

Cyclin-D1 protooncogen expression in prostate cancer

EMİN ÖZBEK¹, BÜLENT MIZRAK², MUSTAFA ÖZBEK³,
SÜLEYMAN BÜYÜKBERBER⁴, MÜRSEL DAVARCI¹

Departments of ¹Urology, ²Pathology and ⁴Internal Medicine, İnönü University
School of Medicine, Malatya-Turkey, ³Department of Internal Medicine,
Dışkapı Social Security Hospital, Ankara-Turkey

Cyclin D1, a cell cycle regulator essential for G1 phase progression, has been implicated in the pathogenesis of certain cancers. High levels of these proteins have been reported in certain human malignancies and have been implicated in aberrant cell division and dysregulated tumor growth. In this study, 30 prostate cancer and 25 benign prostate tissues were studied for cyclin D1 expression using an immunohistochemical technique. All of the primary prostate cancer samples revealed regions of moderate to strongly positive nuclear staining for cyclin D1, however there was no nuclear staining in the benign prostate tissue except weak staining in the stroma. There was a positive correlation between Gleason grade and staining intensity for cyclin D1. The increased expression of cyclin D1 in prostate cancer samples suggests that further studies on the expression of this gene and related genes may be of interest in understanding the pathogenesis of prostate cancer. The positive correlation between Gleason grade and protein expression may be used as a prognostic marker in prostate cancer. Further studies are needed to confirm this suggestion. [Turk J Cancer 2000;30(1):15-23]

Key words: prostate cancer, cyclin D1, immunohistochemistry

Prostate cancer, like many other types of solid tumors, is expected to arise through a series of genetic changes that lead to tumor progression. The identification of the molecular events underlying cell transformation may not only expand our understanding of the natural history of the disease but may also present useful prognostic markers and potential target for therapy.

The neoplastic transformation of prostate cells is a complex process possibly involving the alteration of protooncogenes to oncogenes, inactivation of tumor suppressor gene products such as p53 and pRB (retinoblastoma), and

activation/deletion of other critical genes involved in carcinogenesis (1,2).

Recent progress in the field of cell cycle regulation has allowed us to have more comprehensive knowledge of the cell proliferative mechanisms. The cell cycle is now known to be tightly regulated by several regulatory elements such as cyclins and their associated kinases called cyclin-dependent kinases (cdk's), oncogenes and tumor suppressor gene products (3). It has been also reported that the cell cycle might be modulated at two checkpoints, namely the G1/S and G2/M transitions, where cell cycle progression can be arrested (4). Cyclins are thought to be essential proteins in cell cycle regulation because of their specific and periodic expression during cell cycle progression (5). Binding of the cyclins with cyclin-dependent kinase regulates their activity and contributes to cell cycle regulation (6). As a G1 cyclin, cyclin D1 was identified originally as a putative proto-oncogene, located at chromosome 11q13 as a suppressor of yeast G1 cyclin mutations and as a delayed early response gene induced by colony-stimulating factor 1 (3). Cyclin D1 overexpression has been the subject of several studies, but only few clinical studies on cyclin D1 overexpression have been described (7-10). The aim of this study was to detect the expression of cyclin D1 in primary prostate cancer in different grades and compare the results with benign prostate tissue.

Materials and Methods

A total of 30 primary prostate cancers and benign prostate tissues from 25 patients were examined (mean age 64, range 52-74 years). Cancer tissues were obtained from transrectal biopsy specimen and radical prostatectomy materials and benign prostate hyperplasia (BPH) tissues from transurethral prostatectomy materials. The tumors were graded according to the Gleason's grading system; briefly Gleason 2-4 as well differentiated, Gleason 5-7 moderate and Gleason 8-10 undifferentiated cancer. TNM staging system was used for clinical staging. Serum PSA levels were measured by Chemiluminescence method (Immulite, DPC, CA, USA) (11). The histopathological characteristics, TNM staging and serum PSA levels are summarized in table 1.

All specimens were fixed in 10% buffered formalin for about 24 hours, embedded in paraffin and 5 μ m thick sections were then deparaffinized. After the sections were heated with a microwave oven (containing 0.01 M sodium citrate buffer pH 6.0; 800W), the sections were washed three times with cold 0.5 M tris-buffered saline (TBS). Inhibition of non-specific binding was accomplished by incubation with normal goat serum (Dako, Carpinteria, CA, USA) for 20 minutes. Monoclonal mouse antihuman cyclin D1 oncoprotein antibody (1:100 dilution, Calbiochem, USA) was applied and incubated for one hour. At room temperature an alkaline phosphatase kit was used during link and labeling (Supersensitive, Biogenex). They were then stained with fast red and counterstained with Mayer's haematoxylin. Negative control sections were obtained by incubation with phosphate-buffered saline (PBS) instead of monoclonal cyclin D1 antibody. Staining intensity was assessed as follows: (-), no cancer cells or less than 5% of the cancer cells showed weak or ambiguous staining; (+), less than 50% of the cancer cells were stained; (++) more than 50% of the cancer cells showed positive or strongly positive staining (7,12).

Table 1
Gleason's grade, TNM staging and total serum PSA levels of
patients with prostate cancer

Patient no	Gleason's Grade	TNM staging	PSA (ng/ml)	CD1 staining
1	2	T _{1a} N ₀ M ₀	87	+
2	4	T _{2a} N ₀ M ₀	15	+
3	3	T _{1b} N ₀ M ₀	9	+
4	3	T _{1c} N ₀ M ₀	19	+
5	2	T _{1a} N ₀ M ₀	6	+
6	4	T _{2a} N ₀ M ₀	28	+
7	4	T _{2c} N ₀ M ₀	37	+
8	3	T _{3c} N ₀ M ₀	52	++
9	5	T _{1b} N ₀ M ₀	12	+
10	7	T _{2c} N ₀ M ₀	21	+
11	6	T _{1c} N ₀ M ₀	11	+
12	6	T _{2a} N ₀ M ₀	23	++
13	7	T _{3c} N ₂ M ₁	35	+
14	5	T _{2b} N ₂ M ₁	23	+
15	5	T _{3b} N ₁ M ₀	42	++
16	6	T _{3c} N ₁ M ₀	57	++
17	7	T _{3c} N ₁ M ₀	42	+
18	5	T _{2c} N ₀ M ₀	36	++
19	6	T _{2b} N ₀ M ₀	19	+
20	6	T _{2a} N ₀ M ₀	17	+
21	9	T _{3b} N ₁ M ₀	46	++
22	8	T _{3a} N ₁ M ₀	38	+
23	10	T ₄ N ₃ M ₊	80	++
24	10	T _{3c} N ₂ M ₊	67	++
25	9	T _{3b} N ₁ M ₊	72	++
26	9	T _{1c} N ₀ M ₀	17	+
27	9	T _{3c} N ₂ M ₊	45	++
28	8	T _{3b} N ₁ M ₀	29	++
29	10	T ₄ N ₃ M ₊	92	++
30	8	T ₄ N ₃ M ₊	32	++

Results

There was no nuclear staining in the epithelial cells of benign prostate tissue, which was used as a control, but weak staining (+) in the stroma was observed (Figure 1).

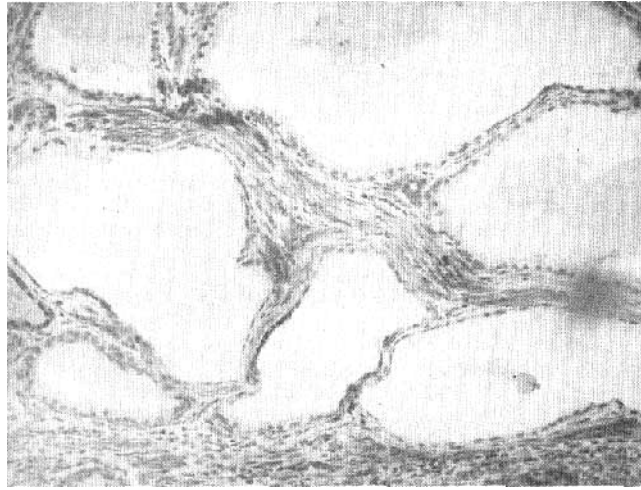
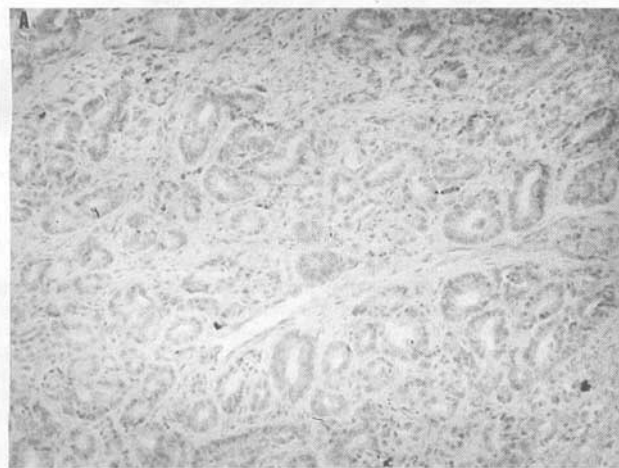


Fig 1. Weak (+) immunohistochemical staining of cyclin D1 in benign prostate tissue. Notice the specific and weak positive stromal staining of the benign tissue and the absence of staining in the nucleus (x200)

Cyclin D1 was positive in all of 30 prostate cancers (100%) and showed a trend of high Gleason's grade (Figures 2A, B, C).



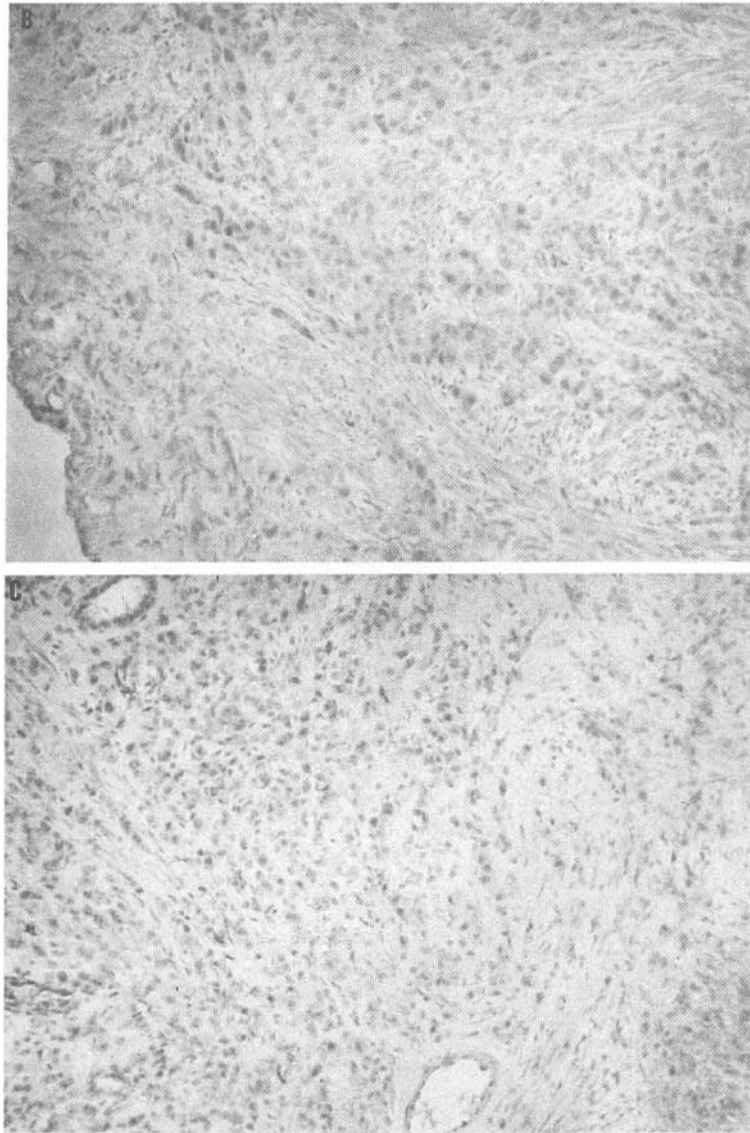


Fig 2. Examples of cyclin D1 expression immunohistochemically stained by the monoclonal mouse antihuman cyclin D1 oncoprotein antibody in well (A), moderately (B) and undifferentiated (C) prostate cancer. Notice the specific nuclear staining and weak stromal staining in the adjacent benign tissue. In cancer tissue there was a positive correlation between staining intensity and tumor Gleason's grade (x200)

There was also positive correlation between clinical staging and CD1 immunoreactivity (Table 1). In the majority of prostate cancers expressing cyclin D1, the adjacent benign prostate acini showed focal, scattered stromal positivity of varying degrees from (+) to (++) staining. Results were shown in tables 1 and 2.

Table 2
Comparison of cyclin D1 expression and Gleason grade in 30 prostate cancer patients

Gleason Grade	n	CD1- (%)	CD1+ (%)	CD1++ (%)
2-4	8	0 (0)	7 (80.75)	1 (19.25)
5-7	12	0 (0)	8 (66.6)	4 (33.3)
8-10	10	0 (0)	2 (20.0)	8 (80.0)

Discussion

Cyclins are involved in the regulation of the cell cycle progression. Overexpression of cyclin D1 is known to shorten the G1-S transition and thus it promotes cell progression and differentiation (13). In many types of human tumor cells, cyclin D1 is deregulated and overexpression of cyclin D1 contributes to oncogenic transformation of cells *in vitro* and *in vivo* (3,14-16). The cyclin D1 gene is amplified and/or overexpressed in several types of human cancers, including cancers of the breast, esophagus and head and neck. However, the role of cyclin D1 in prostate cancer has not been previously studied in detail. In the last one year a few studies have been reported regarding the role of cyclin D1 expression in prostate cancer. Chen et al. (17) reported that overexpression of cyclin D1 increases cell growth and tumorigenicity in human prostate LNCaP cells. In their study, in order to investigate factors involved in human prostate cancer progression, authors studied the effects of cyclin D1 overexpression on human prostate cancer cell proliferation and tumorigenicity by transfecting LNCaP cells with a retroviral vector containing human cyclin D1 cDNA. When compared to the parental and control-vector transfected LNCaP cells, these cyclin D1 transfected cells had more cells in S-shape and lower growth factor requirements. Furthermore these cells grew more in androgen-free medium. Also these authors reported that these tumors were refractory to the androgen-ablation treatment by castration, whereas tumors from parental and vector-control LNCaP cells regressed within 4 weeks after castration. As a result, authors conclude that overexpression of cyclin D1 changes the growth properties, increases tumorigenicity and decreases the requirement for androgen stimulation in LNCaP cells both *in vitro* and *in vivo* (17).

Comparative studies of cyclin D1 expression and clinical features revealed a significant correlation between poor prognosis and overexpression of the cyclin

D1 antigen in certain tumor cells including pancreas, breast and esophageal carcinoma (10,16,17). Gansuage *et al.* (18) reported that cyclin D1 overexpression in tumor cells was associated with shortened survival and may reflect the aggressiveness of pancreatic cancer. Shin *et al.* (12) reported that overexpression of cyclin D1 correlates with early recurrence in superficial bladder cancers. Kallakury *et al.* (19) found cyclin D1 positivity in 31 of 140 prostate cancers (22%) and showed a trend of high Gleason's grade, but it did not reach statistical significance with any of the prognostic variables. Han *et al.* (20) reported the moderate to strongly positive staining for cyclin D1 in twelve of the 50 primary prostate cancer samples (24%) and relatively high levels in all of the six human prostate cancer cell lines examined, but not detected in the cultures of normal human prostate cells. In our study, we found similar results, as positive staining was correlated with high Gleason's grade. We also found cyclin D1 positivity in all patients (100%), but great proportion of staining intensity was low grade and there was a positive correlation between staining intensity and Gleason's grade (Table 1). There was no staining in the epithelial cells of benign prostate tissues, but there was relatively low levels of staining in the stroma.

Perry *et al.* (21) indicated that one of the mechanisms by which growth factors such as EGF may stimulate prostate cell proliferation is through the direct induction of cyclin proteins, which are necessary for entry of cells into mitosis.

Recent evidence suggests that amplification of the 11q13 region is involved in a variety of human tumors including bladder carcinoma, head and neck squamous cell carcinoma and carcinomas of the esophagus and breast (5,8,21,22). In the amplified 11q13 region, several genes have been identified, of which cyclin D1 is most consistently amplified and overexpressed (24). Recent several studies have addressed the clinical and prognostic significance of amplification of 11q13 loci. Amplification of cyclin D1 appears to be correlated with poor prognosis in breast carcinoma, with lymph node involvement and recurrence in head and neck squamous cell carcinoma (22,25). To our knowledge there is no study in the literature regarding the gene amplification in prostate cancer. If this study were made using more and different primary prostate cancer patients showing amplification of the 11q13 region, cyclin D1 overexpression may be used as a molecular prognostic marker in prostate cancer.

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