Immunohistochemical expression of EGF-R in malignant surface epithelial ovarian neoplasms (SEON)

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Summary: EGF-R expression was found to be increased in 40% of malignant epithelial ovarian neoplasms by an immunohistochemical method. No correlation was found between EGF-R expression and clinical stage. There was a suggestion of reduced survival among tumours with positive EGF-R expression.

Key words: Ovarian cancer; Prognostic factors; Growth factor receptors; Immunocytochemistry.

INTRODUCTION

Ovarian carcinoma remains a lethal malignant tumour especially when diagnosed at advanced stages. Thus the identification of new biologic marker related to the tumour's biologic aggressiveness would be useful to allow individualized and possibly more effective therapy.

EGF-R is a member of the tyrosine kinase receptor family with growth promoting effects in many normal and neoplastic cell lines. EGF-R is a transmembrane glycoprotein which consists of three segments a) a glycosylated extracellular domain rich in cysteine, b) a transmembrane segment and c) a cytoplasmic segment with protein kinase activity (1). Studies have shown that EGF-R can bind to two growth proteins EGF and TGF-α which are 40% structurally the same. The binding of EGF with its receptor causes phosphorylation of the tyrosine residue of the EGF-R which leads to the initiation of cellular division (2). EGF-R is overexpressed in squamous cell carcinomas (2), gliomas (1), transitional cell carcinomas of the bladder (1) and in breast cancer (2). Recent studies in breast and urothelial carcinomas have reported a relationship of EGF-R overexpression with poor outcome (3, 4).

Overexpression of EGF-R has been found in surface epithelial ovarian neoplasms (SEON) with rates ranging widely between 31% and 77% depending on methods used (2, 4). Thus far the significance of EGF-R in malignant SEON has not been clarified, with conflicting reports regarding its correlation with prognosis.
The purpose of this study was to describe the expression of EGF-R in advanced malignant SEON and to assess the possible correlation of EGF-R with other parameters, such as histologic type and grade, clinical stage and overall survival.

MATERIALS AND METHODS

A retrospective analysis of tumour tissue obtained from the Department of Pathology, University of Athens Medical School was performed. The tissue specimens were obtained from a total of 40 women with SEON. A retrospective review of the hospital charts provided information regarding age and tumour stage at the time of operation. All patients with malignant SEON were clinically staged according to FIGO classification. At the time of diagnosis, 8/40 (19%) were stage I, 6/40 (16%) stage II, 25/40 (62%) stage III and 1/40 (3%) stage IV. Histological analysis was made according to WHO criteria. After deparaffinization, through graded alcohols, endogenous peroxidase activity was blocked by incubating the slides in 0.1% hydrogen peroxide in methanol for 20 minutes. Immunostaining was performed using the ABC-HRP method (Dakopatts, Denmark). As primary antibody we used the monoclonal antibody EGFRI (6080-1, Bicemator) which recognizes the extracellular domain of the receptor. The primary monoclonal antibody was used at a dilution of 1:80 with a 24 hour (overnight) incubation. Diaminobenzidine Tetrahydrochloride 0.06% in PBS buffer containing 0.03% hydrogen peroxide was used as a chromogen. Tumour sections subjected to the whole procedure, except for incubation with the primary antibody, were used as negative controls, and previously positive for EGF-R breast cancer sections were used as positive controls. All immunostained slides were analyzed and scored in a blinded fashion without knowledge of histologic type and grade or survival data.

Scoring of EGF-R was performed using a standard light microscope. Initially the sections were scanned at low power to determine the areas that were most evenly and heavily labelled. Analysis of the immunostained sections was based on the number of stained cells.

Labelled cells were counted at a magnification of 400 X. In each section 1000 cells were counted and the fraction of positive cells was determined. Two sections per case were evaluated and scored and the average number from both sections was used. Cases with less than 5% positive cells represented no, or very weak expression for EGF-R and were regarded as negative. A scale of I through III (EGF-R index) was used corresponding to estimated percentage of stained cells (I = 0 to 24%; II = 25 to 49%; III = > 50%). Statistical analysis was performed using the T-test. The survival data were analyzed by a computerized program.

RESULTS

EGF-R expression was present in the neoplastic cells with considerable heterogeneity regarding the number of positive cells and the intensity of stain (fig. 1). The main site of EGF-R expression was the cytoplasmic membrane and to a lesser degree the cytoplasm. Neoplastic cells with EGF-R index of I or more were considered as expressing the receptor. EGF-R was expressed in 16/40 (40%) of malignant SEON. EGF-R index was II or more in the majority of cases. EGF-R expression was very low in endometrioid carcinomas (1/8) compared to other histologic types (table 1).

Correlating EGF-R expression with degree of differentiation a tendency for increased expression of the receptor was noted from well to poorly differentiated serous tumours (1/7 of well differentiated, 5/9 of intermediate and 2/4 of poorly differentiated serous tumours). When examining EGF-R expression according to clinical stage no correlation was found. Four of eight cases with clinical stage I (50%), 1/6 with stage II (17%), 8/25 with stage III (30%) and 0/1 were positive for the receptor.

Twenty eight cases were evaluable for survival data. Nine patients with overexpression of EGF-R had a mean survival of 17 months. Eighteen patients without EGF-R expression had a mean survival of 20 months (p = 0.559).

DISCUSSION

The measurement of EGF receptors can be accomplished either biochemically by incubating the cytoplasmic membranes of the neoplastic cells with labelled
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Fig. 1. — Poorly differentiated serous carcinoma: EGF-R expression in the cytoplasmic membrane and the cytoplasm of some tumour cells (ABC × 500).

\(^{125}\text{I-EGF} (\text{a})\), or immunohistochemically, using monoclonal antibodies against the EGF-R. The immunohistochemical method has the advantage that an accurate estimate of the receptor expression on the malignant vs the surrounding normal cells can be made, and the disadvantage that the assessment is subjective and varies among observers depending on the method used. In our study using the monoclonal antibody EGFR1 we found that the EGF receptor was overexpressed in 40% of malignant tumours with a moderate and heavy pattern of EGF-R expression (Index II and III) in the majority of cases (15 of 16).

Endometrioid tumours had the least expression of the receptor.

Koehler et al. using a biochemical assay demonstrated EGF-R expression in 36% of ovarian carcinomas while in a recent study, Scambia et al. found that EGF-R was detected in 54% of advanced ovarian carcinomas \(^{(3)}\). Berchuck et al., using an immunohistochemical method reported 77% of ovarian tumours expressing EGF-R. However in that particular study when the percentage of moderate and heavy stain for EGF-R was considered 41 of 77 cases (53%) were positive for EGF-R expression \(^{(4)}\). The discrepancies that appear among studies can be attributed to methodological differences and/or differences in the cut-off values.

We found that EGF-R expression was present with considerable heterogeneity in the intensity of stain and number of positive cells. Similar heterogeneity in EGF-R expression has been noted in other neoplasms such as glioblastomas and probably represents the end result of evolution from different malignant phenotypic clones \(^{(5)}\). There was a trend for increased EGF-R expression from well to poorly differentiated serous tumours. Similarly Battaglia et al. \(^{(3)}\) found higher levels of EGF-R expression among undifferentiated versus well differentiated ova-
Table 1. — EGF-R expression in Malignant SEON.

<table>
<thead>
<tr>
<th>Histologic type</th>
<th>Mean age</th>
<th>Number of patients</th>
<th>Number of cases</th>
<th>INDEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serous Ca</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Well</td>
<td>7</td>
<td>3</td>
<td>0</td>
<td>2</td>
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<tr>
<td>Intermediate</td>
<td>9</td>
<td>5</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Poorly</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>20</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Mucinous Ca</td>
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<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Clear cell Ca</td>
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<tr>
<td>Mixed</td>
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<td>1</td>
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<tr>
<td>Total</td>
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<td>0</td>
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<tr>
<td>Undifferentiated</td>
<td>55</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total Malignant</td>
<td>61</td>
<td>40</td>
<td>16</td>
<td>1</td>
</tr>
</tbody>
</table>

* 1: 5-24% positive cells; II: 25-49% positive cells; III: >50% positive cells.

No significant correlation between EGF-R expression and clinical stage was found. This result corresponds well with other recent reports (10) and leads to the hypothesis that increased EGF-R expression is not necessarily related to the tumour's biologic aggressiveness or local invasiveness.

In our study only a tendency towards reduced survival was found among patients with EGF-R expression. This finding is in agreement with those of Berchuck et al (9) and Scambia et al (11) who found a statistically significant reduction in survival in patients with EGF-R expression. On the other hand Bauknecht et al (14) and Furugen et al. (7) reported that patients with positive EGF-R expression had a better survival than those whose tumours did not express EGF-R.

Despite the growing reports in the literature, regarding EGF-R expression in malignant SEON its prognostic significance has not yet been clearly defined. Therefore further studies are needed with larger numbers of patients and longer follow-up before a firm conclusion can be reached.

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REFERENCES


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