Apoptosis detection by TUNEL assay in BPH patients

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Abstract

This study presents whether benign prostate growth in aging men correlates with a decrease in apoptotic rate, which were determined with terminal deoxnucleotidyl transferase mediated dUTP biotin nick end labelling (TUNEL). Thirty-eight prostate adenoma were taken from BPH patient who underwent TUR-P. The patient were devided two groups according to their age, 14 of them were under 65 years-old, the other 24 were over 65. Apoptotic indeces, PSA levels, IPSS-LQ, prostate tissue weight were evaluated in these two groups of patients. A significant higher apoptotic rate was observed in second group which contain prostatectomy specimen from patients over 65 age as compared under 65. There were positive correlation between the age and apoptotic rate in BPH patients.

Key words: Apoptosis, prostate, prostatic hyperplasia.

TUNEL yöntemi ile BPH hastalarında apoptoz tespiti

Özet

Bu çalışmada benign prostat hiperplazisine sahip olan yaşlı erkeklerde apoptotik indeks hızındaki azalmayı göstermek amaci ile TUNEL yöntemi kullanılmıştır. Otuz sekiz prostat adenom kitlesi TUR-P sonrasında hastalarda alınmış ve hastaların 65 yaş veya üstü olmalarına göre gruplandırılmıştır. Apoptotik indeks, PSA seviyesi, IPSS ve yaşam kalitesi değerleri, her bir hatta için incelenmiştir. Hasta yaş ve apoptoz arasında pozitif korelasyon saptanmıştır.

Anahtar sözcükler: Apoptoz, prostat, prostat hiperplazisi

Introduction

Benign prostate hyperplasia (BPH) is one of the most common causes of hospitalization in men more than 50 years old. However, many of the characteristics of these conditions, such as their etiology, biology, behavior, prognosis have not fully investigated (Coffey et al., 1999; Kyprianou et al., 1996). The development of BPH is both androgens control and associated with aging. Although epidemiological facts underscore the importance of BPH as a major health problem, suprisingly little is known about or agreed on the cellular and molecular processes involved in the development of disease (Deng et al., 1996; Tahmatzopoulos and Kyprianou, 2004). The intensive research into study of tissue homeostatis over the past 10 years has confirmed that the kinetics of tissue growth in benign or malign conditions, is contingent on two independent parameters, the rate of cell proliferation and the rate of...
cell death. While uncontrolled prostate cancer cell proliferation death comment is very important to control confused events. Generally when cells decides death some molecular pathways changes their products and this situtaion evaluated many times to get true decision. Therefore, there are many important proteins which prevents apoptosis or induces this pathway (Isaacs and Coffey, 1989). After death stimulus, biochemical and morphological great changes occur. In contrast, during necrosis, the cell has a passive role in initiating cell death in responses to pathological changes, hypoxia, extreme temperature, toxins and ionizing radiation, outside the cell (Kyprianou et al., 1996). Apoptosis was originally defined by ultrastructural characteristic features differentiating it from necrosis. Cells undergoing apoptosis all show similar characteristics. Several morphology identifiable stages have been reported, including nuclear apparent changes, exuberant cell surface protrusion and disintegration of the nucleus to form multiple compacted fragments in chromatin material. The nuclear collapse that is the clue of apoptosis has as its biochemical correlate the fragmentation of DNA by endonucleases, producing fragments of 300-5000bp (Claus et al., 1997; Colombel et al., 1998).

Apoptosis refers to programmed cell death different from necrosis, in which individual cells participate in own fragmentation and deletion from living tissue. This process is under genetic control and a defect in apoptosis may result in the development of neoplasms (Berges et al., 1995). This death type of cells are generally seen eucaryotic cells. In general, cells undergoing apoptosis display a characteristic pattern of structural changes in nucleus and cytoplasm, including rapid blebbing of plasma membrane and nuclear disintegration. The nuclear collapse is associated with extensive damage to chromatin and DNA-cleavage into oligonucleosomal length DNA fragments after activation of a calcium-dependent endonuclase. Apoptosis can be induced in a variety of cell types by a wide variety of agents, including hormones, growth factors, chemotherapeutic agents and ionizing radiation. In particular, androgens appear to be important factors influencing apoptosis. Apoptosis was originally defined by ultrastructural characteristic features differentiating it from necrosis (Berry et al., 1984; Arrends and Wyllie, 1991).

Identification of apoptotic structures are highly difficult, but they are more reliable than histopathological examinations. Especially, molecular identification and probing target structures in apoptotic bodies with floresence provides high sensitivity in approaches. One of the apoptosis determination method is in situ cell death detection. This method is highly fast, simple, non-radioactive technique and suitable for routine analysis (Arrends and Wyllie, 1991).

In this study we try to show whether the age-related growth of the prostate may be based on decrease in apoptosis, in patients of different ages with BPH.

Material and methods

Tissues

Thirty-eight prostate adenoma were sampled from BPH patients who underwent prostatectomy (transvesical or transuretral prostatectomy). The patients were divided into two groups according to their age, 14 of them were under 65 years old (age range=46-65, mean age=57.8), the other 24 were over 65 (age range=66-83, mean age=72.3).

Apoptosis determination

Formalin fixed, paraffin embedded pathological specimens were obtained from above patient populations and histological sections (3 µm) were subjected to the following analysis. Tissue sections were analyzed for in situ apoptotic DNA fragmentation using the terminal transferase TdT-mediated dUTP-biotin end-labeling (TUNEL) assay using the in situ cell detection kit. Negative controls consisted of sections in which the terminal transferase was omitted. To strip nuclei of tissue sections from proteins an incubation in 20 µg. Proteinase K (Sigma) per ml 0.01 M phosphate-buffered saline (PBS), pH 7.4 for 15 min. Endogenous peroxidase was inactivated by covering the sections with 0.3% hydrogen peroxide in 50% methanol for 20 min at room temperature. The sections were rinsed three times with PBS for 3 min. Each and immersed in TDT buffer (30 mm Trizma base, pH 7.2, 140 mM sodium cacodylate, 1 mM cobalt chloride). Terminal deoxynucleotidyl transferase (TDT, 0.3 U/µl) and biotinylated uridine triphosphate (6.25 µM), in TDT buffer were then added to cover the sections, and then incubated in a humidified chamber at 370C for 60 min. The reaction was terminated by transferring the slides to TB buffer (300 mM sodium
chloride, 30 mM sodium citrate) for 15 min at room temperature. The sections were rinsed with PBS, and covered with 2% bovine serum albumin (BSA) in PBS for 10 min. Then all of them rinsed in PBS three times for 5 min. Labelled-peroxidase complex (Roche, Istanbul, Turkey), prepared according to the instructions supplied by the manufacturer, was applied to the sections for 30 min, then rinsed in PBS three times for 5 min. Sections were counterstained with ethyl green dehydrated with alcohol and cleared in Histoclear Coverslips were mounted using Histomount (Shandon).

**Statistical evaluation**

All prostate specimens were evaluated by a pathologist to confirm the histological presence of BPH. Pearson correlation test was used to compare these two groups. Age and other parameters for each patient were analyzed for apoptotic index results by SPSS programme. TUNEL-positive, darkly stained nuclei or nuclear fragments with a cytoplasmic halo were recognized as positive apoptotic cells. The apoptotic index was evaluated by counting the number of cells exhibiting TUNEL positivity over the total number of cells in the prostate epithelium (300 to 500), and the apoptotic index was expressed based on this percentage. For all immunostaining procedures, positive staining was evaluated in 3 random fields for each lesion, and the mean values were determined.

**Results**

The patients were divided two groups according to their age. At the first group there were 14 patient ≤65 years old (mean 60). At the second group there were 24 patient over 65 (mean 72) years old. At the first group; mean prostatic tissue weight was 46.63 cc, prostatic specific antigen (PSA) level was 3.65 ng/dl, International Prostatic Symptom Score (IPSS) was 20.0 and Life Quality was 4.1. Statistical comparison between two groups showed no significant difference in prostatic tissue weight, PSA levels, IPSS and life quality assay significant higher apoptotic index was observed in second group which contain prostatectomy specimen from patient over 65 years old (Table 1) as compared under 65 years old. There were negative correlation between the age and apoptotic rate in BPH patient.

**Table 1: Patient characteristics.**

<table>
<thead>
<tr>
<th></th>
<th>≤65 years old</th>
<th>&gt;65 years old</th>
</tr>
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<tbody>
<tr>
<td>Mean age</td>
<td>60</td>
<td>72</td>
</tr>
<tr>
<td>Prostate volume</td>
<td>46.63 cc</td>
<td>53.37 cc</td>
</tr>
<tr>
<td>PSA (ng/ml)</td>
<td>3.65 ng/ml</td>
<td>4.43 ng/ml</td>
</tr>
<tr>
<td>Life Quality</td>
<td>4.1</td>
<td>3.94</td>
</tr>
<tr>
<td>IPSS</td>
<td>20</td>
<td>20.66</td>
</tr>
</tbody>
</table>

<0.05 is considered as significant

TUNEL-positive cells were detected in the benign prostate epithelial cells (Fig 1). The apoptotic index was about 25.6% in BPH for >65 years old patients, but was significantly lower in ≤65 years old patients samples 20.3% (p<0.001). A significant difference in the incidence of apoptosis was detected among prostate epithelial cells in >65 years old group compared with ≤65 years old patients samples (p<0.001) (Table 2).

**Table 2: Apoptotic rates of BPH patients.**

<table>
<thead>
<tr>
<th></th>
<th>≤65 years old (%)</th>
<th>&gt;65 years old (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apoptosis rate</td>
<td>20.3 (p&lt;0.001)</td>
<td>25.6 (p&lt;0.001)</td>
</tr>
</tbody>
</table>

<0.05 is considered as significant

TUNEL-positive slides were evaluated for age, PSA and other factors for each patient. However, when apoptotic index compared with IPSS, PSA and Life quality assays, found not statistically significant (p>0.05). The apoptotic index of the secretory and basal cells of the prostate epithelium was higher in the over 65 aged patients compared with others, whereas there was a significant increase in the proliferative index of the respective cell populations in the hyperplastic prostate. Balancing the apoptotic versus the proliferative activities revealed a substantial net decrease (fourfold) in the total number of cells dying via apoptosis in both the glandular and basal epithelial cell compartments of the hypertrophic prostate (BPH) when compared with the normal gland.
Discussion

BPH is a phenomenon followed by an age-dependent increase in volume of the prostate throughout the entire life of a man. The growth and involution of the prostate depend on the quantitative relationship between the rate of cell proliferation and cell death. Most previous studies have focused on proliferation and apoptotic rates in benign hyperplastic human prostates (Isaacs and Coffey, 1989; Walsh, 1986).

Siegfried et al., (1993) reported that there is an increase in proliferation rate and decrease in apoptotic rate in benign hyperplastic prostate tissue. Therefore they suggest that the growth of the aging prostate results from this disturbance in the balance between cell proliferation and apoptosis (McNeal, 1983).

Claus et al., (1997) studied the apoptotic and proliferative rates in epithelium and in stroma of BPH. They demonstrated the apoptotic and proliferative rate in epithelium of normal prostate tissue. On the other hand the results of this study indicate that the apoptotic rate in stroma of BPH decrease. Because of this results they suggest the decrease of the apoptotic rate in stroma of BPH maybe a reason for growth of the prostate tissue (Berges et al., 1995).

In this study, we found a significant increase in apoptosis in the epithelium and stroma of benign hyperplastic human prostates age dependent. Increase of apoptosis is thought to be due to the induction by the following factors which are the infection caused by the residual urine due to BPH, the bladder neck pressure by the retention and the cateterisation. These results suggest a potential involvement of enhanced expression of some antiapoptotic or apoptotic proteins in deregulation of the normal apoptotic cell death mechanisms in the human prostate with age dependency, thus resulting in a growth imbalance in favor of cell proliferation that might ultimately promote prostatic hyperplasia.

In conclusion, there is a good evidence for a wide range of proliferative diseases that some disturbance of proliferation/apoptosis rate is a factor in tissue growth. Obviously the induction of BPH from normal prostate tissue may be associated with a distinct increase in proliferation rate or a decrease in apoptotic rate. However, our data indicate that the further increase of BPH volume in aging men is not correlated with a decrease in apoptotic rate.

References


