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ARTICLE

Apoptosis in Untreated and Hormone-Treated Prostate Cancer of Various Histological Types

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A total of 102 (66 untreated and 36 hormone-treated) prostate cancers were examined histologically in order to determine their histological grade and the percentage of apoptotic tumor cells. The less differentiated the tumors were, the higher the spontaneous apoptotic activity was. Hormone therapy increased the apoptotic index in the prostate cancers. The increase was of greater significance in grade I than in grade II and grade III tumors. The therapeutic consequences of these findings and the possibility of different oncogene-expressions in various histological types of prostate cancer are discussed. (Pathology Oncology Research Vol 2, No 4, 239–241, 1996)

Key words: apoptosis, prostate cancer, hormone therapy

Introduction

The importance of apoptosis (a form of programmed cell death) in the regression of prostate (and other hormone-sensitive) cancer after hormonal treatment is widely accepted in the literature.[1,2,3,4,5] However, there are substantial differences between the hormone sensitivity of the various histological types of prostate cancer, which may be based on the relative susceptibility to apoptosis of the various tumour cell populations.

The aim of this study was to obtain data on the apoptotic activity of 102 (66 untreated and 36 treated) prostate cancers, also taking into consideration the type and differentiation of the tumors.

Materials and Methods

Prostate cancers from a total of 102 patients was studied. The specimens for histological examination were obtained by needle biopsy, transurethral resection or by total prostatectomy. The mode of sampling may influence the grade assigned, therefore only those needle biopsies were used in which a total of at least 2000 tumor cells could be counted in various sections at various levels of the tissue blocks. The tissue samples came from 66 untreated and 36 treated patients. The treatment was administered for at least 6 months and consisted of antiandrogen therapy combined with orchiectomy. The histological grading and the mode of obtaining the tissue sample in the untreated and treated groups is shown in *Table 1a*. *Table 1b* shows the number of patients undergoing orchiectomy and the various drugs used, as well as the duration of the treatment. In seven of the 102 cases, both untreated and treated tumor tissue of the same patient was examined. The histological classification and grading of the prostate cancers was performed according to Helpap.[7] This grading was used because it also considers cytological features and not purely architecture of the tumour. The tissue samples were fixed in 10% neutral formaline and embedded in paraffin. Standard 6 µm sections were cut serially and stained with HE. In 14 untreated and in 6 treated tumors immunohistochemical reaction was also performed for the TRPM-2 gene product considered to be a marker of apoptosis,[8] and with Apop-Tag (demonstration of nucleosomal fragmentation of DNA) in order to confirm the presence of apoptosis. The Anti-TRPM-2 gene product antibody was the generous gift of Dr. MP Tenniswood (Ottawa, Ontario, Canada); and was used in 1:50 dilution; Apop-Tag was the product of Oncor (Gaithersburg, MD, USA). For immunohistochemical staining, the peroxidase-antiperoxidase method was used. In examining the HE sections, the well-established histological signs of apop-

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Table 1. **a. Mode of tissue sampling in various untreated and treated groups**

Histological grading	*P*	*TUR*	*N*	*Total*	*P*	*TUR*	*N*	*Total*
	Untreated (n=66)				*Treated (n=36)*			
grade I	4	5	1	10	–	1	2	3
grade II	5	3	25	33	–	9	16	25
grade III	5	3	15	23	–	6	2	8

P – prostatectomy, TUR – transurethral resection; N – needle biopsy

b. Mode of androgen ablation and duration of treatment of 36 treated patients

Treatment	*Duration of treatment (months)*			
	6	*7–12*	*13–24*	*>24*
Cyproteronacetate	5	1	1	–
Flutamid	4	–	2	–
Orchiectomy	5	12	1	2
Polyestradiolphosphat				
Orchiectomy + Estramustinphosphat	–	–	–	3

tosis[8] were employed. The percentage of apoptotic cells was determined by examining at least 2000 tumour cells. Thus, at least 200 tumorous gland-like structures or, when glandular structures were not present, 2000 cells were examined. The apoptotic index was determined by two pathologists independently and blinded for the groups.

For statistical evaluation the X^2 test was used.

Results

Apoptosis, characterised by condensation of chromatin, shrinkage of the nucleus and cytoplasm, and presence of intracytoplasmic apoptotic bodies, was easily detected by microscopic examination of the HE stained sections

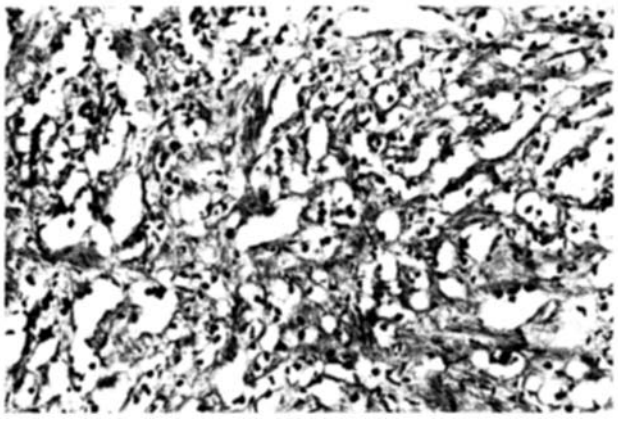

Figure 1. *Prostate carcinoma after androgen ablation of the patient. Most of the tumor cells show the cytological signs of apoptosis (pyknotic nuclei, shrinkage of cytoplasm) HE, x200*

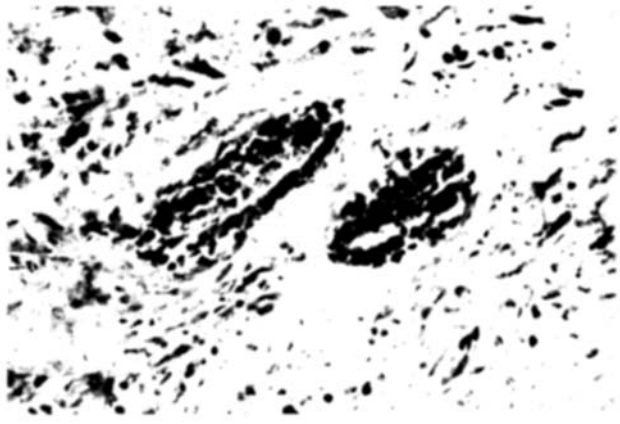

Figure 2. *TRPM-2 immunostaining of a prostate carcinoma after androgen ablation of the patient. See abundance of TRPM-2 positive tumor cells in two gland-like structures. x300*

(*Fig.1*). The immunohistochemical staining with Apop-Tag and for TRPM-2 confirmed the presence of apoptosis (*Fig.2 and 3*).

The average age of the untreated patients was 63.4 years, and that of the treated patients was 66.5 years. The percentage of apoptosis (apoptotic index) is given separately for the untreated and treated group of patients *in Tables 2a* and *b*, respectively.

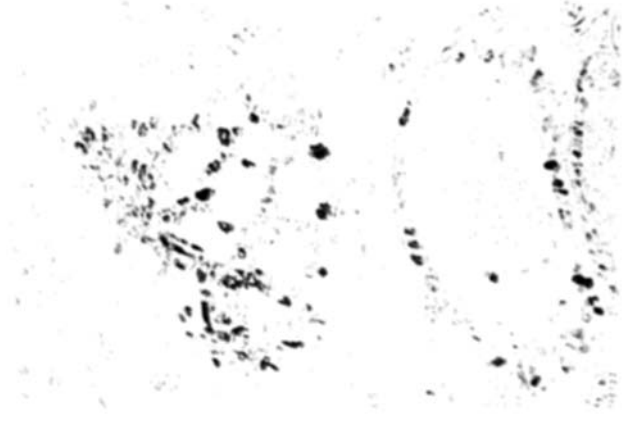

Figure 3. *Apop-Tag immunostaining of a prostate carcinoma after androgen ablation of the patient. Note positive staining of several tumor cells. x400*

The majority of tumors, for both the untreated and treated group, were of grade 2 type. Histologically, all treated and non-treated tumors were so-called usual carcinomas, which are considered as potentially androgen-sensitive. A relatively low apoptotic index (median up to 3.8) was observed in the untreated group and a clear tendency of increasing apoptotic activity was found in parallel with the higher grade of malignancy (*Table 2b*).

Table 2. **Apoptotic index in correlation with histological grade**

grading	a. Treated patients n	apoptotic index %	apoptotic index median	b. Untreated patients n	apoptotic index %	apoptotic index median
grade I	3	27.7 ± 2.3	28.0	10	1.9 ± 0.7	1.7
grade II	25	15.8 ± 4.2	14.9	33	3.2 ± 1.3	2.9
grade III	8	8.7 ± 1.8	8.2	23	4.4 ± 1.6	3.8

In treated (androgen ablated) patients, the apoptotic index increased in all groups (grades I-II-III) (*Table 2a*). The degree of this increase, however, depended on the tumor grade, i.e. the highest increase was observed in grade I cases and the lowest in grade III cases (*Fig.4*).

Discussion

Apoptosis, a form of programmed cell death, also occurs spontaneously in untreated tumors[8] although it is generally believed that the cells of malignant tumors have more or less "forgotten" how to commit suicide in the form of apoptosis. The apoptotic index may be different in various tumors, depending on their histological type and differentiation.

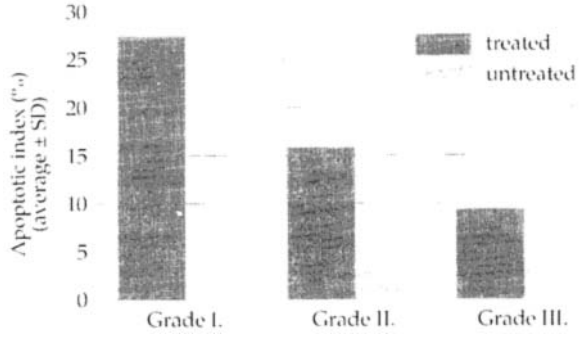

Figure 4. Changes in the apoptotic index of treated and untreated prostate carcinoma patients. Grade I - p<0.01; grade II - p<0.025; grade III - p<0.05

Our recent studies have shown that in untreated prostate cancer the probability of apoptosis increases in parallel with the histological grade of the tumor. Androgen ablation significantly increased the apoptotic activity in all grading groups. This increase was highest (16.5 fold) in the grade I group, lower in the grade II group (5.1 fold) and lowest in the grade III group (1.3 fold). This means that the inducibility of apoptosis decreases with de-differentiation of prostate cancer. Grade I prostate carcinomas are more likely to be influenced by androgen ablation than grade II and grade III tumors. These results suggest, from a practical point of view, that androgen ablation of Grade I prostate carcinomas is justified by the high inducibility of apoptosis, while the grade III tumors should rather be treated by other methods.

Evidently, it is necessary to obtain relevant data on the clinical improvement and survival of patients with prostate cancers of the various histological grades in order to compare the clinical course with the probability of apoptosis. The situation is complicated by the fact that the histology of individual tumors may change with time. A theoretical problem to be examined is whether apoptosis is also related to the presence of various genes, such as TRPM-2, p53 or bcl-2. In fact, the apoptosis suppressing oncoprotein bcl-2 has been detected in hormone refractory prostate cancers.[9] Myers et al[10] have demonstrated that three days after androgen ablation, TGFβ and TRPM-2 expression and enhanced apoptosis can be observed in prostate cancer cells. Sclar et al[11] also found TRPM-2 and TGFβ expression in prostate cancer cells after irradiation, followed by enhanced apoptotic activity. The systematic study of the same tumors before and after hormone therapy in a larger number of cases could provide more pertinent data.

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