The Study of P53immunoexpression In Carcinoma Cervix

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ABSTRACT

Background : Worldwide cervical carcinoma is responsible for about 5% of all cancer deaths in women. Loss of p⁵³ functions is believed to play an important role in the pathogenesis of carcinomas of the uterine cervix. Mutation of the p⁵³ tumor suppressor gene is the most frequent genetic event described thus far in human cancer. Normal p⁵³ protein has a very short half life and thus the protein level is too low to be identified immunohistochemically. In contrast most mutant p⁵³ proteins have a longer half life and are easily detected by immunohistochemical methods.

Aim: The aim of the present study is to examine the over expression of p⁵³ protein in different tumor types of carcinoma cervix.

Materials and methods: This is a retrospective study carried out in the 285 paraffin blocks from the cases of carcinoma cervix in the department of Pathology, Rajah Muthiah Medical College, Chidambaram. Immunohistochemical study using Dextran polymer conjugate-Two step visualization system is used besides H&E staining.

Results: Out of 285 cervical carcinoma biopsies 50 cases were randomly selected and subjected to immunohistochemical analysis for p⁵³ protein.

p⁵³ expression was found to be positive in 42 cases(84%),which include:

- ♦ 39 squamous cell carcinoma
- ♦ 1 adenocarcinoma
- ♦ 1 adenosquamous carcinoma
- ♦ 1 small cell carcinoma

Conclusion: Loss of p53 function is having a key role in squamous cell carcinoma of cervix.

Key words: Carcinoma cervix, p53 protein, immunohistochemistry.

INTRODUCTION:

Cervical cancer is the second leading malignant neoplasm affecting women worldwide. It continues to be a public health problem particularly in developing countries and among socially disadvantaged population¹ Worldwide cervical carcinoma is responsible for about 5% of all cancer deaths in women.²

Loss of p⁵³ function is believed to play an important

role in the pathogenesis of carcinomas of the uterine cervix. Mutation of the p⁵³ tumor suppressor gene is the most frequent genetic event described thus far in human cancer.³

p⁵³ Guardian of the Genome⁴

The P⁵³ gene is located on chromosome 17p 13.1 and it is the most common target for genetic alteration in human tumors. The fact that p⁵³ mutations are common in a variety of human

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tumors suggest that the p⁵³ protein functions as a critical gatekeeper against the formation of cancer, indeed it is evident that p⁵³ protein acts as a “molecular policeman”. The p⁵³ protein is a DNA – binding protein localized to the nucleus; when called into action, it functions primarily by controlling the transcription of several other genes.

Mutation of the p⁵³ tumor suppressor gene is the most frequent genetic event described thus far in human cancer

Role of p53 in cervical carcinogenesis⁵

Loss of p⁵³ functions is believed to play an important role in the pathogenesis of carcinomas of the uterine cervix.

The major functional activities of the p⁵³ protein are cell-cycle arrest and initiation of apoptosis in response to DNA damage. p⁵³ is called in to apply emergency brakes when DNA is damaged by irradiation, UV light or mutagenic chemicals and also in response to changes in cellular redox potential, hypoxia, senescence and other stress conditions that may not directly damage DNA.

Normally cells with wild type p⁵³ are able to delay progression from the G1 to the S phase of the cell cycle while abnormal DNA is repaired. Cells with the inactivated, stabilized or mutant p⁵³ protein cannot, and thus the replication of abnormal DNA is not prevented.³

Once activated p⁵³ can either induce cell growth arrest or apoptosis. The mechanism of p⁵³ activation is crucial for its function as a tumour suppressor and understanding this mechanism may indicate new approaches for anti-cancer therapy and it is identified that the E7 gene of human papilloma virus is the survival factor for human cervical cancer cells (grown in cell culture).

Normal p⁵³ protein has a very short half life and thus the protein level is too low to be identified immunohistochemically. In contrast most mutant

p⁵³ proteins have a longer half life and are easily detected by immunohistochemical methods.⁴

AIM:

The aim of the present study is to examine the over expression of p⁵³ protein in different tumor types of carcinoma cervix.

MATERIALS AND METHODS:

This retrospective study included 285 paraffin blocks from the biopsies diagnosed as carcinoma cervix on H&E stained sections in the Department of Pathology, Rajah Muthiah Medical College-Chidambaram, for a period of 5 years. The study was conducted after obtaining a human ethical committee clearance from the institution.

The biopsy material included cervical punch biopsies, endocervical curettings and hysterectomy specimens. 50 blocks were selected randomly

5-µm thick sections from each paraffin block were subjected to immunohistochemical studies using Dextran polymer conjugate-Two step visualization system, besides H&E staining.

Dextran polymer conjugate-two step visualisation system:

This is an indirect system based on the dextran technology employed in the EPOS (Enhanced polymer one-step staining) labeling system. The primary antibody in the EPOS model is replaced with a secondary antibody, available in either anti-mouse or anti-rabbit format it offers great sensitivity than the traditional indirect system. It is less time consuming than the three-stage avidin-biotin systems and does not react with endogenous biotin.

- a) The sections were dewaxed in xylene.
- b) Deparaffinized sections were treated with 0.3% Hydrogen peroxide in Methanol for 30 minutes to block endogenous peroxidase.

- c) The sections were incubated for 20 minutes with normal serum from the species in which the secondary antibody was made. This was done to eliminate non specific staining.
- d) Excess normal serum was blotted from the slides and incubated with anti-p⁵³ mouse monoclonal antibody, DO-7 from tissue culture supernatant diluted in PBS (phosphate buffered saline) at PH 7.6 containing 1% BSA (biotin labeled secondary antibody) and 0.09% sodium azide.
- e) Incubated at room temperature for 2 hrs.
- f) Sections are cleared in xylol and mounted with DPX (dextrene polyesterene xylene).
- g) Positive control for this antibody is breast carcinoma tissue. Negative controls included replacement of polyclonal primary antiserum with rabbit serum diluted 1:500 whereas negative controls for the monoclonal antibody were performed using mouse myeloma protein of the same subclass and concentration as the monoclonal antibody. All controls gave satisfactory results.

The results were compared with the reports of other workers.

OBSERVATION:

p⁵³ Immuno expression:

Out of 285 cases of carcinoma cervix, p⁵³ protein expression was assessed using standard immunohistochemical technique in 50 randomly selected cases. 39 cases of squamous cell carcinoma out of 47 cases (82.9%) and one each of adenocarcinoma, adeno squamous carcinoma and small cell carcinoma were positive for p⁵³ immuno expression. [table 1]

Table 1. Carcinoma cervix - p⁵³ immuno expression

S.No.	Tumor type	Number of cases	p53 expression(+VE)
1.	Squamous cell carcinoma	47	39 (82.9%)
2.	Adeno carcinoma	1	1(100%)
3.	Adeno squamous carcinoma	1	1(100%)
4.	Small cell carcinoma	1	1(100%)
	Total	50	42(84%)

Tumors in which there was nuclear positivity in at least 10% of the neoplastic cells were regarded as positive. Those tumors with only focal or no nuclear staining were regarded as negative. [Fig.1 to Fig.5]

Fig 1. Large Cell Keratinizing Squamous cell Carcinoma cervix Staining of p53 is seen in the nuclei (magnification-200x, 400x)

Fig 2. Large cell Nonkeratinizing Squamous cell Carcinoma cervix Staining of p53 is seen in the nuclei (magnification200x,400×)

Fig3. Adenocarcinoma cervix Staining of p53 is seen in the nuclei (magnification100x, 200×)

Fig 4. Adenosquamous carcinoma cervix Staining of p53 is seen in the nuclei (magnification100x, 200×)

Fig 5.Smallcell carcinoma cervix-Positive Staining of p53 is seen in the nuclei (magnification200x,400×)

DISCUSSION:

Immunoreactivity for the p⁵³ protein is commonly found in many different human malignancies, p⁵³ point mutations have been demonstrated in human cervical carcinoma cell lines.

Bosari et al (1993)⁶ studied p⁵³ immunoreactivity in 92 lesions of the uterine cervix, including 44 cases of chronic cervicitis, 29 squamous intra epithelial lesions and 19 invasive carcinomas, p⁵³ immunoreactivity was demonstrated in 74 percent of invasive carcinoma

Helland A et al (1993)⁷ conducted a study on genetic alterations of the Tp⁵³ gene, p⁵³ protein expression and HPV infection in Primary cervical carcinomas, 92 patients were investigated for p⁵³ immuno expression. Over expression of the p⁵³ protein was found in 55% (51 /92) of the tumors.

Cooper K et al (1993)⁸ conducted a study on 30 fresh cervical biopsies.²² were HPV positive cervical carcinomas including one adenocarcinoma. 19 (86%) showed expression of p⁵³ protein.

Of the eight HPV-negative squamous cell carcinoma, four showed an even distribution of p⁵³ immunoreactivity, whilst in the other four tumours no p⁵³ was detectable.

Holm R et al (1993)⁹ reported p⁵³ Protein positivity in 62 percent of invasive adenocarcinomas and 7 percent of Squamous cell carcinomas in situ among two hundred and thirty-eight cervical lesions. No staining was found in adenocarcinoma in situ, dysplastic tissue, condyloma and normal tissue.

The results of the study indicate that altered expression of p⁵³ protein may be involved in the progression of cervical carcinomas,

Hunt & Hale (1996)³ conducted a study to assess the over expression of the tumor suppressor gene product p⁵³ in the three main subtypes of carcinoma of the uterine cervix.

Eighty two cases of FIGO stage IB/IIA Uterine cervical carcinoma were studied retrospectively, p⁵³ gene product was over expressed in 17.1% (14/82) of all carcinoma and also in areas of cervical intraepithelial neoplasia grade III adjacent to invasive squamous carcinoma.

Howayda ABD EL ALL¹⁰, Annie RYE & Peierre Duvillard (1999) Studied the p⁵³ immunohistochemical expression of Egyptian cervical carcinoma in an attempt to evaluate its prognostic significance. 38 invasive carcinoma (31 Squamous cell carcinoma and 7 adenocarcinoma ranging from stage 1 B to IVB) were stained with the monoclonal antibody anti p⁵³. Nuclear p⁵³ reactivity was detected in all carcinomas. p⁵³ expression was more pronounced in early clinical stages and in adenocarcinoma compared to squamous cell carcinoma.

Kenneth Webster et al (2000)¹¹ in his study on Human Papillomavirus 16 E2 protein and E7 protein demonstrated that E2 and E7 induce apoptosis via a p⁵³ – dependent pathway.

Horn LC et al (2001)¹² studied about the prognostic value of p⁵³ immunostaining in surgically treated and pathologically staged cervical cancer.

Wang HL and Lu DW (2004)¹³ conducted a study on detection of Human Papillomavirus DNA and expression of p16, Rb and p⁵³ protein in 22 cases of small cell carcinomas of the uterine cervix. The p⁵³ Protein was essentially undetectable in all cases.

Conner MG et al (2002)¹⁴ in his study on clinico pathologic and immunohistochemical analysis of 23 cases found the expression of p⁵³ protein in 10 cases. (43.5%).

Jo Milner (2006)¹⁵ of Yorkshire University p⁵³ research group have shown that the p⁵³ protein is activated in response to DNA damage and in response to agents that induce DNA damage.

In the present study 39 cases of squamous cell carcinoma were positive for p⁵³ immuno expression out of 47 cases (82.9%) one each of

adenocarcinoma, adeno squamous carcinoma and smallcellcarcinoma was having positive p⁵³ immuno expression. This was almost similar to that of K.Cooper et al (1993). [Table-2]

Table-2. Carcinoma cervix - p⁵³ immuno reactivity

S. No	Study	Squamous cell carcinoma		Adeno carcinoma		Adeno. Sq carcinoma		Small cell carcinoma	
		Positive	Neg	Positive	Neg	Positive	Neg	Positive	Neg
1.	S. Bosari et al (1993). n= 19	13(72%) n = 18	5	1 n=1	-	-	-	-	-
2.	A. Helland et al (1993) n= 92	43 (59%) n = 73	30	4 (44%) n = 9	5	4(57%) n =7	3	3 n=3	-
3.	K. cooper et al (1993).n= 30	23(75.9%) n = 29	6	1 n = 1					
4.	R. Holm et al (1993). n= 141	23 (22%) n = 105	82	2 (6%) n = 36	34				
5.	Hunt & Hale (1996) n= 82	5 (22.7 %) n = 22	17	5(14.3%) n = 35	30	4(16%) n = 25	21		
6.	Conner MG et al (2002) n=23							10 n=23	13
7.	Present Study n=50	39(82.9%) n = 47	8	1 n = 1		1 n = 1		1 n = 1	

CONCLUSION:

Loss of p⁵³ function was believed to play an important role in cervical carcinogenesis especially in squamous cell type.

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