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EXPRESSION OF E-CADHERIN, COX-2, P53 AND BCL-2 IN PROSTATE CARCINOMAS: CORRELATION WITH TUMOR DIFFERENTIATION AND METASTATIC POTENTIAL

S. Ozekinci[1], A.K. Uzunlar[2], S. Senturk[3], Abdullah Gedik[4], H. Buyukbayram[1], B. Mizrak[1]
[1]Dicle University, Medical Faculty, Departments of Pathology, Diyarbakir, Turkey
[2]Duzce University, Medical Faculty, Departments of Pathology, Duzce, Turkey
[3]Dicle University, Medical Faculty, Departments of Radiology, Diyarbakir, Turkey
[4]Dicle University, Medical Faculty, Departments of Urology, Diyarbakir, Turkey
Correspondence to: Selver Ozekinci
E-mail: drselver@gmail.com

ABSTRACT

Prognostic factors of prostate cancer such as PSA, stage, Gleason grade, and surgical margins have limitations; they are inable to indicate metastatic potential. The aim of this study was to investigate expression of e-cadherin, cox-2, p53 and bcl-2 in prostate carcinomas, and to search association with tumor differentiation and metastatic potential.

Eighty-seven prostate cancer specimens were retreived from tissue blocks embedded in parafin, and expression patterns of e-cadherin, cox-2, p53 and bcl-2 were investigated by immunoperoxidase method. Gleason scoring system was used for tumor grading, and expression patterns of these antibodies were compared among different tumor grades.

Seven cases (8%) had well-differentiated, 39 (45%) had moderately differentiated, and 41 (47%) had poorly differentiated tumors. Metastases were present in 31 patients (35%). There was a statistically significant association between tumor differentiation and expression of e-cadherin or bcl-2 ($p<0.01$); however no association was found between tumor differentiation and expression of cox-2 or p53 ($p>0.01$). There was no association between expressions of e-cadherin, cox-2, p53 or bcl-2 and metastasis.

E-cadherin expression is less frequent in poorly differentiated tumors; whereas bcl-2 expression is more frequent. However there is no relation with metastatic potential and expression of these markers. Expression of cox-2 and p53 is neither related to the degree of differentiation nor to the metastatic potential.

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Keywords: prostate carcinoma; tumor differentiation; metastasis; immunohistochemistry

Introduction

Prostate carcinoma is the most frequently diagnosed cancer in men in developed countries (13). Serum level of prostate-spesific antigen (PSA) is useful in early detection of prostate cancer and metastasis, however serum PSA levels may also increase in non-malignant prostatic diseases, which is not always satisfactory (2). Currently used prognostic factors such as preoperative PSA, stage, Gleason grade and surgical margins have limitations, and they are not sufficient to indicate metastatic potential of the tumor (21).

E-cadherin, a cell surface glycoprotein in epithelial tissues, is responsible for calcium-dependent cell to cell adhesion. Its basic role is to induce and maintain polarization and organisation of normal epithelium (8, 31). Disorders of epithelial cell adhesion are significant in tumor progression, and they may cause development of more invasive and metastatic phenotypes (29).

Cyclooxygenase (Cox), also known as prostoglandin endoperoxide synthase, is the major enzyme that catalyzes formation of prostoglandin and other eicosanoids from arachidonic acid. The enzyme has two isoforms: cox-1 and cox-2 (6). Cox-2 is synthetised primarily at inflammatory areas under the effect of mitogens, cytokines and growth factors (35). Cox-2 expression is increased in prostate carcinoma, and cox-2 is believed to have an important role in the development and progression of prostate carcinomas and other solid tumor malignancies (37).

P53 is a tumor suppressor gene, also known as the guardian of the genome (8). It plays a critical role in the control of the cell cycle. It mediates the expression of genes involved in cell growth, proliferation and differentiation as a transcription factor (25). Though p53 mutations are not common in prostate carcinomas, inactivation of p53 accelerates tumor progression (11). Mutations of p53 are more common in prostate cancers at a higher tumor stage, higher tumor grade, metastases, or androgen-independent tumors, however the role of p53 in prostate cancer is not very clear (25).

Bcl-2 gene has been initially identified in B-cell leukemia and lymphomas, and the product of the gene inhibits apoptosis. It is expressed at the basal epithelial cells of nonneoplastic prostate tissue (10). Some reports suggest that overexpression of bcl-2 positively correlates with higher tumor stage (4), and it accompanies hormone-resistant disease (1).

The aim of this study is to investigate expression of e-cadherin, cox-2, p53 and bcl-2 in prostate carcinoma specimens and to search for association between these proteins and tumor differentiation or metastatic potential.

Materials and Methods

Patients

Eighty-seven consecutive prostate cancer specimens, obtained from patients who were diagnosed to have prostate cancer in the department of pathology of our institution between 2004 and 2007, were included in this study. Diagnoses of twenty three patients were obtained from prostatectomy materials. Twenty nine patients had transurethral prostatic resection, and 35 patients had needle biopsies. When multiple specimens were available, only the most diagnostic one was sustained. Since the patients were retrieved from pathology archives, and reports of these patients were not sufficient, prostate volume data could not be obtained.

Clinical records and radiologic examinations revealed metastasis in 31 prostate cancers. Metastases were detected within two years after the initial diagnosis.

Patients under 70 years in good general condition and without metastasis were treated by radical prostatectomy after needle biopsy or transurethral prostatic resection. Patients with metastasis and patients that could not be operated were treated by antiandrogens and/or radiotherapy.

Immunohistochemistry

Four μm thick sections were cut from formaldehyde-fixed, parafin-embedded archival tissue blocks. One of the sections was stained with hematoxyline-eosin, and the tumor was graded according to Gleason scoring system. For immunohistochemistry, the sections were picked up on positive charged slides and dewaxed at 70°C for one hour, and then immersed in xylene three times for 5 min. After that they were graded in alcohol three times for 5 min. Then, they were washed in distillated water, and antigen retrieval was carried on in a microwave processor. Sections were heated to 95°C in 0.01M sodium citrate buffer at pH 6.0 for 20 minutes. Incubation at room temperature with 3% hydrogen peroxyde for 10 min followed, and then sections were washed in distilled water two times for five minutes. Sections were incubated with primary antibodies (e-cadherin, cox-2, p53, bcl-2; ready-to-use form, Mouse anti human monoclonal, Labvision, Fremont, CA USA) for 60 minutes, then standard streptavidin-biotin system was used (HRP Detection System, Labvision). Sections were incubated in chromogen AEC and counterstained with hematoxyline, washed with tap water and mounted using an aqueous mounting medium. Positive control tissues used for immunohistochemistry were as follows: colon carcinoma for e-cadherin, skin for cox-2, gastric tissue for p53, and lymph node for bcl-2.

Evaluation

Gleason scoring system was used for tumor grading: scores 4-5 tumors were classified as well-differentiated; 6-7 as moderately differentiated; and 8-10 as poorly differentiated carcinomas (3).

Three patterns of membrane staining were observed with e-cadherin: diffuse positive, diffuse negative and heterogenous staining. Cytoplasmic staining was seen with cox-2 and bcl-2, and it was classified as strongly positive when >50% cells were positive; moderately positive, when <50% cells were positive, and negative (28). p53 nuclear staining was classified according to the percentage of positive cells: strongly positive, when >50% cells were positive; moderately positive, when 5-50% cells were positive; and negative, when <5% cells were positive (28).

SPSS 15.0 software was used for statistical evaluation, and the groups were compared with chi-square test.

Results and Discussion

Mean age of the patients in this series was 64, and varied from 49 to 82. Using Gleason grading system 7 carcinomas (8%) were classified as well differentiated, 39 (45%) as moderately, and 41 (47%) as poorly differentiated. Well differentiated 7 carcinomas (Gleason 4-5) were diagnosed from either prostatectomy materials (3 patients), or transurethral resection (4 patients). There were metastasis in 31 (36%) cases; out of these metastatic cases 2 had well-differentiated, 12 had moderately, and 17 had poorly differentiated carcinomas. **Table 1** shows the distribution of metastatic and non-metastatic cases according to Gleason grading system. No significant difference was found in the degree of tumor differentiation when comparing patients who had metastasis and who did not have metastasis ($p>0.01$).

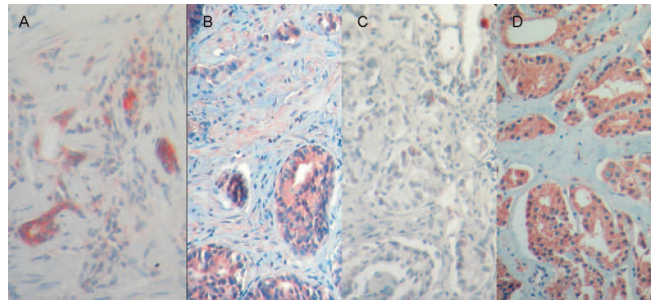

Fig. 1A. x400 Immunoperoxidase diffuse positive staining with e-cadherin

Fig. 1B. x400 Immunoperoxidase moderately positive staining with cox-2

Fig. 1C. x400 Immunoperoxidase modaretely positive staining with p53

Fig. 1D. x400 Immunoperoxidase diffuse positive staining with bcl-2

There was diffuse positive staining in 28 (32%), heterogenous staining in 29 (33%), and negative staining in 30 (35%) tumors, using antibodies to e-cadherin (**Fig. 1A**). Cox-2 staining was positive in 35 (40%) cases, heterogenous in 16 (16%), and negative in 36 (42%) cases (**Fig. 1B**). p53 antibody was negative in 54 (62%) patients, moderately positive (less than 50% of cells) in 15 (18%) cases, and strongly positive

TABLE 1

Distribution of metastatic and nonmetastatic cases according to Gleason grading system

Gleason score	Non-metastatic	Metastatic	Total n (%)
4-5 (well differentiated)	5	2	7 (8)
6-7 (modaretely differentiated)	27	12	39 (45)
8-10 (poorly differentiated)	24	17	41 (47)
Total	56	31	87 (100)

TABLE 2

Distribution of tumors with negative, moderate and strong positive expression of e-cadherin, cox-2, p53 and bcl-2 according to Gleason grading system

Antibody	Well differentiated	Moderately differentiated	Poorly differentiated	Total
e-cadherin[a]				
-	2	7	21	30
+	2	11	16	29
++	3	21	4	28
Cox-2[b]				
-	4	19	12	35
+	1	6	9	16
++	2	14	20	36
P53[c]				
-	5	22	27	54
+	1	8	6	15
++	1	19	8	18
Bcl-2[d]				
-	5	7	1	13
+	1	8	5	14
++	1	24	35	60

[a] membrane staining patterns with e-cadherin

-: diffuse negative staining; +: heterogenous staining; ++: diffuse positive staining

[b,d] cytoplasmic staining patterns with cox-2 and bcl-2

-: diffuse negative staining; +: <50% cells are positive; ++: >50% cells are positive

[c] nuclear staining patterns with p53

-: <5% cells are positive; +: 5-50% cells are positive; ++: >50% cells are positive

(more than 50% of cells) in 18 (21%) cases (**Fig. 1C**). Bcl-2 staining was negative in 13 (15%) cases, heterogenous in 14 (16%) cases, and diffusely positive in 60 (69%) cases (**Fig. 1D**). **Table 2** demonstrates the distribution of cases that had diffuse positive, heterogenous or moderately positive, and negative staining using antibodies to e-cadherin, cox-2, p53 and bcl-2, according to Gleason grading system.

The difference between frequencies of e-cadherin and bcl-2 expression in different tumor grades was statistically significant ($p<0.01$). There was no statistical difference between frequencies of cox-2 and p53 expression in different tumor grades ($p>0.01$).

The difference between frequencies of e-cadherin, cox-2, p53 and bcl-2 expression in metastatic and non-metastatic tumors was not statistically significant ($p>0.01$).

Prostate cancer is the most common malignancy among men, accounting for 29% of all cancer cases, and the second leading cause of cancer-related deaths in western countries (14). Localized carcinomas can be detected with rectal examination and elevated serum PSA levels (8). However, sometimes metastatic potential and prognosis is not related with PSA levels. Despite evaluation of numerous markers such as p53, Bcl-2, Ki-67 and e-cadherin, there is substantial variance between the findings of different studies (20).

E-cadherin is an adhesion molecule which has an important role on normal cell growth and development. It has been reported that when it becomes unregulated, cellular adhesion deteriorates and cells gain invasive properties (33). It has been suggested that e-cadherin inhibits tumor invasion and prevents metastasis (22). There are various reports that emphasize downregulation of e-cadherin expression in prostate carcinomas with loss of differentiation, higher stage and poor prognosis (5, 12). We also observed less frequent expression of e-cadherin in poorly differentiated tumors, supporting these findings. On the other hand some recent articles objected to this issue. Saha et al. (27) reported that loss of e-cadherin gene expression is caused by reversible epigenetic mechanisms, and showed that e-cadherin gene expression was present in metastatic bone lesions of prostate carcinoma. It was hypothesized that e-cadherin gene expression might be the last fact to provide advantage tumor cells to make clusters in metastatic areas (26). These recent studies raise doubt on the suppressor role of e-cadherin on metastasis and invasion.

In this study we observed a statitistically significant difference between immunoreactivity of e-cadherin and tumor differentiation. Although expression of e-cadherin was less frequent in poorly differentiated tumors, presence of heterogenous staining in high grade tumors and negativity or heterogenous staining in lower grade tumors, suggest that there may be different mechanisms of suppresor role of e-cadherin on invasion, or e-cadherin is temporarily downregulated. This study showed no statistically significant association between e-cadherin expression and metastasis, and this finding is compatible with those in the literature (33).

In prostate carcinomas cox-2 immunoreactivity varies from moderate to strong cytoplasmic staining (17), and strong positivity is also observed at blood vessels and stromal cells (9). It is believed that there is an important role of autocrine, paracrine growth factors and angiogenetic factors on progression of prostate carcinomas (18, 35). In tumorogenesis cox-2 acts as an inactivator of tumor supressor genes like p53 (30). It also acts as an angiogenetic agent (32). Moreover cox-2 increases cellular motility (17), and resistance to apoptosis (24). Zha et al. (36) suggest that there is no association betwen Gleason's grade and cox-2 immunostaining. In this study we observed heterogenous cytoplasmic staining in addition to stromal staining. We also did not find association betwen Gleason's grade and cox-2 immunostaining ($p>0.01$). The relation between cox-2 expression and prostate carcinoma may be complicated and it will become clear with larger series and using molecular methods.

Molecular biology of prostate carcinomas is rather complex (11). P53 and bcl-2 are among the markers which may predict metastatic behaviour. In general p53 mutations are seen in a low percentage of prostate carcinomas. Although tumors with higher grade, higher stage and androgen-independent tumors show overexpression, its mechanism is not clearly explained yet (7, 11, 15). There are conflicting reports in the literature on the role of p53; some suggest that it has prognostic value (23), while others report no independent prognostic importance (20, 34). In this study there was no statistically significant relation between p53 immunoreactivity and the degree of tumor differentiation or metastasis. Our finding is not compatible with literature findings which suggest that high stage tumors show overexpression of p53. This incompatibility may be explained by the relatively small number of cases in our series.

In prostate carcinomas overexpression of bcl-2 has also been shown (20). Overexpression of bcl-2 enhances growth of prostate carcinoma by helping tumor cells escape from apoptosis and augmenting angiogenesis (16). Weak staining with bcl-2 has been reported in organ-confined prostate carcinomas unlike e-cadherin and p53 (23). Some reports suggest that it is prognostically unuseful (19), while others paradoxically claim that it is a useful indicator (34). In our series there was statistically significant relation between expression of bcl-2 and tumor differentiation ($p<0.01$). Although higher grade tumors exhibited more staining there was no association between bcl-2 and metastatic behaviour ($p>0.01$).

Conclusions

Overexpression of e-cadherin and bcl-2 was observed more frequently in poorly differentiated prostate carcinomas. These findings support that e-cadherin and bcl-2 might cause loss of differentiation in prostate carcinoma, however they are not useful markers to predict metastatic potential. These results may be explained by different mechanisms on loss of tumor differentiation and gain of metastatic potential. Our results suggest that cox-2 and p53 are not independent markers of loss of differentiation or gain of metastatic behaviour.

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