Prognostic value of p53, c-erb-B2 and MIB-1 in endometrial carcinoma

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Summary

Objective: To assess the immunohistochemical expression of p53 protein, a tumour suppressor gene of the oncogene c-erb-B2 and MIB-1 proliferation marker (Ki-67 antigen) in endometrial carcinoma.

Methods: We studied 29 cases of endometrial carcinoma in which the p53, c-erb-B2 and MIB-1/Ki-67 antigens were investigated by an immunohistochemical method. We evaluated the correlations among the immunohistochemical positivity and the grading, depth of myometrial invasion, stage of the neoplasia and follow-up.

Results: Both p53 and c-erb-B2 were positive in 16 out of 29 cases (55.2%), whereas MIB-1 was positive in 19 out of 29 cases (65.5%). All these three antigens showed a positive correlation with the grading, myometrial invasion and FIGO stage.

Regarding follow-up, p53, c-erb-B2 and MIB-1 were, respectively, positive in 100%, 83.4% and 66.7% of neoplasias of patients who died of disease whereas they were positive in 40%, 40% and 60%, respectively, of tumours of patients with no evidence of disease.

Conclusion: The overexpression of p53, c-erb-B2 and MIB-1 seem to indicate a more malignant tumour phenotype.

Key words: Endometrial carcinoma; p53 tumour suppressor gene; c-erb-B2; MIB-1 proliferation marker (Ki-67 antigen).

Introduction

Recent studies [1, 2] have investigated several oncogenes and tumour suppressor genes that are involved in the onset of endometrial carcinoma.

Oncogenes such as K-ras [3], HER-2/neu gene/c-erb-B2 [4] and p53 gene, a tumour suppressor gene [5], which is localised on the short arm of chromosome 17 and is a determinant in the control of the cellular cycle, are the most studied.

The role of p53 [5-7], in normal cycling cells, consists of blocking cellular growth in the G-1 phase when damage to the genome occurs. Thus this tumour suppressor gene permits the activation of DNA repair mechanisms. The p53 gene is not expressed in normal endometrium whereas in about 20% of endometrial carcinoma this gene is overexpressed and is associated with a high grade and advanced stage of disease [8].

Indeed, today immunohistochemistry allows us to perform the quantitative evaluation of the number of proliferative cells and to identify the Ki-67/MIB-1 antigen which is localised in the nucleus of cells and expressed only in the proliferative phases of the cellular cycle [9]. MIB-1 is a monoclonal antibody to the Ki-67 antigen and is considered a potential prognostic indicator in endometrial carcinoma. In fact, it is related to a higher incidence of relapse and to a higher malignancy rate of the neoplasia [10].

The aim of our study was to clarify the relation between p53, c-erb-B2 and MIB-1 immunostaining profiles of endometrial carcinomas and clinical-pathologic prognostic parameters such as grading, depth of myometrial invasion, clinical stage and follow-up, and thus to evaluate whether these markers could help in the prognostic evaluation of the patient.

Materials and Methods

In this retrospective study we considered 29 cases of endometrial carcinoma treated by surgery (total abdominal hysterectomy and bilateral salpingo-oophorectomy, superior colpectomy and lymph node sampling) in the period 1990-1995 (thus to have an adequate 5-year follow-up) at the Gynaecologic Clinic of Sassari University. The surgical specimens were submitted to pathologic examination at the Institute of Pathology.

At the time of diagnosis (performed with an endometrial biopsy by hysteroscopy), the age of patients ranged from 34 to 90 years (mean 63 years).

The surgical specimens were fixed in 10% neutral buffered formalin, paraffin embedded and cut into 4 μm sections, and then were coloured by hematoxylin-eosin. Some sections were expelled onto glass slides before being treated with 0.1% poly-L-Lysin to increase the adhesiveness. Antigens were investigated in neoplastic tissue by a immunohistochemical method, using antibodies c-erb-B2 against HER-2 (A0485, diluted 1:1600; DAKO Corporation), p53 (clone DO7, diluted 1:200; DAKO Corporation), Ki-67/MIB-1 (Immunotech clone MIB-1, diluted 1:100; Beckman Coulter, Inc., Fullerton, CA). The immunodetermination was performed using immunoperoxidase Avidin-Biotin Complex (ABC method). Endogenous peroxidase was inhibited by the Heyderman and Neville procedure. Diaminobenzidine was the chromogen.

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For the evaluation of p53 we considered the cases with a specific colouration localised only in the nucleus as positive. A threshold value of 10% neoplastic cells was determined for the identification of positive cases.

Regarding the analysis of HER-2, the cases with specific immunoreactivity of cytoplasmic membranes in the majority of cells were considered positive.

Cases with nuclear colouration in more than 15% of neoplastic cells for the analysis of proliferative activity (PA), which was performed with MIB-1, a monoclonal antibody able to react Ki-67 antigen, were considered positive.

All the tumours included in the study showed an endometrial histotype: nine cases had characteristics of squamous differentiation (adenoacantomas), two had a malignant squamous component (adenosquamous), two had partial modifications with clear cells and five had villoglandular aspects.

The grading showed nine well-differentiated neoplasias (G1), 15 moderately differentiated (G2) and five poorly differentiated (G3).

Myometrial invasion showed 13 cases with one-third inner layer infiltration (M1), eight cases with one-third medium infiltration (M2) and eight cases with one-third external involvement (M3).

Lymph node status was available only for nine cases, with only one case of lymph node metastasis histologically demonstrated.

According to the 1988 International Federation of Gynaecology and Obstetrics (FIGO) staging [11], we found 15 cases in stage IB, six IC, one IIA, three IIB, one IIIA, one IIIC and two IVA.

Follow-up was available for 28 out of 29 cases. Two patients died of pathologies not related to the neoplasia and six died of the cancer in a period ranging from 10 to 24 months following the diagnosis. Disease-free survival ranged from 24 months to ten years (mean 69 months) for the remaining 20 patients.

Table 1. — *Correlation with grading.*

	p53	c-erb-B2	MIB-1
G1 (9 cases)	4/9 (44.5%)	5/9 (55.6%)	5/9 (55.6%)
G2 (15 cases)	8/15 (53.4%)	7/15 (46.7%)	9/15 (60%)
G3 (5 cases)	4/5 (80%)	4/5 (80%)	5/5 (100%)
Total (29 cases)	16/29 (55.2%)	16/29 (55.2%)	19/29 (65.5%)

Table 2. — *Correlation with the depth of myometrial invasion.*

	p53	c-erb-B2	MIB-1
M1 (13 cases)	6/13 (46.2%)	6/13 (46.2%)	6/13 (46.2%)
M2 (8 cases)	5/8 (62.5%)	5/8 (62.5%)	6/8 (75%)
M3 (8 cases)	5/8 (62.5%)	5/8 (62.5%)	7/8 (87.5%)
Total (29 cases)	16/29 (55.2%)	16/29 (55.2%)	19/29 (65.5%)

Table 3. — *Correlation with FIGO stage.*

	p53	c-erb-B2	MIB-1
IB (15 cases)	8/15 (53.4%)	8/15 (53.4%)	8/15 (53.4%)
IC (6 cases)	1/6 (16.7%)	2/6 (33.4%)	4/6 (66.7%)
IIA (1 case)	–	–	1/1 (100%)
IIB (3 cases)	3/3 (100%)	3/3 (100%)	2/3 (66.7%)
IIIA (1 case)	1/1 (100%)	1/1 (100%)	1/1 (100%)
IIIC (1 case)	1/1 (100%)	1/1 (100%)	1/1 (100%)
IVA (2 cases)	2/2 (100%)	2/2 (100%)	2/2 (100%)
Total (29 cases)	16/29 (55.2%)	17/29 (58.6%)	19/29 (65.5%)

Table 4. — *Correlation with follow-up.*

	p53	c-erb-B2	MIB-1
DFD* (6 cases)	6/6 (100%)	5/6 (83.4%)	4/6 (66.7%)
NED** (20 cases)	8/20 (40%)	8/20 (40%)	12/20 (60%)
Total (26 cases)	14/26 (53.8%)	13/26 (50%)	16/26 (61.5%)

*DFD = Dead of disease; **NED = No evidence of disease.

Results

Immunohistochemistry showed that p53 was positive in 16 out of 29 cases (55.2%). The evaluation of p53, with regard to typical histopathological parameters, showed a positive correlation with the grading. In fact, it was positive in 44.5% of G1, 53.4% of G2 and 80% of G3 (Table 1). With regard to myometrial invasion p53 was positive in 46.2% of M1, in 62.5% of M2 and in 62.5% of M3 (Table 2). As for FIGO stage, p53 was positive in 53.4% of stage IB, in 16.7% of IC and in 100% of stages IIB, IIIA, IIIC and IVA (Table 3).

C-erb-B2 was positive in 16 out of 29 cases (55.2%). It was positive in 55.6% of G1, in 46.7% of G2 and in 80% of G3 (Table 1). With regard to myometrial invasion c-erb-B2 was positive in 46.2% of M1, in 62.5% of M2 and in 62.5% of M3 (Table 2). As for FIGO stage, c-erb-B2 was positive in 53.4% of stage IB, in 33.4% of IC and in 100% of stages IIB, IIIA, IIIC and IVA (Table 3).

The evaluation of the number of cells proliferating at immunohistochemistry by MIB-1 reported high proliferative activity in 19 out of 29 cases (65.5%). It was positive in 55.6% of G1, 60% of G2 and 100% of G3 (Table 1). Myometrial invasion MIB-1 was positive in 46.2% of M1, 75% of M2 and 87.5% of M3 (Table 2). With regard to FIGO stage, MIB-1 was positive in 53.4% of stage IB, 66.7% of IC and IIB, and 100% of stages IIA, IIIA, IIIC and IVA (Table 3).

Table 4 shows the correlation of these antigens with the follow-up. P53 was positive in 100% of neoplasias of patients who died of disease, whereas it was positive in 40% of tumours of patients with no evidence of disease. C-erb-B2 was positive in 83.4% of neoplasias of patients who died of disease, whereas it was positive in 40% of tumours of patients with no evidence of disease. MIB-1 was positive in 66.7% of neoplasias of patients who died of disease, whereas it was positive in 60% of tumours of patients with no evidence of disease.

Discussion and conclusions

Today the prognosis of endometrial carcinoma is related to several histopathological parameters such as grade, histotype, depth of myometrial invasion, extension to the cervix and presence of lymph nodes or distant metastases [12].

The possibility of studing genetic modifications, which have a role in some tumors of other anatomic sites, has also enhanced the number of potential prognostic indicators in endometrial carcinoma. In particular, the refinement of molecular biological techniques and their use in specimens fixed in formalin and paraffin embedded, have allowed us to study genetic alterations even by patient records. Furthermore the immunohistochemical methods, with the application of the principles of immunology to histology, has permitted the recovery – from common histologic preparations – of proteins which are codified

by oncogenes and/or tumour suppressor genes and are thus directly visualised by optic microscopy.

Our results showed a good correlation between grading, expression of p53, c-erb-B2 and proliferation index, with higher values in the forms of poorly differentiated in comparison to well-differentiated tumours. This correlation was more significant for pathologic stage; in fact, we observed the expression of p53, c-erb-B2 in 100% of cases classified as stages II, III and IV with high proliferative activity.

The comparison of immunohistochemical data with the follow-up pointed out a constant expression of p53 in all tumours of patients who died of disease. Even for c-erb-B2 we reported a higher expression among the patients who died of cancer (83.4%) with respect to that of patients with no evidence of disease (40%). The data relative to MIB-1 were less significant with, respectively, 66.7% and 60% values among the two categories considered.

These results agree with those of other studies [13, 14] which give unfavourable prognostic significance to the amplification and overexpression of c-erb-B2 in endometrial carcinoma and report the association with advanced stages and with death due to the tumour. However these results disagree with those of other authors [15-17] who did not observe a significant association between overexpression of c-erb-B2, stage of disease, and survival.

The correlation, that we reported in our study, between the overexpression of p53 and unfavourable prognosis, confirms the potential role of this tumour suppressor gene in the identification of neoplastic forms which have the most malignant biological behaviour and unfavourable prognosis as already stated in the literature [13].

The evaluation of the number of proliferating cells by the analysis of MIB-1 showed a good correlation with the grade, myometrial invasion and FIGO stage. On the other hand there was no significant association with survival; in fact the reported values (66.7% and 60%) were almost superimposable. These data disagree with the results of some authors [10, 18, 19]. In fact, these authors state that the occurrence of a high proliferation index is associated not only with common histopathological parameters, but also with the possible identification of patients who are at high risk and have an unfavourable prognosis. These differences could be related to the lack of univocal criteria for the definition of threshold values which have been used in different studies with negative effects on the reproducibility of results.

Although our results are limited by a small series and did not achieve statistical significance, the presence of a high proliferation index, associated with the overexpression of p53 and c-erb-B2, seems to indicate a more malignant phenotype.

If the information obtained by these techniques is also used in endometrial biopsies, a more accurate preoperative diagnosis could be achieved. Consequently the surgical approach could be modified and patients who may need neoadjuvant radiotherapic or chemotherapic treatments would be easier identified.

Our data need to be confirmed by studies on larger series, using non-endometrioid histotypes and more sensitive and specific techniques in order to confirm the role of these possible prognostic indicators.

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