Exp Oncol 2003
25, 3, 180–185

Experimental ONCOLOGY

CORRELATION OF E–CADHERIN EXPRESSION WITH CLINICOPATHOLOGICAL DATA IN PATIENTS SUFFERING FROM TRANSITIONAL CELL CARCINOMA OF THE BLADDER

K. Charalabopoulos[1,], S. Tsambalas[2], K. Syrigos[3], X. Giannakopoulos[2], V. Kalfakakou[1], D. Kiortsis[1], J. Alamanos[4], A. Charalabopoulos[1], A. Evangelou[1], N. Sofikitis[2], N.J. Agnantis[5]*
Departments of [1]Physiology, Clinical Unit, [2]Urology, [4]Epidemiology-Statistics and [5]Pathology, Medical Faculty, University of Ioannina, Ioannina, Greece
[3]Oncology-Immunology Unit, Third Department of Medicine, Medical Faculty, University of Athens, Athens, Greece

КОРРЕЛЯЦИЯ ЭКСПРЕССИИ Е–КАДГЕРИНА С КЛИНИКО–ПАТОМОРФОЛОГИЧЕСКИМИ ХАРАКТЕРИСТИКАМИ ПЕРЕХОДНО–КЛЕТОЧНОЙ КАРЦИНОМЫ МОЧЕВОГО ПУЗЫРЯ

К. Чаралабопулос[1,], С. Цамбалас[2], К. Сиригос[3], К. Чиннакопулос[2], В. Калфакаку[1], Д. Кьортис[1], Ж. Аламанос[4], А. Чаралабопулос[1], А. Эвангелу[1], Н. Софикитис[2], Н.Ж. Агнантис[5]*
Отделы [1]физиологии, клиническое подразделение, [2]урологии, [4]эпидемиологии и статистики, [5]патологии, медицинский факультет, университет Янины, Янина, Греция
[3]Подразделение онкологии и иммунологии, третье отделение медицины, медицинский факультет, университет Афин, Афины, Греция

Epithelial cadherin (E-cadherin, E-cad) is a calcium-dependent cell-cell adhesion molecule that binds cells through homotypic fashion interactions. Its role is crucial in the induction and maintenance of cell polarity and differentiation. Downregulation or loss of its function is associated with an invasive and aggressive phenotype in many types of human cancers. 45 male patients (mean age 63 years, age range from 29 years to 87 years) with transitional cell carcinoma (TCC) of the bladder were included in the study. E-cad expression was estimated immunohistochemically in a semiquantitative fashion using light microscopy. An avidin-biotin immunoperoxidase technique was employed using anti E-cad murine monoclonal antibodies. Loss of the E-cad normal surface expression by the bladder cells was found in 32/45 (71%) of patients compared to normal bladder epithelia observed at the intercellular borders ($p < 0.001$). A statistically significant difference between abnormal expression of E-cad and tumor grade and disease stage was also observed ($p < 0.001$). Loss of the surface E-cadherin expression was most frequently detected in grade 3 bladder cancer patients (17/21, 80.95%) than in well differentiated tumors (grade 1) (3/7, 43.86%) ($p < 0.001$). Surface E-cadherin expression was also most frequently lost in bladder cancer patients with lymph node and distant organs metastases (stage 4) (1/1,100%) compared to 13/25 (52%) of patients with lymph node-negative tumors (stage 1) ($p < 0.001$). We conclude that E-cad adhesion molecule is a useful marker and the loss of its expression is associated with high grade and advanced stage in patients suffering from TCC of the bladder.
***Key Words*: bladder cancer, transitional cell carcinoma, E-cadherin, adhesion molecules, invasion, metastasis, differentiation.**

Эпителиальный кадгерин (Е-кадгерин, E-cad) является кальций-зависимой молекулой адгезии, связывающей клетки путем гомотипических взаимодействий. Он выполняет критическую роль в индукции и поддержании полярности и дифференцировки клеток. Подавление или утрата его функции ассоциирована с инвазивным и агрессивным фенотипом различных типов опухолей человека. В исследовании принимали участие 45 мужчин в возрасте от 29 до 87 лет (средний возраст — 63 года) с переходно-клеточной карциномой мочевого пузыря (ТСС). Экспрессию E-cad оценивали в полуколичественной иммуногистохимии с использованием световой микроскопии, авидин-биотин иммунопероксидазного метода и мышиных анти-E-cad моноклональных антител. Утрата нормальной экспрессии E-cad на поверхности опухолевых клеток мочевого пузыря была выявлена у 32/45 (71%) пациентов в сравнении с нормальным эпителием ($p < 0,001$). Обнаружены статистически значимые различия между уровнем экспрессии E-cad и стадией опухолевого процесса ($p < 0,001$). Чаще утрату поверхностной экспрессии E-cad выявляли у больных раком мочевого пузыря III стадии (17/21, 80,95%), чем в высокодифференцированных опухолях (I стадия) (3/7, 43,86%) ($p < 0,001$). Утрату поверхностной экспрессии E-cad также чаще обнаруживали у больных раком мочевого пузыря с метастазами (стадия IV) (1/1, 100%) в сравнении с 13/25 (52%) пациентами без метастазов в лимфатических узлах (стадия I) ($p < 0,001$). Сделан вывод о том, что E-cad может являться надежным маркером, утрата экспрессии которого ассоциирована с прогрессией ТСС мочевого пузыря.
***Ключевые слова*: рак мочевого пузыря, транзиторно-клеточная карцинома, Е-кадгерин, молекулы адгезии, инвазия, метастазирование, дифференцировка.**

Received: May 28, 2003.
*Correspondence: Fax: +30 26510 97850;
E–mail: kcharala@cc.uoi.gr
Abbreviations used: CAMs — cell–cell adhesion molecules; E–cad — epithelial cadherin; TCC — transitional cell carcinoma.

Bladder cancinoma accounts for approximately 51.600 new cases of bladder cancer diagnosed annually, with 9.500 deaths in the United States [1]. Men are affected three times as frequently as women, and the disease usually occurs in patients between fifty and sixty years of age. Bladder cancer is the fifth most common cancer in men and the ninth in women, and the second most frequent urological malignancy in Western society with an incidence rate of 29.8/100.000 males per year. Epidemiologic studies have demonstrated an increased incidence of transitional cell carcinoma (TCC) following exposure to aromatic amines, particularly 2–naphthylamine which probably accounts for the high incidence of urothelial cancers among cigarette smokers and workers in the dye, chemical, and certain rubber industries. Some atmosphere pollutants (e.g. benzo(a)pyrene, found also in cigarette smoke) have also been implicated [2]. In addition, the multiple role of some endogenous substances has been under investigation [3].

Adhesion molecules intermediate cell–cell (cell–to–cell adhesion molecules, CAMs) interactions participating, thus, in normal processes such as embryogenesis, cellular communication and recognition, tissue differentiation, blood coagulation, inflammation, immune response, wound healing and apoptosis, as in various pathologies in which cancer invasion and metastasis are included [4–8]. Signal transduction is also triggered by E–cad. In addition to cadherins involvement in cellular adhesion, the regulated expression of cadherins also appears to control cell polarity, cell sorting and tissue morphology [6]. Adhesion molecules embrace five categories: integrins, cadherins, immunoglobulin gene superfamily (IgSF), selectins, and CD44 [8–12]. Epithelial cadherin (E–cad) is the most important molecule since when it is expressed and functioning, then even the inactivation of all the rest of the adhesion molecules has no significant negative effect on the adhesion reactions. E–cad intermediates in calcium dependent cell–cell adhesion interactions acting in a homotypic, homophilic fashion. E–cad is the main component in desmosomes and adherence junctions [13]. Its role is, therefore, crucial for the epithelial integrity. Loss of the E–cad function leads to a more aggressive and invasive phenotype in many human cancer types. E–cad interacts with a group of undercoated cytoplasmic proteins, named catenins, which link the intercellular portion of the cadherins to the cytoskeleton (alpha, beta and gamma–cat) [14–16]. Plakoglobin, p120, is also regarded to be a new catenin molecule [16, 17]. The loss of E–cad function can also be due to defects in its linkage to the cytoskeleton, which is mediated through catenins [18]. Thus, the E–cad/catenin complex is bound to the actin filaments forming an anchorage to the cytoskeleton. Studies in the last decade have shown that E–cad/catenin complex expression abnormalities have been found in a variety of solid tumors including colorectal cancer [19], head and neck carcinomas [20], breast cancer [21], gastric cancer [22], esophageal cancer [23, 24], pancreatic adenocarcinomas [25], hepatocellular carcinomas [26], lung carcinomas [27]), cervical intraepithelial neoplasia (CIN) [28] as well as prostate and bladder cancer [29–31].

E–cad gene has been identified, mapped on chromosome 16q; it presents a tumor suppressor role. Loss of heterozygocity (LOH) in this region is associated with the development of several tumors [32]. Mutation of the gene, alteration of transcription, post translation modifications or changes in the interaction of the E–cad with the cytoskeleton anchoring proteins, the catenins, (e.g. via phosphorylation) consist possible mechanisms implicated in the cancer development. Thus, an invasion–suppressor role for this gene in neoplastic progression has been demonstrated [33, 34]. Most tumors of the bladder are carcinomas and are associated with dedifferentiation of the epithelial cells and high metastatic capability.

In the present study we investigated the E–cad participation in the carcinogenetic process and its correlation with the disease progression.

MATERIALS AND METHODS

Patients and tumor specimens. In this study, 45 male patients (mean age 63 years, age range 29 to 87 years) with diagnosed bladder cancer recruited from March 1998 to March 2003 were included. Pathological and clinical data are summarized in Table. Tumor volume and size was estimated during endoscopy (mean size of tumors was 3.5 cm, range 2.0–8.0 cm). None of the patients received radiation or chemotherapy preoperatively. 10 patients with acute cystitis were used as control group. Formalin fixed, paraffin embedded samples of normal bladder and TCC of the bladder were graded accordingly to the WHO classification [35]. The disease stage was characterized accordingly to the UICC TNM classification [36].

Immunohistochemistry. In present study, the avidin–biotin indirect immunoperoxidase method was employed using the anti E–cadherin (MAb, HECD–1 human epithelial cadherin–1) murine monoclonal antibody as an undiluted culture supernatant. HECD–1 has been

Table. Clinicopathological data and correlation to E–cad expression in patients with TCC included in the study

Type of tumor	Number of patients	Normal E–cad expression, % (n)	Abnormal E–cad expression, % (n)
TCC	45		
Tumor category			
Ta	8	62.5 (5)	37.5 (3)
T1	17	41.16 (7)	58.84 (10)
Ta + T1	25	48 (12)	52 (13)
T2	15	33.33 (5)	66.66 (10)
T3	4	25 (1)	75 (3)
T4	1	—	100 (1)
Grade category			
G1	7	57.14 (4)	42.86 (3)
G2	17	35.29 (6)	64.71 (11)
G3	21	19.05 (4)	80.95 (17)
Metastasis category			
M0	36	30.55 (11)	69.44 (25)
M1	9	11.11 (1)	88.88 (8)

Notes: Grading and characterization accordingly to WHO and UICC TNM classification, respectively. T1: tumor invasion in subepithelial connective tissue; T2: tumor invasion of superficial muscle; T3: tumor invasion in deep muscle and perivesical fat; T4: tumor invasion of any of the following tissues: prostate, pelvic wall, abdominal wall as well as uterus and vagina in females; G1, G2, G3: the 3–grade histological typing system; M0: no distant metastasis; M1: distant metastasis (details in the text).

previously characterized and its specificity has been reported in the literature [23]. To enhance E–cadherin immunohistochemistry in formalin–fixed, paraffin–embedded tissues, sections were treated with antigen retrieval solution in a microwave oven, according to Pignatelli's described methods [25, 37]. The slides were submerged in 0.01 M citrate buffer at pH 6.0 and heated in a 700 W microwave on full power for 5 × 2 min cycles, pausing to ensure that there was no fluid loss due to evaporation. The slides were then rinsed in phosphate–buffered saline (PBS, x 3) after each stage. 50 μl of anti–E–cadherin primary antibody was then added to the section and incubated overnight at 4 °C. An avidin–biotin complex immunoperoxidase technique was utilized to amplify epitope recognition (ABC kit, Dako Ltd, High Wycombe, UK) and subsequent colorific visualization was achieved by 50 μl of DAB solution at a concentration of 0.3 μg/ml (Dako Ltd., High Wycombe, UK). The slides were then washed and mounted for microscopic examination. Positive control tissue sections known to be of a homogeneous phenotype were used to ensure accurate and reproducible staining; normal transitional bladder epithelial cells present in the tumor slides were used as internal positive controls. Negative controls were duplicate sections similarly stained in which the primary antibody was omitted and replaced by normal mouse immunoglobulins.

Evaluation and statistical analysis. The apportionment of cellular staining of E–cad was classified according to its localization in the cell membrane or cytoplasm by two independent investigators on two separate occasions and scored as follows: if the staining was identical to that of normal bladder epithelium, then tumors were classified as normal (i.e. membranous immunoreactivity, means surface expression of E–cad in > 90% of the urothelial cells stained positively with high density) and score 3 was given; heterogeneous staining (i.e. < 90% positive tumor cells) and score 2 was given; cytoplasmic only and score 1 was given , and negative (absence of staining, excessively considerable fraction of E–cad negative cells) and score 0 was given. For statistical analysis purposes, tumors were divided in two groups: one with the score 3 (normal group, membranous expression of E–cad) and a second one including patients' tumor specimens with scores 0, 1, and 2 (heterogeneous, cytoplasmic, and negative staining). Tumors were regarded by another observer who had no knowledge of the staining results. Fisher's test was used for the correlations. *P*–values less than 0.05 ($p < 0.05$), were regarded to be statistically significant.

RESULTS

Table, except the clinicopathological data of patients studied, additionally summarizes the E–cad immunoreactivity (normal or abnormal) according to tumor stage, grade and metastases.

Fig. 1 and 2 show the expression pattern of E–cad in heterogeneous and cytoplasmic types of staining in bladder cancer cases. In normal bladder epithelium only the cell–cell borders are attained, thus, a membranous expression is observed. In Fig. 3 a normal expression of E–cad in normal urothelium is observed simultaneously to cancer epithelium which presents absolute absence of E–cad expression. The part of the cells in contact with the basement mebrane does not react with the anti–E–cad antibody. Tumors of 13 out of 45 patients (13/45, 28.88%) showed membranous E–cad immunoreactivity regarded as normal staining. Loss of the normal membranous staining pattern was observed in 32 (32/45,71.1%) of the 45 patients and the abnormal (heterogeneous, cytoplasmic, downregulation/absence) of E–cad expression was associated with both high grade and advanced stage in bladder cancer as was shown by the statistical analysis ($p < 0.001$). Furthermore, a statistically significant difference between abnormal expression of E–cad and tumor grade and disease stage was also observed ($p < 0.001$). Loss of membranous E–cad expression was most frequently

Fig. 1. Abnormal (heterogeneous) expression of E–cadherin in bladder carcinoma

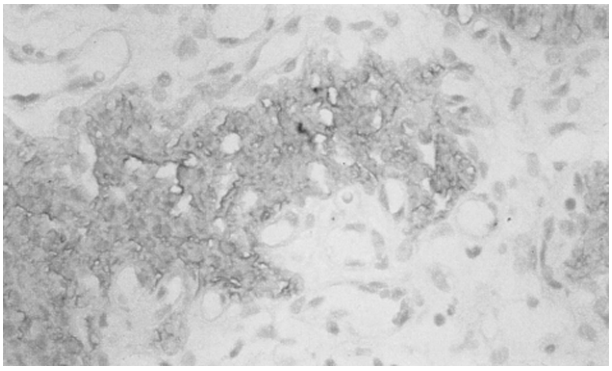

Fig. 2. Abnormal (cytoplasmic) expression of E–cadherin in bladder carcinoma

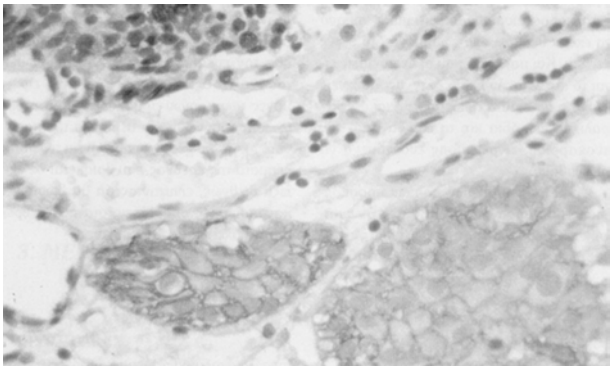

Fig. 3. Normal E–cad expression in normal urothelium adjacent to cancer epithelium without E–cad expression

detected in grade 3 bladder cancer patients (17/21, 80.95%) than in well differentiated tumors (grade 1) (3/7, 43.86%), ($p < 0.001$). 4 of 7 G1 tumors appeared membranous E–cad expression ($p < 0.001$), whilst 28 of 38 G2 +G3 tumors appeared abnormal staining pattern ($p < 0.001$). Correlation with tumor stage category showed that 17 of 40 Ta + T1 + T2 stages (17/40, 42.5%) showed normal E–cad expression comparing to only 1/5 (20%) patients with T3 + T4 stages ($p < 0.001$). Membranous E–cad expression was also most frequently lost in bladder cancer patients of T4 tumor category (1/1, 100%) compared to 13/25 (52%) of Ta + T1 stage tumor category ($p < 0.001$).

DISCUSSION

Up to 95% of bladder tumors are transitional carcinomas with adenocarcinomas and small cell tumors consisting the rest of the cases. Overall, 75% of primary bladder tumors are present as superficial lesions. Hematuria is the first sign in the majority of patients, followed by urinary frequency or irritative symptoms. Until now, histologically determined depth of infiltration and differentiation grade has been the most important prognostic variables for tumor progression. Unfortunately, they can not predict accurately the behavior of most bladder tumors. A significant degree of tumor heterogeneity remains even within various prognostic subgroups. The abovementioned parameters fail in up to 36% of bladder cancer patients, even those with superficial carcinoma [38]. The major problem in clinical praxis is the selection of those patients who are at high risk from cancer recurrence and progression and who may benefit from adjuvant treatment modalities. Thus, grade I lesions (highly differentiated tumors) rarely progress to a higher stage. Controversely, a Ta grade III tumor presents a higher risk of progression to more advanced stage. The development of reliable prognostic markers for TCC of the bladder will accurately predict not only the course of the disease but also the response of a tumor to therapy dictating thus treatment strategies. Though, accurate and reliable prediction of tumor aggressiveness (invasiveness and metastatic potential) is difficult to be determined.

E–cad is a transmembrane calcium dependent protein involved in cell–cell adhesion at the level of adherent junctions in the epithelium [39]. Continued expression and functional activity of E–cad are required for cells to remain tightly associated in the epithelium, and in its absence many other proteins involved in this process become incapable of supporting intercellular adhesion. Owing to its capacity to maintain the state of adhesion between epithelial cells, this molecule is important in tissue differentiation and maintenance, and it is regarded to act as an important suppressor, of epithelial tumor cell invasion and metastatic spread [33, 40]. Furthermore, E–cad triggers signal transduction. Loss of E–cad is an important step in the progression of many carcinomas and is believed to lead to a decreased cell adhesiveness in tumors [41]. Cell detachment from the epithelial sheet is the initial step in this process. Cellular adhesion molecules may be associated with invasion and metastasis in a wide variety of human malignancies including TCC of the bladder.

The use of molecular markers may guide the decision making process in the treatment of superficial bladder cancer [42, 43]. The ability to stratify superficial tumors with invasive or metastatic capabilities and those unlikely to become invasive or clinically treating would be of great clinical benefit. Superficial bladder tumors that maintain a more malignant phenotype may be better treated with early aggressive intravesical therapy or cystectomy. On the other hand, muscle invasive bladder cancer is notorious for its potential clinical virulence and is ideally treated with surgical extirpation. Despite this aggressive form of therapy, a significant incidence of recurrence and disease progression remains in some patients who may ultimately benefit from some adjuvant form of therapy. The desire to predict which superficial tumors will recur or progress and which invasive tumors will metastasize has led to the development of a variety of bladder cancer prognostic markers. In this study, we demonstrated a significant association between E–cad expression and bladder cancer grade, stage and metastases. Thus, the normal membranous E–cad expression was lost in 32/45 (71%) of patients ($p < 0.001$), whilst an association between aberrant expression of E–cad with tumor grade, advanced stage, and metastases was observed. Loss of the membranous E–cad expression was most frequently detected in grade 3 bladder cancer patients (17/21, 80.95%), than in well differentiated tumors, grade 1, (3/7, 43.86%) with a p value less than 0.001. p–value less than 0.001 was found when the E–cad expression was correlated between stage 4 and stage 1 patients. These findings are similar and in accordance with those previous reported by some members of our research group [31]. In a study by Bringuier et al [30] reported in 1993 it was shown that the decreased E–cad immunoreactivity correlates with poor survival in 49 patients suffering from bladder cancer. This finding was confirmed in several additional studies [38].

In conclusion, our data suggests that E–cad is a reliable diagnostic and prognostic marker in TCC of the bladder. The ability of taking bladder biopsy specimen during cysteoscopy, which is a routine examination to urologists, makes the hole procedure of approaching as was described above, very useful in the daily clinical praxis. Treatment modalities may also dictated. In addition, since a substantial amount of knowledge on molecular alterations observed in TCC cases is nowadays available, we hopefully believe that this will lead to even better ways to prevent, diagnose, treat, and follow–up bladder cancer.

REFERENCES

1. **Heiken JP, Forman HP, Brown JJ.** Neoplasms of the bladder, prostate, and testis. Radiol Clin North America 1994; **32**: 81–98.

2. **Charalabopoulos K, Karkabounas S, Charalabopoulos A, Papalimneou V, Ioachim E, Giannakopoulos X.** Inhibition of benzo(a)pyrene induced carcinogenesis by

vitamin C alone, and by pairs of vitamin C/vitamin E and selenium/glutathione. Biol Trace Elem Res (in press).

3. **Giannakopoulos X, Charalabopoulos K, Baltogiannis D, Chatzikiriakidou A, Alamanos Y, Georgiou I, Evangelou A, Agnantis NJ, Sofikitis N.** The role of N-acetyltransferase-2 and glutathione S-transferase on the risk and aggressiveness of bladder cancer. Anticancer Res 2002; **22**: 3801–4.

4. **Hynes RQ.** Integrins: versatility, modulation, and signalling in cell adhesion. Cell 1992; **69**: 11–25.

5. **Eidelman GM, Crossin KL.** Cell adhesion molecules: implication for a molecular histology. Ann Rev Biochem 1991, **60**: 155–90.

6. **Takeichi M.** Cadherin cell adhesion receptors as a morphogenetic regulator. Science 1991; **251**: 1451–5.

7. **Charalabopoulos K, Binolis J, Karkabounas S.** Adhesion molecules in carcinogenesis. Exp Oncol 2002; **24**: 249–57.

8. **Cohen MB, Griebling TL, Ahaghotu CA, Rokhlin OW, Ross JS.** Cellular adhesion molecules in urologic malignancies. Am J Clin Path 1997; **107**: 56–63.

9. **Takeichi M.** Cadherin in cancer: implications for invasion and metastasis. Curr Opin Cell Biol 1993; **5**: 806–11.

10. **Charalabopoulos K, Pignatelli M.** Adhesion molecules and cancer. Arch Hell Med 2001; **18**: 16–9.

11. **Williams AF, Barclay AN.** The immunoglobulin superfamily domains for cell surface recognition. Ann Rev Immunol 1988; **6**: 381–405.

12. **Haynes BF, Liao HX, Patton KL.** The transmembrane hyalouronate receptor (CD44): multiple functions, multiple forms. Cancer Cells 1991, **3**: 347–50.

13. **Takeichi M.** The cadherins. Cell-cell adhesion molecules controlling animal morphogenesis. Development 1988; **102**: 639–55.

14. **Furukawa Y, Nakatsuru S, Nagafuchi A, Tsukita S, Muto J, Nakamura Y, Horii A.** Structure, expression and chromosome assignment of the human catenin (cadherin associated protein) alpha 1 gene (CTNNA1). Cytogenet Cell Genet 1994; **65**: 67–74.

15. **Kraus C, Liehr T, Hulsken J, Behrens J, Birchmeier W, Grzeschnik KH, Ballhausen WG.** Localization of the human beta catenin gene (C-TNNB 1) to 3p21: a region implicated in tumor development. Genomics 1994; **23**: 272–4.

16. **Piepenhagen PA, Nelson WJ.** Defining E-cadherin associated protein complexes in epithelial cells: Plakoglobin, beta-and gamma catenin are distinct components. J Cell Sci 1993; **104**: 751–62.

17. **Butz S, Stappert J, Weissing H, Kemler R.** Plakoglobin and beta-catenin. Distinct but closely related. Science 1992; **257**: 1142–4.

18. **Shimazui T, Schalken JA, Giroldi LA, Jansen CF, Akara H, Koizo K, Debryne FM, Bringuier PP.** Prognostic value of cadherin-associated molecules (alpha-, beta-, and gamma-catenins and p120 cas) in bladder tumors. Cancer Res 1996; **56**: 4154–8.

19. **Kinsella AR, Green B, Lepts GC, Hill CI, Bowie G, Taylor BA.** The role of the cell-cell adhesion molecule E-cadherin in large bowel tumour cell invasion and metastasis. Br J Cancer 1993; **67**: 904–9.

20. **Mattijssen V, Peters HM, Schalkwijk L, Manni JJ, vant Hof-Grootenboer B, de Mulder PH, Ruiter DJ.** E-cadherin expression in head and neck squamous cell carcinoma is associated with clinical outcome. Int J Cancer 1993; **55**: 580–5.

21. **Oka H, Shiozaki H, Kobayashi K, Inoue M, Tahara H, Kobayashi T, Takatsuka Y.** Expression of E-cadherin cell adhesion molecule in human breast, cancer tissues and its relationship to metastasis. Cancer Res 1993; **153**: 1696–701.

22. **Mayer B, Johnson JP, Leitl F, Jauch KW, Heiss MM, Schildberg FW, Birchmeier W, Funke I.** E-cadherin expression in primary and metastatic gastric cancer. Downregulation correlates with cellular dedifferentiation and grandular disintegration. Cancer Res 1993; **53**: 1690–5.

23. **Jankowski J, Newham PM, Kandemir O, Hirano S, Takeichi M, Pignatelli M.** Differential expression of E-cadherin in normal, metaplastic and dysplastic oesophageal mucosa: a putative biomarker. Int J Oncol 1994; **4**: 441–6.

24. **Syrigos K, Karayiannakis AJ, Charalabopoulos K, Katirtzoglou N.** Abnormal expression of E-cadherin in dysplastic Barrett's oesophagus. Ann Gastroenterol 2000; **13** (Suppl 1): 92.

25. **Pignatelli M, Ansari JQ, Gunter P, Liu D, Hirano S, Tekeichi M, Kloppel G, Lemoine NR.** Loss of membranous E-cadherin expression in pancreatic cancer: correlation with lymph node metastasis, high grade and advanced stage. J Pathol 1994; **174**: 243–8.

26. **Charalabopoulos K, Syrigos K, Papalimneou V.** Role of E-cadherin/catenin complex in primary liver tumors. Ann Gastroenterol 2000; **13**: 110.

27. **Charalabopoulos K, Charalabopoulos A, Papalimneou V.** Adhesion molecules in lung cancer. Exp Oncol 2003; **25**: 16–21.

28. **Vessey CJ, Wilding J, Folarin N, Hirano SMT, Soutter P, Stamp GWH, Pignatelli M.** Altered expression and function of E-cadherin in cervical intraepithelial neoplasia and invasine squamous cell carcinoma. J Pathol 1995; **176**: 151–9.

29. **Umbas R, Schalken JA, Aalders TW, Carter BS, Karthaus HF, Schaafsma HE, Debruyne FM, Isaacs WB.** Expression of the cellular adhesion molecule E-cadherin is reduced or absent in high grade prostatic cancer. Cancer Res 1992; **52**: 5104–9.

30. **Bringuier PP, Umbas R, Schaafsma HE, Karthaus HF, Debruyne FM, Schalken JA.** Decreased E-cadherin immunoreactivity correlates with poor survival in patients with bladder tumors. Cancer Res 1993; **53**: 3241–5.

31. **Syrigos KN, Krausz T, Waxman J, Pandha H, Rowilinson-Busza G, Verne J, Epenetos AA, Pignatelli M.** E-cadherin expression in bladder cancer using formalinfived, paraffin embedded tissues: correlation with histopathological grade, tumour stage and survival. Int J Cancer 1995; **64**: 367–70.

32. **Takeichi M.** Cadherin cell adhesion receptors as a morphogenetic regulator. Science 1991; **251**: 1451–5.

33. **Vleminckx K, Vakaet LJr, Mareel M, Fiers W, van Roy F.** Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. Cell 1991; **66**: 107–19.

34. **Frixen VH, Behrens J, Sachs M, Eberle G, Voss B, Warda A, Lochner D, Birchmeier W.** E-cadherin mediated cell-cell adhesion prevents invasiveness of humam carcinoma cells. J Cell Biol 1991; **113**: 173–85.

35. **Mostofi FK, Sobin LH, Torloni H.** Histological typing of urinary bladder tumours. In: Histological typing of urinary bladder tumours. International histological classification of tumours, 10, Geneva, World Health Organisation, 1973.

36. UICC, TNM Classification of malignant tumours. Hermanek P, Sobin LH, eds. Berlin: Sprieger – Verlag, 1987; 65–7.

37. **Gagliardi G, Kandemir O, Lin D, Guida M, Benvestito S, Ruers TGM, Benjamin IS, Northover JMA, Stamp GWH, Pignatelli M.** Changes in E-cadherin im-

munoreactivity in the adenoma-carcinoma sequence of the large bowel. Virch Archiv 1995; **426**: 149–54.

38. **Otto T, Birchmeier W, Schmidt V, Hinke A, Schipper J, Roebben H, Raz A.** Inverse relation of E-cadherin and autocrine motility factor receptor expression as a prognostic factor in patients with bladder carcinomas. Cancer Res 1994; **54**: 3120–3.

39. **Garrod D, Colins J.** Intercellular functions and cell adhesion in epithelial cells. In: Epithelial organisation and development. Fleming TP, ed. London: Chapman and Hall, 1992; 1–53.

40. **Birchmeier W, Beherens J.** Cadherin expression in carcinomas: role in the formation of cell junctions and the prevention of invasiveness. Biochem Biophys Acta 1994; **1198**: 11–26.

41. **Geiger B, Ayalon O.** Cadherins. Ann Rev Cell Biol 1992; **8**: 307–32.

42. **Malkowicz BS.** Superficial bladder cancer: the role of molecular markers in the treatment of high-risk superficial disease. Sem Urol Oncol 1995; **15**: 169–75.

43. **Orntoft TF, Wolf H.** Molecular alterations in bladder cancer. Urol Res 1998; **26**: 223–33.