

The E-Cadherin Expression vs. Tumor Cell Proliferation Paradox in Endometrial Cancer

IRENE GONZÁLEZ-RODILLA¹, LAURA ALLER², JAVIER LLORCA³, ANA-BELÉN MUÑOZ², VIRGINIA VERNA², JOSÉ ESTÉVEZ² and JOSÉ SCHNEIDER²

¹Department of Pathology, Marqués de Valdecilla University Hospital, Cantabria University, Santander, Spain;

²Department of Obstetrics & Gynecology, Hospital Universitario "Marqués de Valdecilla", Santander, Spain;

³Department of Epidemiology & Public Health, Cantabria University, Santander, Spain

Abstract. *Background: E-Cadherin is a putative marker of good prognosis in endometrial cancer. Paradoxically, in a previous study of endometrial carcinoma we found that E-Cadherin is significantly co-expressed with molecular markers of proliferation, usually associated with a worse prognosis in most tumor types. Patients and Methods: The expression of estrogen (ER) and progesterone receptors (PR), Ki67, Human Epidermal Growth Factor Receptor 2 (HER-2, c-ERB-B2), p53 and E-Cadherin was studied by means of immunohistochemistry in 126 endometrial carcinoma samples. The results were correlated with patient survival and included in a multivariate model, in order to identify factors independently associated with the patient outcome. Results: E-Cadherin overexpression was associated with a significantly better overall survival in the whole group of patients with endometrial carcinoma ($p=0.012$), as well as in the group of patients exclusively harboring endometrioid tumors ($p=0.004$). In a restricted multivariate model, only tumor stage and E-Cadherin expression retained their independent prognostic power, both for the whole group of tumors ($p=0.04$), as well as for the subgroup of endometrioid carcinomas ($p=0.05$). Conclusion: E-Cadherin is an independent predictor of survival in endometrial carcinoma, regardless of histological variety. Proliferation, on the other hand, does not seem to play a prominent role in this same context. This may explain why E-Cadherin retains its prognostic power, despite being significantly co-expressed with all tested molecular proliferation markers.*

In a previous report about the expression of biological markers of apoptosis in endometrial carcinoma (1), we obtained an

intriguing collateral finding, namely that the expression of the intercellular adhesion marker E-Cadherin, a putative indicator of a better prognosis, was significantly co-expressed with the proliferation marker Ki67 and the product of the mutant p53 gene. The latter are usually associated with a worse prognosis for most tumor types and indicate an increased proliferation rate and the loss of proliferation control, respectively. Moreover, in that same study, at variance with a previous report about an inverse correlation between E-Cadherin and mutant p53 expression in advanced endometrial cancer (2), we also reported that the direct correlation between E-Cadherin and p53 expression we found was restricted to early-stage endometrial cancer.

In the present study, we attempted to clarify this issue by investigating the correlation of E-Cadherin expression and of proliferation-associated molecular markers with the survival of the patients, and included them in a multivariate model, in order to identify those independently associated with patient outcome.

Patients and Methods

We analyzed 126 endometrial carcinoma samples from patients treated at Marqués de Valdecilla University Hospital, Santander, Spain, between 2004 and 2010. Some of them belong to our previous study on biological markers of apoptosis in endometrial cancer (1).

The histological subtypes were as follows: Pure endometrioid, $n=103$; clear cell, $n=11$; papillary serous, $n=8$; other types, $n=4$.

According to Bokhman's classification (3), 48 of them were type I, and 78 type II tumors. Type II tumors, according to this classification, comprise all clear-cell and papillary serous carcinomas, together with undifferentiated, grade III endometrioid tumors. If we consider the latter separately, 44 out of 95 were grade I-II, and 51 grade III carcinomas.

Out of the 126 patients, 121 were operated upon at our center. The remaining 5 patients had inoperable disease due to advanced stage or other medical reasons, and were treated conservatively, either by means of radiotherapy, hormonal therapy, or a combination of both. The surgical stage of the 121 operated patients, according to the 2009 FIGO classification (4) was as follows: I, 83; II, 12; III, 24 and IV, 2. The mean age of the patients was 65.9 years (range=43-88 years).

Correspondence to: Professor J. Schneider, Department of Obstetrics & Gynaecology, Marqués de Valdecilla University Hospital, Cantabria University, Avenida Herrera Oria S/N, 39011 Santander, Spain. Tel: +34 942203303, Fax: + 34 942202610, e-mail: jschneider@humv.es

Key Words: Endometrial carcinoma, E-cadherin, proliferation.

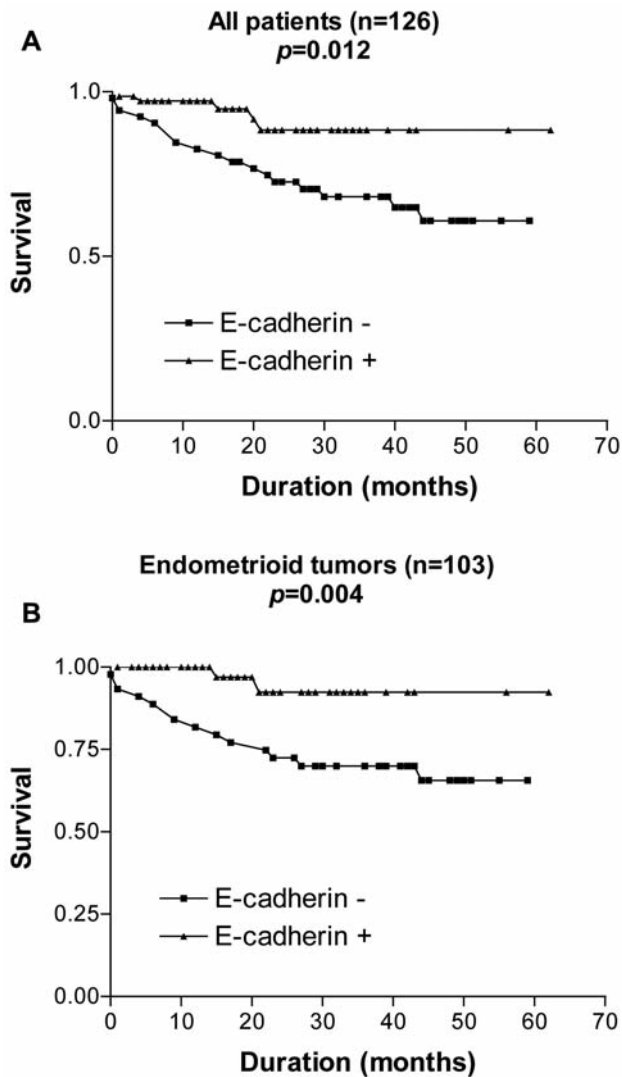


Figure 1. Influence of E-cadherin expression by endometrial carcinomas on patient survival. A: All patients. B: Only patients with endometrioid tumors.

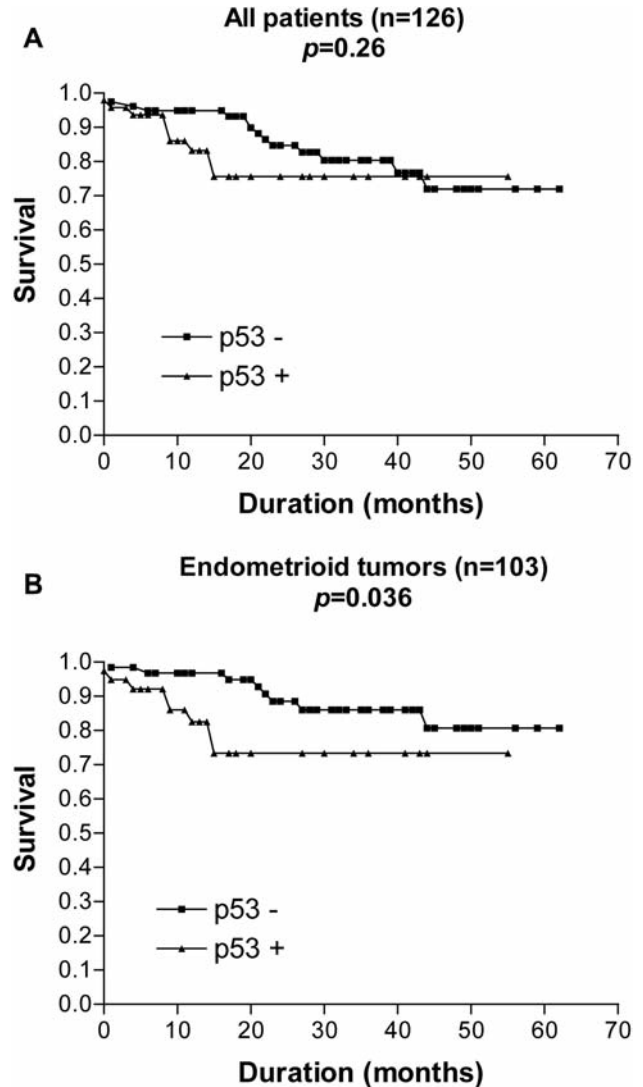


Figure 2. Influence of mutant p53 expression by endometrial carcinomas on patient survival. A: All patients. B: Only patients with endometrioid tumors.

By means of immunohistochemistry, we studied the expression of the following molecular markers by the tumors: estrogen (ER) and progesterone receptors (PR), Ki67, c-ERB-B2, p53 and E-Cadherin.

The techniques employed, as well as the scoring system used for all markers (with the exception of Ki67) have been described extensively elsewhere (1, 5, 6). The final score ranges between 0 and 6, which allows for correlation analyses between continuous, nonparametric variables to be performed. The Ki67 score was expressed directly as the percentage of stained cells. For the survival analyses, the expression of these markers was dichotomized into positive and negative, using the following cut-offs, based on our previous studies (1): ER, PR and E-Cadherin, upper tertile (scores 5 and 6); p53 and c-ERB-B2, lower tertile (scores 1 and 2); Ki67, 10%. All antibodies were purchased from Dako, Glostrup, Denmark

in a pre-diluted form, with the exception of the antibody to c-ERB-B2, which was diluted to 1:50. The immunohistochemical procedure was carried out in an automatic autostainer using the EnVision amplification system (both from Dako). All slides were interpreted by the same pathologist (IGR), thus ensuring uniformity of criteria throughout the series.

Statistics. The correlation between continuous variables was analyzed by means of Spearman's rank correlation test for non-parametric variables.

A survival analysis was carried out considering death as failure and survivorship at the end of follow-up as censored data. Kaplan Meier curves were estimated for histological type, tumor stage and any of the biological markers (positive vs. negative, taking the cut-

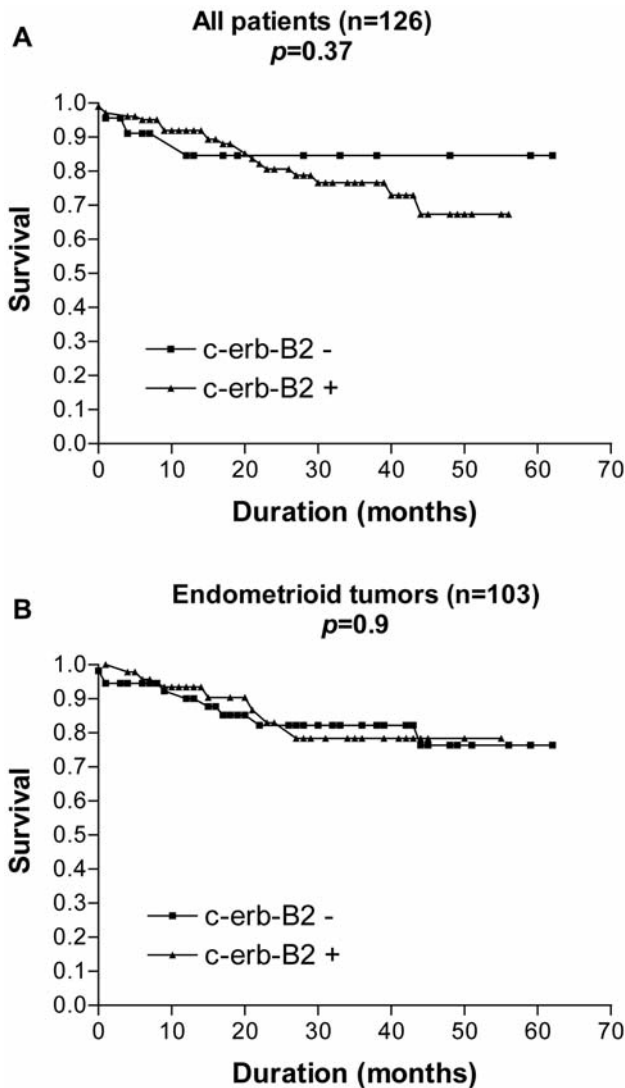


Figure 3. Influence of *c-erb-B2* (HER-2) expression by endometrial carcinomas on patient survival. A: All patients. B: Only patients with endometrioid tumors.

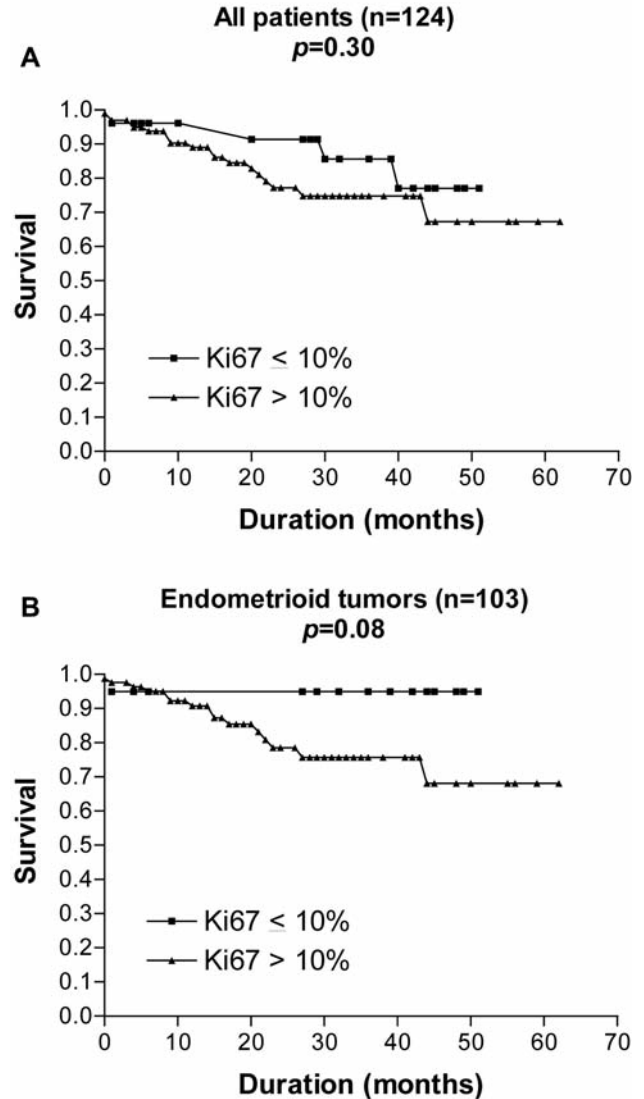


Figure 4. Influence of Ki67 expression by endometrial carcinomas on patient survival. A: All patients. B: Only patients with endometrioid tumors.

offs as detailed above). A multivariate model was obtained by means of Cox regression analysis.

All obtained values were considered significant when the corresponding *p*-value was less than 0.05.

Results

Expression of E-Cadherin in the whole group of endometrial carcinomas was significantly positively associated with estrogen receptor- ($r=0.22$, $p=0.015$), Ki67 ($r=0.27$, $p=0.0027$), c-ERB-B2 ($r=0.18$, $p=0.04$) and p53 ($r=0.24$, $p=0.008$) expression, and inversely with patient age ($r=-0.26$, $p=0.003$). Considering only endometrioid carcinomas, the findings are

virtually identical, with the only difference that in this subgroup, a significant association between E-cadherin expression and PR expression also existed ($r=0.25$, $p=0.001$).

E-Cadherin overexpression was associated with a significantly better overall survival in the whole group of patients with endometrial carcinoma (Figure 1A), as well as in the group of patients exclusively with endometrioid tumors (Figure 1B). In the multivariate model, only tumor stage and E-cadherin expression retained their independent prognostic power, both for the whole group (Tables I and II), as well as for the subgroup of endometrioid carcinomas (Tables III and IV).

Table I. Influence of biological and clinical features on survival of patients with endometrial carcinoma: Whole study population, regardless of histology (n=126). Cox proportional hazards model, complete model.

Variable	Crude hazard ratio	p-Value	Adjusted hazard ratio*	p-Value
ER pos. vs. neg.	0.45 (0.20-1.01)	0.053	0.55 (0.20-1.53)	0.25
PR pos. vs. neg.	0.34 (0.15-0.81)	0.01	0.58 (0.21-1.63)	0.31
Age >57 vs. ≤57	7.36 (1.00-54.4)	0.05	3.78 (0.49-28.9)	0.20
c-ERB-B2 pos. vs. neg.	1.14 (0.52-2.52)	0.75	0.57 (0.21-1.52)	0.26
p53 pos. vs. neg.	2.41 (1.04-5.58)	0.04	1.40 (0.49-4.00)	0.53
E-Cadherin pos. vs. neg.	0.29 (0.11-0.80)	0.02	0.27 (0.08-0.95)	0.04
Ki67 >10% vs. ≤10%	2.02 (0.69-5.94)	0.20	1.89 (0.50-7.15)	0.35
Endometrioid histology vs. rest	0.54 (0.25-1.19)	0.13	0.65 (0.25-1.74)	0.39
Histological grade 3 vs. grades 1&2	4.24 (1.45-12.4)	0.008	3.93 (0.87 - 17.7)	0.08
Stage I	1 (Reference)			
II	3.78 (0.85-16.9)	0.08		
III	12.2 (3.79-39.0)	<0.001		
IV	7.93 (0.88-71.2)	0.06		

*Adjusted by stage.

Regarding the proliferation-related molecular markers tested for their relationship with survival, only p53 expression was associated with a worse survival, but only in the subgroup of patients with endometrioid tumors (Figure 2). The expression of c-ERB-B2, at variance with other gynecological, hormone-dependent tumors, most notably breast cancer, was not associated with a difference in survival, neither for the whole group of patients (Figure 3A), nor for those with purely endometrioid carcinomas (Figure 3B). The same applied to the expression of Ki67, a pure proliferation marker, which was not associated with survival for either group of patients (Figure 4).

Discussion

In the present study we once more found a significant positive relationship between the expression of E-Cadherin by endometrial carcinoma and the expression of all the tested molecular markers of cell proliferation (Ki67, c-ERB-B2, p53). This is in apparent contradiction with the fact that, at the same time, E-Cadherin expression was also associated with a significantly better patient survival, both in the univariate and the multivariate analysis, where in fact it was the only factor besides tumor stage to retain its independent prognostic power. This is in agreement with four previous studies about the relationship between E-cadherin expression and survival in endometrial carcinoma, one of them restricted to stage IV tumors only (2, 7, 8, 9). Furthermore, the prognostic value of E-Cadherin expression was not linked to a particular histological variety, since the results did not change after excluding non-endometrioid carcinomas from the model. The paradox arises from the fact that although clearly defining a better prognosis of the patients, E-cadherin expression was also significantly associated with the expression of molecular markers associated with increased tumor cell proliferation, a feature usually defining a worse prognosis in virtually all other

Table II. Influence of biological and clinical features on survival of patients with endometrial carcinoma: Whole study population, regardless of histology (n=126). Cox proportional hazards model, best restricted model after stepwise regression.

Variable	Hazard ratio	p-Value
E-Cadherin	0.27 (0.08-0.95)	0.04
Stage I	1 (reference)	-
II	2.71 (0.60-12.3)	0.20
III	10.4 (3.23-33.8)	<0.001
IV	6.43 (0.71-57.8)	0.10

known cancer types. Interestingly, and somewhat surprisingly, from our results, proliferation does not seem to play a significant role in endometrial cancer, at least as far as patient survival is concerned. In fact, neither a high Ki67 proliferation index, nor the expression of c-ERB-B2, were associated with a worse survival of the patients in our series. Mutant p53 expression, a known regulator of proliferation, but also of apoptosis, was associated with a significantly worse survival only in the subgroup of endometrioid carcinomas. This lack of association between proliferation and prognosis for patients with this tumor might, thus, explain the paradox: since proliferation seems to be largely irrelevant for patient survival, the correlation of E-Cadherin expression with proliferation does not impair its beneficial effect on survival. Alternatively, an attractive hypothesis might be that, as control of proliferation is lost in endometrial carcinoma cells, E-cadherin expression is up-regulated to counteract this, so that the tumor may well grow, but as long as its cells do not acquire the possibility of detaching themselves from their neighbors, they are not capable of metastasizing and killing the host. Surprising as it may seem, the finding that proliferation does not seem to be

Table III. Influence of biological and clinical features on survival of patients with endometrial carcinoma: Only patients with endometrioid histology (n=103). Cox proportional hazards model, complete model.

Variable	Crude hazard ratio	p-Value	Adjusted hazard ratio*	p-Value
ER pos. vs. neg.	0.75 (0.25-2.23)	0.60	0.76 (0.21-2.71)	0.67
PR pos. vs. neg.	0.39 (0.14-1.12)	0.08	0.54 (0.15-1.93)	0.34
Age >57 vs. ≤57	does not converge		does not converge	
c-ERB-B2 pos. vs. neg.	1.83 (0.62-5.38)	0.27	1.44 (0.38-5.53)	0.59
p53 pos. vs. neg.	2.43 (0.73-8.04)	0.15	1.44 (0.34-6.13)	0.63
E-Cadherin pos vs. neg.	0.21 (0.04-0.95)	0.04	0.21 (0.04-0.99)	0.049
Ki67 >10% vs. ≤10%	4.59 (0.60-35.5)	0.14	does not converge	
Histological grade 3 vs. grades 1&2	2.41 (0.75-7.75)	0.14	3.03 (0.62-14.7)	0.17
Stage I	1 (reference)			
II	2.07 (0.22-19.9)	0.53		
III	9.80 (2.43-39.5)	0.001		
IV	8.11 (0.84-78.3)	0.07		

*Adjusted by stage.

a key feature in the natural history of endometrial carcinoma is not new. In fact, in a relatively recent report on advanced, inoperable endometrial cancer, already mentioned above, Singh *et al.* (2) found that a high Ki67 proliferation index is not associated with survival in this tumor type, and also found a relatively weak association of p53 with survival, which was lost in the multivariate analysis. In contrast, E-cadherin retained its prognostic significance in both the general multivariate model, and that excluding non-endometrioid histologies. Although these findings were obtained in a very specific series of patients with inoperable tumors treated by means of hormonal manipulation, and therefore not representative of the bulk of endometrial cancer cases usually found in the clinic, they are largely superimposable on our own, and reinforce our proposed explanation for this paradox.

In conclusion, E-cadherin emerges as an independent predictor of survival in endometrial carcinoma, regardless of the histological variety. Furthermore, proliferation does not seem to play a prominent role in this same context. This may explain why E-Cadherin retains its prognostic power, despite being significantly co-expressed with all the molecular proliferation markers tested here.

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Table IV. Influence of biological and clinical features on survival of patients with endometrial carcinoma: Only patients with endometrioid histology (n=103). Cox proportional hazards model, best restricted model after stepwise regression.

Variable	Hazard ratio	p-Value
E-Cadherin	0.21 (0.04-0.99)	0.049
Stage I	1 (reference)	-
II	1.40 (0.14-13.6)	0.77
III	10.5 (2.58-42.7)	0.001
IV	6.84 (0.71-65.9)	0.10

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Received September 7, 2013

Revised October 14, 2013

Accepted October 15, 2013