mimic showed maximal specific effect on miR-1207-3p expression. And this concentration was used to make the novel discovery that increased expression of microRNA-1207-3p in PCa cells significantly inhibits migration, and proliferation, and induces apoptosis via direct molecular targeting of FNDC1^[1]. These functional experiments clearly demonstrate a mechanism by which miR-1207-3p regulates key cellular processes dysregulated in the development and progression of PCa.

Our novel pathway has important clinical implications as miRNAs possess several key features that make them attractive prostate cancer biomarkers^[13, 14, 38]. Therefore, we investigated the clinical relevance of miR-1207-3p in human PCa patients. Two independent publicly available gene expression data sets that had been deposited in the OncoPrint database were analyzed^[39, 40]. Our analysis of the OncoPrint database demonstrated convincing novel evidence of concurrent overexpression of FNDC1, FN1, and AR in the most clinically significant PCa, metastatic PCa, in comparison to primary tumors in both data sets^[1]. In another independent study that our team conducted at the Moffitt Cancer Center, we discovered that FN1 overexpression in prostate tumors positively correlated with overall death and PCa-specific death^[1]. To our knowledge this is the first description of FN1 as a prognostic biomarker in human clinical PCa. Thus, we can conclude that miR-1207-3p correlates with tumor aggressiveness. Further, we independently investigated prostate cancer RNA-seq datasets deposited at the Array Express archive of the European Bioinformatics Institute (EBI). The RNA-seq dataset was analyzed in the RNA-sequencing pipeline implemented on the bioinformatics web platform, Galaxy. The data revealed that a subset of prostate cancers demonstrated concurrent increased FN1 and AR expression in PCa samples as compared to normal prostate samples^[1]. Based on these findings, we strongly believe our miRNA-1207-3p is a promising candidate biomarker for aggressive prostate cancer and further research is being pursued.

In summary, as shown in Figure 1, the findings of our work collectively describe a miR-1207-3p/FNDC1/FN1/AR

novel regulatory pathway in PCa, for the first time. Overall, we identified that miR-1207-3p is significantly underexpressed in PCa cells, and demonstrated that miR-1207-3p is a vital regulator of cellular proliferation, migration and apoptosis of PCa cells [1]. Notably, convincing clinical data was discovered indicating that this novel molecular pathway may be an important predictor of cancer recurrence and metastasis in PCa [1]. Consequently, miR-1207-3p may have potential diagnostic, prognostic, and therapeutic applications in PCa.

Conflicting interests

The authors have declared that no conflict of interests exist.

Acknowledgements

Work in Dr. Ogunwobi's laboratory is supported by the NIMHD/NIH grant to Hunter College: 8 G 12 MD007599.

Author contributions

O.O. conceived the experiments, D.D. conducted the experiments, and O.O. and D.D. analyzed the results.

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