

Expression of p53 Protein by Immunohistochemistry in Urothelial Neoplasm: A Hospital-based Study from Eastern India

Roy Chowdhury Anadi¹, Ranjan Kumar Dey²

¹Associate Professor, Department of Pathology, R.G. Kar Medical College, Kolkata, West Bengal, India, ²Associate Professor, Department of Urosurgery, R.G. Kar Medical College, Kolkata, West Bengal, India

Abstract

Background: It is difficult to predict which urothelial neoplasm would subsequently recur or progress to muscle invasive tumors or produce metastasis.

Objectives: The aim and objective of the study were to evaluate the scope of immunohistochemical grading of p53 inurothelial neoplasms with regard to grade and histopathological pattern.

Materials and Methods: Forty-five consecutive patients were taken, and samples were obtained from transurethral resection. Histopathological examinations were performed, and the grading was done according to the World Health Organization/International Society of Urological Pathology consensus classification of urothelial neoplasms. Immunohistochemical staining for p53 was performed on formalin-fixed paraffin-embedded tissue sections with appropriate positive and negative control.

Results: We found 5 patients of papillary urothelial neoplasm of low malignant potential (PUNLMP), 12 cases of non-invasive low-grade papillaryurothelial carcinoma, and 18 patients with non-invasive high-grade papillaryurothelial carcinoma including 10 cases of invasive urothelial carcinoma. All 5 PUNLMP cases showed negative results. 3 of 12 low-grade papillary urothelial carcinoma had high nuclear p53 accumulation, while all of the 16 papillary high-grade carcinomas had high p53 index. All the invasive urothelial carcinomas were high p53 expressor.

Conclusion: A trend of expression of p53 in urothelial neoplasm supports the notion that mutation of p53 gene might be unrelated to the development of urothelial neoplasms but definitely play a crucial role in progression of the malignancy.

Key words: Urothelial neoplasm, p53 index, immunohistochemistry

INTRODUCTION

The p53 gene is located on chromosome 17p13.1, and it is the most common target for genetic alteration in human tumors. The name of the gene is TP53 and the protein is p53, but for the sake of simplicity, we refer to both as p53. A little over 50% of human tumors contain a mutation of this gene. p53 is a transcription factor that is at the center of a large network

of signals that sense cellular stress such as DNA damage, shortened telomeres, and hypoxia. P53 thwarts neoplastic transformation by three interlocking mechanisms: Activation of temporary cell cycle arrest, induction of permanent cell cycle arrest, or triggering programmed cell death. p53 activates transcription of the mir34 family of micro RNAs. Targets of mir34 include proproliferative genes such as cyclins and antiapoptotic genes like BCL2. With loss of function of p53, DNA damage goes unrepaired; mutations accumulate in dividing cells and the cell marches along a one-way street leading to malignant transformation.^[1]

It is well documented that many mutations like heat shock protein have been associated with mutant form of p53 and lead to an increased half-life of the p53 protein. Mutated p53 may bind to wild-type p53 and change it to the mutated

Access this article online



www.ijss-sn.com

Month of Submission : 09-2017
Month of Peer Review : 10-2017
Month of Acceptance : 10-2017
Month of Publishing : 11-2017

Corresponding Author: Dr. Ranjan Kumar Dey, 10, Gurusaday Road, Ajanta Apartments, Flat 8b, Kolkata - 700 019, West Bengal, India.
Phone: +91-9831046873.
E-mail: ranjankdey@rediffmail.com

conformation.^[2] Since conformation and oligomerization of p53 are putatively important for its function, the function is probably changed in these cases as well.^[3] p53 immunohistochemistry has been suggested as an aid in the diagnosis of malignancy. P53 mutations are common in a variety of human tumors, and homozygous loss of p53 occurs in carcinoma of the lung, colon, breast, and others. The frequency of p53 mutation is lower in endometrial and thyroid carcinomas. To shed some light on p53 mutation in urothelial carcinoma, we analyzed 30 cases in our institution correlating immunohistochemical expression of p53 with regard to grade, stage, and outcome of the patient. Urinary bladder carcinoma is the seventh most common cancer worldwide, representing 3.2% of all adult cancers.^[4] Urothelial carcinoma is a recurrent neoplasm with a significant number of cases progressing to an infiltrating and very aggressive disease. Identifying the prognostic factors of progression is a challenge so that high-risk patients who may be a candidate for a radical cystectomy may be identified.

The aim of our study was to perform grading and staging of urothelial neoplasms and semiquantitative analysis of p53 immunoreactivity in paraffin-embedded sections. Then, statistical correlation was done between p53 positive immunostaining, grade, and stage of urothelial neoplasm.

MATERIALS AND METHODS

It was a prospective study of 45 consecutive patients of urothelial neoplasms attending the Department of Urosurgery from March 2011 to April 2012. The patients were informed and consents were obtained. Urothelial neoplasms of different grades were diagnosed according to the World Health Organization/International Society of Urological Pathology (WHO/ISUP) consensus classification of urothelial neoplasm of the urinary bladder. Anti-p53 mouse monoclonal antibody (Clone-DO7, Cell Marque, Rocklin, CA, USA) was used which reacted with mutant as well as a wild form of p53. The immunohistochemical stain was applied on 4 μm sections of formalin-fixed paraffin-embedded tissue. Epitope retrieval was done in conjunction with a microwave. Immunostaining was evaluated by counting 500 tumor cells for each case at high power (x400, magnification). Breast carcinoma cells were taken as positive control.

Samples demonstrating at least 10% nuclear reactivity were considered to be positive for p53. A high p53 index was defined when more than 50% or more nuclei were stained. Nuclear positivity between 10 and 50% was considered as low nuclear positivity.

RESULTS

The total of 45 cases of transurethral bladder resection specimens was processed for histopathological and

immunohistochemical analysis. The cases were tabulated according to the WHO/ISUP 2004 Classification, and we found a clear trend of high-grade lesions (total 28 of 45) in Indian community because of the habit of late presentation [Table 1]. p53 analysis showed high expression in all the 10 cases of invasive carcinomas and 16 of 18 cases of non-invasive high-grade urothelial carcinomas [Figure 1]. None of the high-grade cases showed negative expression. However, only two cases of non-invasive high-grade urothelial carcinoma showed low expression because of squamous metaplasia. 9 of 12 non-invasive low-grade urothelial carcinomas were low express or of p53. All the cases of PUNLMP were negative [Table 2]. Trend analysis showed that expression of p53 was high in terms of higher grade and invasiveness, i.e., mutation of p53 gene might be unrelated to the development of urothelial neoplasms but definitely play a crucial role in the progression of the malignancy [Chart 1].

DISCUSSION

Mutated p53 gene is a common genetic abnormality in transitional cell carcinoma of the bladder.^[5] Wild-type p53 protein has a short half-life, but the protein encoded by mutated p53 remains active for a long period. Therefore, mutation of

Table 1: Histopathological categories of all cases

Category	Number (%)
PUNLMP	5 (11.11)
Non-invasive low-grade urothelial carcinoma	12 (26.67)
Non-invasive high-grade urothelial carcinoma	18 (40.00)
Invasive urothelial carcinoma	10 (22.22)
Total case	45

PUNLMP: Papillary urothelial neoplasm of low malignant potential

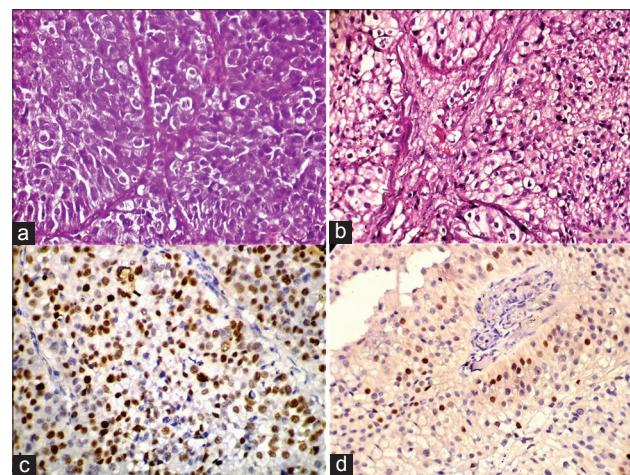


Figure 1: (a) Invasive urothelial carcinoma (Hematoxylin-Eosin Stain, x400). (b) Non-invasive papillary urothelial carcinoma (Hematoxylin-Eosin Stain, x400). (c) High expression of p53 in invasive urothelial carcinoma (p53 IHC, x400). (d) Low expression of p53 in non-invasive papillary urothelial carcinoma (p53 IHC, x400)

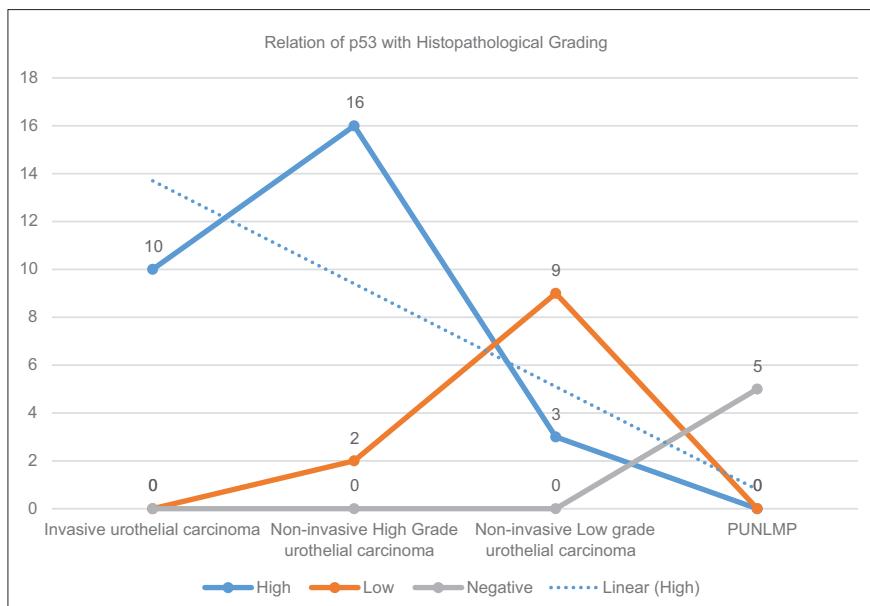


Chart 1: Trend analysis of p53 index in different histopathological categories

Table 2: p53 index in different categories according to the diagnosis

p53 index	Invasive urothelial carcinoma	Non-invasive high-grade urothelial carcinoma	Non-invasive low-grade urothelial carcinoma	PUNLMP
High	10	16	3	0
Low	0	2	9	0
Negative	0	0	0	5
Total	10	18	12	5

PUNLMP: Papillary urothelial neoplasm of low malignant potential

p53 gene results in p53 accumulation in the cells nuclei. This accumulation is detectable with immunohistochemical methods and correlates with p53 gene mutation.^[6] Several works have already been done in this regard.

Soni *et al.* worked on p53 immunohistochemistry in transitional cell carcinoma and dysplasia of the urinary bladder and found more p53-positive cases in Grade II–III tumors than in Grade I tumors ($P = 0.004$) and significantly more p53 positivity in stage T2–T4 tumors than in stage T1 tumors.^[7] Serth *et al.* recommended p53 immunohistochemistry as an independent prognostic factor for superficial transitional cell carcinoma of the urinary bladder, since they found that 85.7% urothelial tumors with more than 20% of cells positive for p53 had disease progression with muscle-invasive growth ($P < 0.001$) compared to the proliferative rate by immunostaining for proliferating cell nuclear antigen ($P = 0.0033$). In their study, the positivity of p53 was a significant indicator for relapse of bladder cancer ($P = 0.0029$).^[8] Our results also revealed high p53 positivity in high-grade papillary urothelial carcinomas and invasive carcinomas.

Malloffé *et al.* studied immunohistochemical expression of CK20, P53, and Ki67 as objective markers of urothelial

dysplasia. In their series, CK20 was positive through the full thickness of urothelium in 72% of cases, p53 was positive in 80% of cases, and Ki67 in 94% of cases.^[9] Thus, immunohistochemistry was found to be a useful tool to distinguish between dysplastic changes from reactive atypia. Unfortunately, we could not find any isolated case of urothelial dysplasia or CIS possibly because most of our patients reported late in the course of their disease which is the natural trend in developing countries.

Recently, Kalantari and Ahmadnia conducted a study on p53 overexpression in bladder urothelial neoplasms and suggested that the difference of nuclear p53 accumulation between PUNLMP and low- and high-grade TCC (invasive or non-invasive) was statistically significant ($P < 0.001$), whereas the difference between invasive high-grade and non-invasive low-grade carcinoma was not statistically significant.^[10] From this standpoint, they justified the importance of PUNLMP in the new WHO/ISUP classification system. We observed that most of the PUNLMPs showed negative results for p53, but there was a significant difference in p53 positivity in low-and high-grade urothelial neoplasm.

Gonzalez *et al.* worked on the prognostic value of combined p53 and survivin in PT1G3 urothelial carcinoma of the

urinary bladder. Tumors with the invasion of the lamina propria above the level of muscularis mucosa (or the large vessels present at this level) were subclassified as pT1a and tumors with infiltration of deep lamina propria beyond the muscularis mucosa were subclassified as pT1b, and it was seen that patients with pT1b tumors had a significantly increased risk of local progression and metastasis. They reported that percentage of stained nuclei with p53 was higher for patients with tumor progression than for patients without tumor progression.^[11]

Microvessel counts are strongly associated with recurrence and length of survival in patients with bladder cancer. There is a significant correlation between increased nuclear accumulation of p53 and increased microvessel counts. One of the mechanisms of p53-mediated tumor progression may be the promotion of an angiogenic response, which could have therapeutic applications.^[8]

In our series, we found that two patients of non-invasive high-grade papillary urothelial carcinoma had low expression of p53 and these two tumors showed prominent squamous differentiation. However, it is reported in literature that in 15–20% of tumors, despite p53 gene mutation, its product does not accumulate in the nucleus.^[6] Some p53 gene point mutations may result in lack of or severe decrease in p53 protein synthesis. On the other hand, in a proportion of tumors, despite the nuclear accumulation of protein, there is no mutation of p53 gene.^[5] It has been shown that some cellular oncogenic products like mouse double minute 2 lead to overexpression of p53 without any detectable p53 mutation.^[12,13] Thus, a p53 mutation, even though present in a large number of transitional cell carcinomas, it represents only one event in the putative pathway of the evolution of these tumors. In early stages of bladder cancer, deletion of chromosome 9 may be the only genetic abnormality, suggesting an initial role in the development of the urothelial cancer.^[14,15] Carcinomas with only chromosome 9 aberration do not show progression. Since p53-positive immunohistochemistry is associated with aggressive and recurrent tumors, it possibly plays a role in the evolution of the tumors to a higher grade and indicates the potential for progression.

CONCLUSION

Our results show that p53 protein expression is associated with high-grade urothelial neoplasm and advanced stage

of the disease, and it possibly plays a role in the evolution of the tumors to a higher grade. Our data were on a small sample size, but the significant differences we observed in tumor progression encourage us to perform future research. If the high prognostic value of p53 protein expression in urothelial neoplasm is confirmed in larger prospective trials, more aggressive therapeutic strategies could be discussed for patients with p53-positive tumor specimens.

REFERENCES

- Stricker TP, Neoplasia KV. In: Kumar V, Abbas AK, Fausto N, Aster JC, editors. Robbins and Cotran Pathologic Basis of Disease. 8th ed. Philadelphia, PA: Elsevier; 2010. p. 259-330.
- Hainaut P, Milner J. Interaction of heat-shock protein 70 with p53 translated *in vitro*: Evidence for interaction with dimeric p53 and for a role in the regulation of p53 conformation. EMBO J 1992;11:3513-20.
- Vogelstein B, Kinzler KW. P53 function and dysfunction. Cell 1992;70:523-6.
- Lopez-Beltran A, Sauter G, Gasser T, Hartmann A, Schmitz-Drager BJ, Helpapet B, et al. Tumours of the urinary system. In: Eble JN, Sauter G, Epstein JI, Sesterhenn IA, editors. World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs. Lyon, France: IARC Press; 2004. p. 89-123.
- Esrig D, Elmajian D, Groshen S, Freeman JA, Stein JP, Chen SC, et al. Accumulation of nuclear p53 and tumour progression in bladder cancer. N Eng J Med 1994;331:1259-64.
- Esrig D, Spruck CH 3rd, Nichols PW, Chaiwun B, Steven K, Groshen S, et al. P53 nuclear protein accumulation correlates with mutations in the p53 gene, tumor grade, and stage in bladder cancer. Am J Pathol 1993;143:1389-97.
- Soini Y, Turpeenniemi-Hujanen T, Kamel D, Autio-Harmainen H, Risteli J, Risteli L, et al. P53 immunohistochemistry in transitional cell carcinoma and dysplasia of the urinary bladder correlates with disease progression. Br J Cancer 1993;68:1029-35.
- Serth J, Kuczyk MA, Bokemeyer C, Hervatin C, Nafe R, Tan HK, et al. P53 immunohistochemistry as an independent prognostic factor for superficial transitional cell carcinoma of the bladder. Br J Cancer 1995;71:201-5.
- Malloffre C, Castillo M, Morente V, Solé M. Immunohistochemical expression of CK20, p53, and ki-67 as objective markers of urothelial dysplasia. Mod Pathol 2003;16:187-91.
- Kalantari MR, Ahmadnia H. P53 overexpression in bladder urothelial neoplasms: New aspect of world health organization/International society of urological pathology classification. Urol J 2007;4:230-3.
- Gonzalez S, Aubert S, Kerdraon O, Haddad O, Fantoni JC, Biserte J, et al. Prognostic value of combined p53 and survivin in pT1G3 urothelial carcinoma of the bladder. Am J Clin Pathol 2008;129:232-7.
- Oliner JD, Kinzler KW, Meltzer PS, George DL, Vogelstein B. Amplification of a gene encoding a p53-associated protein in human sarcomas. Nature 1992;358:80-3.
- Cordon-Cardo C, Latres E, Drobnyak M, Oliva MR, Pollack D, Woodruff JM, et al. Molecular abnormalities of mdm2 and p53 genes in adult soft tissue sarcomas. Cancer Res 1994;54:794-9.
- Takahashi T, Habuchi T, Kakehi Y, Mitsumori K, Akao T, Terachi T, et al. Clonal and chronological genetic analysis of multifocal cancers of the bladder and upper urinary tract. Cancer Res 1998;58:5835-41.
- Gonzalez-Zulueta M, Ruppert JM, Tokino K, Tsai YC, Spruck CH 3rd, Miyao N, et al. Microsatellite instability in bladder cancer. Cancer Res 1993;53:5620-3.

How to cite this article: Anadi RC, Dey RK. Expression of p53 Protein by Immunohistochemistry in Urothelial Neoplasm: A Hospital-based Study from Eastern India. Int J Sci Stud 2017;5(8):142-145.

Source of Support: Nil, **Conflict of Interest:** None declared.