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ORIGINAL PAPER

Expression of $p21^{waf1/cip1}$, $p27^{kip1}$, p63 and Androgen Receptor in Low and High Gleason Score Prostate Cancer

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Abstract The aim of this study was to investigate the expression of $p21^{waf1/cip1}$, $p27^{kip1}$, p63 and androgen receptor proteins in relation to serum prostate specific antigen levels in low and high Gleason score prostate cancers. Biopsies of patients suffering from prostate adenocarcinoma of low (3+3 to 3+4) and high (5+4 to 5+5) Gleason scores (13 cases each group) were immunostained for positive regulators of cell cycle control ($p21^{waf1/cip1}$ and $p27^{kip1}$), and essential markers of normal prostate gland ontogeny (p63) and growth (androgen receptor) to find differentially expressed markers of malignant progression. Serum prostate specific antigen levels were also monitored at the time of biopsy and following anti-androgen therapy. All cases except one in each group were androgen receptor positive. P63 and $p21^{waf1/cip1}$ proteins detected in normal basal cell nuclei were lost in all but one studied tumors respectively. $P27^{kip1}$ protein, however, was detected in all low Gleason score prostate cancers, but it was found in only 7/13 high score cases. Prostate specific antigen levels, either pre- or post-treatment, did not show strict correlation with the $p27^{kip1}$ results. The low to high grade dedifferentiation of prostate adenocarcinoma is accompanied with the down-regulation of $p27^{kip1}$ protein, which may be an important molecular sign of the lost cell cycle control.

Keywords High Gleason score · Loss of $p27^{kip1}$ · Prostate adenocarcinoma

Abbreviations

AR	androgen receptors
H&E	Haematoxylin–eosin
PSA	prostate specific antigen
CAB	complete androgen blockade

Introduction

Antiandrogen therapy of prostate carcinoma is based on the fact that most of these tumors are androgen dependent [1]. Androgens exert their growth promoting effect through androgen receptors (AR) [2]. Immunohistochemical demonstration of AR-s is successful in the majority of prostate carcinomas. However, AR positivity may be seen in antiandrogen-therapy resistant cases, too, which observation led to better understanding of mutations in the structure of AR-s. Androgens are also in close interaction with several tumor-suppressor and cell proliferation promoter genes [3–5]. These interactions may be manifested in up-regulation or suppression of such genes, upon the effect of androgens or deprivation of androgens by any type of antiandrogen therapy [6].

The aim of our study was to investigate the expression of growth regulating factors $p21^{waf1/cip1}$, $p27^{kip1}$, p63 and AR in tumor cells of prostate carcinomas, showing high (5+4 to 5+5) and low (3+3 to 3+4) Gleason score.

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Materials and Methods

Prostate biopsies of 13 patients suffering from 5+5 or 5+4 (Group 1) as well as 13 patients suffering from 3+3–3+4 (Group 2) Gleason score prostate carcinoma were investigated histologically. The primary indication of the biopsy was to confirm the diagnosis suggested by rectal digital examination and/or elevated prostate specific antigen (PSA)-level. The samples were used for immunohistochemical studies after informed consent of the patients and permission of the Local Ethical Committee.

Patients' age, PSA values at diagnosis and on average 29.96 (3–42) months after diagnosis, therapy, tumor–node–metastasis (TNM) stage and Gleason score are indicated in Tables 1 and 2. In addition to the routine H&E staining androgen receptor, p21, p27 and p63 expression was investigated using immunoperoxidase reactions.

The needle biopsy samples were fixed in 4% neutral-buffered formalin, embedded into paraffin. Five μm thick sections were cut and stained with H&E, and proceeded for immunoperoxidase staining. Monoclonal mouse primary antibodies against human-p21, -androgen receptor (Dako, Glostrup, Denmark), -p27 (Novocastra, Newcastle upon Tyne, UK) and p63 (BioGenex, San Reno, CA, USA) antigens were used in 1:100 dilutions at 37°C overnight. Heat induced antigen/epitope retrieval before immunostaining included microwaving of dewaxed sections in TRS buffer (pH 6, Dako) at 400 W power for 30 min. Endogenous peroxidases were inactivated in methanol-H_2O_2. Ready-to-use EnVision Plus horseradish peroxidase conjugate was used for 30 min incubation to detect primary antibodies and the reaction product was developed using diaminobenzidine-H_2O_2 chromogen-substrate system.

AR, p21, p27 and p63 indices were established by analyzing 1000 tumor cells. A tumor was considered positive for AR and all other gene products if more than 50% of the tumor cells showed obvious staining even if it was of moderate intensity.

Results

All tumors were adenocarcinomas, one of them in group1 was mucin producing. The age of the patients and the clinical data are shown in Tables 1 and 2. Thirteen prostate carcinomas were of 5+5 or 5+4 (Group 1) and 13 of 3+3–3+4 (Group 2) Gleason score.

Group 1

Elevated or highly elevated PSA values were found at the time of diagnosis, which markedly decreased upon therapy, except in one case. Five patients died on average 14.6 (3–28) months after diagnosis six patients are still alive on average 37 (31–42) months after diagnosis and no data about two patients are available. The TNM staging shown in the tables is that observed at the time of diagnosis. Six patients received complete androgen blockade (CAB). Radical prostatectomy was carried out in three cases, amended with CAB and bicalutamide therapy in one case, with CAB in another case, and with bicalutamide in the

Table 1 Group I. age, PSA value, Gleason score, TNM stage, therapy, clinical status, AR, $p21^{waf1/cip1}$, p63, and $p27^{kip1}$ expression of patients with high Gleason score

No	Age (years)	PSA cc at the time of biopsy (ng/ml)	Last PSA level (ng/ml)	TNM	Therapy	AR	p21	p63	p27	Gleason-score	Status (months after biopsy)
1	77	15.6	7.6	T2cN0M0	CAB	+	−	−	+	5+5	Alive, 42
2	78	500	92.4	T3aNxM1b	CAB	+	+	−	+	4+5	Bone metastasis, died, 13
3	62	2116	504	T4NxM1b	Estramustine	+	−	−	−	4+5	Bone metastasis, died, 28
4	81	30.5	N. D.	T3aNxM0	Castration	+	−	−	+	5+5	N. D.
5	75	250	15.4	T3aN1M1b	Castration, estramustine, bisphosphonate	+	−	−	+	5+5	Died, 10
6	64	50	0.1	T2bNxM0	rad. prost., bicalutamide, CAB	+	−	−	−	4+5	Alive, 37
7	61	17	0.0	T3aN0M0	rad. prost., CAB	+	−	−	−	5+5	Alive, 39
8	74	118	76	pT2cNxM0	Castration	+	−	−	+	5+5	Alive, 37
9	64	27.1	0.3	T3bN0M0	rad. prost., bicalutamide,	+	−	−	−	−	4+5	Alive, 36
10	65	89	60	T2cNxM1b	Estramustine	+	−	−	+	4+5	Died, 19
11	72	133	8.9	T2cNxM0	CAB	+	−	−	−	5+5	Alive, 31
12	74	1000	N. D.	T2cNxM0	Castration	+	−	−	−	4+5	N. D.
13	77	38	38	T2cNxM0	CAB	−	−	−	+	5+5	Died, 3

Table 2 Group 2. Age, PSA value, Gleason score, TNM stage, therapy, clinical status, AR, p21[waf1/cip1], p63, and p27[kip1] expression of patients with low Gleason score

No	Age (years)	PSA cc at the time of biopsy (ng/ml)	Last PSA level (ng/ml)	TNM	Therapy	AR	p21	p63	p27	Gleason-score	Status (months after biopsy)
1	78	39.4	0.0	T2bNxM0	Monoantiandrogen, castration	+	−	−	+	3+3	Alive, 37
2	65	12.7	0.0	T1cN0M0	rad. prost.	+	−	−	+	3+3	Alive, 35
3	62	16.1	0.0	T2cN0M0	rad. prost.	−	−	−	+	3+3	Alive, 41
4	71	131.4	3.7	T3aNxM1b	CAB	+	−	−	+	3+4	Bone metastasis, alive 32
5	80	173.9	0.0	T3aNxM0	CAB	+	−	−	+	3+3	Alive, 38
6	60	25.5	1.6	T2aN0M0	rad. prost.	+	−	−	+	3+3	Alive, 30 Local recurrence
7	68	102.9	11.2	T2bNxM0	CAB	+	−	−	+	3+4	Alive, 36
8	77	16.2	7.5	T2aNxM0	CAB	+	−	−	+	3+3	Alive, 40
9	68	50	2.7	T3bNxM1b	Castration	+	−	−	+	3+4	Died, 20
10	71	5.3	0.0	pT2bN0M0	rad. prost.	+	−	−	+	3+3	Alive, 31
11	83	14.5	0.1	T3aNxM0	CAB	+	−	−	+	3+3	Died, 18
12	76	148.1	0.3	T3bNxM0	Monoantiandrogen	+	−	−	+	3+3	Alive, 31
13	56	4.6	0.8	pT2cNxM0	rad. prost.	+	−	−	+	3+3	Alive, 35

CAB complete androgen blockade, *rad. prost.* radical prostatectomy

third case. In three cases castration, in another two cases estramustine treatment was the only therapy applied. One patient was treated with castration, estramustine and bisphosphonate.

The prostate carcinoma cells of all but one patient were androgen receptor positive. The patient with AR negative tumor died 3 months after the biopsy.

All tumors were p63 negative. P63 positivity was found in the cells of normal or hyperplastic glands, occasionally as a part of biopsy samples containing tumor tissue as well. p21 positivity was only found in the mucin-producing adenocarcinoma in our material.

p27 positivity was detected in seven of 13 cases (Fig. 1), four of whom died during the follow up period, two are still alive and we have no data from one patient. One of the patients in this group was AR negative (Table 1).

Group 2

Moderately or highly elevated PSA levels were found at the time of diagnosis, which were significantly decreased upon therapy. Two patient died 18 and 20 months after diagnosis. Eleven patients are still alive 35 (30–41) months after diagnosis. The TNM staging showed T1c–3bNxM0–1b at

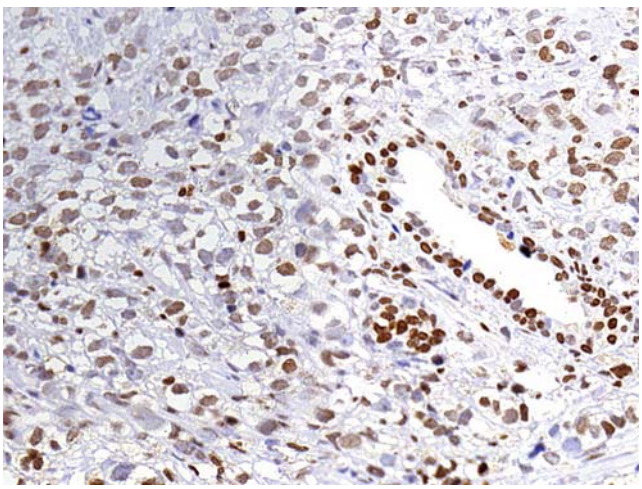

Fig. 1 Weak nuclear immunostaining for p27 antigen in Gleason score 5+5 prostate adenocarcinoma cells. Note the strong staining in the cell nuclei of a pre-existing duct on the *right* (p27 immunoperoxidase, ×600)

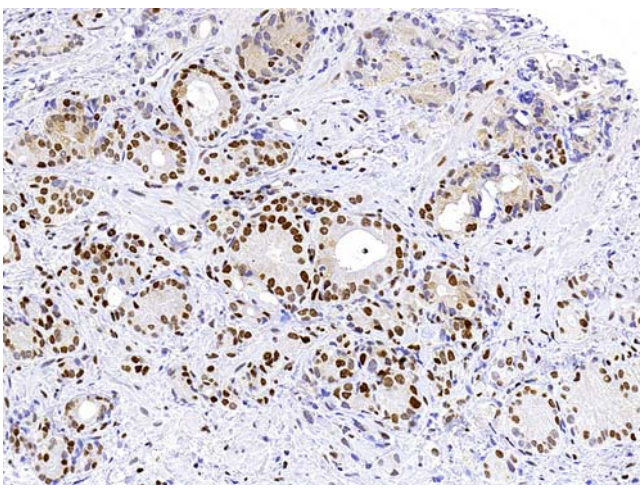

Fig. 2 Moderate to strong nuclear immunostaining for p27 antigen in Gleason score 3+4 prostate adenocarcinoma cells (p27 immunoperoxidase, ×300)

the time of diagnosis. Five patients received complete androgen blockade. Radical prostatectomy was applied to five patients, monoantiandrogen to one patient alone and monoantiandrogen+castration in one and one patient was castrated.

Androgen receptor positivity was found in 12 cases and only one prostate carcinoma was AR negative. Like in group 1 all tumors were negative for p21 and p63, but all studied cases in this group were positive for p27 protein (Table 2; Fig. 2)

Discussion

Compared to normal prostate epithelium several genes are expressed differentially in prostate carcinoma. Genes playing a role in signal transduction (c-myc, HER-2/neu) and apoptosis (fas, bcl2) are usually up-regulated, whereas tumor-suppressor genes like p53 and p27 show down-regulation. The low expression of tumor-suppressor genes, together with decreased apoptotic and increased proliferative activity, may be considered as predictor of unfavorable outcome of the disease [7, 8]. Experimental studies showed that castration-induced atrophy was associated with an increase in p27 expression and androgen treatment decreased p27 levels [9]. Also, p27 can mediate growth controlling effects in tumor cell lines e.g. that exerted by forced connexin43 expression [10, 11]. Gorbe et al. (2005) detected evenly high levels of both p27 and p21 proteins in aligned myoblasts, which left cell cycle just preceding fusion [12].

P63 is essential for prostate development and is selectively expressed in adult prostate basal cells. However, p63 expression is lost in prostate carcinoma [13].

The action of the cell senescence inhibitor p21 is based on the inhibition of cyclin dependent kinases which mediate the expression of tumor-promoting factors [14, 15]. Upregulation of p21 has been shown to play an important role in promoting myoblast differentiation, proliferation and viability [16]. Accelerated senescence leads to decreased ability of cell proliferation. Increased p21 activity seems to counteract this process. Antiandrogen treatment appears to suppress the activity of p21 [17].

All tumors in our series were p63 negative, in accordance with previous studies [18].

Similarly, no p21 nuclear positivity could be detected in the tumor cells in either studied groups, except one, mucin-producing adenocarcinoma.

Androgen receptor positivity was found in nearly all prostate carcinomas, except of two cases. No difference was observed for androgen receptor expression between the two groups we studied.

However, difference between the high Gleason score (Group 1) and low Gleason score (Group 2) tumors occurred regarding nuclear positivity for p27. Only seven of 13 tumors showing high Gleason score showed positive reaction for $p27^{kip1}$, whereas all low-grade tumors were $p27^{kip1}$ positive. Cordon-Cardo et al. [19] observed distinct altered patterns of $p27^{kip1}$ gene expression in benign prostatic hyperplasia and prostatic carcinoma already in 1998. Correlation between loss of p27 protein in prostate cancer and tumor grade was reported by Guo et al. [20]. Kuczyk et al. [21] attributed predictive value to the decrease of p27 expression for the recurrence-free and long-term survival of prostate cancer patients. Recently, Doganavsargil et al. [22] have shown that higher p21 and lower p27 expression is correlated with adverse prognostic factors in case of prostate carcinoma.

In this study, the low to high grade progression of prostate adenocarcinoma was accompanied with the down-regulation of $p27^{kip1}$ protein, which may be an important molecular sign of the lost cell cycle control. Regarding differential diagnosis, neither positivity nor negativity for nuclear $p27^{kip1}$ protein exclude or prove the existence of prostate carcinoma.

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