

Full Length Research paper

Molecular characterization of fusion transcripts in prostate cancer patients: A tool having diagnostic implications in Pakistan

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Accepted 15 March, 2011

Substantial fraction of information has been added with reference to fusion transcripts in hematological malignancies. However these fusion transcripts have been characterized in epithelial cells of prostate and are documented to gear up the process of neoplasia. TMPRSS2-ERG and related transcripts are key offenders in the subversion of core biological systems driving oncogenic transformation. In an RT PCR based study, 90 samples of tissue specimen of prostate cancer patients and 20 “prostate cancer negative” tissue specimens from different hospitals of Lahore and Islamabad were analyzed. There was a prevalence of atleast one isoform in 65 patients. There is no pre-existing accurate and precise assay to determine or evaluate the aggressiveness of prostate cancer in Pakistan. This study underscores that prostate cancer tissue specimen harbor hormonally regulated TMPRSS2-ERG and NDRG1-ERG fusions. This study represents a different approach in the clinical management of prostate cancer in Pakistan. It has to be included in diagnosis and prognosis to improve the treatment of prostate cancer. It seems pragmatic that PSA based evaluations do not guarantee a proper and scrutinized analysis of prostate cancer patients.

Key words: Fusion transcript, prostate cancer, gene, RNA.

INTRODUCTION

Prostate cancer is a serious molecular anomaly that arises because of versatile regulators that play a dominant role in disease exacerbation. Since the discovery of the first fusion transcript, documented by Tomlins et al., there is an overwhelming number of the chimeric transcripts that have been characterized and continuously being added into the list uninterruptedly [Tomlins et al., 2005]. TMPRSS2 and ERG gene reside about 3 megabases apart on chromosome 21. The most recurrent and widespread fusion is between 5' untranslated regions of TMPRSS2 and 3' ERG [Yoshimoto et al., 2006; Joost et al., 2009]. Solute carrier family 45, member 3 (SLC45A3), also referred to as prostestin, is a prostate-specific, androgen-regulated gene that has been shown to be a 5' partner.

It has recently documented that these chimeric transcripts have heterogeneous expression moreover it is

robustly stimulated in prostate associated molecular anomalies [Rickman et al., 2009]. Another key player of family of chimeric genes is NDRG. These family members with dual biological actions have been characterized in prostate cancer. NDRG1 is involved in cellular differentiation and is typically downregulated in prostate cancer cells [Mostaghel et al., 2007]. In accordance with the concept of NDRG1 overexpression resulted in a reduction of metastatic potential. Contrarily, NDRG3 of the same family is an oncogene that is actively expressed in prostate cancer cell line [Wang et al., 2009]. It is interesting to note that hormone-induced overexpression of NDRG1-ERG fusion leads to an increased risk of metastasis due to the disruption of NDRG1 [Pflueger et al., 2009].

We have documented some fusion transcripts individual or in co-occurrence in prostate cancer patients. These findings have broader implications for the fusion transcript accurate detection. Currently molecular diagnosis is unavoidable for defining the molecular and pathological linkage of the fusion in tumor specimens.

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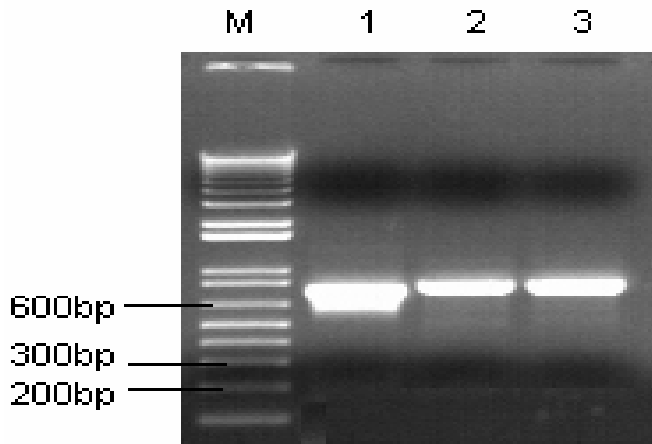


Figure 1. Displays the fusion Transcript NDRG-ERG in lanes 1, 2 and 3 (Left to Right). Amplicon size is of 677 bp.

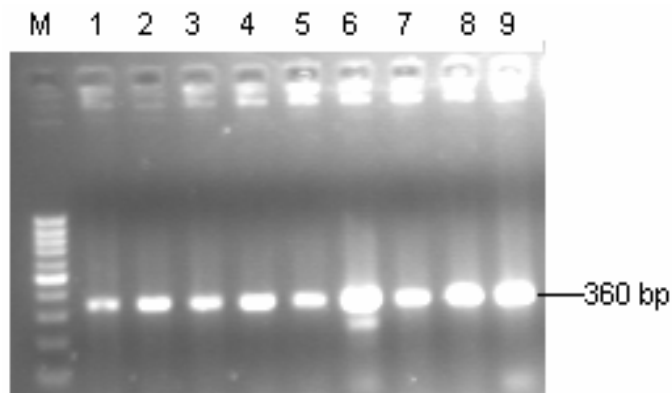


Figure 2. Shows RT based amplification of chimeric RNA transcripts. Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9 are positive for fusion transcripts. Lane 9 shows amplicon from LNCaP cell line. Lane 5 has two chimeric transcripts amplified (TMPRSS2 exon 1 and ERG exon 2) (360 bp).

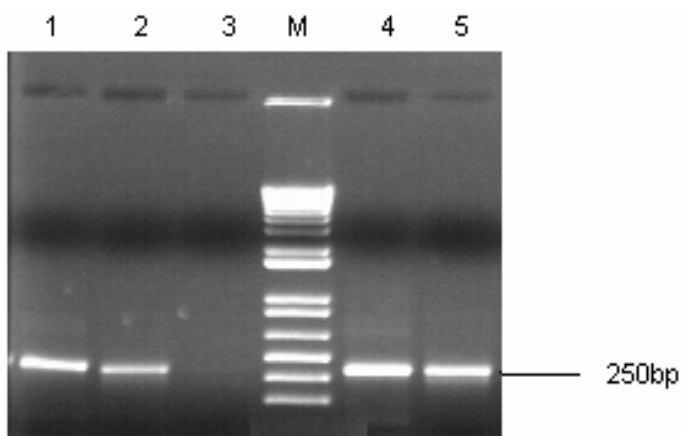


Figure 3. Shows RT based amplification of chimeric RNA transcripts. Lanes 1, 2, 4, 5, indicate variant fusion transcript is present of TMPRSS2 exon 2 and ERG exon 4 (251bp).

These techniques have to be used for new directed therapies targeting the oncogenic fusion.

MATERIALS AND METHODS

Tissue specimen and RNA extraction

90 Prostate tissue samples were taken from prostate cancer patients. For control we used LNCaP, androgen sensitive prostate cancer cell line. Fusion transcripts were generated in the cell line after treatment with androgen and radiation. RNA was extracted using Trizol reagent according to manufacturer's instructions. The amount and quality of RNA were measured by spectrophotometric analysis at 260 nm (The amount of RNA from tissue ranged between 30 and 100 ng).

RT-PCR for detection of fusion transcripts in the tissue specimen

Total RNA after pretreatment with DNase was subjected to reverse transcription with SuperScript II RNase H- Reverse Transcriptase (Invitrogen). The cDNA synthesis was done according to manufacturer's protocol. Primers used for the study were already reported by Hessels et al. (2007). RT PCR based amplifications were done with slight modifications in the protocol documented by [Hessels et al., 2007].

RESULTS AND DISCUSSION

The Figure 1 displays the fusion Transcript NDRG-ERG in lanes 1, 2 and 3 (Left to Right). Amplicon size is of 677 bp. Figure 2 shows RT based amplification of chimeric RNA transcripts. Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9 are positive for fusion transcripts. Lane 9 shows amplicon from LNCaP cell line. Lane 5 has two chimeric transcripts amplified (TMPRSS2 exon 1 and ERG exon 2) (360 bp). Figure 3 shows RT based amplification of chimeric RNA transcripts. Lanes 1, 2, 4, 5, indicate variant fusion transcript is present of TMPRSS2 exon 2 and ERG exon 4 (251bp).

In this particular study we were able to characterize two fusion transcripts (TMPRSS2-ERG and NDRG-ERG) which previously had not been analyzed in prostate cancer patients in Pakistan. Moreover the statistical data of the prevalence of these fusion transcripts in population of Pakistan is still not available. This is the first study which has emerged and addresses the frequency of these chimeric transcripts.

In previous studies, the frequency of TMPRSS2-ERG fusion transcripts, evaluated either by fluorescence in situ hybridization or a RT-PCR-based approach, was 40 to 59% [Demichelis et al., 2007; Cerveira et al., 2006; Perner et al., 2006]. Consistent with the same observation, 29 radical prostatectomy specimens, screened by Hessels et al, 2007 TMPRSS2-ERG fusion transcripts were detected in 59% of the cases. This indicated that 50% of the prostate cancers harbor TMPRSS2-ERG fusion transcripts. In this particular study, the most

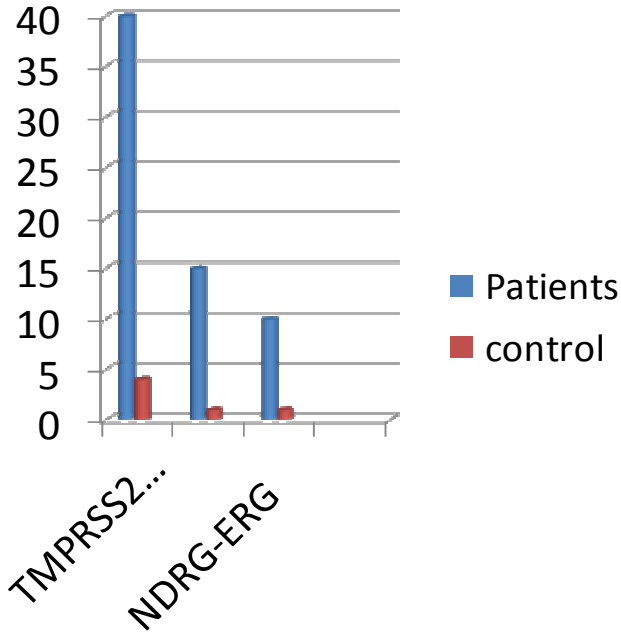


Figure 4. Indicates prevalence of fusion transcripts TMPRSS2-ERG and NDRG-ERG in prostate cancer patients and controls.

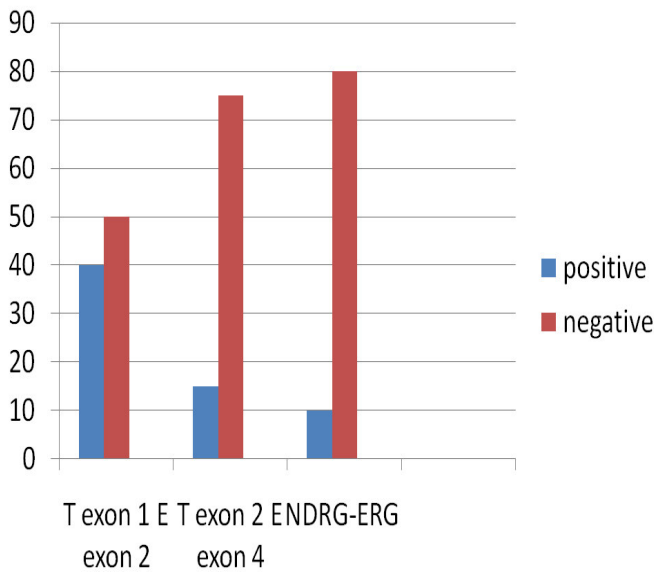


Figure 5. indicates prevalence of fusion transcripts TMPRSS2-ERG and NDRG-ERG in prostate cancer patients suggesting a comparison between patients positive and negative for fusion transcripts.

prevalent fusion transcript is of TMPRSS2-ERG (TMPRSS2 Exon 1 and ERG Exon 2) which was characterized in 40 patients out of 90 as depicted in Figure 4. Furthermore TMPRSS2-ERG (TMPRSS2 Exon 2 and ERG Exon 4) was prevalent in 15 patients and NDRG-ERG was prevalent in 10 patients (data indicated

in Figure 4). Additionally Figure 5 describes prevalence of fusion transcripts TMPRSS2-ERG and NDRG-ERG in prostate cancer patients suggesting a comparison between patients positive and negative for fusion transcripts TMPRSS2: ERG fusion gene plays an imperative role in prostate cancer (PC) development or progression, but the extent to which TMPRSS2: ERG is down-regulated in response to androgen deprivation therapy (ADT) can be determined by checking the expression profile of the fusion transcript of the patient.

Our findings indicate that prostate cancer tissue harbours a miscellany of fusion transcripts which are instrumental in prostate cancer disease aggressiveness. These fusion transcripts have to be evaluated at transcriptional level to unfold the heterogeneity of the disease. In Pakistan there are no reliable markers for prostate cancer detection other than prostate specific antigen (PSA). Because prostate cancer is a heterogeneous disease, it is clear that a combination of markers will become important in prostate cancer diagnosis. It although is androgen regulated but the fusion transcripts belong to another molecular state that needs a reliable assessment which still does not exist in Pakistan.

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