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Role of MDR1 During Prostate Cancer Development

Satyanarayana Rentala[1,*] and Lakshmi Narasu Mangamoori[2]

[1]Department of Biotechnology (Engg.), GITAM Institute of Technology, GITAM University, Rushikonda, Visakhapatnam, India.
[2]Centre for Biotechnology, Institute of Science and Technology, Jawaharlal Nehru Technological University, Hyderabad, India.
[*]Corresponding Author E-mail: s.rentala@yahoo.com

Abstract

Despite advancements in detection and in treatment of this disease, prostate cancer death rates remain largely unchanged. This is largely due to the heterogeneous biology of prostate cancer, which is characterized by a wide spectrum of clinical behavior ranging from indolent to a rapidly progressing disease. In this manuscript we studied about molecular markers like p53 and MDR1 in prostate cancer patients.

Keywords: RAPD, RT-PCR, Prostate cancer tissues, Multi drug resistance 1 (MDR1) and p53.

Introduction

Over the past 20 years, technological advances in molecular biology have proven invaluable to the understanding of the pathogenesis of human cancer[1-5]. The application of molecular technology to the study of cancer has not only led to advances in tumor diagnosis, but has also provided markers for the assessment of prognosis and disease progression[6-9]. Most of the time, prostate cancer grows slowly. Autopsy studies show that many older men (and even younger men) who died of other diseases also had prostate cancer that never caused a problem during their lives[10-15]. These studies showed that 7 or 8 out of 10 men had prostate cancer by age 80. But neither they nor their doctors even knew they had it. Prostate cancer is one of the most commonly diagnosed cancers in men in United States. Over 234,000 new cases will be diagnosed and over 27,000 men will die from this disease. Screening for the prostate specific antigen (PSA) was initiated in the 1990's and resulted in a sharp

increase of prostate cancer incidence. Since 1992 rates of prostate cancer incidence were slowly declining. Widespread use of PSA screening allows for more frequent detection of early stages of prostate cancer, a period when radical prostatectomy is considered curative. Despite advances in detection and in treatment of this disease, prostate cancer death rates remain largely unchanged since the 1950's. This is largely due to the heterogeneous biology of prostate cancer, which is characterized by a wide spectrum of clinical behavior ranging from indolent to a rapidly progressing disease. The challenge is to differentiate between the subpopulation of patients who may benefit from more aggressive treatment and those who may opt to avoid additional therapy and its side effects. New predictors of disease progression and patients' survival are necessary because the use current predictors of prostate cancer progression such as PSA, Gleason grade and tumor stage are often insufficient to accurately identify more aggressive disease. Early detection and accurate diagnosis are essential to provide the most appropriate treatment course and novel biomarkers are necessary to more accurately predict prostate cancer progression and patient survival.

This manuscript will first review the characteristics of prostate cancer and discuss the importance of tumor vascularization of prostate cancer progression and then explores relationship between p53 and Multi drug resistant (MDR1) genes. All together we elucidated the importance of angiogenesis and tumor vascularity in prostate carcinogenesis and how this process may be affected by therapies providing ground for future investigations.

Materials and Methods

Isolation Genomic DNA from the mammalian tissues using Bangalore Genie kit

The DNA was isolated from the samples using Bangalore Genei kit, as prescribed on the leaflet. The integrity of the DNA was also checked using agarose gel electrophoresis. The same DNA was used to investigate RAPD analysis of MDR1 and p53.

RNA Isolation - cDNA synthesis

RNA was isolated using Trizol reagent. First strand of cDNA is synthesized using an oligo(dT) adapter primer and M-MLV H-reverse transcriptase. After RNA hydrolysis, 2nd strand of cDNA is primed with a specially designed randomized N6 adapter primer. The adapter sequences on both ends of the cDNA are then used to amplify the cDNA with long and accurate PCR. They also allow directional cloning of the cDNA. The Primers for MDR1 are Forward 5'-CCC ATC ATT GCA ATA GCA GG-3' and Reverse 5'-GTT CAA ACT TCT GCT CCT GA-3'.

BLAST

Here we used Nucleotide-nucleotide BLAST (blastn) to study the relationship between MDR1 promoter and p53. This program, given a DNA query, returns the most similar DNA sequences from the DNA database that the user specifies.

Results and Discussion

Integrity of DNA in prostate cancer samples: The DNA integrity was checked using Agarose gel electrophoresis technique. Figure 1 shows the integrity of the DNA in all given prostate cancer samples, which shows isolated DNA is intact throughout the experiments. We used this DNA for other experiments to study about various markers in these prostate cancer samples.

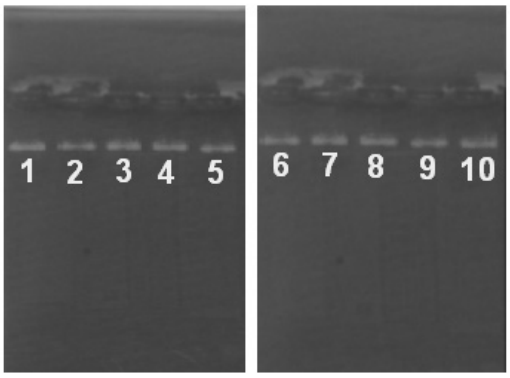

Figure 1: Integrity of DNA in Prostate cancer samples. DNA was isolated from the samples using Bangalore Genie Mammalian genomic DNA isolation kit. The integrity was checked using Agarose gel electrophoresis.

RAPD analysis of p53 in Prostate cancer samples: The isolated genomic DNA from the samples was amplified for p53 using the given primers. Out of 10 samples, 2 samples showed variations in the gene profile for p53. For further analysis we isolated RNA from these samples.

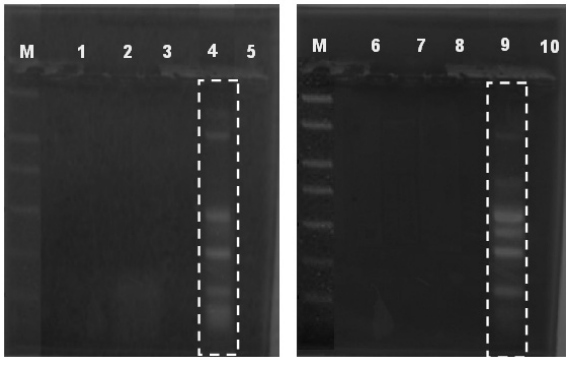

Figure 2: RAPD analysis of p53: Out of 10 samples, two patients showed p53 expression. Surprisingly all other samples did not show any band pattern for p53.

RAPD analysis of MDR1 in Prostate cancer samples: The isolated genomic DNA from the samples was amplified for MDR1 using the given primers. Out of 10

samples, 2 samples showed variations in the gene profile for MDR1. For further analysis we isolated RNA from these samples

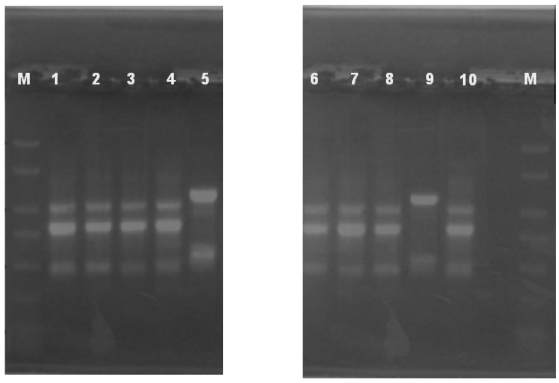

Figure 3: RAPD analysis of MDR1: Out of 10 samples, Two patient samples showed MDR1 expression. Surprisingly all other samples showed similar band pattern for MDR1.

RT-PCR analysis of MDR1: The RNA isolated from the cancer tissues was subjected to cDNA synthesis. This cDNA is subjected to Polymerase chain reaction using MDR1 primers.

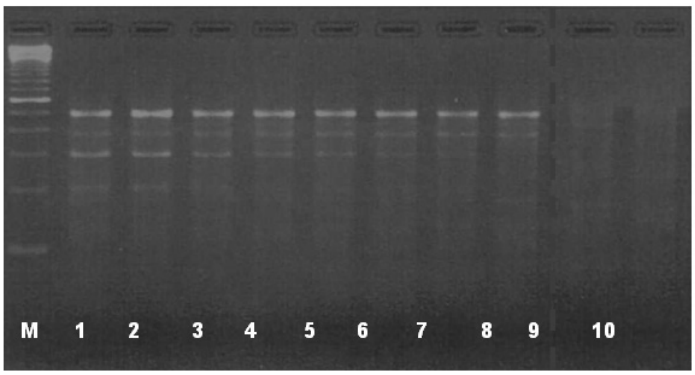

Figure 4: The figure shows the RT-PCR analysis of MDR1.

Conclusion

The RAPD analysis of Genomic DNA from all the prostate cancer tissue s showed that p53 was not expressed in adenocarcinoma patients, but well expressed in early detected or first stage cancer patients (Sagaraiah and A. Ankineedu). Our previous publication suggests that MDR1 expressing bone marrow progenitor cells easily migrate from the bone marrow to the damaged tissues during hemostasis. Except above two patients, in the other samples, for cancer growth, it needs angiogenesis. So

the angiogenesis part is taken by migration of MDR1 expressing cells from bone marrow to the nearest blood vessels of the cancer tissue, and these cells use the angiogenic growth factors like EGF, FGF and VEGF and make angiogenic vessels. An interesting article published in International Journal of Pharmaceuticals Research by *Mahadeo Sukhai g*roup , showed us a way to conclude that, p53 negatively regulates MDR1 expression. So whenever P53 protein binds to MDR1 promoter it stops MDR1 expression. Since in case of the other patient samples the p53 is highly mutated and the p53 protein is absent at MDR1 promoter binding site, so the MDR1 is concurrently expressed its protein ABCP1, a protein which effluxes all the drug molecules given to the patient. But two patient samples. whose tumour was detected at initial stage so we can see p53 in considerable amounts. Hence they lost MDR1 at RNA-cDNA level. To treat cancer at this level, we need to design new drugs for stopping this MDR1+ cells migration towards cancer tissues. We also need to develop the new diagnostic kits based on MDR1 expression in these cancerous tissues.

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