

Role of microRNA-7-1-3p in Prostate Cancer Progression

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Abstract

Prostate cancer (PCa) is one of the most common malignant diseases in western world and now emerging as a challenging disease in developing countries. Although much progress has been done to control of androgen sensitive PCa in recent years, the early diagnosis and treatment for Prostate cancer are not yet satisfactory and, thus the metastatic progression control/treatment is still poor. MicroRNAs (miRNAs) are well known regulatory factor of physiological and developmental processes, it has been revealed that many miRNAs contribute the initiation and progression of various cancers. In the present preliminary study, we evaluated the expression status of miR-7-1-3p on normal prostate cell line (PNT1A), androgen sensitive prostate cancer cell line (LNCaP) and androgen insensitive prostate cancer cell lines (DU145). The results of this study revealed that miR-7-1-3p was up-regulated in metastatic form of prostate cancer cell line DU145, but no changes were noticed in miR-7 expression on other cell lines (LNCaP and PNT1A). Thus our preliminary studies suggested that miR-7-1-3p may serve as a novel microRNA for the diagnosis and as a new therapeutic target in prostate cancer.

Keywords: Prostate cancer; miRNAs; miR-7-1-3p; Epigenetics; hypermethylation; qPCR

Introduction

Prostate cancer is the most frequent solid cancer in older men in the Western world, representing one of the most frequent causes of cancer deaths, and is now emerging in developing countries as well [1]. Pathological diagnosis of prostate cancer is usually obtained by prostate-specific antigen (PSA) analysis, biopsy and Gleason pathological scoring of the tissue samples. The PSA blood test has been used in various stages of prostate cancer management, including screening and the assessment of future risk of prostate cancer development, detection of recurrent disease after local therapy and in the management of advanced disease. PSA-based decision-making in prostate cancer has significant shortcomings [2]. The majority of prostate tumours are dependent on androgens for growth in the initial stages, and are effectively treated by androgen-ablation therapy; however, in most cases the tumour eventually progresses to an androgen-independent phenotype [3]. Clinical management of prostate cancer needs novel approaches to correctly assess, monitor its progression and predict its outcome. In spite of being insensitive to hormone-withdrawal therapy, the majority of these tumours continue to express the androgen receptor (AR), and androgen-regulated genes such as PSA, indicating that the AR pathway is active. Androgen-independent prostate cancer (AIPC) tends to progress and metastasize, and has a low survival rate. There is currently no consensus on therapy for such tumours [4]. At present androgen

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ablation therapy, surgery and radiation therapy are effective for the treatment of local prostate cancer but not for the metastatic prostate cancer.

MicroRNAs (miRNAs) are known to control a wide range of biological functions such as cellular proliferation, differentiation and apoptosis [5-7]. Recent reports showed strong evidence that miRNAs can act as oncogenes or tumor suppressors, having key roles in cancer initiation and progression [8, 9]. MicroRNAs are being reported in body fluids, such as serum, plasma, and urine, and can be readily used as non-invasive biomarkers of prostate cancer as novel diagnostic and prognostic tools [10]. 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.

Few studies that have been carried out for miR-7 in the context of cancer but there is no evidence that proves miR-7 involved in prostate tumour formation. MicroRNAs (miRNAs) repression via methylation of CpG Island in its promoter region may be an important mechanism in carcinogenesis [11]. Although DNA methylation constitutes an important mechanism for microRNA upregulation in cancer, this field largely remains unexplored. The present study is to focus the gene expression status of miR-7-1-3p on prostate cancer, which could be useful to unravel molecular mechanism of miR-7-1-3p in tumour formation and its aggressiveness. Based on above information the present preliminary study was planned to understand the role of miR-7-1-3p in human prostate cancer.

Materials and Methods

This study examined the expression of mature miR-7-1-3p in human prostate cancer cell lines (androgen sensitive – LNCaP; androgen insensitive - DU145) and prostate epithelium cell line (PNT1A). 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. Total RNA was extracted using RNeasy Mini Kit (Qiagen) and the expression pattern of the miR-7-1-3p was analysed as described by Balcells et al. [12], where poly (A) tailing of the miRNAs is followed by reverse transcription with a tagged poly (T) primer.

Briefly, the reaction mix (10 µl) consisting of 100 ng of total RNA, 1 µl of 10x poly(A) polymerase buffer, 0.1 mM of ATP, 1 µM of reverse transcription poly(T) primer (adaptors), 0.1 mM of dNTP mix, 100 units of MuLV reverse transcriptase (New England Biolabs) and 1 unit of poly(A) polymerase (New England Biolabs) was incubated at 42°C for 1 hour followed by enzyme inactivation at 95°C for 5 minutes. Quantification of microRNA was performed by PCR using the following primers Forward: 5-AGTATTGCCCCGCCGTGA-3 Reverse: 5-AGGTCCATTTTTTTTTTTTTTTTCCA-3. MiR-7 levels were normalized to 18sRNA levels using the $2^{-\Delta\Delta C_t}$ model.

Results and Discussion

The result of this study revealed that epigenetic modification in genes encoding the miRNA is the main factor aggressiveness of the prostate cancer and the results were discussed in detail. The expression level (Fold Induction) of miR-7 in both the androgen insensitive cell lines (DU145) was higher than the androgen sensitive LNCaP and normal prostate epithelial cell line PNT1A (Figure 1). Differential expression of miR-7-1-3p in aggressive prostate cancer cell lines suggested that the up-regulation of miR-7-1-3p is relevant to the genesis and development of metastatic prostate cancer. The recent investigations also confirmed experimentally that miR-7 involved in cell proliferation, migration, apoptosis, and promote tumour growth [13].

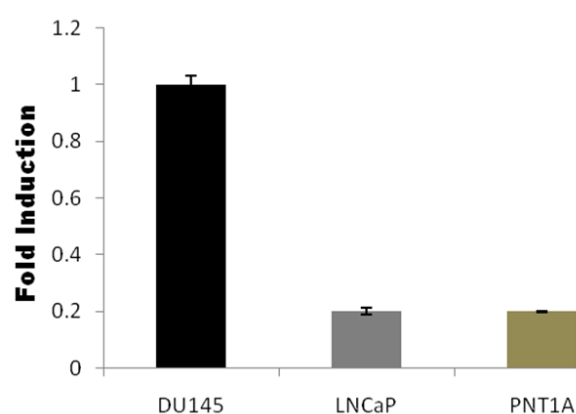


Figure 1: Represents Expression status of miR-7-1-3p in different prostate cancer and normal prostate cell lines quantitative real time PCR (qPCR).

Conclusion

The improvement of early PCa detection and stage migration as well as reduced PCa mortality were not fully achieved and up-to-date PSA represents the gold standard biomarker of PCa diagnosis together with clinical findings. Nonetheless, PSA shows weakness in discriminating between malign and benign prostatic disease or indolent and aggressive cancers [14]. MiRNAs have received increasing attention as targets for cancer therapy, as they can target multiple signaling pathways related to tumor progression, metastasis, invasion, and chemo-resistance. Emerging evidence suggests that aberrant expression of miRNAs can lead to the development of resistant prostate cancers [15]. MicroRNAs (miRNAs) repression via methylation of CpG Island in its promoter region may be an important mechanism in carcinogenesis [11]. Although DNA methylation constitutes an important mechanism for microRNA upregulation in cancer, this field largely remains unexplored. Although few studies reported on miR-7 expression status [13] but the positive correlation of miR-7-1-3p with cancer progression, mechanism behind the phenomenon has not yet elucidated. Further investigations will need to support miR-7-1-3p is a potential biomarker and target for prostate cancer.

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