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Original Article

p53 IMMUNOSTAINING IN BENIGN AND MALIGNANT EFFUSIONS

Gul Naz Akhtar [1], Mahfooz ur Rahman [2],
Saeed Akhtar Khan [3], Naseer Ahmed Chaudrhy [4]

ABSTRACT:

Objective: To evaluate p53 immunostaining as a marker of malignancy in pleural and peritoneal effusions and to compare the results with HE staining.

Design: Pleural and peritoneal effusion samples were obtained from patients suffering from benign and malignant diseases. H&E staining and p53 immunostaining were performed on smears prepared from these samples.

Setting: The samples were obtained from patients admitted in Mayo Hospital, Services Hospital, Gulab Devi Chest Hospital and Institute of Nuclear Medicine and Oncology (INMOL).

Subjects: One hundred cases having either pleural or peritoneal effusions were selected. Fifty of these cases were positive for malignant cells on H&E staining. Fifty cases contained only mesothelial cells.

Main outcome measures: To compare the specificity and sensitivity of p53 immunostaining with HE stain as a diagnostic marker of malignancy.

Results: Out of the 50 malignant cases, 31 (62%) were found to be p53 positive. None of the benign cases showed positive staining. p53 was found to have a specificity of 100%, sensitivity of 62%, a positive predictive value of 100% and a negative predictive value of 72.4%.

Conclusions: p53 is a highly specific and moderately sensitive marker of malignancy.

KEY WORDS: Cytology, p53 immunostaining.

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INTRODUCTION

The nuclear protein p53 was first described by Lane and Crawford in 1979. The p53 gene, located on the short arm of chromosome 17, is a common site of mutation or deletion in human tumours including those of breast, colon, lung, liver, mesenchyme, bladder and myeloid origins[1]. The p53 protein plays an important role in regulating cell proliferation. The product of the wild type p53 (wt p53) gene is involved in several cellular functions including transcription, regulation of the cell cycle, and induction of apoptosis in response to DNA damage, DNA repair and the preservation of genetic stability [2,3].

It also plays an important role in the prevention of malignancies and its regular functioning is lost in most human cancers due to mutation, insertions or deletions in the p53 gene. It accomplishes its two main functions namely, induction of cell cycle arrest and induction of apoptosis by upregulating the expression of p21 gene which is a potent inhibitor of most cyclin dependant kinases regulating cell cycle.[4]

1. Children Hospital & Institute of Child Health, Lahore, Pakistan
2. Institute of Haematology & Blood Transfusion Service, Punjab
3,4. Postgraduate Medical Institute, Lahore, Pakistan

Correspondence:

Dr. Gul Naz Akhtar,
216 B, PCSIR Housing Society Phase-I,
Right Canal Bank,
Lahore, Pakistan
E-mail: drmur@brain.net.pk

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The mutated p53, on the other hand, acts as an oncogene and promotes growth of tumours. The resultant mutated p53 gene products have a longer half life than the wild type proteins and the increased stability of aberrant p53 proteins renders them more readily detectable by immunohistochemical means.[5] Thus the highly increased levels of the mutated gene product in malignant cells differentiates them from benign cells.

This study was undertaken to assess the potential role of immunostaining for p53 protein in distinguishing non neoplastic cells from malignant cells in effusion smears and its comparison with the results obtained with HE stain.

PATIENTS AND METHODS

One hundred cases of effusions were selected. 50 of these were positive for malignant cells and the other set of 50 contained reactive mesothelial cells.

The non-malignant effusions had developed in the pleural and peritoneal cavities due to the following conditions:

Clinical diagnosis in non malignant cases	*Number of Cases*
Pulmonary Tuberculosis	17
Cirrhosis	14
Congestive cardiac Failure	10
Chronic Renal Failure	7
Acute peritonitis	2

Malignant effusions had developed due to the following causes:

Clinical diagnosis in malignant effusions	*Number of Cases*
Ca. Lung	14
Ca. Ovary	11
Ca. Breast	6
Ca. Liver	5
Ca. Colon	4
Ca. Stomach	4
Adeno Carcinoma (Primary Unknown)	2
Ca. Endometrium	2
Ca. Cervix	1
Non Hodgkin Lymphoma	1

Effusions were labelled as benign or malignant on the basis of H&E staining, and no cases having any doubt were included in the study.

For p53 staining the NovoCastra p53_ABC kit obtained from Novo Castra Laboratories Ltd. (UK) was employed. Smears were prepared on poly-L lysine coated glass slides. They were immersed in 1.5% hydrogen peroxide in methanol for 10 minutes to block endogenous peroxidase activity. Smears were washed in TBS and normal rabbit serum was applied for 10 minutes to reduce non specific antibody binding. The primary antibody was then employed for 60 minutes. Following further washing with TBS, smears were incubated with a secondary antibody diluted 1:500 times, for a period of 30 minutes at room temperature. The smears were again washed with TBS. The ABC reagent (100μl TBS, 1μl reagent A, 1μl reagent B. Allow to stand for 30 minutes before use) was then applied and slides were incubated for a further 30 minutes at room temperature. Visualization of the reaction product was achieved with 3,3 diamino-benzidine tetrahydrochloride. After further washing with TBS, smears were counterstained with haematoxylin and mounted in DPX.

Cases which had mutation of the p53 gene showed over expression of the protein due to its stabilization within the nucleus. The protein thus became detectable by IHC and imparted a brownish colour to the nucleus. Positively staining nuclei were counted and the smears having more than 5% positively staining nuclei were taken as positive.[6]

RESULTS

Out of 50 malignant effusions, 31(62%) showed positive staining. Table 1 shows percentage of staining in effusions arising from different tumours. It was difficult to ascertain whether the development of the effusions were due to the primary source or because of metastasis. Further, none of the non-malignant effusions showed positivity.

The specificity, sensitivity and predictive value of p53 as a diagnostic marker were also

investigated in this study. p53 had a 100% specificity, 64% sensitivity, a positive predictive value of 100% and a negative predictive value of 72.4%.

TABLE-I

Percentage of p53 positivity in tumours of different origin

Origin of Tumour	*p53 Positivity*
Lung	64.2%
Ovary	54.2%
Liver	60%
Breast	60.6%
Colon	75%
Stomach	75%
Endometrium	50%
Adeno Carcinoma *	100%
Cervix	0%
Non Hodgkin Lymphoma	0%

* primary unknown

DISCUSSION

Identifying malignant cells in serous effusions is a well known diagnostic problem. Reactive mesothelial cells may be difficult to distinguish from malignant cells particularly when the former cells occur in clumps. Similarly, the characteristic features of malignant cells may undergo substantial modifications in effusions.[7] Thus, the availability of techniques that would enhance the diagnostic accuracy of routine cytological methods could be of great clinical value.

p53 overexpression at the protein level using immunohistochemical techniques in a wide range of human malignant tumours has been performed in various studies. p53 immunostaining can be performed on frozen sections and formalin fixed paraffin embedded tissues. For staining of cells present in effusions, cell blocks of fluid embedded in formalin can also be prepared. However it has been reported that antigenicity may be better preserved in freshly prepared cytological preparations.[8]

Hall et al. conducted a study on samples of pleural and ascitic fluids, sputum, bronchial washings and fine needle aspirations from diverse sites; they concluded that out of 35 morphologically positive cases, 71% were p53 positive.[9] Mullick et al. demonstrated p53 positivity in 55% cases of pleural effusions.[10]

The present study demonstrates the possible usefulness of p53 staining in diagnostic cytopathology as a marker of neoplasia. p53 immunostaining is a highly specific but moderately sensitive costly marker of malignancy in serous fluids. It can be especially helpful where clinical information, H&E and other cytological techniques fail to provide an unequivocal answer regarding the malignant potential of cells in the effusions.

REFERENCES

1. Porter PL, Gown AM, Kramp SG, Coltrera MD. Wide spread p53 over expression in human malignant tumours. An immunohistochemical study using methacarn fixed, embedded tissue. Am J Pathol 1992; 140(1):145-153.
2. Harris CC. Structure and function of the p53 tumour suppressor gene: clues for rational cancer therapeutic strategies. J NCI 1996; 88:1442-1454.
3. Levine AJ. p53, the cellular gatekeeper for growth and diversion. Cell 1997; 88:323-331.
4. El-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW, Vogelstein B. WAF 1, a potential mediator of p53 tumour suppression. Cell 1993; 75:817-825.
5. Harris AL. Mutant p53-The commonest genetic abnormality in human cancer? (Editorial) J. Pathol 1990;162: 5-6.
6. Soini Y, Vahakangas K, Nuorva K, Kamel D, Lane DP, Paakko P. p53 immunohistochemistry in malignant fibrous histiocytomas and other mesenchymal tumours. J Pathol 1992; 168: 29-33.
7. Derenzini M, Nardi F, Farabegoli F, Ottinetti A, Roncaroli F, Bussolati G,. Distribution of silver stained interphase nucleolar organizer regions as a parameter to distinguish neoplastic from non neoplastic reactive cells in human effusions. Acta Cytol 1989; 33(4): 491-498.
8. Walts AE, Said JW, Koeffler HP. Is immunoreactivity for p53 useful in distinguishing benign from malignant effusions? Localization of p53 gene product in benign mesothelial and adenocarcinoma cells. Modern Pathology 1994;7(4):462-468.
9. Hall P A, Ray A, Lemoine N R, Midgley C A, Krausz T, L. p53 immunostaining as a marker of malignant disease in diagnostic cytopathology [Letter] Lancet 1991; 338 (8765): 513.
10. Mullick SS, Green LK, Brown RW, Brown RW, Smith D, Gondo MM, et al. p53 gene product in pleural effusions. Practical use in distinguishing benign from malignant cells. Acta Cytol 1996;40(5):855-860.