



The Expression Of P53 In Human Urothelial Carcinoma

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The Expression of P53 in Human Urothelial Carcinoma

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Abstract

“Background:”

There is no clear cut way of predicting which urothelial

carcinomas would subsequently recur or progress or which muscle-invasive tumours would progress following treatment.

“Objectives:”

To study the immunohistological expression P53 in human urothelial carcinoma with regard to grade category (stage) and outcome.

“Patients, Materials and Method:”

The expression of p53 in human urothelial cancer was studied using an immunohistological (ABC) method. Staining characteristics of the 86 tumours studied were analysed with regards to grade, stage and outcome.

“Results:”

Thirteen out of 45 G1 tumours, 10 out of 15 G2 tumours and 21 out of 26 G3 tumours were positive for p53. Twenty out of 55 pTa and pT1 tumours were positive for p53. In comparison 24 out of 31 muscle invasive tumours were p53 positive. Regarding outcome of pTa and pT1 tumours most of the tumours that were p53 negative had no recurrence. Recurrences of higher grade and higher stage were more commonly associated with p53 positive tumours. On the other hand the difference in the outcome of p53 positive and p53 negative muscle invasive tumours was not significant even though p53 positive tumours had slightly higher inferior outcome.

“Conclusions:”

Higher grade tumours were more commonly associated with p53 positivity in comparison with lower grade tumours. A higher proportion of muscle-invasive tumours were found to be positively stained for p53 in comparison with superficial (pTa and pT1) tumours. Recurrences of higher grade and stage were more common in p53 positive superficial tumours in comparison with p53 negative superficial tumours.

KEY WORDS. Urothelial carcinoma, P53, Immunohistochemistry. Bladder Cancer.

Introduction

P53 is a 393 amino acid nucleo-phosphoprotein first identified as a result of binding of the large T antigen of DNA virus SV40.[1] The p53 gene product binds

strongly to the alpha unit of DNA polymerase.[2] and E1B antigen of adenovirus and the E6 antigen of polyoma virus. It has been shown to be a candidate gene for the control of cellular proliferation.[3] The p53 (TP53) gene which is located on chromosome 17p13.1 has been described as the most commonly altered gene in human cancer. It codes for a 53 kDa stress-induced nuclear phosphoprotein whose main functions are cell cycle arrest, programmed cell death (apoptosis), inhibition of tumour growth, protection against viral infection and preservation of genetic stability. These activities are mediated by transcriptional transactivation, transcriptional suppression and inhibition of DNA replication. [4] Currently there is no accurate way to predict which superficial urothelial cancers will subsequently become muscle invasive or which muscle invasive urothelial tumours will subsequently progress and result in death. Studies regarding the expression of p53 by urothelial cancers are few and these studies have suggested varying rates of expression. This study was initiated to test the hypothesis that expression of p53 in urothelial cancer is more commonly associated with tumours of high grade and high category and that the expression of p53 is associated with inferior outcome.

PATIENTS AND METHODS.

Between 1990 and 1994, 86 patients (49 male and 37 female), with urothelial carcinomata, mean age 69.5 years (range 20 to 95 years) who were treated in Dryburn Hospital were enrolled in the study. These patients had a mean follow up time of 55.7 months (range 1-444) months.

Urothelial tumour samples were obtained from all the 86 patients requiring surgical excision or transurethral resection of their tumours. 55 of these patients required transurethral resection of bladder tumours and 31 patients had resection of bladder tumours followed by radiotherapy and / or laparotomy and cystectomy. In each case the tumours were staged based upon the TNM classification (UICC 1987), by a careful bimanual examination under anaesthesia at the time of surgery in combination with the histology report. The tumours were graded according to the system of Bergkvist et al., 1965 [1], using routine haematoxylin and eosin (H&E) stained sections of formalin fixed-paraffin embedded tumour. The patients were followed up at regular intervals and any recurrent or persistent tumour carefully graded and staged (categorised). In the case of pTa and pT1 tumours these patients had 3 monthly check cystoscopies initially for 2 years and in the absence of recurrence, check cystoscopies were carried out at 6

monthly intervals for 2 years following which the patients were followed up at yearly intervals in the case of no recurrence but when a recurrent tumour was found the follow up interval was then reduced to 3 monthly intervals. Intravenous urography was performed at 2 yearly intervals and any recurrent or persistent tumour carefully graded and staged. The patients who had cystectomy were followed up in the out patients department (these patients had careful clinical examinations and appropriate investigations as was indicated for example; bone scan, chest x-ray, liver function tests, intravenous urography, biopsy of any recurrent tumour as well as any other investigation and management that was necessary). Those patients who had transurethral resection of their tumours and subsequent radiotherapy were followed up by regular check cystoscopies and bimanual examination. In the case of the patients with superficial bladder tumours who had frequent superficial recurrences these patients were treated by intravesical chemotherapy following trans urethral resection of their tumours.

Routinely formalin fixed paraffin wax embedded blocks of urothelial cancer were cut at 5µ and attached to poly-L-lysine coated slides. The sections were allowed to dry overnight at room temperature. The following Avidin-Biotin peroxidase (ABC) immunocytological procedure was then carried out: The sections were deparaffinised, rehydrated, rinsed in tap water for 5 minutes and then rinsed in distilled water. Endogenous peroxidase activity was blocked by placing the sections in 1% hydrogen peroxide/methanol solution for 20 minutes. The sections were next rinsed in running tap water for 5 minutes. In order to allow for 'batch' runs to be performed and to provide consistent reproducible results Shandons sequenza immunostaining centre and its cover plate assembly (figure 1.) was used for the subsequent intermediate steps of the immunohistochemistry. In fact the slides were put in the Shandons sequenza immunostaining centre and then rinsed in phosphate buffered saline (PBS) PH 7.2. Incubation was then carried out in normal rabbit serum (DAKO X902) diluted 1/20 with PBS for 10 minutes. The slides were next transferred to the primary antisera (p53 antibody) diluted 1/100 with PBS [Novo castra NCL-DO7] and incubated overnight at 4 degrees centigrade. The slides were next rinsed in PBS for 5 minutes and then incubated in secondary antisera (DAKO E354 Rabbit anti-mouse immunoglobulins/Biotinylated) diluted with PBS for 5 minutes and then incubated in AB complex (DAKO K 355 AB Complex/HRP) diluted 1/50 with PBS for 45 minutes and rinsed in PBS for 5 minutes. Immunoreactivity was visualised with Diaminobenzidine Tetrachloride dihydrate (DAB)

solution for 1 to 5 minutes. The slides were rinsed in PBS for 5 minutes and then removed from the sequenza immunostaining centre and rinsed in running tap water for 10 minutes. The cell nuclei were counter stained lightly in Mayers Haematoxylin. The slides were washed, dehydrated, cleared and mounted in DPX.

Sections of formalin fixed paraffin wax embedded known p53 positive breast cancer specimens were also cut at 5u and stained simultaneously with the urothelial tissues using the same steps as above for use as control slides. For purposes of negative control, sections of tumour specimens were processed and stained as above apart from the omission of the primary antisera.

Microscopy (immunohistochemistry for p53)

Routine microscopy of the immunohistochemistry slides was performed in order to assess each slide for the expression of p53 (staining for p53). Positive staining was demonstrated by brownish coloration of the nucleus (Figure 2).

Assessment of staining for p53

Staining was assessed taking into consideration the intensity of positive staining through out the section. Staining intensity was scored on a 4 point scale: Negative stain (No staining) (0), weak (1), moderate (2), and strong (3). The extent of staining was based upon the proportion of tumour cells positively stained: 0-25% (+), 26-50% (++), 51-75% (+++), 76-100% (++++). The results of the extent of staining were summarised as: 'a' for (+) and (++) and 'b' for (+++) and (++++). In the final analysis of data all tumours showing weak, moderate and strong staining were recorded as positive and those showing no staining (0) were recorded as negative. Tumour nuclear staining was recorded as positive. Staining characteristics of the tumours were agreed upon by at least 2 people involved with the study. Results of the immunohistochemical and histological analysis were recorded without knowledge of the clinical outcome of the patients. The results of the immunohistochemical analysis were recorded independently without knowledge of the routine histological findings of the tumours.

Statistical analysis

Statistical analysis of the results was done by calculating chi square tests of the various tumour groups and the outcome.

Results

The results of the histological and immunohistochemical analysis of the tumours are summarised in tables 1 (a and b), 2 and 3. Forty five tumours were well differentiated (G1), 15 tumours were moderately differentiated (G2) and 26 tumours were poorly differentiated (G3). Thirty one tumours were muscle invasive tumours and the remaining 55 tumours were pTa and pT1 tumours. Positive staining of any intensity (mild, moderate and strong staining) irrespective of the proportion of tumours stained was obtained in 44 of the 86 tumours.

Grade

Thirteen of the 45 G1 tumours were positive for p53 and the remaining 32 were scored as negative for p53. Ten of the 15 G2 tumours were scored as positive and the remaining 5 negative. Twenty one of the 26 G3 tumours were positive for p53 and the remaining 5 were scored as negative. The differences in the staining characteristics of the three grades of tumour were significant using chi-square test with Yates' correction. (G1/G2 $P < 0.01$, G1/G3 $P < 0.001$).

Category (Stage)

Twenty of the 55 pTa and pT1 tumours were scored as positive for p53 and the remaining 35 were negative. In comparison, 24 of the 31 muscle invasive tumours were positive for p53 and 7 were negative. The difference in the staining characteristics of the two groups of tumours were significant using chi-square test with Yates' correction. (pTa-pT1 / T2-T4, $p < 0.001$)

Outcome (pTa and pT1 tumours) and (T2 - T4 tumours)

Regarding the 20 pTa and pT1 tumour that did not recur, 2 (10%) were p53 positive and 18 (90%) were p53 negative. In the case of the 21 tumours in which the recurrences were of the same grade and stage, 7 (33%) were p53 positive and 14 were p53 negative. Regarding the 8 tumours in which the recurrences were of higher grade but the same stage, 7 (86%) were p53 positive and 1 was p53 negative. Out of the 6 tumours in which the recurrences were of higher stage (category) 4 were p53 positive and 2 were p53 negative. Eighteen out of 20 p53 positive tumours (90%) recurred and 17 out of 35 p53 negative tumours (47%) recurred. In comparison with p53 negative tumours, p53 positive tumours had a significantly higher proportion of recurrences of higher grade and higher stage and a significantly lower proportion of tumours that did not recur or the recurrences were of the same grade and stage. (chi

square with Yates' correction $p < 0.01$).

Of the 31 patients with T2 - T4 tumours who were treated, 24 died as a result of their tumours and 7 were alive without evidence of tumour at the end of the study. Two of the 7 patients with p53 negative tumours were alive and the remaining 5 patients died as a result of their tumour progression. On the other hand 5 out of the 24 patients with p53 positive tumours were alive and the remaining 19 had died as a result of their tumours. The difference in the outcome of the two groups of tumours was not significant using chi-square test.

Discussion

Wright and co-workers [5] found eighteen percent of bladder tumours expressed p53 protein strongly and a further 36% exhibited weakly positive staining giving a total of 54% positively stained. Reports have demonstrated higher rates of p53 (TP53) gene mutations in invasive compared to superficial bladder tumours. In a series of 30 patients with superficial bladder tumours the incidence of TP53 mutations was 13% (4/30) [6], whereas Spruck and co-workers [7] reported mutations in 3% (1/36) of superficial tumours and in 51% (25/49) of muscle-invasive tumours. In another study no mutations were found in superficial bladder tumours (0/21) but 33% (8/24) of muscle-invasive tumours exhibited mutations and all mutations were found in grade 3 tumours.[8] The incidence of nuclear p53 accumulation detected by antibody Pab1801 in patients with cis was 45% (20% tumour cells positive) and was the only independent marker of tumour progression and death in univariate and multivariate analysis.[9] The high level of p53 expression in cis may probably explain its propensity to progress.

Immuno-histological detection of p53 assessed by polyclonal antibody CM1 (10% nuclear staining) was highly significant in predicting progression in 25 pT1 bladder tumours, although grade remained the most statistically significant predictor of progression.[10] Nevertheless, a similar study using three primary antibodies (CM1, Pab1801 and DO-7) did not find that p53 immunoreactivity was associated with outcome.[11] These studies highlight the discrepancies of p53 immuno-reactivity in superficial disease. In another study in which 243 patients were treated by radical cystectomy, p53 immuno-reactivity using monoclonal antibody Pab1801 was significantly associated with an increased risk of recurrence and decreased survival.[12] In contrast patients with Transitional cell carcinoma treated by radical

cystectomy and adjuvant chemotherapy had significantly decreased recurrence and increased survival if their tumours possessed altered p53 determined immuno-histochemically. Correspondingly, for patients without p53 immuno-reactivity, adjuvant chemotherapy conferred no recurrence or survival benefit [13]

The results of this prospective study revealed positive p53 immuno-reactivity (nuclear staining of any intensity, mild, moderate or strong staining) using primary antibody DO-7 in 44 out of 86 urothelial carcinomas. The results of this study confirm the suggestion that higher grade and higher category tumours had a significantly higher proportion of tumours with positive immuno-reactivity to p53. Regarding outcome of pTa and pT1 tumours, it was observed that most of the tumours that did not recur were p53 negative (18/20). In addition out of a total of 41 pTa and pT1 tumours in which there was no recurrence or the recurrences were of the same grade and stage (a low risk group), 32 were p53 negative (32/41). In contrast positive p53 immuno-reactivity was observed in most tumours in which the tumour recurrences were either of higher grade or of higher stage (11/14). This difference in behaviour pattern was statistically significant. In view of this it could be said that pTa and pT1 tumours with the potential of progressing to a higher category are likely to be p53 positive. Higher grade superficial (pTa and pT1) tumours are known to be associated with a higher rate of recurrence in comparison with lower grade tumours. In this study there were few G2 and G3 superficial tumours (9 and 1 respectively) out of the 55 studied. In view of this it was not possible to find out whether the higher rate of recurrence in p53 positive tumours is independent of grade.

A higher proportion of patients with p53 positive T2 to T4 tumours died as a result of their tumours in comparison with patients with p53 negative tumours. The difference in outcome was not statistically significant at $p < 0.05$. The reason for this could perhaps be that the numbers involved were small or perhaps p53 immunoreactivity may not be a factor in the outcome of T2 to T4 tumours. To clarify this it may be useful to have another study recruiting a large number of patients with muscle-invasive tumours. Esrig and co-workers [12]

studied a larger number of patients and found that expression of p53 in T2 to T4 to be associated with inferior outcome. However, based upon the results of this study alone it could be concluded that it is possible that the immuno-histological expression of p53 may be associated with inferior prognosis but this

has not been satisfactorily proven in the case of muscle-invasive tumours (T2-T4).

Conclusion

It would be concluded that: Higher grade tumours were found to be more commonly associated with p53 positivity in comparison with lower grade tumours. A higher proportion of muscle-invasive tumours were found to be positively stained for p53 in comparison with superficial (pTa and pT1) tumours. Recurrences of higher grade and stage were more common in superficial tumours positive for p53 in comparison with p53 negative superficial tumours. The difference in the outcome of with regard to p53 positive and p53 negative muscle-invasive tumours was not statistically significant in that even though muscle-invasive p53 positive tumours had worse outcome in comparison with p53 negative tumours the difference was not statistically significant.

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Dr Thompson Sarkodie-Djan, formerly Senior Lecturer in Engineering at Teesside University in Middlesbrough in the United Kingdom for his help with the statistical analyses.

Previous presentation of the paper

This paper was presented at a previous Bi-Annual meeting of the South African Urological

Surgeons Association meeting in Pretoria South Africa in 1994

Ethical Approval:

Ethical approval for this research project was granted by Durham Health Ethical Committee (North Durham Ethics Committee).

Competing Interests.

There are no competing interests to declare.

Authors Contribution

Authors Contribution:

Anthony Kodzo-Grey Venyo designed the study, applied for and obtained ethical approval for the project, recruited patients for the study, was involved in the following: assessment, investigation, management and follow-up of the patients including clinical staging of all the tumours. He also collected all urothelial tumour specimens for storage, cutting and staining. He studied the technique of cutting and staining of specimens under Mr Harold Greenwood. He also undertook microscopic examination of all immunohistologically stained slides for scoring of the staining characteristics of the tumours and the control specimen. Additional tasks performed by Anthony Kodzo-Grey Venyo include: Microscopic examination of all the tumour specimens that had already been graded and staged by Dr Douglas John Lindsay Maloney; He analyzed the results of all the histological data and correlated them with the clinical data; the entire write up of the paper.

Mr David Herring TD FRCS agreed to be the a co-supervisor for the project. He was involved with the assessment, investigation and management of the patients from the beginning to the end of the project and he read all the report and agreed with all the findings.

Mr Harry Greenwood collected all the tumour specimens from Anthony Kodzo-Grey Venyo, stored and processed all the specimens for the routine haematoxylin and eosin staining and wells as the immunohistological staining of all the specimens. (He stained / supervised all the staining). He also examined and cross checked the immunohistological findings of Anthony Kodzo-Grey Venyo. Mr Greenwood also read the paper which has summarized our findings.

Dr Douglas John Lindsay Maloney agreed to be a joint supervisor for the project. Dr Maloney did the histological grading and pathological staging of all the tumours and he also checked the immunohistologically stained specimens for their staining characteristics as well as he read the paper which has summarized our findings.

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Illustrations

Illustration 1

The Expression of p53, histological grade and tumour category.

Illustration 1 (Tables 1 a and b):

The Expression of p53, histological grade and tumour category

1a: Grade

Staining	Grade 1 (G1)	Grade 2 (G2)	Grade 3 (G3)	Totals
p53 positive	13	10	21	44
p53 Negative	32	5	5	42
Totals	45	15	26	86

1 b: Category

Staining	pTa and pT1 tumours	T2 - Tumours(muscle	Totals

Illustration 2

The Expression of p53 and outcome of pTa and pT1 tumours.

Illustration 2 (Table 2)

The Expression of p53 and outcome of pTa and pT1 tumours

Outcome	p53 positive	p53 negative	Totals
No recurrence	2	18	20
Recurrence of same grade and stage	7	14	21
Recurrence of higher grade & same stage	7	1	8
Recurrence of higher stage +/- higher grade	4	2	6
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Totals	20	35	55

Illustration 3

The Expression of p53 and outcome of T2 - T4 tumours

Illustration 3 (Table 3)

The Expression of p53 and outcome of T2 - T4 tumours.

Staining	p53 positive	p53 negative	Totals
Alive	5	2	7
Died as a result of their tumours	19	5	24
Totals	24	7	31

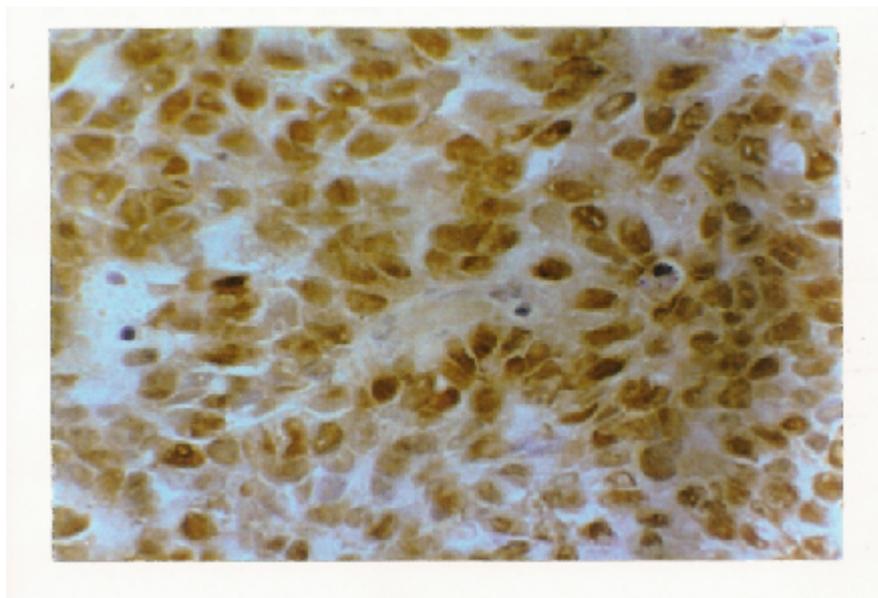
Illustration 4

Shandons Sequenza Immunostaining centre.



Illustration 5

Bladder carcinoma strongly positively stained for p53



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