Bcl-2 and p53 Overexpression as Associated Risk Factors in Transitional Cell Carcinoma of the Bladder

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Bcl-2 and p53 genes are implicated in cell cycle regulation with roles on programmed cell death. Consequently, presence of Bcl-2 and nuclear accumulation of p53 were proposed to confer a growth advantage tumour cells.

We have investigated their role as prognostic factors in fresh tumour samples from a cohort of twenty patients with transitional cell carcinoma of the bladder by immunohistochemical analysis in paired specimens.

Expression of Bcl-2 was observed in 11 cases (69%) and nuclear p53 accumulation in 9 (45%). In the presence of Bcl-2 protein expression, tumours showed a slightly higher rate of recurrence (55% vs. 40%) and significantly more progression (36% vs. 0%). Recurrence and progression rates were not significantly different in tumours with and without nuclear p53 overexpression (recurrence rates 56% vs. 55% and progression rates 33% vs. 27%, respectively). Grade and stage appeared as important prognosticators since 75% of grade 3 tumours showed recurrence and 50% progressed in contrast to 44% and 13%, respectively, of grades 1 and 2 tumours. Similarly, 50% of Ta-T1 tumours recurred and 20% progressed, while these rates were 75% and 75% for T2-T3 tumours. Also, expression of Bcl-2 and nuclear accumulation of p53 correlated with grade. In grade 3 tumours, 75% showed nuclear p53 overexpression and 80% cytoplasmic Bcl-2 protein. These figures were 25% and 64% for grades 1 and 2 tumours.

In conclusion, Bcl-2 protein expression in transitional cell carcinoma appears to be associated with a poorer prognosis and together with nuclear p53 overexpression they are associated with tumour de-differentiation.

Introduction

Tumorigenesis is thought to result from a series of cellular events, including activation of oncogenes and inactivation of tumour suppressor genes. The p53 tumour suppressor gene mutations are the most frequent genetic abnormality seen in human tumours [1].

The p53 gene encodes for a nuclear protein that regulates expression of genes which are important in DNA repair, cell division and apoptosis [2]. This protein functions to arrest cell division and allowing time to DNA repair. Mutation of this gene leads to a dysfunctional abnormal protein which has a longer half life. Detection of mutant p53 gene in human malignancies including breast, colon, lung and prostate indicates the importance of p53 in carcinogenesis.
A group of genes other than discussed above are thought to be important in tumour progression and programmed cell death. One of these, the Bcl-2 gene encodes for a protein which is responsible to block apoptosis [3].

The aim of this study was to investigate expression of Bcl-2 and p53 oncoproteins in bladder tumours and to determine the association of their expression with progression and recurrence.

Materials and methods

In this study we evaluated surgical samples of 20 patients with transitional cell carcinoma (TCC) of the bladder which were diagnosed and treated during 1994 through 1997.

Immunohistochemical techniques

Fresh tumour samples were prepared by the “touch-print” technique, air dried and fixed in 10% neutral buffered formalin.

Bcl-2 immunostaining

Tissues were incubated with TBS for 10 minutes. Endogenous peroxidase activity was blocked with 2% H$_2$O$_2$ for 10 minutes. Samples were then subjected to antigen retrieval by heating in microwave oven within 0.01 M sodium citrate for a total of 10 minutes. After cooling at room temperature, samples were incubated with blocking sheep serum for 60 minutes. Then murine monoclonal antibody (Clone 124, Signet Laboratories Inc., Massachusetts, USA) raised against Bcl-2 protein was applied and specimens were incubated at 4 °C for 16-18 hours. Consequently, incubating with biotinylated secondary was followed by incubating with streptavidin-horseradish peroxidase for another 30 minutes. 3-3’ Diaminobenzidine (DAB) was used as the chromogen and specimens were counterstained with haematoxylin. After dehydration with ethanol series of 70–80–100%, respectively, each for 2 minutes, all specimens were air dried and covered with balsam. Bcl-2 staining was accepted positive when there was cytoplasmic staining. Intensity of staining was scored as follows: (0) no staining, (1+) weak, (2+) moderate, (3+) intense staining. The first two groups were accepted as negative and the latter two as positive staining for Bcl-2.

p53 immunostaining

The above mentioned protocol was utilized for p53 immunostaining. Mouse monoclonal antibody (pAb 1801, Oncogene Science Inc., London) to p53 protein was used. Results were considered positive when there was distinct nuclear staining.