Gene Reports 4 (2016) 70–73

Contents lists available at ScienceDirect

Gene Reports

journal homepage: www.elsevier.com/locate/genrep

Methylation dependent miR-510 in prostate cancer: A novel upcoming candidate for prostate cancer

Durairaj Sekar [a,b,*], Venugopal Basam [b], Ramalingam Krishnan [a], Panagal Mani [c], Pethanen Sivakumar [d], Vincent Gopinath [d]

[a] Narayana Medical College and Hospital, Chinthareddy palem, Nellore-524002, India
[b] Stem Cell Division, Cryovault Biotech India Pvt Ltd, Bangalore-560018, India
[c] Department of Biotechnology, Annai College of Arts and Science, Kumbakonam, Tamil Nadu, India
[d] Department of Zoology, Arumugam Pillai Seethai Ammal College, Tiruppattur, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 26 December 2015
Received in revised form 29 March 2016
Accepted 6 April 2016
Available online 23 April 2016

Keywords:
Prostate cancer
miRNAs
miR-510
Epigenetics
Hypermethylation

ABSTRACT

Over the years, considerable progress has been achieved in the management of androgen sensitive prostate cancer. However, inadequacies in the early diagnosis and treatment of prostate cancer remain, resulting in poor treatment and ineffective control of metastatic progression. MicroRNAs (miRNAs) are well known regulatory factor for the cellular and developmental processes and it has been revealed that many miRNAs contribute the initiation and progression of various cancers including prostate cancer. In the present study we have analyzed the expression and methylation status of miR-510 in normal prostate cell line (PNT1A and HPrEC), primary prostate cancer cell line (LNCaP and VCaP), advanced prostate cancer cell lines (DU145 and MDA PCa 2b) as well as in human prostate cancer biopsies and benign prostatic hyperplasia (BPH) samples. Our studies revealed that miR-510 was up-regulated in metastatic form of prostate cancer cell lines (DU145 and MDA PCa 2b) and in human prostate cancer biopsies but no significant changes were noticed in miR-510 expression in other cell lines (LNCaP, VCaP and PNT1A) as well as in BPH samples. At the same time, miR-510 promoter region was found to be methylated in LNCaP and PNT1A cell lines, but in case of DU145, PC-3 cell lines and prostate cancer biopsies, the miR-510 promoter region was found to be unmethylated. Thus the miR-510 may serve as a novel candidate for the diagnosis and as a new therapeutic target for prostate cancer treatment.

1. Introduction

Prostate cancer (PCa) is a most frequent solid cancer in men, representing one of the most frequent causes of deaths in the world, and is now budding in developing countries as well (Jemal et al., 2011). Clinical and pathological diagnosis of PCa is usually obtained by prostate specific antigen (PSA) test, biopsy and Gleason scoring of the tissue samples (Humphrey, 2004). The PSA in blood was widely used in various stages of prostate cancer management, including diagnosis and the appraisal of future risk for prostate cancer development, the PSA also used in the detection of frequency of the disease after local therapy and in the management of advanced stages of the disease but it has a number of significant shortcomings (Pezaro et al., 2014). The majority of PCa is dependent on the hormones, androgens for the initial growth and are successfully treated by androgen ablation therapy, however, in most cases the tumor in due course progresses to an androgen independent phenotype (Feldman and Feldman, 2001). Clinical management of PCa needs novel approaches to correctly assess, monitor its progression and predict its outcome. Even though these tumors are insensitive to hormone withdrawal therapy, they continue to express the androgen receptor (AR), and androgen-regulated genes such as PSA, indicating that the AR pathway is dynamic. Androgen independent PCa tends to progress and metastasize, and patient has a low survival rate. There is currently no compromise on therapy for such tumors (Ghosh et al., 2005). At present androgen ablation therapy, surgery and radiation therapy are effective for the treatment of primary or local PCa but not for the metastatic PCa.

MicroRNAs (miRNAs) are considered to be major regulatory molecules known to control a wide range of biological functions such as cellular proliferation, differentiation and apoptosis (Sekar et al., 2016, 2015, 2014; Saravanan et al., 2015). Recent reports showed strong evidence that miRNAs can either act as oncogenes or tumor suppressors, having key roles in cancer initiation and progression (Cho, 2007; Tili et al., 2007). It has been suggested that miRNAs also behaves as circulating microRNAs for the treatment of major diseases like Acute

Abbreviations: miRNAs, MicroRNAs; BPH, Benign prostatic hyperplasis; PCa, Prostate cancer; PSA, Prostate specific antigen; AR, Androgen receptor; PRDX1, Peroxiredoxin 1; q RT-PCR, Quantitative real time PCR; M, Methylated; U, Unmethylated.

* Corresponding author at: Advance Research Centre, Narayana Medical College and Hospital, Cinnthareddy Palem, Nellore, India. Tel.: +91 9791294770.
E-mail address: duraimku@gmail.com (D. Sekar).

http://dx.doi.org/10.1016/j.genrep.2016.04.005
2452-0144/

Myocardial Infarction (Wang et al., 2010) and miRNAs are being reported in body fluids, such as serum, plasma, and urine, and can be readily used as non-invasive biomarkers of PCa as a novel diagnostic and prognostic tools (Srivastava et al., 2011). It acts as an important post transcriptional regulator of gene expression in many types of cancer and their up and down regulations occurs commonly in prostate cancer.

It has been known that miR-510 has been demonstrated to be involved in a number of types of malignancy and downregulated miR-510 functioned as a tumor suppressor by reducing cellular proliferation and migration, and inducing apoptosis in renal cell carcinoma (Chen et al., 2015). Interestingly, another study revealed that miR-510 directly binds to the 3′UTR of peroxiredoxin 1 (PRDX1) and blocks its protein expression, thereby suppressing migration of human breast cancer cells (Guo et al., 2013). Few studies have been carried out for miR-510 in the context of cancer, but there is no evidence that proves miR-510 is involved in prostate tumor formation. miRNA repression via methylation of CpG island in its promoter region may be an important mechanism in carcinogenesis (Formosa et al., 2013). Although DNA methylation constitutes an important mechanism for microRNA upregulation in cancer, this field largely remains unknown. The present study is focused mainly on epigenetic regulations and the gene expression status of miR-510 in PCa, which could be useful to unravel molecular mechanism of miR-510 in tumor formation and aggressiveness. Based on the above information the present study was planned to understand the mechanism of miR-510 in human PCa.

2. Materials and methods

2.1. Cell culture and tissue sample collection

This study examined the expression of mature miR-510 in human prostate cancer cell lines (androgen sensitive—LNCaP and VCaP; androgen insensitive—DU145, PC-3 and MDA PCa 2b) and normal prostate epithelium cell line (PNT1A, HPrEC). The above cell lines were received from NCCS, Pune, India and grown at 37 °C with ATCC recommended media (DMEM) supplemented with 10% fetal calf serum. A total of 10 genetically unrelated (age group between 45 and 70 years) prostate cancer samples and 10 benign prostatic hyperplasia (BPH) samples were both collected from 2014 to 2015 at Narayana Medical College and Hospital, Nellore, India. None of the patients received androgen deprivation treatment, chemotherapy or radiational therapy prior to radical prostactomy. All the samples obtained through transurethreal resection of the prostate (TURP) and the following biochemical and pathological parameters are checked for prostate cancer and BPH patients: Prostate Specific Antigen (PSA), Gleason Score and the pathological stages. Data collected from each subject as clinical variables including age, height, weight, body mass index, and family history and this study was approved by Institutional ethical committee (IEC).

2.2. Quantitative real time PCR (q PCR)

Total RNA were extracted using RNeasy Mini Kit (Qiagen) and the expression pattern of the miR-510 was analyzed as mentioned by Ingrid Balcells et al. (2011), where the first step is adding poly (A) tail to the total miRNAs followed by reverse transcription with a specific microRNAs tagged poly (T) adaptors. The overall reactions were initiated using the reaction mixer consisting of 100 ng of total isolated RNA, 10× poly (A) polymerase buffer, 0.1 mM of ATP, 1 μM of reverse transcription poly (T) primer (adaptors), 0.1 mM of dNTPs mix, 100 units of MuLV reverse transcriptase and 1 unit of poly(A) polymerase (New England Biolabs) were immediately incubated at 42 °C for 1 h followed by 95 °C for 5 min for enzyme inactivation. Quantification of microRNA was performed by qPCR using the following primers Forward: 5-AGTA TGGCCCGGCCGTGA-3 Reverse: 5-AGGTCCATTTTTTTTTTTTTTCCT-3. miR-510 levels were standardized using 18sRNA levels using the $2^{\Delta\Delta Ct}$ model. Quantification of PRDX1 was performed by qPCR using the following primers Forward 5 ATTCTCTACCAAATTGCACA-3 and Reverse 5-TACGAGATATCCACATCAAA 3. All the data were normalized to GAPDH using the primer sequences Forward 5-AGCCACATCGCTCAGA CAC-3 and Reverse 5′-GCCCAATACGACCAAATCC-3.

2.3. Determination of CpG island methylation

The epigenetic regulation of miR-510 was studied using 5′-Aza-dC treatment, bisulfite conversion and methylation analysis as described by Mudduluru and Allgayer (2008). CpG islands upstream to the transcription start site of pri-miR-510 were determined with the CpG island searcher (http://www.uscnorris.com/cpgislands2/ cpg.aspx) and PCR primers were designed using the Methprimer software (http://www.urogene.org/methprimer). Primer sequences for mir-510 includes Methylated Forward (5′TCGTACGTAGATTTTTTTTGGTATC3′), Methylated Reverse (5′ ATAAATCACACTCCCCACACG′), Unmethylated Forward (TTGTATGTAGATTTTTTTTGGTATT3′) and Unmethylated Reverse (5′ TGTATGTAGATTTTTTTTGGTATTG 3′). Hypermethylation status of miR-510 was analyzed in all above mentioned prostate cancer cell lines and normal prostate cell line using methylation specific PCR. We also evaluated expression and methylation status of miR-510 in tumor samples and BPH samples by using the above methods.

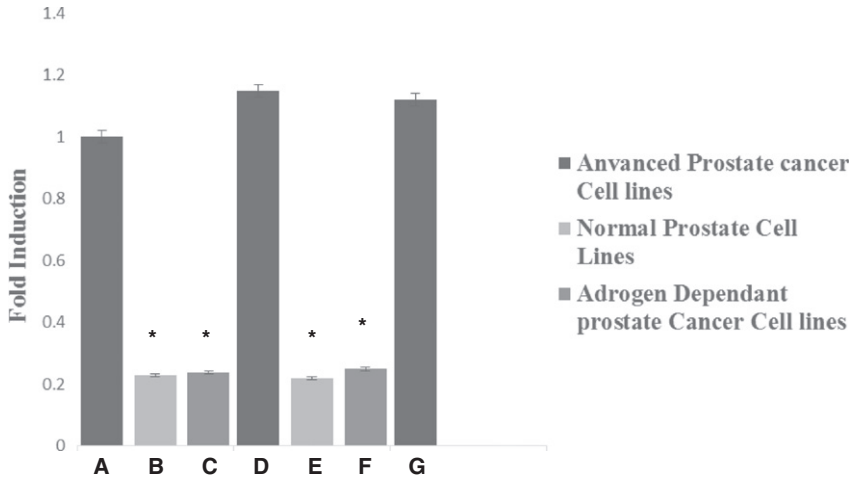

Fig. 1. Shows the expression status of miR-510 in different prostate cancer and normal prostate cell lines by quantitative real time PCR (q RT-PCR). P* value < 0.05 Vs DU145 PC-3 and MDA PCa 2b. Data are expressed as mean values ± SE. A—DU145. B—PNT1A. C—LNCaP. D—MDA PCa 2b. E—HPrEC. F—VCaP. G—PC-3.