

Apoptosis and Cancer: Mutations Relationship within Caspase 2 Genes in Turk Male Patients

Orcun İŞLER¹, Nesrin TURAÇLAR² and Hasibe Cingilli VURAL¹

¹Selcuk University, Science Faculty, Department of Molecular Biology, 42079 Selçuklu, Konya, Turkey,

²Vocational School of Health Services, Selcuk University, Konya, Turkey, dmesrinturaclar@yahoo.com

Address for Corresponding : Dr. Hasibe Cingilli VURAL, hcvural@gmail.com

Abstract

Prostate cancer is the most frequently diagnosed male cancer in developed countries. Prostate cancer is the most common cancer diagnosed among men in Turkey, accounting for 42.5% of all cancer cases in men. It ranks second after lung cancer as the underlying cause of cancer death in Turk men. If cancer is a disease where too little apoptosis occurs there are other diseases where too much apoptosis is thought to be part of the problem. Caspases and apoptotic process very important role in modern cancer treatment. For this reason, we study and analyzed 2 exons of the human caspase 2 gene, using a strategy combining RT-PCR amplification and HRM, followed in the second study, considered to investigate the associations between prostate cancer and caspase 2 gene expression and nucleotide sequence analysis in 12 individuals with prostate cancer and healthy persons. As a result of working, variant-1 and variant-2 of caspase-2 gene mutation was identified in the tissues of the prostate using HRM method. For variant-1, 4 number one mutation was detected in the samples, while for variant-2, 2 numbered examples of mutation has been identified. According to the statistical evaluation in our study, there was significant difference in CSP2 mutations. At the same time, it has been evaluated with one-to-one correspondence tests whether presence of CSP2 mutation has relationship with the development of any clinical symptoms while diagnostic criteria.

Keywords: Apoptosis, Caspase, Mutation, RT-PCR

Introduction

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 [1], and second leading cause of cancer-related deaths among men in the Turkey. On average, one out of every four people will have cancer in their lifetime. Although inherited cancers account for only a small fraction of all tumours, most cancers are caused by a mix of hereditary and environmental factors [2].

Multicellular organisms employ two main mechanisms for the elimination of cells: necrosis and apoptosis [3]. Necrosis may be triggered by the rupture of the plasmatic membrane and may be accompanied by formation of an inflammatory process. On the contrary, apoptosis involves a “cleaner” type of death, in which the chromatin is condensed; the DNA becomes

fragmented forming vesicles known as “apoptotic bodies”. Biochemically, the main features of apoptosis include caspase cascade activation and DNA fragmentation. Upon receiving specific signals instructing the cells to undergo apoptosis a number of distinctive changes occur in the cell. A family of proteins known as caspases are typically activated in the early stages of apoptosis [4]. Apoptosis is coordinated by a family of cysteine proteases, which are activated by frequently divergent proapoptotic stimuli [5].

Activation of the caspase cascade has been correlated with the onset of apoptosis [6]. Caspases are synthesized as relatively inactive precursors that require proteolytic processing for activation [6,7]. Caspases are cysteine proteases that primarily serve two functions: the processing and activation of proinflammatory cytokines, and the cleavage of multiple proteins during apoptosis to allow the ordered dismantling of cells that are undergoing death [8, 9].

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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 [10]. Thus, it has been suggested that caspases can be viewed as tumor-suppressor proteins. Caspase family of cysteinyl-proteases plays the key role in the initiation and execution of programmed cell death.

Impaired apoptosis is involved in tumor initiation and progression, since apoptosis normally eliminates cells with increased malignant potential such as those with damaged DNA or aberrant cell cycling [11,12]. Most prostate cancer cells have a protracted history of development, suggesting that prostate cancer cells must have evolved various mechanisms to subvert the apoptotic program [13]. Impaired apoptosis signaling and extended cell survival seem to be closely associated with prostate tumor initiation, metastasis and progression to the androgen-insensitive state [14]. Caspase-2 was reported to be involved in a number of apoptotic pathways triggered by various stimuli. The caspase-2 gene generates two main splice variants (Casp-2L and Casp-2S) that fulfill either pro- or anti-apoptotic functions, respectively [15]. Binding of Casp-2 pre-mRNA to RBM5 protein results in the accumulation of Casp-2L, whereas deletion or mutation of the RBM5-binding site in the Casp-2 pre-mRNA leads to accumulation of the Casp-2S isoform. Another mechanism that might lead to accumulation of Casp-2S is its transcriptional regulation by TAp73 and DNp73 [16]. It is possible that survival promoted by the caspase-2 splice variant might be responsible for regulation of its tumor-suppressor function. Furthermore, novel roles of caspase-2 in non-apoptotic processes, such as cell cycle regulation and DNA repair have been suggested [17]. Caspase-2 may have more distinctive properties, and understanding the function of caspase-2 is challenging. The function of caspase-2 is still poorly understood. While containing the long prodomain and being able to respond to a variety of apoptotic stimuli, and plays an important role for DNA damage induced apoptosis [18]. Caspase-2 is not really essential for most physiological cell deaths. Caspase-2, the most conserved member of the caspase family, has long been recognized as an important protein in the regulation of apoptosis. In addition, a tumor-suppressor function has been suggested for caspase-2. While not expressed in individuals suffering from cancer in healthy individuals show caspase expression. In addition, a tumor-suppressor function has been suggested for caspase-2. Furthermore, 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. In order to control the role of caspases in apoptotic and non-apoptotic processes, these enzymes might have acquired distinct activation mechanisms. Indeed, the specific activation of caspase-2 in different complexes, located in various intracellular compartments, might explain its distinct functions. Despite the early discovery of caspase-2, its physiological function has long remained an enigma. A number of recent publications now suggest not just one, but multiple functions, including roles in apoptosis, DNA repair and tumor suppression. How can one enzyme have so many talents? Considering the diversity of interaction partners and the specific mode of pro-apoptotic action proposed in these studies, caspase-2 might in fact represent a primordial protease serving numerous pathways, established before the advent of a more elaborate functionally diversified caspases system.

This article discusses various observations that relate to caspase-2 functions in apoptosis, the DNA damage response and tumour suppression, and attempts to reconcile reports that have often been contradictory and controversial. It has been evaluated with one-to-one correspondence tests whether presence of CSP2 mutation has relationship with the development of any clinical symptoms while diagnostic criteria. According to the statistical evaluation in our study, there was significant difference in CSP2 mutations. At the same time, it has been evaluated with one-to-one correspondence tests whether presence of CSP2 mutation has relationship with the development of any clinical symptoms while diagnostic criteria.

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 Necmettin Erbakan, Turkey. Namely, Paraffine-embedded prostate cancer tissue specimens of 12 prostate cancer patients were used in this study. Age range was 50-74 years were males. 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 specimens were from archived paraffin blocks that were collected specifically for this study. Control

samples were obtained from natural or healthy tissue.

Molecular Material

Variant-1 and variant-2 belonging to caspase-2 of primers were obtained from the Teknik Kimya, from Turkey (Table 1).

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

Caspase-2 Variant-1	F: 5'-CAC CTC CCC ATC ATC CAC-3' R: 5'-GAG ATT CCC ACA TCC CTG AG-3'
Caspase-2 Variant- 2	F: 5'- GCA CTT ATC AAG GAT CGG GA-3' R: 5'- ATG GGC ACA GGA AAC AAG TC-3'

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. Xylene was used to remove paraffin effectively from the tissue sample without adversely affecting DNA isolation. Xylene is the 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 [19].

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.

Table 2: 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
2	58	5	NA	No	No
13	49	5	NA	No	No
19	69	6	T _{1a}	testes	No
20	59	5	T _{1a}	No	No
23	70	8	T _{2b}	No	No
28	89	5	NA	No	No
30	62	8	T _{1c}	Bladder	No
31	51	5	T _{2a}	No	No
35	58	7	T _{1c}	Bladder	No
36	65	7	T _{2a}	Testis, colon	Colon
42	72	9	N ₀	Kidney	Lung
48	67	7	T _{1c}	Bladder	No

NA; normal and close to normal, healthy (According to the data of total)

N₀; tumor cells absent from regional

T_{1a}; benign prostate tumor (5% cancer, 95% good-natured of prostate tissue)

T_{1c}; the tumor have been identified at this stage

T_{2a}; tumor localized one side of prostate tissue

T_{2b}; the cancer has started to metastasize (between individuals is observed under the age of 71)

No; not observed in other types of cancer (individual and familial)

DNA Isolation Procedure

Formalin-fixed paraffin samples were cut into 10- μ m sections. The sections were pulverized under liquid nitrogen condition using microdismembrator (B. Braun, Melsungen, Germany) of each sample, 0.1 g of pulverized tissue powder was resuspended in 1 ml of xylene and left for 15 minutes at 55°C. The suspension was then centrifuged at 14000 g for 5 minutes. The pellet was suspended in 0.1 ml of xylene and processed as above for the second time. The resulted sediment was mixed with 100% ethanol and processed with xylene lysis buffer [Tris, sodium dodecyl sulfate, ethylenediamine tetraacetic acid (EDTA)]. A lysis buffer containing 300 μ g/ml of proteinase K was added to the pellet, mixed and incubated at 55°C for an overnight period. The DNA was extracted following the use of phenol-chloroform procedure, then dissolved in TE buffer (Tris-HCl and EDTA) and stored at 4°C. Furthermore, Genomic DNA was isolated from paraffin-embedded prostate cancer tissue and the normal tissue using the tissue kit (Roche) [20].

HRM

Mutation scanning techniques are used to detect sequence variants without the need for prior knowledge of the identity or precise location of the variant, in contrast with genotyping techniques, which determine the status of a specific variant. High-resolution melting is a recently developed method that shows great potential as a mutation scanning technique [21].

Results

The apoptosis process is mediated by sequential activation of caspases. To understand apoptotic cell death, therefore, many studies have examined the caspase activation mechanism. Despite intense structural and biochemical investigations, there are still many unknown features of the caspase and caspase activation mechanism. This article summarizes the most recent structural studies on the caspase-activating complexes. As a result of working, variant-1 and variant-2 of caspase-2 gene mutation was identified in the tissues of the prostate using HRM method. For variant-1, 4 number one mutation was detected in the samples, while for variant-2, 2 numbered examples of mutation has been identified.

These data show that loss of caspase-2 results in an increased ability of cells to acquire a transformed phenotype and become malignant. This suggests that the role of caspase-2 in cell death is redundant or is compensated by other caspases. However, there is indirect evidence to suggest that caspase-2 may play some role in cancer. Our data provide strong support for a context-dependent function of caspase-2 in apoptosis.

This study investigates the role of caspase-2 in apoptotic signaling of human male prostate cells. In addition, we were also evaluated samples from individuals with prostate cancer the most conserved member of the caspase family and caspase-2, known to act as a tumor suppressor with HRM the method of analysis aimed to identify single point mutations.

As a result of working, variant-1 and variant-2 of caspase-2 gene mutation was identified in the tissues of the prostate using HRM method. For variant-1, 4 number one mutation was detected in the samples, while for variant-2, 2 numbered examples of mutation has been identified (Figure 1 and Figure 2).

This article reviews the current knowledge on mutations of apoptosis genes involved in the pathogenesis of human diseases and summarizes the gradual transformation of discoveries in apoptosis research into benefits for the clinical management of diseases.

We can summarize that significant caspase-2 activation is associated with apoptosis induction tested in samples of patients with prostate cancer cells an least partially, for the induction as well.

Figure 1: Variant-1 to caspase-2 image analysis of the HRM (WT: wild type)

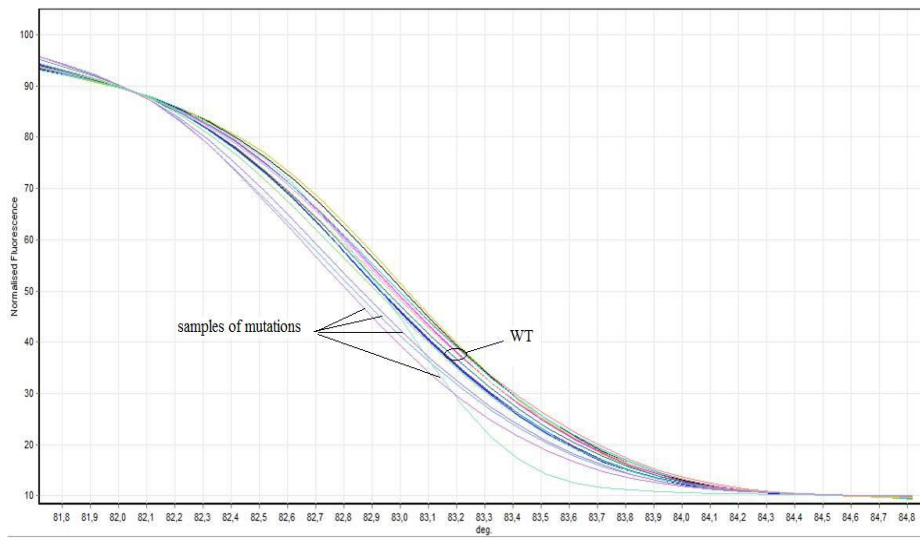


Figure 2: Variant-1 to caspase-2 image analysis of the Melting curve (WT: wild type)

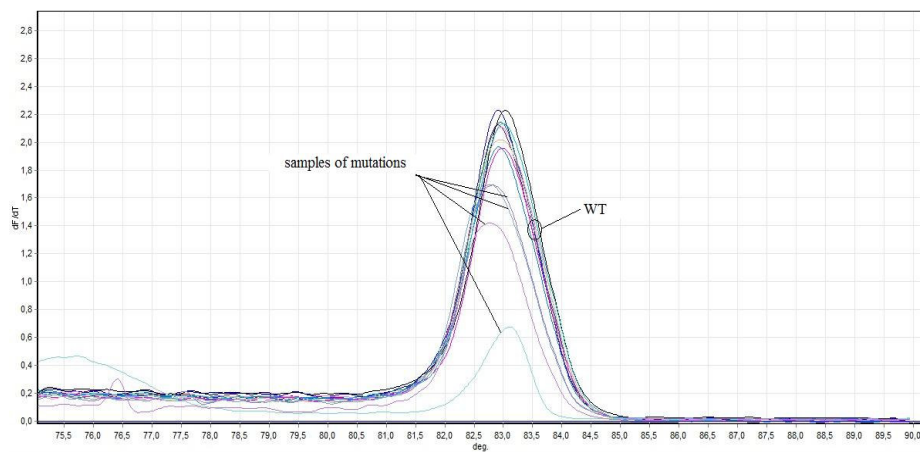


Figure 3: Variant-2 to caspase-2 image analysis of the HRM (WT: wild type)

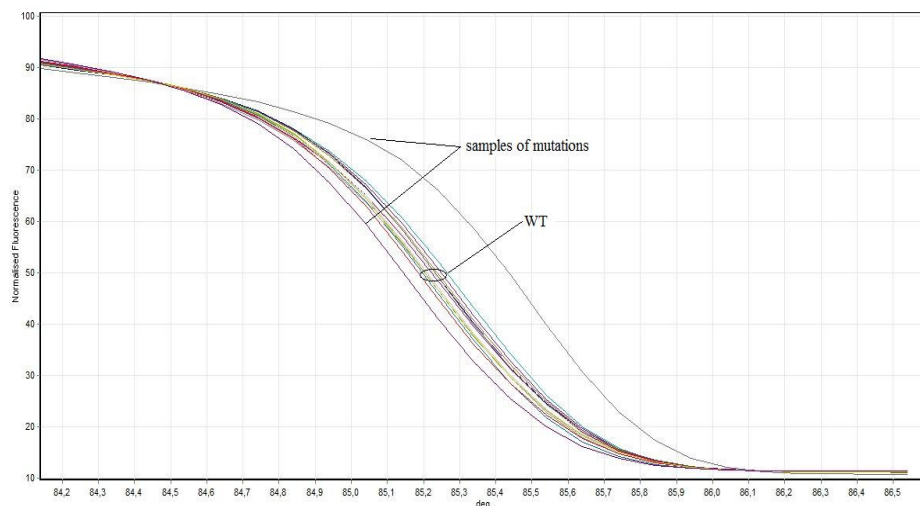
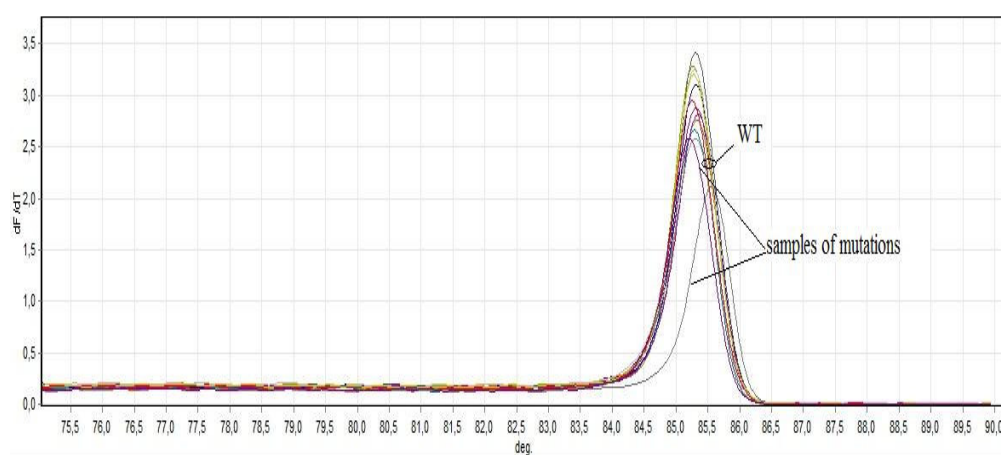


Figure 4: Variant-2 to caspase-2 image analysis of the Melting curve (WT: wild type)

Discussion

The caspases have been divided into two groups: initiators and effectors. First group of initiator or apical caspases (caspases-2, -8, -9, -10, and, probably, -11) activate the second-group of caspases (caspases-3, -6, and -7). The effector or downstream caspases are able to directly degrade multiple substrates including the structural and regulatory proteins in the cell nucleus, cytoplasm, and cytoskeleton [22]. Proteolysis of cellular substrates by caspases (cysteine-dependent aspartate-specific proteases) is one of the hallmarks of apoptotic cell death [23]. Although the activation of apoptotic caspases is considered a 'late-stage' event in apoptosis signaling, past the commitment stage, one caspase family member, caspase-2, splits the cell death community into half - those searching for evidence of an apical initiator function of this molecule and those considering it as an amplifier of the apoptotic caspase cascade, at best, if relevant for apoptosis at all. This review screens past and present biochemical as well as genetic evidence for caspase-2 function in cell death signaling and beyond [24]. Caspase-2 is the second identified caspase. It contains a CARD domain and recruit multi-protein complex "PIDDosome2 through CARD binding with RAIDD (RIP associated ICH-1/ECD3 homologous protein with a death domain) [25].

The adaptor proteins RAIDD and PIDD (p53 induced protein with death domain) in this complex are essential components for the activation for caspase-2 [26]. Caspase-2 appears to act upstream of mitochondrial permeabilisation by cleaving and activating Bid, and plays an important role for DNA damage induced apoptosis [27]. A recent study demonstrated that disruption of caspase-2 has a significant impact on mouse aging, suggesting that caspase-2 deficiency

compromised the animal's ability to clear oxidative damaged cells [28]. Caspase-2 may have more distinctive properties, and understanding the function of caspase-2 is challenging. Given that comparatively few substrates have been found for caspase-2, as well as its unique characteristics, the exact apoptotic and non-apoptotic function of caspase-2 has been the subject of much debate.

As described above, a number of anticancer therapies are being tested that influence the expression and/or activity of factors that regulate apoptosis.

Targeting caspases and apoptotic machinery will play an increasingly important role in future modern cancer therapy, and approaches are being developed that allow "on demand" activation of expression.

Caspase family proteases are key factors in apoptosis, and the related research can help us to obtain the essence of the above phenomena at the molecular level and enable us to make breakthroughs in the therapy of tumors, immune system diseases and nervous system diseases using the artificial control of apoptosis [29].

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