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Original Paper

Abnormal Expression of p120 Correlates with Poor Survival in Patients with Bladder Cancer

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p120 is a cytoplasmic molecule closely associated with the Ca^{2+}-dependent cell–cell adhesion molecule E-cadherin, by forming complexes between the cytoplasmic domain of E-cadherin and the cytoskeleton. Although it has been shown that loss or downregulation of E-cadherin is associated with an invasive and poorly differentiated phenotype in several tumours, there is very little information available concerning p120 expression in malignant disease. We used an avidin–biotin immunoperoxidase technique to examine the immunoreactivity and cellular localisation of p120 and E-cadherin in 68 transitional cell carcinomas (TCC) and 14 normal bladder biopsies and correlated these results with pathological and clinical parameters. E-cadherin and p120 were expressed in a normal membranous pattern in all normal bladder epithelium specimens. Loss of normal surface E-cadherin and p120 expression was found in 52/68 (76%) and 57/68 (84%) tumours, respectively. There was a significant correlation between the loss of normal membranous expression of p120 and increased grade ($P < 0.001$) and T stage ($P < 0.001$). The abnormal expression of p120 was correlated with poor survival ($P < 0.05$). Our data indicate that the E-cadherin–p120 complex may be a useful prognostic marker in bladder cancer.

Key words: E-cadherin, p120, bladder cancer, prognostic marker
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INTRODUCTION

EPITHELIAL CADHERIN (E-cadherin) is the prime mediator of intercellular adhesion in epithelial cells. This transmembrane glycoprotein, localised mainly in the zonula adherens junctions, mediates by its extracellular domain cell–cell adhesion through calcium-dependent homotypic interactions [1]. Its cytoplasmic domain associates with a group of undercoat proteins termed catenins (α-, β-, γ-catenin and p120) [2]. Both β- and γ-catenin bind directly to the cytoplasmic domain of E-cadherin while α-catenin links the bound β- or γ-catenin to the actin microfilament network of the cellular cytoskeleton [3], binding which is essential for the formation of stable cell–cell adhesion. The p120 protein, which was originally identified as a substrate of *src* and several other receptor tyrosine kinases, is another member of the catenin family [4, 5]. p120 binds directly to the cytoplasmic domain of E-cadherin and *in vitro* studies have demonstrated that p120 acts as a regulatory molecule of the adhesive function of E-cadherin [5, 6].

A number of clinicopathological studies have demonstrated that loss of E-cadherin expression is commonly associated with high grade and advanced stage in a variety of malignancies, including breast carcinomas [7], colorectal tumours [8], prostatic adenocarcinomas [9], pancreatic tumours [10] and squamous carcinomas of the head and neck [11, 12]. In bladder cancer in particular, we and others have reported that abnormal expression of E-cadherin occurs frequently in high grade and advanced stage tumours and is correlated with a more aggressive phenotype [13–15].

There is, however, evidence that alterations in E-cadherin expression in bladder cancer are associated with abnormal expression of the α-, β- and γ-catenins [16], whilst the role of p120 in the E-cadherin–catenin function has not been fully investigated in bladder cancer. Therefore, we evaluated the p120 expression and cellular localisation in superficial and invasive bladder cancer and correlated these results with pathological and clinical parameters.

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MATERIALS AND METHODS

Patients and tumour specimens

Eighty-two human bladder samples in formalin-fixed, paraffin embedded tissue blocks were obtained from the Histopathology Department of Hammersmith Hospital, London, U.K. These consisted of 68 transitional cell carcinomas (50 males and 18 females, mean age 67.9 years) and 14 normal bladder samples (9 males and 5 females, mean age 64.3 years) obtained by cystoscopic biopsy. The malignant tumours were classified and re-graded by one pathologist using the three-grade system according to the WHO classification [17]: grade I, $n = 18$; grade II, $n = 19$; grade III, $n = 31$. The T stage of the primary tumour was defined by cystoscopy, biopsy, bimanual examination and radiological imaging (computed tomography (CT) and magnetic resonance imaging (MRI)). The nodal status was not assessed. The presence or absence of metastatic disease was assessed by CT scan of the liver and chest radiography in all patients. Nuclear medicine bone scans were performed if clinically indicated. Treatment and length of survival following diagnosis were obtained from hospital and general practice records.

Immunohistochemistry

Commercially available mouse monoclonal IgG antibody, against p120 (Transduction Laboratories, Lexington, Kentucky, U.S.A.), packaged at 0.25 mg/ml, was used at a dilution of 1:1000. Mouse monoclonal antibody to E-cadherin (HECD-1) was used as undiluted culture supernatant, as previously described [13]. The antibodies are active in frozen and formalin-fixed paraffin-embedded tissue samples (data not shown) and, therefore, we did not include any frozen material in our study. To enhance the immunoreactivity, sections were treated with an antigen retrieval solution in a microwave oven, according to our previously described methods [10]. Briefly, the slides were submerged in 0.01 M citrate buffer at pH 6.0 and were heated in a 700 W microwave on full power for 5×2 min cycles pausing to ensure there was no fluid loss due to evaporation. The slides were rinsed in phosphate buffered saline (PBS) (three times) after each stage. Fifty microlitres of anti-E-cadherin and p120 primary antibody were then added to the section and incubated overnight at 4°C. An avidin–biotin complex immunoperoxidase technique was used to amplify epitope recognition (ABC kit, Dako Ltd, High Wycombe, U.K.) and subsequent colorific visualisation was achieved by 50 µl 3,3′-diaminobenzidine tetrahydrochloride dihydrate (DAB) solution, at a concentration of 0.3 mg/ml (Dako). The slides were then washed and mounted for microscopic examination. The above procedure has been proven to be an accurate and reproducible method of antigen retrieval [10, 13]. Positive control tissue sections known to be of a homogenous phenotype were used to ensure accurate and reproducible staining and included normal small and large intestinal mucosa. Normal epithelial bladder tissue present in the tumour slides was used as an internal positive control. Negative controls were duplicate sections similarly stained in which the primary antibody was omitted and replaced by normal mouse immunoglobulins.

Evaluation

The sections were examined under light microscopy by three independent observers. The proportion of stained cells, the intensity and cellular localisation of immunostaining were used as criteria for the evaluation, using the adjacent normal urothelium as an internal positive control. Tumours were classified as exhibiting normal staining if the pattern of immunoreactivity was identical to that of the membranous staining of normal bladder epithelial cells. Abnormal staining was considered if tumours showed heterogenous staining (i.e. less than 90% positive tumour cells), weak or altered distribution (i.e. cytoplasmic or nuclear) of immunostaining, or complete absence of staining.

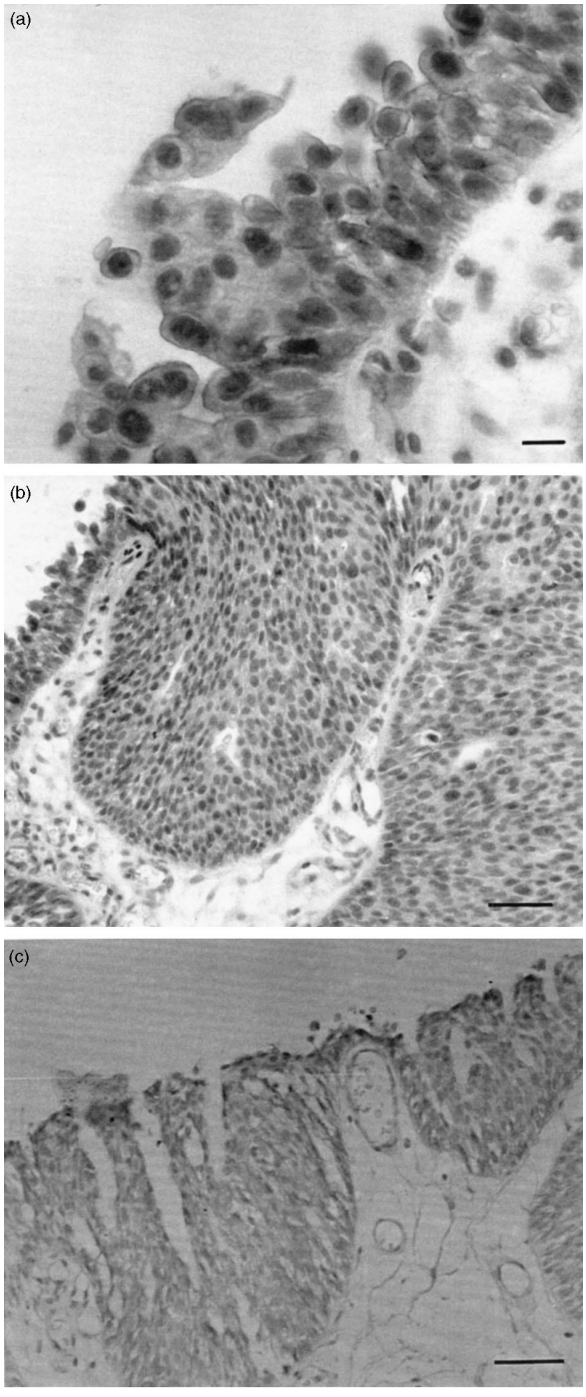

Figure 1. p120 immunoreactivity in normal bladder mucosa (a) and transitional cell carcinoma of the bladder (b and c). Preserved membranous expression in normal bladder mucosa (a); cytoplasmic (b) and heterogeneous (c) staining in poorly differentiated transitional cell carcinoma (avidin–biotin indirect immunoperoxidase staining; bar 150 µm).

Table 1. *Correlation of abnormal E-cadherin and p*120 *immunoreactivity with tumour grade and T stage* (P < 0.001)

	Number ($n = 68$)	Abnormal E-cadherin	Abnormal p120
Grade			
I	18	6 (33%)	11 (61%)
II	19	15 (79%)	16 (84%)
III	31	31 (100%)	30 (97%)
T stage			
1	15	4 (27%)	9 (60%)
2	9	7 (78%)	8 (89%)
3	27	27 (100%)	24 (89%)
4	17	14 (82%)	16 (94%)

Tumours were classified as normal if staining was similar to that of normal transitional epithelial cells (i.e. membranous immunoreactivity). Abnormal tumours were those that showed heterogeneous staining (i.e. when tumours were composed of positive and negative areas), only cytoplasmic staining or negative staining (i.e. complete absence of immunoreactivity).

Statistical analysis

For statistical analysis, antigen expression was considered either as normal or abnormal. Correlations between antigen expression and clinicopathological variables were evaluated by Fisher's exact test. $P < 0.05$ was considered statistically significant.

The log rank test was applied to examine survival and the relevant Kaplan–Meier curves are presented for each patient group. A P value of less than 0.05 was accepted as statistically significant.

RESULTS

All 14 normal bladder specimens showed homogenous membranous E-cadherin and p120 localisation (Figure 1a). Membranous staining was observed at the intercellular borders of histologically normal bladder epithelium present in the bladder tumour specimens, although no staining was seen in the most superficial umbrella cells.

16 of 68 tumours (23.5%) showed normal membranous E-cadherin immunoreactivity, whilst normal membranous immunoreactivity for p120 was seen in 11/68 (16.1%) tumours. Loss of membranous E-cadherin and p120 immunoreactivity (Figure 1b, c) was associated with advanced tumour grade and T stage, as shown in Table 1. All P values of the described differences had a significance < 0.001. With regard to the co-expression of p120 and E-cadherin, all cases with normal E-cadherin expression also showed normal p120 expression.

In four cases (6%) reduced p120 expression was not associated with altered E-cadherin expression. However, in most cases (94%) both molecules were expressed at an equivalent level according to our classification. The correlation between E-cadherin and p120 expression was highly significant ($P < 0.001$).

The analysis of survival data revealed that abnormal E-cadherin and p120 expression were correlated with poor outcome ($P < 0.05$) (Figure 2), although multivariate survival analysis demonstrated that their prognostic significance was not independent of the grade and stage (data not shown).

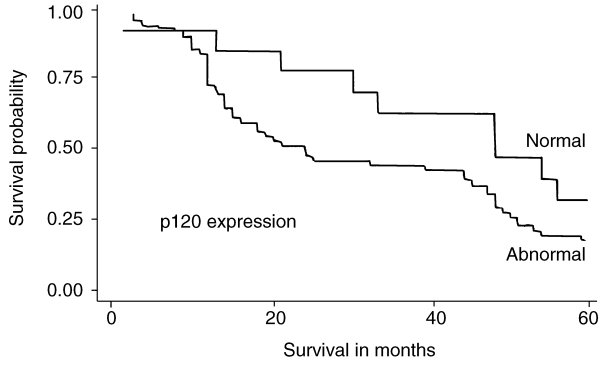

Figure 2. Overall survival for 68 patients with transitional cell carcinoma of the bladder with normal or abnormal expression of p120. Abnormal p120 expression was correlated with poor survival (P<0.05).

DISCUSSION

With an estimated 52 000 new cases and almost 10 000 deaths every year in the U.S.A., carcinoma of the urinary bladder accounts for approximately 2% of all malignant tumours [18, 19]. Several biological and molecular parameters have been considered as potential prognostic markers for bladder cancer, but up to now tumour grading and staging have been the most important prognostic variables [20]. There is, however, significant intra- and interobserver variation in the reporting of tumour grade and stage [21, 22] and as a result, more reliable and objective indicators of prognosis are required.

In this study we investigated the expression of p120, a component of the E-cadherin–catenin adhesion complex, in bladder tissue samples. Normal bladder epithelium showed membranous expression of the E-cadherin–p120 complex at the cell–cell borders, which reflects the normal localisation of intercellular adhesion molecules. Abnormal staining patterns (either negative, heterogeneous or cytoplasmic) of either E-cadherin or p120 were seen in 84% (57/68) of bladder cancers. The abnormal staining pattern is likely to reflect a reduction or loss of adhesion, as in other human malignancies. Since the E-cadherin–catenin complex is known to be the prime mediator of intercellular cohesion and epithelial tissue integrity, these alterations may lead to loss of cell differentiation and allow cells to detach from the primary site, invade surrounding tissues and metastasise to lymph nodes and distant organs. Acquisition of cell dissociation and motility, introduced by aberrations of the E-cadherin–catenin complex could enhance the release of cancer cells from the primary site and affect the initial steps in the metastatic process [2]. Our findings are of particular interest in bladder cancer since the high recurrence rate of this malignancy could be attributed to intra-epithelial expansion of tumour cells or shedding and subsequent implantation of tumour cells elsewhere in the bladder [23]. The strong correlation seen in bladder cancer between loss of membranous immunoreactivity and high grade seems to indicate that disturbances in E-cadherin–p120 complex expression and function play an important role in the progression of this tumour. Previous reports have shown that loss of membranous E-cadherin expression in bladder cancer is associated with advanced stage and progression [13, 24] and that it is independently related to recurrence-free survival [25]. Our data suggest that immunostaining for p120 is a more accurate indicator of dysfunction since it more directly reflects loss of cell–cell adhesion than does E-cadherin alone.

It is of note that abnormal p120 expression almost always coexisted with altered E-cadherin expression. The binding site of p120 on E-cadherin is in close proximity but distinct from that of catenins [26]. p120, like β- and γ-catenin, is phosphorylated in response to a number of growth factor receptors upon their ligand binding activation [6]. Tyrosine phosphorylation of the catenins appears to be a possible mechanism for the functional modulation of the E-cadherin/catenin adhesion system. Epidermal growth factor (EGF) as well as hepatocyte growth factor/scatter factor (HGF/SF) induce tyrosine phosphorylation of β- and γ-catenin and p120 with subsequent cellular redistribution of E-cadherin, cell dissociation and increased cell motility [6, 27–29]. Furthermore, the tyrosine phosphorylation of p120 by the *src* tyrosine kinase is associated with cell transformation [6]. The above data suggest that p120 may participate in the modulation of E-cadherin-mediated cell adhesion, perhaps independently from the catenins.

It is of particular interest that p120 forms distinct, cadherin-independent complexes with the adenomatous polyposis coli (APC) tumour suppressor gene product [30, 31]. This observation is important if normal APC function is critical in maintaining the growth of several epithelia, including that of bladder [32].

In conclusion, we demonstrated that loss of membranous p120 immunoreactivity occurs in 84% of bladder carcinomas and correlates with high grade, advanced stage and poor prognosis. Further work is in progress to establish whether normal membranous E-cadherin–p120 expression can be restored by gene transfer or biological therapy to induce a less invasive and metastatic phenotype.

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