p53 expression in carcinoma of the cervix

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Abstract

Aim—To assess overexpression of the proposed tumour suppressor gene product p53 using the mouse monoclonal antibody DO-7 in the three main subtypes of carcinoma of the uterine cervix and to evaluate its value as a prognostic indicator.

Methods—Eighty two cases of FIGO Stage IB/IIA uterine cervical carcinoma were studied retrospectively. The tumours had been previously typed into adenocarcinomas, squamous carcinomas and adenosquamous carcinomas after the tissue had been fixed in formalin and embedded in paraffin wax. p53 protein expression was assessed using a standard immunohistochemical technique and the findings were correlated with tumour type, lymph node status and clinical outcome.

Results—In total, the p53 gene product was overexpressed in 17.1% (14/82) of all carcinomas and also in areas of cervical intraepithelial neoplasia grade III adjacent to invasive squamous carcinoma. Where present, the normal epithelium was uniformly negative. No association was found between p53 overexpression and tumour subtype, lymph node status or clinical outcome.

Conclusions—It seems unlikely that p53 analysis will be of value in determining prognosis in carcinoma of the uterine cervix.

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cial ways: after (1) somatic point mutation¹⁰; (2) loss of heterozygosity¹¹; and (3) following human papillomavirus (HPV) infection, as the viral oncoprotein E6 is known to bind to, stabilise and ultimately degrade wild type p53.¹²

In this study, we have used a mouse monoclonal antibody (DO-7) to examine the overexpression of the p53 gene product in stage IB/IIA cervical carcinomas and have correlated our findings with lymph node status and clinical outcome in order to assess the value of p53 as a prognostic indicator.

Methods

Eighty two cases of FIGO stage IB/IIA cervical carcinoma, which had been treated by Wertheim's hysterectomy without prior radiotherapy, were selected from the files of the Department of Reproductive Pathology at St Mary's Hospital, Manchester, UK.

The tissue had been fixed in formalin, routinely processed and embedded in paraffin wax. The carcinomas were typed according to the criteria of Buckley and Fox¹³ using haematoxylin and eosin stained sections and periodic acid Schiff/alcan blue staining, with and without diastase predigestion.

A microwave antigen retrieval method was used and sections were irradiated at 750 W for 20 minutes in 1 litre of citrate buffer (pH 6.0). For immunohistochemical staining, sections were dewaxed in xylene and a standard avidin biotin immunoperoxidase technique was applied. The sections were first incubated with normal goat serum at room temperature for 20 minutes, to reduce background staining, and then with anti-p53 mouse monoclonal antibody, DO-7 (Novocastra Laboratories, Newcastle upon Tyne, UK), diluted 1 in 100 in phosphate buffered saline at room temperature for 60 minutes. A colorectal adenocarcinoma with known overexpression of the p53 gene product was used as a positive control. Negative controls, involving substitution of the primary antibody with rabbit serum, were included for all cases.

The percentage of tumour cells staining positively was assessed using a semiquantitative technique: tumours in which there was nuclear positivity in at least 10% of the neoplastic cells were regarded as positive; those tumours with only focal or no nuclear staining were regarded as negative. Note was made of lymph node status (lymph node metastases absent or present) and clinical outcome (alive and well; alive with recurrent disease; died of disease). In all cases a minimum of five years follow up was available. The results were analysed using a χ^2 test, with Yates's correction where appropriate, and Fisher's exact test with the SAS statistical

The p53 gene is located on chromosome 17p13.1 and is thought to act as a tumour suppressor.¹ It is involved in the regulation of cell proliferation by stimulating the transcription of other specific cell cycle control genes.² 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, stabilised or mutant p53 protein cannot, and thus the replication of abnormal DNA is not prevented. Consequently, inactivation of wild type p53 gene product represents the most common genetic alteration in human carcinogenesis³⁻⁵ and its overexpression, as detected by immunohistochemistry, has been proposed to indicate a worsened prognosis in some malignancies.⁶⁻⁸

Although the exact sequence of molecular events has not been elucidated fully, loss of p53 function is believed to play an important role in the pathogenesis of carcinomas of the uterine cervix,⁹ where it has been shown that the p53 gene product may be inactivated in three prin-

Figure 1 Positive nuclear staining for p53 in a well differentiated adenocarcinoma of the uterine cervix.

Table 1 Patterns of p53 staining in the main subtypes of uterine cervical carcinoma

Tumour type	p53 staining (%)	
	Positive	Negative
Squamous carcinoma (n = 22)	5 (22.7)	17 (77.3)
Adenosquamous carcinoma (n = 25)	4 (16.0)	21 (84.0)
Adenocarcinoma (n = 35)	5 (14.3)	30 (85.7)
Overall (n = 82)	14 (17.1)	68 (82.9)

Table 2 Percentage death rate with respect to p53 staining in patients with and without lymph node metastases in each tumour type and overall

Tumour type	p53 staining	
	Positive	Negative
<i>Lymph node metastases present</i>		
Squamous carcinoma	25% (1/4)	50% (3/6)
Adenosquamous carcinoma	100% (1/1)	22% (3/11)
Adenocarcinoma	0% (0/1)	55% (6/11)
Overall	33% (2/6)	43% (12/28)
<i>Lymph node metastases absent</i>		
Squamous carcinoma	100% (1/1)	22% (3/11)
Adenosquamous carcinoma	67% (2/3)	10% (1/10)
Adenocarcinoma	0% (0/4)	5% (1/19)
Overall	38% (3/8)	13% (5/40)
<i>Total</i>		
Squamous carcinoma	40% (2/5)	35% (6/17)
Adenosquamous carcinoma	75% (3/4)	19% (4/21)
Adenocarcinoma	0% (0/5)	23% (7/30)
Overall	36% (5/14)	25% (17/68)

package. A significant correlation between two parameters was taken at the 95% confidence limit, where $p < 0.05$.

Results

Each of the three most frequent tumour subtypes (squamous, adenosquamous and adenocarcinoma) were represented as different tumour types having different behaviour patterns.¹⁴ Twenty cases of squamous carcinoma, 25 adenosquamous carcinomas and 35 adenocarcinomas were studied. Overall, there was p53 overexpression in 17.1% (14/82) of the carcinomas, demonstrated by granular nuclear positivity (fig 1). There was no evidence of cytoplasmic or membranous posi-

tivity in any of the tumours. The number of the tumour subtypes containing more than focal positivity is shown in table 1. Most of the cases deemed to be negative contained no positive cells. Scattered, focal nuclear positivity was present, however, in 10 cases (three squamous carcinomas, three adenocarcinomas and four adenocarcinomas). Only three tumours in total (two adenocarcinomas and one adenosquamous carcinoma) contained more than 50% positive cells.

p53 overexpression was seen in epithelium which contained cervical intraepithelial neoplasia (CIN) grade III adjacent to invasive squamous carcinoma, but not in epithelium containing CIN grade I or II, cervical glandular intraepithelial neoplasia (CGIN), or in any normal epithelium present. In the foci of CIN III, isolated positive cells were present at all levels within the surface squamous epithelium. The stromal cells were negative.

No association was found between p53 overexpression and cervical carcinoma overall, the individual tumour subtypes, lymph node status or clinical outcome (table 2).

Discussion

The pathogenesis of carcinoma of the uterine cervix is thought to occur through a multistep process but further work is still required to clarify the exact mechanism. Attention has been focused on a number of possible co-factors including cigarette smoking,¹⁵ immunodeficiency,¹⁵ HLA subtypes,¹⁶ certain HPV subtypes,¹⁷ and the proposed tumour suppressor gene p53,¹⁸ as loss of p53 function is the commonest genetic alteration involved in human malignancies.

The mouse monoclonal antibody DO-7 was chosen to examine p53 overexpression in uterine cervical carcinoma as it had been demonstrated to be the most specific and sensitive antibody directed against p53 protein when used on paraffin wax embedded tissue.¹⁹

p53 gene product has been shown to be overexpressed in cervical carcinoma as a whole and in its three main tumour subtypes to varying extents.²⁰⁻²³ Our value of 17.1% (14/82) obtained for the total number of carcinomas containing more than 10% immunoreactive tumour cells is in keeping with the majority of the published data—for example, 25.5% (Oka *et al*),²⁴ 20.2% (Kainz *et al*)²⁵ and 14.0% (Busby-Earle *et al*).²⁶ However, a wide range of figures does exist, with one study claiming up to 74% positivity in squamous carcinomas.²⁷ Within the individual tumour subtypes of our study, 22.7% of squamous carcinomas, 16.0% of adenosquamous carcinomas and 14.3% of adenocarcinomas were regarded as positive. Assessment of p53 overexpression by immunoreactivity has only been described once previously in adenosquamous carcinoma where 57% (four of seven) of tumours were stated as being positive.²¹ In adenocarcinomas of the uterine cervix, 11% (four of 36),²⁰ 44% (four of nine)²¹ and 100% (one of one)²⁷ have been shown to contain cells overexpressing p53 protein. In the present study there was no sig-

nificant difference between the individual tumour subtypes with respect to p53 protein expression.

Only three of the tumours studied (two adenocarcinomas and an adenosquamous carcinoma) contained more than 50% positive cells. Using the same antibody and a similar method, Lambkin *et al*²² found that seven of 55 cervical squamous carcinomas had more than 50% positive cells. They stated that microwave pretreatment significantly increased both the numbers of tumours and the number of individual cells within any particular tumour showing p53 immunoreactivity.

We were able to demonstrate p53 immunoreactivity in those areas of CIN III adjacent to invasive components, as has been described,²⁷ but foci of CGIN and the normal epithelium included were negative. Holm *et al*²⁰ found all grades of CIN and CGIN to be negative for p53 and suggested that alteration in p53 protein expression is not an early event in the pathogenesis of cervical neoplasia. Bosari *et al*²⁷ observed that the "distribution of p53-immunoreactive cells paralleled the extension of dysplastic changes in the squamous epithelium" and suggested that p53 immunoreactivity may precede the development of tumours with a fully malignant and invasive phenotype. The presence of focal positive cells in an otherwise negative tumour may be due to increased p53 expression in cells undergoing DNA repair.²⁸ Another explanation is alteration in mdm-2 gene expression.²⁹ Wild type p53 is known to be bound by mdm-2 oncogene product in vitro and it is possible that overexpression of mdm-2 has resulted in accumulation of detectable p53 gene product.

The degradation of the p53 gene product after binding to the E6 viral protein in cells infected with high risk HPV subtypes may play a fundamental role in the development of cervical carcinoma.¹² It may therefore be considered a deficiency of this study that HPV analyses were not performed. Alternatively, Butz *et al*²⁰ have demonstrated that p53 does not seem to be regularly inactivated in HPV positive cancer cells. It has also been shown that p53 mutations are rare in some HPV negative cervical carcinomas³¹ and that there is no consistent correlation between p53 mutations and HPV status.²⁶ These conflicting findings indicate that mechanisms other than HPV infection and p53 mutation must come into play to cause functional inactivation of p53. Further studies are therefore necessary to clarify the exact role of the p53 gene product and high risk HPV subtypes in the pathogenesis of cervical carcinoma.

To our knowledge, only two previous studies have examined the prognostic value of p53 protein overexpression in surgically treated early stage carcinomas of the uterine cervix.^{21 25} Our findings were similar; there was no relation between p53 overexpression and prognosis in the uterine cervix. Kainz *et al*²⁵ used the monoclonal antibody BP53-12 on 109 cases of squamous carcinoma and found no relation to prognosis. Work from the same institute also failed to demonstrate that p53 overexpression

was of prognostic value in advanced, stage III squamous cervical carcinoma.³² Similar findings were obtained by Oka *et al*²⁴ using the polyclonal antibody CM1 on stage III squamous carcinoma. Helland *et al*²¹ found no relation between p53 protein expression and prognosis in 92 stage I-IV cervical carcinomas, also using the CM1 antibody. The latter is the only other study to correlate p53 protein expression with prognosis in adenocarcinoma and adenosquamous carcinoma by immunohistochemistry, although smaller numbers were involved and the disease stage for tumour subtypes was not specifically stated. Helland *et al*²¹ found that 55% (51/92) of carcinomas were positive, but p53 protein expression was only detected in less than 5% (45/51) of the tumour cells in the majority of cases. As false tumour positivity does occur³³ and there is a close relation between high levels of p53 overexpression and neoplasia,³⁴ the validity of such observations based on results from tumours with only a small number of positive cells is difficult to assess.

These differences in interpretation highlight the problem of correlating results from different immunohistochemical studies involving antibodies directed against p53. As protocols have not been standardised, it is perhaps not possible to draw conclusions from comparisons of studies where the tumour stages, antibodies, fixation processes, antigen unmasking techniques, the determination of tumour cell positivity qualitatively and quantitatively, and statistical analysis have varied considerably.

In a recent review of the value of p53 overexpression as an indication of poor prognosis in human malignancies, Dowell and Hall³⁵ concluded that while p53 is of undisputed importance in our understanding of oncogenesis and neoplasia, it is probably only a weak prognostic factor; observations in keeping with our findings. The p53 protein does indeed seem to play a pivotal role in the pathogenesis of carcinoma of the cervix but, despite the relatively small numbers involved in this series, there is no evidence to suggest that its overexpression may be useful as a prognostic indicator. Traditional parameters such as tumour size and lymph node status¹⁴ still seem to be the most important determinants of prognosis in carcinoma of the uterine cervix.

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