ORIGINAL ARTICLE

YY1-miR-146a-YY1 Regulatory Circuitry: A Potential Mechanism of Prostate Cancer Formation and Progression

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ABSTRACT

MicroRNA-146a (miR-146a) has been demonstrated as a tumour-suppressor in different malignancies including prostate cancer. As an endogenous small RNA, miR-146a regulates several gene expressions at the post-transcriptional level. Previous studies indicate that miR-146a is highly expressed in normal prostate tissue and significantly down-regulated in prostate cancer tissue and even worse in castration-resistant prostate cancer (CRPC) tissues. Over-expression of miR-146a in prostate cancer cell lines results in a marked reduction of cell proliferation, invasion and tumorigenesis. However, the regulating mechanism of miR-146a expression in the different stages of prostate cancer remains unclear. Yin Yang 1 (YY1), a critical regulator in prostate cancer development and progression, is predicted to be a direct target of miR-146a and binds to the promoter region of miR-146a, which is partly confirmed by the inverse expressions of miR-146a and YY1 in prostate cancer. Therefore, we hypothesize that YY1-miR-146a-YY1 regulatory circuitry contributes to prostate cancer and may serve as a future intervention target.

Keywords: MicroRNA-146a (miR-146a), prostate cancer, regulatory circuitry, Yin Yang 1 (YY1)

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INTRODUCTION

Prostate cancer is a common cause of cancer mortality and one of the most frequently diagnosed malignancies in males. In 2012, about 238 590 new patients were diagnosed with prostate cancer and 29 720 deaths were attributed to the disease in the United States of America [USA] (1). In the early stage of prostate cancer, patients are often sensitive to androgen ablation therapy; however, most often, androgen-dependent prostate cancer (ADPC) inevitably progresses to an androgen independent stage [castration-resistant prostate cancer (CRPC)] (2). Patients with CRPC often have a very poor prognosis since no curative treatment is currently available. It has been a major clinical challenge for doctors to reverse the transformation from ADPC to CRPC.

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MicroRNAs (miRNAs) are a class of small noncoding regulatory RNAs (about 19–25 nucleotides) which exert their function by binding to the 3′-untranslated region (3'-UTR) of a subset of mRNAs resulting in their degradation or repression of translation. Recent estimates suggest that one-third of human mRNAs may be regulated by miRNAs (3). During the progression of prostate cancer, several miRNAs and their targets have been discovered to express in an aberrant manner, which leads to the development, invasion and metastasis of this disease (4). Moreover, the aberrant expression of miRNAs has been found useful as biomarkers for diagnosis as well as prognosis of prostate cancer.

Many recent studies have suggested a role for miR-146a in the development and maintenance of neoplastic processes as papillary thyroid carcinoma, pancreatic carcinoma, gastric cancer, breast cancer and prostate cancer. Lin *et al* (5) performed an miRNA array exhibiting a differential expression in CRPC as compared to ADPC cell lines, and found that miR-146a was one of the eight miRNAs repressed in CRPC cell lines. Further *in situ* hybridization analysis of miR-146a expression in prostate cancer tissues demonstrated that this miRNA is highly expressed in normal prostate tissue and significantly down-regulated in prostate cancer tissue and even worse in CRPC tissues. Over-expression of miR-146a in prostate cancer cell lines resulted in a marked reduc-

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tion of cell proliferation, invasion and metastasis to bone marrow through ROCK1. In our previous study (6), we found that miR-146a expression was significantly decreased in CRPC tissues compared to ADPC tissues. Functional analyses showed that ectopic over-expression of miR-146a in CRPC cell lines not only inhibited cell growth, colony formation, and migration *in vitro*, but also reduced tumorigenicity and angiogenesis *in vivo*. Mechanistic studies revealed that miR-146a repressed the expression of epidermal growth factor receptor (EGFR) through binding to the 3'-UTR in a dual phosphorylated extracellular signal-regulated kinase (p-ERK) dependent manner. The consistent evidence showed that miR-146a might act as a tumour suppressor in prostate cancer progression from ADPC to CRPC through a complicated regulating network. However, the regulating mechanism of miR-146a expression in different stages of prostate cancer remains unclear.

HYPOTHESIS

The microRNA miR-146a impacts CRPC cell survival, thus it must be critically involved in CRPC formation and progression. Moreover, another important regulator in prostate cancer named Yin Yang 1 [YY1] (7, 8) is found not only as a direct target of miR-146a but also as a regulatory factor binding upstream of pri-miR-146a gene through bioinformatic analyses (9, 10). Based on these findings, we suppose that a YY1-miR-146a-YY1 regulatory circuitry may contribute to CRPC.

EVIDENCE AND DISCUSSION

MicroRNAs are a class of small, non-coding, single-stranded RNAs that negatively regulate gene expression by mainly binding to the 3'-UTR region of target mRNAs at the post-transcriptional level. MicroRNAs are required for many biologic processes, including cancer formation and progression. Recent work has demonstrated that miRNAs themselves were regulated by transcriptional factors directly binding to the sequence upstream pri-miRNA. For example, miR-221/222, two closely related miRNAs encoded in cluster from a genomic region on chromosome X, were strongly upregulated in several forms of human tumours. It was identified that two separate distal regions upstream of miR-221/222 promoter were directly bound by the NF-kB subunit p65 and c-jun and both drove efficient transcription. Moreover, either the site-directed mutagenesis disrupting p65 binding sites or the ectopical inhibition of NF-kB activity significantly reduced transcriptional activity (11). Schiffgen and colleagues also found that the underlying mechanisms of microRNA deregulation in cancer cells include epigenetic modifications, which play a crucial role in carcinogenesis (12). They demonstrated that numerous miRNAs are induced in renal cell carcinoma cell lines after treatment with inhibitors of the DNA-methyltransferase (5-aza-2'-deoxycytidine) and the histone-deacetylase (suberoylanilide hydroxamic acid). Moreover, the enrichment of H3 and H3K18 acetylation at the miR-9 promoter led to re-expression of miRNA, while DNA hypermethylation remains unchanged (12).

Human miR-146a resides in the LOC285628 gene on human chromosome 5. The LOC285628 transcript contains no significant open reading frame (ORF), implying that it probably belongs to a class of noncoding RNAs. There are two-exon structures of the miR-146a primary transcript (pri-miR146a), and the mature miR-146a sequence situated in the second exon. We performed a bioinformatic search for transcription factor binding sites (TFSearch software, http://www.cbrc.jp/research/db/TFSEARCH.html) in the human genomic sequence (20kbp) upstream of miR-146a transcriptional unit and identified predicted six YY1 binding sites embedded in two regions highly conserved in mammals, as evidenced from Human Mar. 2006 (NCBI36/hg18) assembly UCSC Genome Browser [http://genome.ucsc.edu/] (Fig.1).

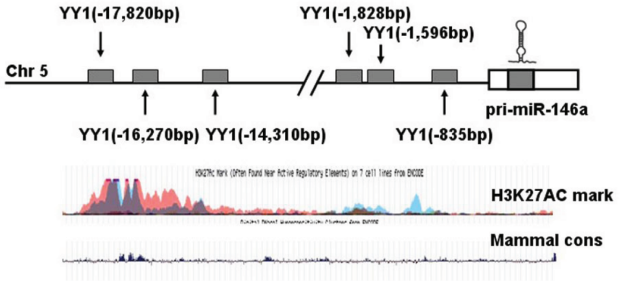

Fig. 1: The diagram illustrates the position of Yin Yang 1 (YY1) binding sites in miR-146a promoter.

Yin Yang 1 was discovered as a transcription factor and its transcriptional activity can be a repressor or an activator in different situations. As a ubiquitously expressed transcription factor, YY1 has attracted the interest of researchers not only because of its essential role in normal cell growth, but also due to its aberrant expression and potential regulatory function in different cancers. Yin Yang 1 has been reported to physically interact with a number of proteins regulating cell proliferation and apoptosis, such as p53, Mdm2, Ezh2, Rb, caspases and HDACs (13), indicating that YY1 works not only as a transcriptional factor but also as a critical joint of epigenetic modification. Therefore, as a multifunctional mediator of different signalling pathways, YY1 potentially acts as a critical regulator in cancer development and progression and likely plays a proliferative or oncogenic role in these processes.

Yin Yang 1 overexpression has been detected in prostate cancer, and YY1 expression was found to be correlated with malignant histological phenotypes (14). In mechanism research, YY1 inhibited prostate cancer Fas-induced and TRAIL-induced apoptosis through suppression of Fas and the death receptor 5 [DR5] (14). Moreover, YY1 is shown to be a coactivator of androgen receptor in promoting prostate-specific antigen (PSA) transcription, especially in androgen-deprived circumstances, which indicates that YY1 potentially

regulates prostate cancer development and progression through stimulating androgen receptor function. The aberrantly activated androgen receptor-signalling pathway in prostate cancer directly leads to CRPC transformation, which confirms that YY1 is associated with prostate cancer progression. The expression of YY1 has been reported to be controlled by a series of miRNAs like miR-7 (16), miR-29 (17), miR-34a (18) *et al*. Of interest, YY1 is also predicted to be the direct target of miR-146a in a bioinformatic miRNA target software [Fig. 2] (10), which is partly confirmed by the inverse expressions of miR-146a and YY1 in prostate cancer (5, 6).

Fig. 2: Predicted target site of miR-146a in the 3'-untranslated region (3'-UTR) of Yin Yang 1 (YY1).

CONCLUSION

We present strong evidence indicating that both YY1 and miR-146a are involved in prostate cancer formation and progression though several means. Yin Yang 1 is over-expressed and miR-146a is found down-regulated in prostate cancer. Moreover, in bioinformatic analyses, YY1 is not only a direct target of miR-146a but also binds to the promoter region of miR-146a, which confirms the hypotheses that YY1-miR-146a-YY1 regulatory circuitry contributes to prostate cancer and may serve as a future intervention target.

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