

# A Study on Evaluation of Caspase Gene Expression Profile in Turk Patients with Primer Prostate Cancer

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## Abstract

Harmful cells', such as cancerous cells, growth depends on evasion of apoptosis, which is considered as one of the hallmarks of cancer. Apoptosis is ultimately carried out by the sequential activation of initiator and executioner caspases, which constitute a family of intracellular proteases involved in dismantling the cell in an ordered fashion. In cancer, therefore, one would anticipate caspases to be frequently rendered inactive, either by gene silencing or by somatic mutations. From clinical data, however, there is little evidence that caspase genes are impaired in cancer. Executioner caspases have only rarely been found mutated or silenced, and also initiator caspases are only affected in particular types of cancer. There is experimental evidence from transgenic mice that certain initiator caspases, such as caspase-8 and -2, might act as tumor suppressors. Caspase-2, the most conserved member of the caspase family, has long been recognized as an important protein in the regulation of apoptosis. Caspase-2 is activated upon genotoxic stress in a large protein complex termed the PIDDosome (Tinel and Tschopp, 2004). For this reason, we study and analyzed 2 exons of the human caspase-2 gene, using a strategy combining gene expression based RT-PCR amplification to investigate the associations between prostate cancer and caspase-2 gene expression in 9 individuals with prostate cancer. As a result of working, the level of variant-1 and variant-2 of caspase-2 gene expression in prostate cancer tissues was shown to be lower than in the control specimens and  $\beta$ -actin gene. Expression levels of  $\beta$ -actin were used as internal positive control. It has been demonstrated that the restoration of caspase-2 deficient cancer tissues augments their sensitivity to undergo apoptosis in response to chemotherapeutic agents and to other apoptotic inducers. Consistent

with this, evidence is accumulating for potential roles of caspase-2 in non-apoptotic processes, including cell cycle regulation and DNA repair. In addition, a tumor-suppressor function has been suggested for caspase-2. Here we discuss the various defects in caspases dependent cell death machinery identified in the prostate cancer specimens.

**Keyword:** apoptosis, cancer patient, caspases, gene expression

## Introduction

Cancer results from the accumulation of several genetic and epigenetic events, arising over a long time interval. It is clear that identifying the molecular alterations that distinguish cancer cells from normal cells will ultimately help to define the nature and understand the pathologic behavior of a cancer cell. Prostate cancer is the most frequently diagnosed male cancer in developed countries. Despite recent advancements in the treatment and management of prostate cancer, it still remains the most common malignancy and second leading cause of cancer-related deaths among men in the Turkey. Development of effective therapeutic modalities for the treatment of human cancer relies heavily upon understanding the molecular alterations that result in initiation and progression of the tumorigenic process. Many of the molecular changes identified in human prostate tumorigenesis so far play key roles in apoptosis regulation. Apoptotic signaling pathways are dependent on the activation, by proteolytic cleavage after key aspartic acid residues, of a family of cysteine proteases, termed caspases, which mediate cleavage and functional destruction of various essential intracellular proteins. This gene encodes a member of the cysteine-aspartic acid protease (cas-

pase) family. Caspases play important roles in regulating apoptotic signaling pathways. The encoded protein may function in stress-induced cell death pathways, cell cycle maintenance, and the suppression of tumorigenesis. Interestingly, each caspase-2 mRNA is initiated from separate promoter regions, and the casp-2L promoter is much stronger than the casp-2S promoter, in agreement with the respective transcript levels of the two variants (Logette et al., 2003).

The present studies were initiated in order to determine the mechanism of activation of caspase-2 in apoptosis of cancer cells. The aim of this article is to summarize the various defects in caspases dependent cell death machinery identified in the prostate cancer specimens. Development of effective therapeutic modalities for the treatment of human cancer relies heavily upon understanding the molecular alterations that result in initiation and progression of the tumorigenic process. Many of the molecular changes identified in human prostate tumorigenesis so far play key roles in apoptosis regulation. As a result, the overall activity of caspases in malignant tissues decreases and the activation threshold increases, thus leading to the prevention of apoptosis.

## Experimental procedures

### Material and Methods

#### Tumor Samples

Paraffine blocks of prostate pathologies were derived from the archives of the Department of Pathology in Faculty of Medicine at the University of Konya, Turkey. Namely, paraffine-embedded prostate cancer tissue specimens of 9 prostate cancer patients were used in this study. Age range of all patients was 50-74 years. These patients went to physicians to be demonstrate a variety of serious symptoms of prostate cancer, e.g, difficulty in voiding, urodynia, urgent and frequent urination, and hematuria. Their prostates were examined by one or more of the following means: rectal ultrasound detection, digital rectal examination, computed tomography, and magnetic resonance imaging. Biopsy was performed for the patients who were suspected to have prostate cancer, and all speci-

mens were from archived paraffine blocks that were collected specifically for this study. Control samples were obtained from natural or healthy tissue of the same cases.

#### Molecular material

Variant-1 and variant-2 belonging to caspase-2 of primers and  $\beta$ - actin primer were obtained from the Primer design (**Table 1** and **Table 2**).

#### Deparaffinization of Samples

Deparaffinization removes the bulk of paraffin from the paraffin-embedded sample. A number of techniques for deparaffinization are known and any suitable technique can be used with the present invention. In this study, we used a method of the invention utilizes washing with an organic solvent to dissolve the paraffin. A xylene was used remove paraffin effectively from the tissue sample without adversely affecting DNA isolation. A xylene is the

**Table 1.** Variant-1 and variant-2 belonging to caspase-2 of primers.

Gene		Oligonucleotide series
Caspase-2 Variant- 1	F	5'-CGC GGG GTC TTG GTC C-3'
	R	5'-AGG ATG CAT GCC ACA CAC T-3'
Caspase-2 Variant- 2	F	5'- GGA AGA AAT CTG CTG CAC CAC-3'
	R	5'- TTC TAA CAA TTC GCT CAA CAA CAG-3'

**Table 2.**  $\beta$ - actin primer.

Gen	No		Oligonucleotide series	Tm	bp
$\beta$ - actin	NM_001101.2	F	5'- CGCAAAGACCTGTACGCCAAC -3'	63	164
		R	5'- GAGCCGCCGATCCACACG -3'	63	

**Table 3.** Prostate parameters.

Prostate Cancer Characteristics					
Case No	Patient Age	Gleason Score (ng/dl) (PSA)	TNM (Tumor-lymph nodes-metastasis)	Family history of cancer and other cancers	Personal history of cancer and the other cancers
13	49	5	NA	No	No
19	69	6	T1a	testes	No
28	89	5	NA	No	No
30	62	8	T1c	Bladder	No
31	51	5	T2a	No	No
35	58	7	T1c	Bladder	No
36	65	7	T2a	Testis, colon	Colon
42	72	9	N0	Kidney	Lung
48	67	7	T1c	Bladder	No

preferred solvent for use in the methods of the invention. Paraffin was typically removed by washing with xylene vigorous mixing followed by centrifugation. Samples are centrifuged at a speed sufficient to cause the tissue to pellet in the tube, about 20,000×g. After centrifugation, the organic xylene supernatant is discarded. And then, tissue samples were passed a series of alcohol (Absolute alcohol and 75% ethyl alcohol) and were centrifuged in each of steps. Thus, the paraffin was removed from tissue samples.

### Patient Anamnesis

The study was approved by the local ethics committee, all patients gave written informed consent for participation in the study. Patients with either prostate cancer and other primary cancer, and family history of cancer in terms of patient age and gleason score (see Table 2) and healthy individuals without prostate cancer were included in the study. In addition to working in an individual's age and

eligibility criteria in terms of gleason scores already published in previous studies. Tissue samples taken from patients according to pathological findings were recruited between 2010 and 2011.

### RNA extraction

Total RNA from 1 dpp prostate was extracted using the RNeasy Plus Mini Kit (Qiagen) Absorbance measurements at 260 nm in water were used to adjust the stock concentration of all RNA samples to 1 µg/µl assuming an absorbance of 1 is equivalent to RNA at 40 µg/ml. Quality control standards were applied to all RNA samples in this study. Briefly, these were that the purity (A260:A280) was at 1.7.

### Reverse Transcription

5 µl of total mRNA were reverse transcribed using the Precision nanoScript™ Reverse Transcription kit (Primer Design Ltd.) according to the kit instructions.

## RT-PCR

The amount and quality of RNA were measured by Nanodrop Photospectrometer (NanoDrop 2000c THERMO, USA). 5 µg of total mRNA were reverse transcribed using the Precision nanoScript™ Reverse Transcription kit (Primer Design Ltd.) After incubation at 55 °C for 30 second, the reactions were stopped at 95 °C for 10 min. The resulting total cDNA was then used to determine expression levels of variants of caspase 2. Expression levels of β-actin were used as internal positive control. Samples were heated for 10 min at 95°C (enzyme activation), followed by 50 cycles of 15 s at 95°C (denaturation), 30 s at both of 55-60°C (annealing) and then 15 s 72°C (binding). The statistical significance of differences in mRNA expression was analyzed by the Relative Expression Software Tool (REST). PCR efficiencies for caspase-2 and b-actin were 2.07, 1.98 and 1.89, respectively.

## Statistical Analyses

Cell viability, apoptosis and necrotic in rates of changes were evaluated "one-way ANOVA" test using SPSS 15.0 statistical program. With the level of caspase-2 gene expression in primer prostate cancer patients and healthy examples of differences in levels of beta-actin gene expression "REST (2009 V2.0.13)" compared with the statistical program. P values that is less than 0.05, was considered statistically significant. In addition, with increased caspase-2 mRNA expression levels in prostate cancer cells showed apoptosis while using the caspase cascade.

## Results and Discussion

RT-PCR analysis of caspase-2 expression was carried out using a primer set for detection of caspase-2L and -2S. The two forms of caspase-2 mRNAs were

detected in the control blood, but we observed that caspase-2L mRNA predominated, and caspase-2S mRNA was barely detectable. This was true whether caspase-2 expression was normalized according to beta-actin. Our results suggest that testing caspase-2 expression by RT-PCR technique seems to be a reliable method in detection of patients with primer prostate cancer because of its high specificity. Apoptosis-related genes down regulated in NSCLC compared to non tumor lung tissue ( $p < 0.05$ ) included representatives of the tumors. The potential of apoptosis-related genes as prognostic and predictive markers should be validated in future studies. A larger group of patients is needed in order to confirm the above results, while their clinical significance as potential prognostic and predictive markers should be validated in future studies.

Apoptosis occurs via an extrinsic and an intrinsic pathway. The extrinsic pathway is initiated by binding of cell surface death receptors (via death domains) to adaptor proteins [e.g., Fas-associated via death domain (FADD)] in a death-induced signaling complex. The intrinsic pathway acts through generation of mitochondrial permeability transition leading to the establishment of the 'apoptosome' protein complex. Both pathways converge into a common cascade that consists of proteolytic enzymes-caspases (Shinno et al., 2005).

Caspase-2 is the earliest identified caspase in mammals. This enzyme is unique for its features of both initiator and effector caspases. Caspase-2 appears to be necessary for the onset of apoptosis triggered by several insults, including DNA damage, administration of TNF, and different pathogens and viruses (Logette et al., 2003). Many studies have shown that caspase-2 serves as an apoptosis inducer in some types of cells. Read et al. (2002) reported the spontaneous recruitment of procaspase-2 into a protein complex without cytochrome c or Apaf-1 in some cells.

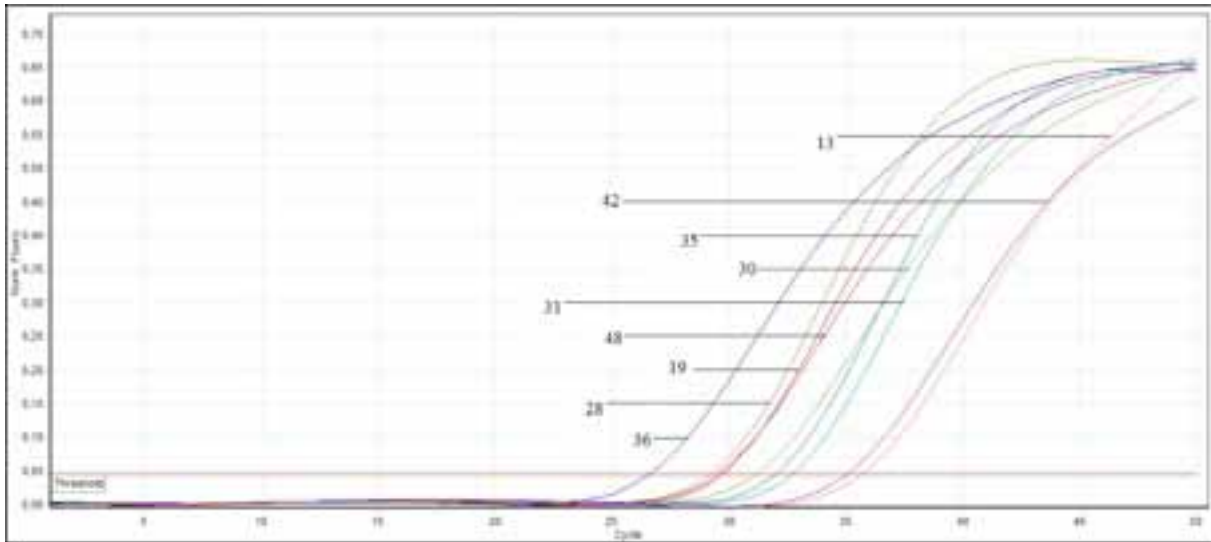


Figure 1. Prostate Ca samples at 55 ° C in the image of  $\beta$ -actin gene expression

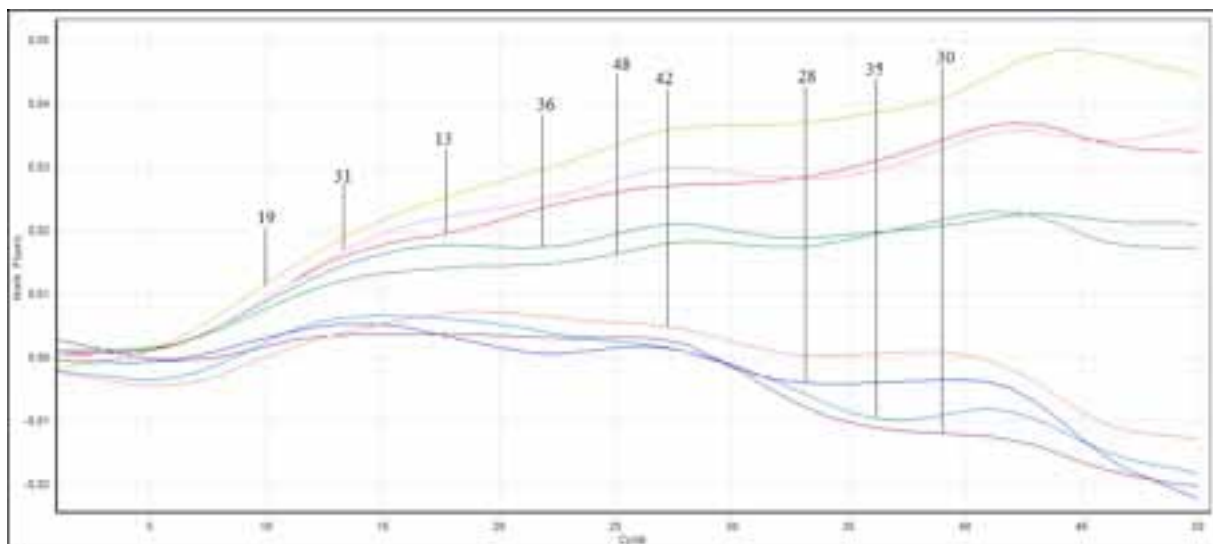
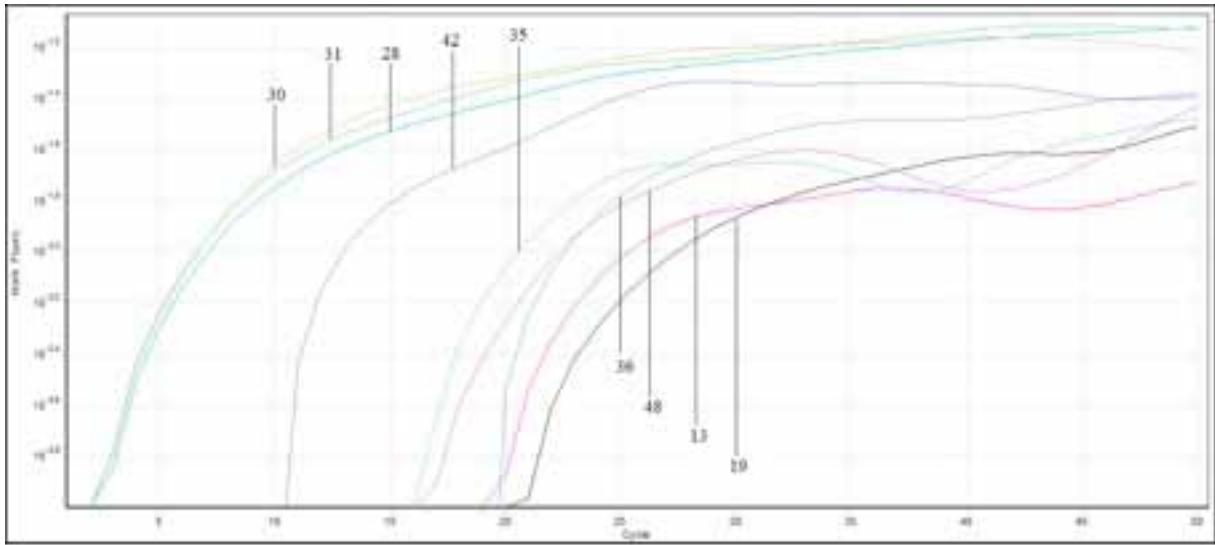
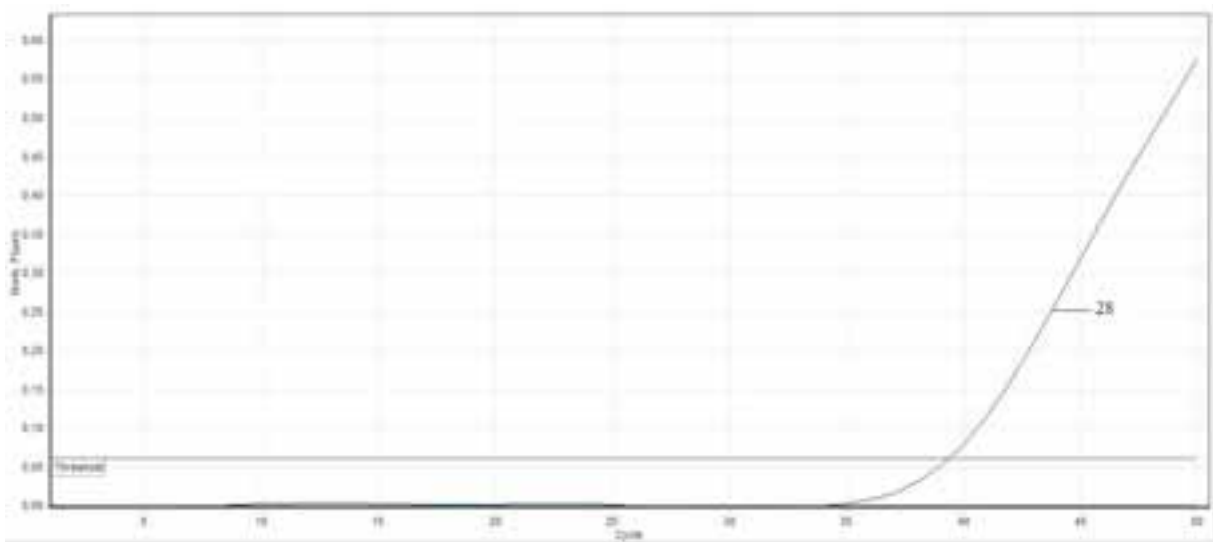


Figure 2. Prostate Ca samples at 55 ° C in the image of caspase-2 Variant 1 gene expression



**Figure 3.** Prostate Ca samples at 55 °C in the image of caspase-2 Variant 2 gene expression



**Figure 4.** Prostate Ca samples at 60 °C in the image of caspase-2 Variant 1 gene expression.



In the absence of active caspases cells do not undergo caspase independent cell death and instead could survive an insult and promote clonogenic tumor growth (Fischer et al., 2007). Thus, it has been suggested that caspases can be viewed as tumor-suppressor proteins (Schulz, 2005). Although the effector caspase-6 was suggested as a putative candidate tumor suppressor gene, the question of whether other caspases (i.e. the initiator caspases) are acting as tumor suppressors remained to be specifically addressed.

The caspase-2 gene generates two main splice variants (caspase-2L and caspase-2S) that fulfill either pro- or anti-apoptotic functions, respectively (Cote et al., 2001). Binding of caspase-2 pre-mRNA to RBM5 protein results in the accumulation of caspase-2L, whereas deletion or mutation of the RBM5-binding site in the caspase-2 pre-mRNA leads to accumulation of the caspase-2S isoform (Fushimi et al., 2008).

Over the last few years intense investigation of the function and activation mechanisms of caspase-2 by several groups has clearly implicated this enzyme in apoptosis (Krumschnabel et al., 2009; Zhivotovsky and Orrenius, 2005), but despite the large amount of data accrued, inconsistencies in findings have not allowed solid conclusions to be drawn. In addition, several recent publications have described previously unknown mechanisms relating the activation of caspase-2 to its potential functions, and that might shed light on the roles played by this enzyme (Andersen et al., 2009; Nutt et al., 2005). These new

findings suggest the involvement of several kinases in the regulation of caspase-2 activation, as well as the presence of various activation platforms for this enzyme, some of which contain the p53-inducible death domain-containing protein, PIDD. Furthermore, novel roles of caspase-2 in non-apoptotic processes, such as cell cycle regulation and DNA repair have been suggested (Shi et al., 2009).

The absence of a loss-of-function phenotype of caspase-2 does not necessarily indicate that these proteins have trivial functions. It is possible that certain phenotypes of any of these deficiencies could be observed in certain pathological conditions, such as cancer, deficient fertilization, or premature aging. In fact, a tumor-suppressor function for caspase-2 has now been described. In addition, caspase-2 was recently implicated in cell cycle regulation. Because many of the tumor-suppressor proteins are known to play a role in cell cycle regulation, it is likely that these two newly assigned functions for caspase-2 are interrelated (Vakifahmetoglu-Norberg and Zhivotovsky 2010).

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