p53 expression in tissue adjacent to endometrial carcinoma

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Summary

Objective: Since several investigations did not demonstrate the presence of altered p53 in endometrial hyperplasias, it has been concluded that these alterations constitute a relatively late event in endometrial carcinoma.

The aim of the present study was to assess the presence of p53 in the tissue adjacent to endometrial carcinoma in attempt to elucidate the relationship between these tissues.

Methods: New slides were prepared from paraffin-embeded tissue blocks of 49 endometrial endometrioid carcinoma hysterectomy specimens so that in each case tumor tissue and adjacent uninvolved endometrium were represented. Immunohistochemical staining for p53 detection was then performed.

Results: In 43 of the 49 hysterectomy specimens evaluated, the tissue adjacent to the endometrial carcinoma was non hyperplastic and in six it was hyperplastic. Positive immunohistochemical staining was found in 22 (44.9%) of endometrial carcinomas and in eight (16.3%) of the adjacent tissues. A statistically significant higher percentage of hyperplastic adjacent tissues than non-hyperplastic adjacent tissues were immunohistochemically p53 positive (50.0% vs 11.3%; p = 0.047).

Conclusions: Our findings may indicate that p53 alterations are not necessarily a late event in endometrial endometrioid carcinogenesis. Since a large proportion of tissues adjacent to endometrial carcinoma do not show p53 alterations, other early cellular events may also play a role.

Key words: p53; Endometrial carcinoma; Adjacent tissue.

Wild type p53 protein product has a short half-life and therefore is not demonstrable by immunohistochemical staining. Altered p53 gene protein has a prolonged half-life and accumulates to detectable immunohistochemical levels.

Overexpression of p53 has been reported in 17-52% of endometrial carcinomas and is considered to be associated with poor prognosis [1-5]. It is generally accepted that endometrial hyperplasias are a precursor of endometrial carcinoma. Yet, several investigations did not demonstrate the presence of altered p53 in endometrial hyperplasias [6-9]. It has been therefore concluded that alterations of p53 constitute a relatively late event in endometrial carcinoma.

The aim of the present study was to assess the presence of p53 in the tissue adjacent to endometrial carcinoma in an attempt to elucidate the relationship between these tissues.

Material and methods

Paraffin-embeded tissue blocks of unselected 49 hysterectomy specimens with endometrial endometrioid carcinoma diagnosed during a 4-year period (1996-1999) were examined.

Formalin-fixed hematoxylin-eosin stained 6 μm slides from the tumor tissue of the same patients were newly performed and reviewed by two senior pathologists (YF and IA) in order to verify the diagnosis.

An additional 4 μm unstained slides were prepared from each case for immunohistochemical staining. In each case tumor tissue and adjacent uninvolved endometrium were represented in the stained slides. For p53 detection we used mouse anti-p53 clone Pab-1801 CA-18-0170 antibody (Zymed Laboratories Inc., San Francisco, USA), in dilution of 1:300, with an amplification Kit (Ventana, Tucson Arizona, USA). Immunohistochemistry was performed by deparaffinization of slides in Xylene, absolute ethyl alcohol and degradated alcohols 96% and 70%. Microwave mediated epitope retrieval was performed for 15 minutes in EDTA buffer pH-8 (Zymed). Immunoperoxidase stains were performed using a modified labeled streptavidin technique and run on an automated system (Ventana Autostainer, NexES-Ventana) using a commercially available chromogen (AEC Ventana). All sections were counterstained with Meyer's hematoxylin. Sections of cervical squamous carcinoma known to contain altered p53 protein served as positive controls.

Two parameters in each case were then evaluated. The percentage of tissue area stained in 10 to 12 high power fields and its intensity graded as 0, 1, 2, 3, (negative, weakly positive, intermediate positive and strongly positive staining, respectively).

Cancer tissue and adjacent tissue with staining of more than 50% of the area examined and an intensity of 2.3 was considered as positive.

Differences were compared by the two-tailed Fisher's exact test. A p value of < 0.05 was considered significant.

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Results

The median age of the patients was 65.5 years (range 32-91) and 39 (79.6%) of them were postmenopausal. At the time of diagnosis, 43 (87.7%) patients had FIGO Stage I and 24 (48.9%) patients had grade 1 tumors. The remaining patients had Stage II-III and grade 2-3 tumors.

In 43 of the 49 hysterectomy specimens evaluated, the tissue adjacent to the endometrial carcinoma was non hyperplastic (40 atrophic and 3 proliferative) and in six it was hyperplastic.

Table 1 shows the rate of positive immunohistochemical staining for p53 in endometrial cancer and adjacent tissue by adjacent tissue type.

Positive immunohistochemical staining was found in 22 (44.9%) of endometrial carcinomas and in eight (16.3%) of the adjacent tissues. A statistically significant higher percentage of hyperplastic adjacent tissues than non-hyperplastic adjacent tissues were immunohistochemically p53 positive (50.0% vs 11.6%; p = 0.047). Both the adjacent and the tumor tissue were p53 positive in three of 43 (6.9%) cases with non hyperplastic adjacent tissue and in three of six (50.0%) adjacent hyperplastic tissue. This difference was also statistically significant (p = 0.02).

Table 1. — *Immunohistochemical staining for p53 in endometrial endometrioid cancer and adjacent tissue.*

			Adjacent tissue			
	Tumor tissue		Non hyperplastic		Hyperplastic	
	No.	%	No.	%	No.	%
Total	49	100.0	43	100.0	6	100.0
p53 negative	27	51.1	38	88.4	3	50.0
p53 positive	22	44.9	5	11.6	3	50.0

Discussion

Loss of p53 suppressor gene function may cause unrestrained cellular proliferation leading to malignancy. It is known that malignant transformation is a multistep process. p53 mutations are considered to be a relatively late event in colon and breast carcinoma [10, 11] and an early event in lung and esophageal carcinogenesis [12, 13].

It is well established that hyperplasias are precursors of endometrial endometrioid carcinoma. Kohler *et al.* [7] using single stranded plymorphism analysis found no p53 mutations in any of the hyperplasias examined, whereas 10% of 30 endometrial carcinomas had mutations. Eissa al *et al.* [8] also found no mutant p53 proteins in endometrial hyperplasias and Ioffe *et al.* [14] found no p53 overexpression in simple and complex hyperplasias. These findings led to the conclusion that p53 alterations are a late event in endometrial carcinoma. However, contrary results have been obtained by other investigators. Yu *et al.* [15] found positive immunostaining in 10 % of simple and 12% of atypical hyperplasias. Similarly, Riethdorf *et al.* [16] found p53 alterations in 9% to 18% of various types of hyperplasia and Enomoto *et al.* [17] reported the presence of p53 allelic loss in one of four (25%) informative cases of endometrial atypical hyperplasia and the presence of p53 mutations in 8% (1/13) of such hyperplasias. The inconsistency of results may stem from different techniques used for identification of altered p53 or its protein product. In addition, imunohistochemical staining does not always indicate the presence of mutations [18, 19] nor does every mutation lead to accumulation of p53 protein [20]. It should be pointed out that all these investigations have been performed on hyperplastic tissues without coexistant carcinoma.

Only a few studies have dealt specifically with tissue adjacent to the endometrial carcinoma. Kaku *et al.* [6] mention that in 23 of 26 cases, foci of endometrial hyperplasia adjacent to endometrial carcinoma were recognized in representative sections for immunohistochemical staining and were non reactive for p53. Sherman *et al.* [21] also reported that non-hyperplastic and hyperplastic endometria in their study were nonreactive for p53. However, as noted by the authors themselves the exact anatomic relationship of the tissue sections examined to the coexistant invasive tumors was not known.

In the present study, we have specifically assessed the p53 immunohistochemical staining status in endometrial tissue adjacent to the malignancy; p53 immunohistochemical staining was observed in 50% of hyperplastic endometrium adjacent to tumor. In spite of the small number of adjacent tissues with hyperplasia, this rate was significantly higher than that of positive staining in nonhyperplastic adjacent tissue (11.3%). We also found that the percentage of positive staining in both the tumor and the adjacent endometrial tissue was significantly higher in cases with adjacent hyperplastic tissue. In addition, as opposed to results reported by Kaku *et al.* [6], we found no statistical difference in the proportion of positive tumor staining in neoplasms with or without hyperplasia. In this context it is noteworthy that Sherman *et al.* [21] reported that in serous endometrial carcinoma, 86% of concomitant endometrial intraepithelial carcinomas, a recognized precursor, were immunoreactive for the p53 protein.

Our findings do not negate the notion [6, 21] of a dualistic pathway of endometrial carcinogenesis. Namely that in one pathway, the neoplasm develops from estrogen-associated hyperplasias while in the other it arises from estrogen-independent atrophic endometrium.

Conclusions

Our findings, mainly the significantly higher rate of positive staining in hyperplastic adjacent tissue, may indicate that p53 alterations are not necessarily a late event in endometrial endometrioid carcinogenesis. Obviously, since a large proportion of tissues adjacent to endometrial carcinoma does not show p53 alterations, other early cellular events may play a role in the development of endometrial carcinoma.

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