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Original Paper

Superficial Bladder Cancer: Study of the Proliferative Nuclear Fraction as a Prognostic Factor

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The expression of the anti-proliferating cell nuclear antigen (PCNA) was examined in bladder specimens from 48 patients with superficial transitional carcinoma, with the use of the PC10 monoclonal antibody. In vesical tumours with good clinical behaviour, we found a median PCNA positivity of 7.1% with a range of 5–25%. In vesical tumours with high incidence of recurrence, the median was 36.6% with a range of 15–80%. In vesical tumours with a strong tendency to invasion, the median positivity for PCNA staining was 68% with a range of 40–92%. In conclusion, we believe that using PC10 immunostaining to determine a nuclear proliferative fraction is a quick and simple method of studying the prognosis of patients who have vesical tumours of low grade and low stage.

Key words: bladder cancer, proliferating cell nuclear antigen
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INTRODUCTION

THE DEGREE of histological differentiation and stage of bladder carcinomas constitute two most valuable prognostic factors in establishing the aggressiveness of these tumours. Bladder cancers can be essentially divided into two groups, superficial tumours, which are generally low grade and low stage, and invasive carcinomas, which are usually high grade, muscle-invasive tumours. Although the majority of the superficial transitional cell carcinomas (TCC) are low stage and low grade, there are a number of tumours that will behave more aggressively than the usual superficial cancers, displaying high incidence of recurrence and rapid transformation into a higher grade malignancy with deep invasion of the bladder wall [1].

To determine which low grade, low stage tumours will progress into more aggressive tumours, there have been numerous investigations reporting on markers which help to predict this transformation [2, 3]. Loss of expression of the blood group antigens in low grade superficial tumours indicates a propensity for transformation to a high grade malignancy [4, 5].

Newer techniques, including the study of the proliferative activity of the tumour measured by flow cytometry, image analysis, tritiated thymidine or bromodeoxyuridine labelling, or immunohistochemical staining using monoclonal antibodies such as Ki-67 have been utilised to screen low grade malignant tumours in an attempt to predict their transformation.

The purpose of this study was to investigate the value of the monoclonal antibody PC10 anti-proliferating cell nuclear antigen (PCNA) immunostaining for determination of risk factors in superficial bladder TCCs.

PATIENTS AND METHODS

The bladder specimens from 48 patients with superficial TCCs were studied. The cases were divided into three groups.

Group A comprised 15 patients with initial diagnosis of low grade superficial TCC (A-I) who, after cystoscopic resection, were free of disease for at least 3 years.

Group B comprised 18 patients with initial diagnosis of low grade superficial TCC (A-I) who, in the subsequent years after diagnosis, developed recurrences of the same histological grade.

Group C comprised 15 patients with initial low grade, low stage TCC (A-I) who, within 2 years, had invasion of the muscle wall. There was no sign of concurrent high grade tumour at the time of original biopsies.

For immunohistochemistry, an indirect immunoperoxidase technique was utilised. Sections were deparaffinised, rehydrated, washed with Tris buffer and treated with methanol solution and peroxidase to eliminate endogenous peroxidase. Following washing, the sections were incubated with the monoclonal antibody PC10 anti-PCNA (Dako) for 1 h. They were then washed and incubated with rabbit antimouse immu-

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noglobulin with peroxidase (Dako). Another wash and incubation with goat antirabbit tagged with peroxidase (Nordik) were then followed. The staining was done with diaminobenzidine. Harris haematoxylin was utilised as a counterstain. For positive controls, sections of lymph nodes with reactive germinal centres were used.

RESULTS

The most common pattern of staining that we observed with monoclonal antibody PC10 (PCNA) was the presence of diffuse nuclear positivity, although many cells showed a granular arrangement in the nucleus. The intensity of the staining varied from cell to cell; all nuclei that stained with PCNA were considered positive, irrespective of the pattern or the intensity of the staining.

The percentage of positivity for the PC10 immunostaining was determined by counting the number of cells with positive nuclei in relation to the total number of tumour cells which were present in 10 representative high-power fields (400x). A minimum of 2000 cells were counted. Since positive staining of PCNA is present in normal tissue, we considered a tumour to be positive when there was at least 5% positive PCNA tumour cells.

In group A tumours with good clinical behaviour, we found a median PCNA positivity of 7.1% with a range of 5–25%. The majority of the positive nuclei were scattered in groups in all the reviewed fields of the tumour (Figure 1).

In group B tumours with high incidence of recurrence, the median was 36.6% with a range of 15–80%. The most striking finding in this group was the tendency to find zones of the tumour with high PCNA positivity while in the remainder of the tumour the positive tumour nuclei were scattered in groups. Five of the 18 tumours studied in this group showed diffuse positivity in more than 50% of the tumour cells.

In group C tumours with a tendency to invasion, the median positivity for PCNA staining was 68% with a range of 40–92%. The pattern of staining observed was homogenous with large areas of tumour positivity (Figure 2). 14 of the 15 cases in this group showed positivity in more than 50% of the tumour cells counted (Figure 3).

DISCUSSION

Low grade, low stage superficial bladder tumours have, in general, a good prognosis, but unfortunately there is a small percentage of these tumours which progress and behave aggressively [6]. Routine histological examination cannot distinguish the low grade tumours with good behaviour from those that will progress. In order to resolve these problems, there have been numerous investigations which can be classified into two types: the study of blood group antigen alteration, and the analysis of proliferative cellular fraction of the tumour. In the latter, flow cytometry [7], image analysis [8], incorporation of tritiated thymidine and bromodeoxyuridine [9, 10] and immunohistochemical staining using different monoclonal antibodies that are directed against the cell cycles are used [11]. All these methods have advantages and disadvantages.

Numerous works have shown that the analysis of cellular DNA measured by flow cytometry is an important system for the prediction of prognosis of bladder tumours and for the monitoring of progression of the disease and their response to treatment [12]. Similarly, a high level of correlation between DNA content, histological grade and clinical behaviour has been observed [13, 14]. However, the data obtained may be influenced by the amount of necrotic tissue or normal stroma present and by the presence of lymphocytes. In addition, it is necessary to have specialised trained personnel and expensive machinery.

Figure 1. Positive nuclei in a superficial vesical tumour with good clinical behaviour (group A); 15% positive PCNA tumour cells.

Figure 2. Homogeneous positivity pattern of staining in a superficial vesical tumour with a strong tendency to muscle invasion (group C); 82% positive PCNA tumour cells.

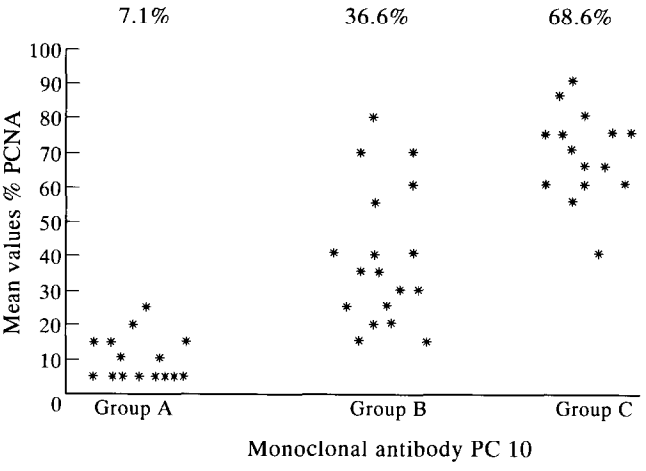

Figure 3. PCNA expression in superficial bladder cancer.

The incorporation of thymidine and/or bromodeoxyuridine has demonstrated that there is a good correlation between histological grade and clinical behaviour, but the technique is complicated.

There have been several papers published demonstrating the utility of the monoclonal antibody Ki-67 as a marker of proliferation in different types of tumours [15–18]. In our study, we utilised the monoclonal antibody PC10 (PCNA) directed against a nuclear antigen associated with cellular proliferation. PCNA has been identified as an auxillary factor to DNA polymerase. The synthesis of PCNA occurs in late G1 phase, presumably after the restriction point, and throughout S-phase. The advantage of PC10 is that it can be used in formalin-fixed paraffin-embedded tissue, including archival tissue, making it useful for retrospective studies [19, 20].

Our results demonstrate that the monoclonal antibody PC10 is an excellent marker for aggressive superficial bladder tumours of low stage and low grade, distinguishing these from those which have good clinical behaviour. It may also have independent prognostic value when tumours show more than 50% nuclear positivity, since in our study no tumours in group A, but four of 18 in group B and 14 of 15 in group C had more than 50% positivity in tumour nuclei, but further studies are required to confirm this observation.

In conclusion, we believe that the use of monoclonal antibody PC10 immunostaining as a marker of proliferation is a quick, inexpensive method for the study of prognosis of vesical tumours of low grade and low stage.

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