Journal of Cell and Molecular Biology 3: 89-93, 2004.
Haliç University, Printed in Turkey.

E-cadherin immunohistochemistry for prostate cancer early diagnosis and monitoring of illness

Serdar Arisan*, Cem Sönmez, İbrahim Duman, Turhan Çaşkurlu, Ayhan Dalkılıç, Erbil Ergenekon

**Şişli Etfal Research and Training Hospital, 1st Urology Clinics, Şişli-Istanbul, Turkey (* author for correspondence)*

Received 7 June 2004; Accepted 25 June 2004

Abstract

Prostate cancer (pCA) is the most commonly diagnosed noncutaneous malignancy in men world wide. The natural history of prognostic factors involved in prostate cancer are not clearly defined. Hence, molecular parameters able to accurately assess the aggressiveness and the metastatic potential of the cancer are urgently needed. The E-cadherin gene, on chromosome 16q22 encodes for transmembran 120 kDa glycoprotein belonging to the group of Ca^{2+} depending homophilic cell adhesion molecules (CAMs). Loss of E-cadherin function in cancer cells likely plays at least two different roles in tumor development and progression. In this study after prostatectomy procedure, the prostate specimens were analyzed for E-cadherin activity and compare with Gleason scores. It is aimed to reveal the prognostic importance of E-cadherin molecules in pCA.

Keywords: e-cadherin, prostate cancer, metastasis

Prostat kanseri erken tanı ve hastalığın izlenmesinde e-cadherin immunohistokimya uygulanması

Özet

Prostat kanseri, hemen hemen en sık rastlanılan erkek kanserlerinden bir tanesi olup, çok yaygındır. Halen doğal prognostik faktörleri prostat kanseri için aydınlatılamamıştır. Bu yüzden acilen agresivite ve prognoz ile moleküler mekanizmaların bulunmasına ihtiyaç vardır. E-cadherin geni 16q22 kromozomu üzerinde 120kDa büyüklüğünde ve Ca^{2+} bağlı adezyon molekülleri ailesindendir. E-cadherinin fonksiyonel kaybı kanser hücrelerinde tumor gelişimi ve progresyonuna neden olur. Bu çalışmada prostatectomy sonrasında Gleason skorlamaları ve e-cadherin aktivitesi karşılaştırılmış ve prostat kanseri için E-cadherin moleküllerinin önemi ortaya konmaya çalışılmıştır.

Anahtar sözcükler: e-cadherin, prostat kanseri, metastaz

Introduction:

Prostate cancer (pCA) is the most commonly diagnosed noncutaneous malignancy in men in USA and also world wide (American Cancer Society, 2002; DeMarzo et al., 1999). Mortality in prostate carcinoma is associated with metastasis and metastasis in prostate carcinoma is usually associated with perineural invasion, for it is the preferred mechanism of prostate cancer metastasis. One of the current theory states this form of metastasis follows the path of least resistance offered by the perineural sheath. Understanding

specific mechanisms of this carcinoma/nerve interaction is key to potential therapeutics targeted to this process. Death from prostate cancer is the result of metastasis and local spread, not from organ-confined disease. Hence, understanding the mechanism of prostate cancer spread and metastasis is the key to treating this disease successfully and increasing survivability (Umbas et al., 1997; Yagi and Takeichi, 2000).

Currently, however, prostate spesific antigen (PSA) in combination with digital rectal examination (DRE) are undergoing evaluation as screening modalities for prostate cancer. Because of a lack of sensitivity and spesificity markers are rarely of use in early diagnosis of cancer. Also they can be used as monitoring disease evaluation with therapy. The goal of future research should be that development of the most specific, cheap and easy markers for common cancer types as prostate cancer (Gao et al., 1997; Buyuktuncer et al., 2003).

The natural history of prognostic factors involved in prostate cancer are not clearly defined. Hence, molecular parameters able to accurately assess the aggressiveness and the metastatic potential of the cancer are urgently needed (Arisan, 2003). Since vascular invasion and the spread of prostate tumour cells to the blood represent preliminary steps in the metastatic process, blood-borne detection of circulating prostate epithelial cells (CPC) could be an early marker of invasiveness (Tomita et al., 2000; Mason et al., 2002). One of the prominent features of the development and progression of prostate cancer is the development of abnormalities in cell adhesion in prostate epithelium and prostate cancer cells. These abnormalities extend to inter-cellular adhesion structures and cell–matrix adhesion molecules.

The E-cadherin gene, on chromosome 16q22 encodes for transmembran 120 kDa glycoprotein belonging to the group of Ca^{2+} depending homophilic cell adhesion molecules (CAMs). These molecules involved in developmental morphogenesis and maintenance of the epithelial phenotype and they are almost exclusively expressed in (adult) epithelial tissues. E-cadherin is bound *via* a series of undercoat proteins, the catenins to the actin cytoskeleton. This linkage between transmembranous cadherins and actin filaments of the cytoskeleton is necessary to form strong cell-cell adhesion. Furthermore, deletion of the a-catenin gene in *vitro* results in the inactivation of E-cadherin-mediated cell–cell adhesion in prostate cancer cells (Nathke et al., 1994). Dysfunction or mutation of β-catenin may also result in cell–cell disengagement, and in subsequent invasion (Vleminckx et al., 2001).

Loss of E-cadherin function in cancer cells likely plays at least two different roles in tumor development and progression. Most of the studies on E-cadherin and cancer have focussed on its role in suppression of metastasis. Loss of E-cadherin expression was demonstrated to be associated withincreased invasiveness in many human tumor types. Furthermore, re-expression of E-cadherin in cell culture results in reversion of the invasive phenotype (Poser et al., 2001; Chen CL et al., 2003). These results strongly suggest that E-cadherin plays a major role as a suppressor of invasion in human tumors.

Immunohistochemical studies in human cancers, including prostate cancer have requently shown that a proportion of invasive carcinomas and carcinomas *in situ* show aberrant levels of E-cadherin and/or catenin expression in comparison to their related normal tissue (El Hariry et al., 2001). In this study after prostatectomy procedure, the prostate specimens were analyzed for E-cadherin activity and compare with Gleason scores. It is aimed to reveal the prognostic importance of E-cadherin molecules in pCA.

Materials and methods

The study comprised 44 selected patients (median age= 68,9, range 48-74) treated in the Urology Department of Sisli Etfal Research and Training Hospital from 1995 to 2001. They were diagnosed as having clinically localized pCA. Before radical retropubic prostatectomy (RRP), distant metastases were excluded by abdominal CT, X-rays of the lungs and bone scans. The patients also were followed detailed anammes, physical examination, PSA amount, TRUS biopsy. None of the patients were received preoperatively *hormon* therapy or radiotherapy. RRP were followed up according to Walsh tecnique.

Tumor specimens were pathologically classified according to TNM system by the same pathologist. All specimens were classified 2 groups for Gleason score <7 and Gleason score>7 and followup ranged 6 to 84 months and included at 3 months postoperatively and over 0,4ng/ml PSA was accepted as nux. Specimens were selected to represent a spectrum of Gleason scores ranging from 2 to 10 with a wide spectrum of pathologic stages.

Pathological determination and immunohistochemistry

Formalin fixed, paraffin embedded archival tissues containing RRP specimens were obtained from Pathology Department of Sisli Etfal Research and Training Hospital.

After dewaxing, paraffin embedded sections were cut serially at 2-5 mm thick, respectively, and stained for E-cadherin. Endogeneous peroxidase activity was blocked by 10% hydrogen peroxide solution in methanol in 20 min. After rinsing in distiled water tumour bearind slides were kept in 10 mM citrate buffer (pH 6) four times for 5 min. They treated with blocking serum (Horse serum, NHS-Novacastra) for prevent of non-spesific binding. The spesific primer antibody (Novacastra 36B5) for the detection of E-cadherin was then added. The anti E-cadherin antibody was applied for 10 min. After rinsing with PBS for three times, standart streptavidin-peroxidase complex was used according to the manufacturer's instructions.

Classification of immunohistochemical responses: Depending on the percentage of cells showing membrane-bound positive immunohistochemical staining for E-cadherin, the tumours were classified into three groups, i.e. no staining <5% (negative distribution), 5-95% heteregenous distribution and over 95% is called as positive distribution.

Positive distribution was accepted as normal E-cadherin distribution, negative and heteregenous distribution called as abberant E-cadherin distribution.

Statistically all data were analyzed with SPSS statistical software. Fischer exact test and probability analyses were done in this study.

Results

In this study RRP operated 44 prostate cancer patiens were investigated pathologically and they were classified as pT2 (14 patients), pT2b (13 patients), pT3a (8 patients) and/or pT3b (9 patients). These classes were investigated for E-cadherin activity. 27 patients (61%) had lymph, vesicle seminalis and organ confined disease (pT2). 8 of the total patients (18%) had no lymph and vesicle seminalis invasion and they were classified as pT3a. 9 patients (21%) have no lymph and have seminal vesicle invasion (pT3b) (Table1).

Table 1: Patient profile

Patient Characteristics	
Patient number	44
Age	
Average	63 years
Range	48-78 years
Pre-op PSA levels	
Average	17.28 ng\ml
Range	4.14-41.4 ng\ml
PIN presented case number with adenocarcinoma	33 patients (73%)
Gleason score	
>7	6 patients (13%)
7	17 patients (39%)
<7	21 patients (48%)
Stages	
pT2a	14
pT2b	13
pT3a	8
pT3b	9
Followup period	
Average	31.6 months
Range	9-84 months
Recurrence according to post op PSA value	5 patients

The average age of the patients were 63.35, median was 64 and range is 48 to 78. The average of PSA levels of patients before operation was 17.28 ng/ml and median was 16.42 ng/ml and range was 4.14-41.4 ng/ml. Reinvestigated 33 of 44 specimens (73%) had adenocarcinoma and PIN. 6 patients (13%) Gleason score was higher than 7, 17 patients (39%) Gleason score was 7 and 21 patients (48%) was smaller than 7. The average survey was 31.6 months, median was 35 months and range was 9 to 84 months. 5 patients (10%) showed biochemical (PSA levels) nux in average 19.11 months, median 18 months and range 6 to 35 months. One of the patient died from lung metastasis at 38th month (Table 1).

pT2 classified 11 of 27 patients (41%) had normal E-cadherin distribution, 16 had (59%) aberrant distribution. pT3 classified 2 of 17 patients (11%) E-cadherin distributed normal and 15 patients (89%) had distribution aberrant. The results of pT2 and pT3 classified groups were not determined significant as statistically (p>0,05).

E-cadherin was distributed in 2 of 23 patients (8%) whose Gleason score was higher than 7 normally, 21 of

them showed aberrant distribution. Beside of these 7 of 21 patients (34%) whose Gleason score was smaller than 7 had normal distribution and 14 of them (66%) aberrant distribution. The results of the E-cadherin distribution due to Gleason Score classification were not significant as statistically (p>0,05).

6 of 17 patients (35%) which was classified as pT3 had Gleason Score smaller than 7 and 1 of them (17%) had normal E-cadherin distribution. 6 patients (83%) had aberrant E-cadherin distribution. 12 patients showed Gleason Score higher than 7 and one of them (8%) had normal E-cadherin distribution and 11 of the group (92%) had aberrant E-cadherin distribution. The results of the E-cadherin distribution due to Gleason Score classification were not significant as statistically (p>0,05).

The Gleason Score of pT2 classified 23 of 27 patients (82%) was smaller than 7 and 9 of them (39%) had normal E-cadherin distribution and 13 of the group (61%) had aberrant distribution. In the same classification 5 patients (18%) had higher Gleason Score than 7 and one of them (20%) had normal E-cadherin distribution and remain part of the group (80%) had aberrant E-cadherin distribution. In this comparion between E-cadherin distribution and Gleason Score level in pT2 was not significant as statistically (p>0,05) (Table 2).

Table 2: E-cadherin distribution according to stage and Gleason score rates.

Stage	E-cadherin distribution
pT2	11\27 patients (41 %)
7 and higher values of Gleason score	1\4 patients (25 %)
Gleason score smaller than 7	9\23 patients (39 %)
pT3	2\17 patients (11 %)
7 and higher values of Gleason score	1\11 patients (9 %)
Gleason score smaller than 7	1\17 patients (17 %)
E-cadherin aberrant distribution	
pT2	16\27 patients (59 %)
7 and higher values of Gleason score	3\4 patiens (75 %)
Gleason score smaller than 7	13\23 patients (61 %)
pT3	15\17 patiens (89 %)
7 and higher values of Gleason score	10\11 patients (91 %)
Gleason score smaller than 7	5\17 patients (83 %)
p>0.05	

After the RRP operation 5 patients (10%) had biochemically (PSA level) nux and all of these patients had aberrant E-cadherin distribution. One of the patients who showed recurrence had positive E-cadherin distribution but, it is accepted as aberrant for intracytoplasmic distribution (Figure 1).

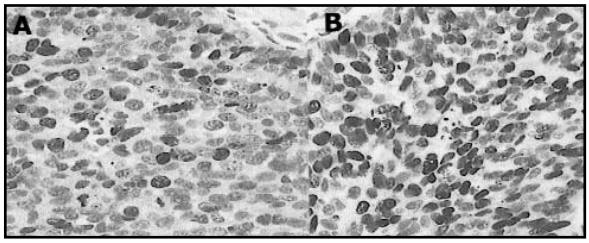


Figure 1: E-cadherin immunostaining in normal (A) and adenocarcinoma prostate (B) tissue.

Discussion

E-cadherin expression is a promising marker of prostate cancer progression that a large, controlled clinical trial or the marker is warranted. Abnormal expression of E-cadherin has been correlated in several human carcinomas with the pathological characteristics of the tumor, such as tumor stage, grade of differentiation, invasiveness, lymph node involvement and distant metastases (Yagi and Takechi, 2000). E-cadherin expression in the primary tumor might be a powerful tool in clinical practice as a marker to predict the metastatic potential in individual patients with prostate. Of course, other factors like histological grade of disease can be detected by E-cadherins (Hirohashi, 1998).

E-cadherin expression correlates with epithelial differentiation whereas loss of E-cadherin expression promotes epithelial dedifferentiation and invasiveness of human carcinoma cells (Jiang et al., 1994). Restoration of wild-type E-cadherin function prevents invasiveness of epithelial tumour cells (Vleminckx et al., 1991). Loss of expression of E-cadherin at chromosome band 16q22 may be associated with a biallelic mutation mechanism characteristic of tumor suppressor gene inactivation. Downregulation of E-cadherin expression is often accompanied by methylation of 5' CpG island of E-cadherin in lung, liver, bladder and gastric carcinoma cell lines, further suggesting that the mechanisms by which E-cadherin

is inactivated in cancer are analogous to those observed for classical tumour suppressor genes (Buyuktuncer et al., 2002). Some studies showed that in human prostate cancer, E-cadherin immunostaining was reduced or absent in 46 of 92 primary and metastatic cases whereas benign non-malignant tissue stained uniformily positive (De Marzo et al., 1999).

In another study decreased E-cadherin immunostaining was correlated with increased invasive potential: a spectrum of staining was found between well-differentiated tumours (Gleason score 4) and undifferentiated tumours (Gleason score > 10) which stained positive and negative, respectively. Between these two extremes, mixed populations of E-cadherin positive and E-cadherin negative cells were associated with moderately differentiated (Gleason score 5–7) and poorly differentiated (Gleason 8–9) tumours (De Marzo et al., 1999; Umbas et al., 1997).

In conclusion, classical prognostic factors are not enough for true determination in prostate cancer studies. Therefore more reliable markers are needed for clinical usage. E-cadherin is one of the most studied molecules for this reason. Distribution of e-cadherin status of patients can show a new approach for prognosis of illness.

References

American Cancer Society: Cancer Facts and Figures-2002. Atlanta, Ga: American Cancer Society, 2002

Arısan S. Prostate cancer and importance of tumor marker studies. *J of Cell and Mol Biol.* 1: 49-51, 2003.

Buyuktuncer D, Arisan S, Ozdilli K. Ecadherin molecular mechanism in prostate cancer. *J of Cell and Mol Biol.* 2: 57-64, 2003.

Cano AMA, Perez-Moreno I, Rodrigo A, Locascio MJ, Blanco MG, del Barrio F, Portillo MA, Nieto. The transcription factor snail controls epithelial mesenchymal transitions by repressing E-cadherin expression. *Nat Cell Biol.* 2: 76–83, 2000.

Chen CL, Liu SS, Ip S-M. E-cadherin expression is silenced by DNA methylation in cervical cancer cell lines and tumours. *Eur J Cancer.* 2: S0959 8049(02)00175-2, 2003.

DeMarzo AM, Knudsen B, Chan-Tack K and Epstein JI. E-cadherin expression as a marker of tumor aggressiveness in routinely processed radical prostatectomy specimens. *Urology.* 53: 707–713, 1999.

El-Hariry I, Pignatelli M, Lemoine NR. FGF-1 and FGF-2 modulate the E-cadherin/catenin system in pancreatic adenocarcinoma cell lines. *Br J Cancer.* 84: 1656–1663, 2001.

Gao X, Porter AT, Grignon DJ, Diagnostic and prognostic markers for human prostate cancer. *Prostate.* 31: 264–281, 1997.

Hirohashi S. Inactivation of the E-cadherin-mediated cell adhesion system in human cancers. *Am J Pathol.* 153: 333–339,1998.

Jiang WG, Puntis MCA, Hallett MB. The molecular and cellular basis of cancer invasion and metastasis and its implications for treatment. *Br J Surg.* 81: 1576–90, 1994.

Mason MD, Davies G, Jiang WG, Cell adhesion molecules and adhesion abnormalities in prostate cancer, *Critical Reviews in Oncology/Hematology.* 41: 1–28, 2002.

Nathke IS, Hinck L, Swedlow JR, Papkoff J and Nelson WJ. Defining interactions and distributions of cadherin and catenin complexes in polarized epithelial cells. *J Cell Biol.* 125: 1341–1352, 1994.

Perl AK, Wilgenbus P, Dahl U, Semb H and Christofori G. A causal role for E-cadherin in the transition from adenoma to carcinoma, Nature. 392: 190–193, 1998.

Poser I, D. Dominguez, A.G. de Herreros, A. Varnai, R. Buettner, A.K. Bosserhoff, Loss of E-cadherin expression in melanoma cells involves up-regulation of the transcriptional repressor Snail, *J Biol Chem.* 27: 24661–24666, 2001.

Tomita K, van Bokhoven A, van Leenders GJLH, Ruijter ETG, Jansen CFJ, Bussemakers MJG, Schalken JA. Cadherin switching in human prostate cancer progression. *Cancer Res.* 60: 3650–3654, 2000.

Vleminckx KL, Vakaet J, Mareel M. Genetic manipulation of E-cadherin expression by epithelial tumour cells reveals an invasion suppressor role. *Cell.* 66: 107-119, 2001.

Umbas R, Isaacs WB, Bringuier PP, Xue Y, Debruyne FM and Schalken JA. Relation between aberrant a-catenin expression and loss of E-cadherin function in prostate cancer. *Int J Cancer.* 74: 374–377, 1997.

Yagi T and Takeichi M. Cadherin superfamily genes: Functions, genomic organization, and neurologic diversity. *Genes Dev.* 14: 1169–1180, 2000.