

p53 and bcl-2 Expression in Invasive and Pre-invasive Uterine Papillary Serous Carcinoma and Atrophic Endometrium

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Abstract

Introduction: Uterine papillary serous carcinoma (UPSC), a high-grade tumour, is known to be associated in some cases with an identifiable intraepithelial neoplasia (IEN) component. Biomarker studies incorporating this latter component are not well documented. One aim of the present study was to compare levels of immunohistochemical (IHC) expression of p53 tumour suppressor gene and bcl-2 oncoprotein between UPSC and IEN, as well as normal endometrium to determine its biologic significance. The other major aim was to determine if these IHC results have any bearing on survival data in this tumour. **Materials and Methods:** An immunoreactivity score was assigned for examination of p53 and bcl-2 expression in a total of 21 cases of UPSC, 9 with an evaluable IEN component and 11 with associated non-neoplastic endometrium. Statistical analysis of IHC results was performed, in addition to correlation with survival data and disease stage. **Results:** p53 was identified in 16/21 cases of UPSC (76%) and 8/9 cases of IEN (89%), and no cases of normal endometrium. By contrast, bcl-2 was positive in all normal endometria with less expression in UPSC leaving 15/21 (71%) cases positive, and in IEN, leaving 5/9 (55%) of cases positive. Differences in immunoreactive scores for both p53 and bcl-2 between UPSC and benign glands, as well as between IEN and benign glands reached statistical significance with *P* values of 0.006 and 0.014 for p53, and 0.003 and 0.027 for bcl-2 respectively. There was no statistical significance between values for UPSC and IEN. Cox regression analysis found no statistically significant relationship between patient survival time in early and late stages of disease, and p53 and bcl-2 immunoscores. **Conclusions:** The lack of a significant difference between the bcl-2 and p53 values for both UPSC and IEN suggests that these molecular alterations occur at an early stage of tumour pathogenesis. A potential advantage of the use of immunohistochemical markers is their application to routinely processed surgical specimens. In this case, bcl-2 and p53 were applied in UPSC to determine any potential significance, but neither marker proved to be a useful predictor of survival time or disease stage.

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Introduction

Major differences at clinicopathologic and molecular genetic levels are known to exist between the commonest variety of endometrial carcinoma, endometrioid adenocarcinoma (EC) and uterine papillary serous carcinoma (UPSC). The p53 tumour suppressor gene is expressed in the majority of cases of UPSC, in contrast to the usual situation in EC. Expression of the bcl-2 oncoprotein, which acts as an anti-apoptotic factor to

prolong cell survival, has been less well-characterised than p53 in endometrial malignancies, and shown to have wide variability of expression. For both markers, the invasive phase of carcinoma, as well as normal and hyperplastic endometrium, have been examined, but the early stages of neoplasia for UPSC have not been as extensively analysed. To determine if p53 and bcl-2 have any role or possible significant interaction in the early stages of pathogenesis of

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UPSC, immunohistochemical (IHC) evaluation of 9 cases of intraepithelial neoplasia (IEN) phase of tumour was performed, and compared with findings in 21 cases of invasive tumour and 11 cases of non-neoplastic endometrial glands. Statistical correlation with the clinical parameters of disease stage and survival time was also performed.

Materials and Methods

Tumour Samples

A total of 21 cases of primary UPSC were extracted from the files of Kandang Kerbau Women's and Children's Hospital over the time period 1991 to 1998 inclusive. Curettings with only a scant amount of material available for examination were excluded from the study. Two patients were not staged at the primary institution and were not included in the stage-related statistical analysis. International Federation of Gynecology and Obstetrics (FIGO) criteria (1988) staging for the remaining 19 patients established 4, 1, 12, and 2 cases of Stage I, II, III and IV disease, respectively. The age at diagnosis ranged from 42 to 75 years, with a median age of 62 years. The range of survival time was 1 to 62 months, with a median follow-up time of 21.5 months. All 19 patients with known follow-up underwent surgery. Twelve of these patients had chemotherapy, 6 with additional radiotherapy. One other patient received radiation alone following surgery.

Twenty-one cases were examined, comprising 15 hysterectomy specimens and 6 endometrial curettage specimens. Nine hysterectomy specimens were associated with sufficient adjacent areas of IEN as to be immunohistochemically evaluable. Eleven hysterectomy specimens included evaluable non-neoplastic endometrial glands, comprising 10 cases of atrophic endometrium, and 1 case of secretory phase glands.

Immunohistochemical Analysis

Immunoperoxidase studies were all performed on formalin-fixed paraffin-embedded tissue sections using mouse monoclonal antibodies directed against p53 (1:50 dilution; Dako, Denmark), and bcl-2 oncoprotein (1:50 dilution, Dako, Denmark) utilising the labelled streptavidin biotin method (Dako LSAB kit K0675).

Sections were cut at 3 microns onto silanised slides, deparaffinised and rehydrated. Endogenous peroxidase was blocked with 0.5% hydrogen peroxide for 10 minutes, followed by thorough washing in running water. Heat-mediated antigen retrieval was performed as follows: microwaving for 5 minutes in 0.01M citrate buffer (pH 6.0) for p53, and pressure cooking for 2 minutes in 0.01M citrate buffer (pH 6.0) for bcl-2.

The sections were incubated in turn with optimally diluted antibody (Ab) for 30 minutes, biotinylated Ab for

15 minutes, then peroxidase-conjugated streptavidin complex for 15 minutes. Sections were washed and developed with diaminobenzidine for 10 minutes before being counterstained with Harris' haematoxylin, dehydrated, cleared and mounted.

Positive control sections for p53 consisted of a case of colonic adenocarcinoma which showed diffuse strong nuclear staining, and for bcl-2, reactive tonsils which showed strong staining of mantle zone lymphocytes.

p53 stained slides were assessed for nuclear reactivity, and bcl-2 slides for cytoplasmic and membrane reactivity. Less than 5% of cells stained was considered a negative result.

Immunoreactivity score for p53 and bcl-2 in non-neoplastic endometrial glands, IEN, and invasive UPSC was devised as the product of the percentage of tumour cells positive, multiplied by the staining intensity. The immunoreactivity profile for each marker was scored as follows: percentage of cells stained, less than one-third (1+), less than two-thirds (2+) and more than two-thirds (3+). Intensity was scored as weak (1+), moderate (2+) or strong (3+). Thus, the maximum score obtainable for an individual case is 9.

Statistical Analysis

Statistical analyses were performed using the Wilcoxon signed rank test, Cox regression analysis and the Mann-Whitney test as appropriate. As is conventional, *P* values of less than 0.05 were considered to be significant, although we refer to *P* values close to 0.05 (arbitrarily defined to include values in the range 0.04 to 0.06) as being marginally significant or insignificant as appropriate.

Results

Immunohistochemical Staining

Of a total of 21 cases of UPSC investigated for p53, only 5 were completely negative (24%), and only 1 of 9 IEN cases was negative (11%), with most positive cases in both situations showing high levels of immunoreactivity (Fig. 1). All normal endometrial glands were negative. No discrepant cases of staining were seen between IEN and invasive components (Table 1). By contrast, the highest immunoreactive scores for bcl-2 were seen in normal endometrial glands, all of which (100%) showed positive scoring (range, 2 to 9). A lower positive rate (71%) was seen in UPSC, with 15/21 cases positive, whilst 5 of 9 IEN cases were positive (55%). Immunoreactivity scores were highest in normal glands with diminution of scoring levels in both UPSC and IEN (Fig. 2). Scoring levels between the invasive and IEN components were generally similar with the exception of 1 case, which scored 9 for IEN and 4 in UPSC (Table 2).

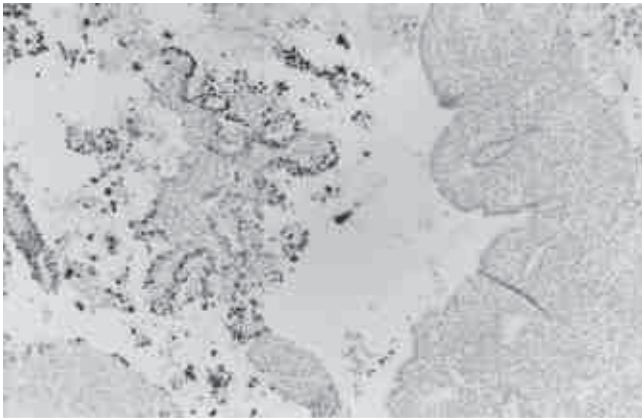


Fig. 1. Uterine papillary serous carcinoma showing nuclear p53 positive reaction and lack of staining in normal endometrium.

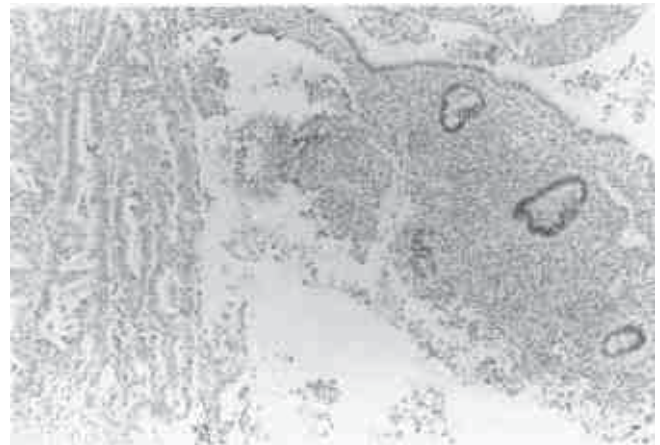


Fig. 2. Strong positive bcl-2 staining reaction in normal endometrial glands on the right contrasting with a negative reaction in uterine papillary serous carcinoma.

Statistical Analysis

For both p53 and bcl-2, no statistically significant differences were found between the results of the UPSC and IEN categories. However, between the neoplastic and benign categories, statistically significant differences were found for both p53 and bcl-2 (Table 3).

The relationship between survival time (in months) with stage of disease, p53 and bcl-2 scores was investigated using Cox regression. For the purpose of this analysis, stage of disease was classified as being either “early stage” (stage 1 or 2) or “late stage” (stage 3 or 4). Similarly, the p53 scores were classified as being either “low” (score of 0 to 4) or “high” (score of 5 to 9), and likewise for the bcl-2 scores (“very low”: 0, 1 or “moderately low”: 2, 3, 4). No statistically significant relationship was found between survival and the immunoreactive scores, $P = 0.827$ for p53 and $P = 0.273$ for bcl-2 (Table 4). As for stage, this resulted in a P value of 0.056, which was not significant.

The difference in p53 and bcl-2 scores between early- and late-stage samples was also investigated. There was no

evidence of a significant difference in the p53 or bcl-2 scores between these categories ($P = 0.183$). As for p53, this was marginally not significant ($P = 0.050$).

Discussion

The UPSC variant accounts for approximately 7% to 10% of all endometrial carcinoma cases, and generally behaves as an aggressive, non-oestrogen dependent neoplasm. This is putatively related to early vascular dissemination. Clinico-biologically it appears distinct from endometrial carcinoma. However, the stages at which molecular changes occur are less well characterised and would be intriguing to chart in view of some unusual features that may result in early serous neoplasia. It is recognised that IEN may arise without coexistent invasive tumour in the uterus, yet be associated with invasive serous carcinomatosis in extrauterine sites.¹ The importance of this phenomenon lies in its role in predicting patient survival.²

Table 1. p53 Immunoreactive Scores

Case No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
UPSC invasive carcinoma	9	9	6	9	9	9	9	6	0	9	9	0	6	0	6	1	0	2	9	6	0
IEN component (total = 9)	9				9	6	9					0	9		9	1			9		
Endometrial glands (total = 11)		0	0		0	0	0						0	0	0	0			0		0

IEN: intraepithelial neoplasia; UPSC: uterine papillary serous carcinoma

Table 2. bcl-2 Immunoreactive Scores

Case No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
UPSC invasive carcinoma	0	4	0	1	2	1	0	2	4	2	1	1	2	0	4	1	4	0	0	2	3
IEN component (total = 9)		0			2	0	0					1	2		9	1			0		
Endometrial glands (total = 11)		6	3		9	4	6						9	2	9	6			6		6

IEN: intraepithelial neoplasia; UPSC: uterine papillary serous carcinoma

Table 3. *P* Values for Comparative Immunoreactive Scores between UPSC, IEN and Benign Categories

Categories compared	Sample size*	<i>P</i> value	
		p53	bcl-2
UPSC and IEN	9	0.564	0.655
UPSC and benign	11	0.006 [†]	0.003 [†]
IEN and benign	7	0.014 [†]	0.027 [†]

* Sample size refers to the identical number of cases in each category available for comparison.

[†] Statistically significant results (*P* < 0.05).

IEN: intraepithelial neoplasia; UPSC: uterine papillary serous carcinoma

p53 is a tumour suppressor gene located on the short arm of chromosome 17. In the wild form, the p53 protein induces effects through the transcriptional activation of oncogenes involved in the modulation of cell cycle progression. The protein is also involved in cell growth control and DNA repair and can induce apoptosis.³ In the malignant cell however, p53 mutation acts as an anti-apoptotic agent to favour cell survival of abnormal cells.⁴ p53 mutation has been reported in the majority of cases of invasive UPSC. Relatively few cases of IEN have been examined, the largest single series amounting to 21 patients.⁵ Typically, where invasive tumour is positive, the IEN is correspondingly positive, with the same level of immunoreactivity. Only 1 case of a total of 36 found in the literature⁵⁻⁷ showed discrepant IHC staining with invasive tumour positive and IEN component negative.⁶ p53 is not normally expressed in normal or hyperplastic endometrial glands.

bcl-2 oncoprotein product of the gene, located on chromosome 18, is known to block physiologically programmed cell death and prolong cell survival. In a similar fashion, it may also assist the survival of abnormal neoplastic cells.⁸ bcl-2 is under oestrogenic influence, and is normally detected in cycling proliferative endometrium, disappearing at the onset of secretory phase.⁹ Wide variations in positivity rates for bcl-2 in invasive carcinoma have been described, ranging from 25%⁹ to 85.7%.⁷ To our knowledge, the findings in IEN have not been previously reported.

Our results suggest that there is a strong relationship between p53 expression and neoplasia, with statistically significant differences observed between normal and neoplastic glands. For bcl-2, a moderate diminution in positivity between normal and neoplastic glands was also statistically significant. Thus, acquisition of p53 mutation in neoplasia is associated with corresponding loss of bcl-2 expression. For each antibody, minimal differences were observed in expression between IEN and UPSC.

Our study found little evidence of the usefulness of p53 or bcl-2 immunoreactive scores in predicting survival time

Table 4. Results of Cox Regression Analysis and Comparison of Immunoreactive Scores between Early- and Late-stage Patients

		<i>P</i> value
Cox regression on survival time	p53*	0.827
	bcl-2 [†]	0.273
	Disease stage [‡]	0.056
Difference in scores between early (1,2) and late (3,4) disease stage	p53	0.050
	bcl-2	0.183

* p53 scores classified as being either "low" (score of 0 to 4) or "high" (score of 5 to 9).

[†] bcl-2 scores classified as being either "very low" (score of 0,1) or "moderately low" (score of 2,3,4).

[‡] Disease stage classified as being "early stage" (0,1) or "late stage" (3,4).

or disease stage. However, the small number of early-stage samples (5 cases) makes it difficult to draw any firm conclusions. Several studies have attempted to correlate the effects of bcl-2 and p53 on cell survival with apoptotic index and proliferative activity. Ioffe^{10,11} found that in endometrioid carcinoma, p53 expression correlated with both apoptosis and cellular proliferation rates, in contradistinction to other authors. In the case of bcl-2, high levels have been reported to be inversely related to apoptotic index (AI) in non-serous endometrial carcinoma.^{9,12} Other tumour types may show^{13,14} or lack any such correlation.¹⁵⁻¹⁷ When the relationship between AI and cellular proliferation rate is examined in some of these studies, the findings are also contradictory. In the case of non-serous EC, bcl-2 and Ki-67 expressions have been reported as showing no significant relationship.

Our study demonstrates that loss of bcl-2 expression, commensurate with p53 expression, occurs during the IEN phase of malignancy in UPSC. p53 mutations in malignancy, including UPSC, act in general to favour cell survival by an anti-apoptotic effect. Diminution in levels of bcl-2 would, however, suggest less inhibition of apoptosis. Theoretically, prevention of apoptosis could facilitate proliferative activity.¹⁸ The unexpected finding of our study was that similar effects on apoptosis are associated with the loss of bcl-2 expression. The link between apoptosis and cell proliferation is not entirely clear, nor is it known at what stage the 2 processes become mutually exclusive,¹⁹ but may be associated with caspase activity. Up-regulation of the cellular apoptosis susceptibility (CAS) gene in association with Bax and caspase-3, and down-regulation of bcl-2 may be involved in the complex regulation of proliferation and apoptosis, leading eventually to malignancy.²⁰ Interaction between these bcl-2 protein family members also plays a role in the balance between proliferation and apoptosis. Bax IHC expression has been found in UPSC and inversely correlated with bcl-2.²¹ The role of other potential anti-apoptotic factors such as bcl-XL and Mcl-1 has not been

elucidated for UPSC. Furthermore, bcl-2 mechanisms of action may be influenced by p53 itself, acting as a transcriptional activator of bax and negative transregulator of the bcl-2 gene.²⁰

The molecular and genetic alterations relating to cellular proliferation and apoptosis in the early stages of carcinogenesis are obviously complex and diverse, the relationship between these 2 components not having been previously described for the situation of serous IEN.

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