Expression of the Apoptosis-related Genes Bcl-2 and p53 in Clinical Samples from Endometrial Carcinoma Patients

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Abstract. Background: Although alterations in the mechanisms of apoptosis are an integral part of the tumor phenotype, their precise role in endometrial carcinoma is still obscure. The aim was to determine whether Bcl-2 plays a similar biological role in endometrial cancer as in breast cancer, endometrial cancer being also a hormone-dependent tumor. Materials and Methods: The expression of the apoptosis-related Bcl-2 and p53 genes, together with Ki67, E-cadherin, c-erb-B2 and estrogen and progesterone receptors were studied in 136 formalin-fixed, paraffin-embedded endometrial carcinoma samples by means of immunohistochemistry. Results: Bcl-2 expression correlated directly and significantly with E-cadherin (r=0.22, p=0.011) estrogen receptor (r=0.18, p=0.04) and progesterone receptor expression (r=0.30, p=0.0006), and inversely with surgical stage (r=–0.20, p=0.024). Mutant p53 expression was directly and significantly associated with increasing patient age (r=0.25, p=0.007), tumor grade (r=0.37, p<0.001), Ki67 (r=0.47, p=0.0001), c-erb-B2 expression (r=0.21, p=0.012) and with E-cadherin expression (r=0.19, p=0.026). Conclusion: Bcl-2 and p53 are independently and significantly co-expressed with E-cadherin in endometrial carcinoma. Furthermore, the expression of Bcl-2 is also significantly associated with the expression of both progesterone and estrogen receptors, in that order, suggesting that, analogously to breast cancer, apoptosis is hormonally regulated to some degree also in endometrial cancer.

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papillary serous, 7; clear cell, 5; undifferentiated, solid, 2; endometrioid papillary, 2; mixed, 19; other, 6. Out of the 136 patients, 124 were subsequently operated upon at our hospital. The remaining 12 patients were considered inoperable at the time of diagnosis, either for medical reasons, or because of advanced stage and were subject to primary radiotherapeutic or combined radiotherapeutic and medical treatment. Following surgery, the classification by surgical stage was as follows: stage I, 83; stage II, 15; stage III, 23 and stage IV, 3.

The expression of the apoptosis-related Bcl-2 and p53 genes, together with Ki67, E-cadherin, c-erb-B2 and the estrogen and progesterone receptors were studied by means of immunohistochemistry. The p53 protein detected by means of immunohistochemistry is the dysfunctional product of the mutant gene, which is more stable than the product of the wild-type gene, and therefore more easily detectable by means of the technique. Thus, a positive immunohistochemical result for p53 reflects a defect in the genetic machinery of the cell, and consequently an ominous prognostic factor. The antibodies were purchased from Dako, Glostrup, Denmark in prediluted form, with the only exception of the c-erb-B2 antibody, which was diluted to 1:50. All the reactions were carried out under standard conditions using the Dako EnVision system in an automatic Dako Autostainer (Dako), which assured the uniformity of results. As further variables, tumor stage and grade were also included in the statistical analysis.

The same pathologist (I.G.R.) interpreted all the results. The scoring system described in previous reports (2-4) was used. It assesses both the number of reactive tumor cells (less than 10%, 10-50%, >50%) and the intensity of staining (none, less than the positive control, as high as the positive control). This results in a semiquantitative score ranging from 0 to 6, which allows for statistical correlation analyses to be carried out.

The statistical analysis was performed using the GraphPad Prism (GraphPad Software, San Diego, CA, USA) biostatistical package. The correlation between continuous variables was studied by means of Spearman’s rank correlation test for nonparametric variables. The association of dichotomic variables was studied by means of contingency tables and the Chi-square test. The results were considered significant when the p-value was <0.05.

Results

Bcl-2 expression correlated directly and significantly with E-cadherin (r=0.22, p=0.011) estrogen receptor (r=0.18, p=0.04) and progesterone receptor expression (r=0.30, p=0.0006), and inversely with surgical stage (r=-0.20, p=0.024). The results are summarized in Table I.

In order to test whether the lowest levels of Bcl-2 expression levels were biologically significant from the lowest levels upwards., as, according to our previous experience (4) is the case in breast carcinoma, the semiquantitative Bcl-2-expression results were dichotomised into low levels of expression, corresponding to the lower tertile of the applied score (“0-2”) vs. the rest. Their association was studied by means of contingency tables with the levels of expression corresponding to the highest tertile (“5-6”) of E-cadherin and progesterone receptors, for which the strongest degree of correlation had been found. Indeed, the association of low Bcl-2 expression with high E-cadherin and progesterone receptor expression was statistically significant in both cases (p=0.012 and p=0.0002, respectively), indicating that, as in the case of breast cancer, Bcl-2 expression levels seem to be biologically significant from the lowest level upwards also in endometrial carcinoma.

Out of all the other studied genes, the one most prominently associated with apoptosis is p53. Therefore, the correlation of its expression with all other variables was also analyzed. Mutant p53 expression was directly and significantly associated with growing patient age (r=0.25, p=0.007), tumor grade (r=0.37, p<0.001), Ki67 (r=0.47, p<0.0001) and c-erb-B2 expression (r=0.21, p=0.012), as expected, and somewhat surprisingly also with E-cadherin expression (r=0.19, p=0.026) (Table II), which is considered a molecular marker of lower tumor aggressiveness.

Discussion

The correlation found in this study between Bcl-2 and hormone receptor expression in endometrial carcinoma has been described previously in a very similar study carried out at the Mayo Clinic, USA (5).
being a hormone-dependent tumor, it is not surprising that these findings largely duplicate those previously described for the paradigm of endocrine-related carcinomas, \textit{i.e.} breast cancer (1, 4).

On the other hand, the significant correlation of Bcl-2 expression with E-cadherin expression in endometrial cancer has not been described before, to the best of our knowledge. The expression of both genes has been linked before in a study carried out on MDA-MB-435 cells (6), originally thought to be breast cancer cells, but ultimately identified as melanoma cells (7). In that study, blocking E-cadherin expression by means of interference plasmids resulted in Bcl-2 down-regulation, and in a modulation of the cells’ sensitivity to staurosporine. The present results with tumor specimens seemed to corroborate a direct link between Bcl-2 and E-cadherin expression, and that this association seems to play a definite role in tumor biology.

In contradiction to the present results, E-cadherin and p53 expression were found to be significantly inversely correlated (8) in stage-IV and recurrent endometrial cancer. In fact, in the present study p53 and E-cadherin were found to be significantly directly correlated. However, the present series was largely composed of early-stage endometrial carcinoma patients, and interestingly, the correlation index between E-cadherin and p53 expression substantially increased when the stage-I tumors were analyzed separately ($r=0.37, p=0.0008$), whereas the subanalysis of the other stages showed an insignificant trend towards an inverse correlation ($-0.06, p=0.72$). Thus, apparently, as tumor stage increases, the positive correlation found between p53 and E-cadherin expression seems to be inverted for some still obscure reason, which may explain the discrepant results of this study and that reported by Singh \textit{et al.}

In conclusion, Bcl-2 and p53 are independently and significantly co-expressed with E-cadherin in endometrial carcinoma. Furthermore, the expression of Bcl-2 is also significantly associated with the expression of both progesterone and estrogen receptors, in that order, suggesting that, analogously to breast cancer, apoptosis is hormonally regulated to some degree also in endometrial cancer.

**References**


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