Epidermal Growth Factor Receptor (EGFR) Mutation Does Not Correlate with Platinum Resistance in Ovarian Carcinoma. Results of a Prospective Pilot Study

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Abstract. Background: Different studies have demonstrated epidermal growth factor receptor (EGFR) status as an independent prognostic factor for ovarian cancer (OC). Recent studies in non-small cell lung cancer suggest that the presence of a clinical response to tyrosine kinase inhibitors correlates with somatic mutations in the kinase domain of EGFR, exons 18-21. For patients with OC, data are not available on EGFR gene mutation. Materials and Methods: Shock-frozen samples from 32 patients with OC were screened for L858R deletion mutations of EGFR within exon 21 of the kinase domain and 15 bp deletion in exon 19. Additionally, nine commercially available OC cell lines and 32 established OC lines were analysed. Results: In cell lines, as well as in tumor samples, stratified to platinum-free therapy interval, no mutation of the EGFR gene was observed. Conclusion: Mutations in the kinase domain of the EGFR, exons 19 and 21, are absent or very infrequent in patients with OC.

Ovarian cancer (OC) is the leading cause of death of all gynecological malignancies (1). The overall 5-year survival rate remains poor although surgical treatment and chemotherapy have improved significantly (2, 3).

In more than 50% of all ovarian cancer patients recurrence develops despite radical cytoreductive surgery and adjuvant chemotherapy with carboplatin and paclitaxel (3, 4). The response rate and overall survival are reduced in the relapse situation (4).

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was present in almost all the tumors of patients with non-small cell lung cancer who responded to gefitinib therapy (14-16). There are no data on the prevalence of these gene mutations in patients with ovarian cancer.

Taking into consideration the indications that EGFR gene mutations may have demographic, ethnic or geographic features (14-16), a prospective pilot study of deletion mutations in exon 19 and the L858R missense mutation in exon 21 of the EGFR gene in tumor specimens of ovarian cancer patients as well as in ovarian cancer cell lines was conducted.

Materials and Methods

Tissue samples and cell lines. Shock-frozen tumor specimens from 32 ovarian cancer patients obtained from the systematic Tumor Bank Ovarian Cancer Network. “TOC” (17) were screened for deletion mutations in exon 19 and the L858R missense mutation in exon 21 of the EGFR gene. There were 17 patients who responded to platinum- and taxane-based chemotherapy and 15 patients who did not. There was no known history of kinase inhibitor treatment for any of the patients. Table I summarizes all relevant patient characteristics.

All patients signed informed consent, approved by the Clinical Review Board and Ethics Committee of the Medical University Berlin, Charité, Germany. The tumor specimens were collected according to the Tumor Bank Ovarian Cancer standard operating procedures. A new documentation tool “IMO” (Intraoperative Mapping of Ovarian cancer) was used for the documentation of the surgical procedures (17). Platinum-resistant patients were defined as those who progressed or relapsed during or within six months after surgical procedures (17). Platinum-resistant patients were defined as those who progressed or relapsed during or within six months after surgical procedures (17). Only Caucasian patients were enrolled into this study.

In addition, nine commercially available ovarian cancer cell lines (TOV-90, TOV-112D, TOV-21G, OVCAR-3, A2780, A2780 ADR, ES-2, SK-OV-3 and CaOV-3) and 32 established ovarian cancer cell lines obtained from the University of Ulm were screened for deletions in exon 19 and the L858R missense mutation in exon 21 of the EGFR gene.

DNA extraction. DNA was isolated from tumor specimens and ovarian cancer cell lines using commercially available kits (DNA Extraction System II; ViennaLab, Vienna, Austria).

Mutation analysis. To analyse the deletions in exon 19 and the L858R mutation in exon 21 of c-erbB-1, the EGFR gene, extracted DNA samples were amplified. Each reaction mixture contained 25 ng of DNA, 12.5 pmol of both sense and antisense primers, 200 µM dNTPs (Amersham Biosciences, Uppsala, Sweden), 2.5 µl of 10X Amplification Buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.01% w/v gelatin, 1.5 mM MgCl₂, 0.1% Triton X-100 [ViennaLab]) and 1.4 units Super Taq Polymerase (HT Biotechnology Ltd., Cambridge, UK). The polymerase chain reaction (PCR) was performed on a GeneAmp PCR system 2700 (Applied Biosystems, Foster City, CA, USA). For the exon 19 fragment, the PCR cycling parameters were: an initial denaturation step at 94°C for 1 min, 40 cycles of 94°C for 30 sec, 53°C for 30 sec and 72°C for 30 sec, followed by a hold of 72°C for 7 min. For the exon 21 condition, the PCR conditions only differed in the annealing temperature (54°C). The primers for amplification were for exon 19, 5’-GCCAGTTAACGTTCCCTTC-3’ (sense), 5’-GAGGTTCAAGCCATGGAC-3’ (antisense), and for exon 21, 5’-TGATCTGTCCCTACAGC-3’ (sense), 5’-TGACCTAAAGCCACTCC-3’ (antisense).

For mutation analysis, the PCR products were purified using Centri Sep 96 (Princeton Separations, NJ, USA) and then sequenced using Big Dye Terminator Cycling Sequencing Kit, version v3.1 and the ABI Prism 310 Genetic Analyzer (Applied Biosystems) according to the manufacturer’s recommendation. Sequencing was conducted with exon 19 sense and exon 21 sense primers.

Detection systems for both mutation types were validated using DNA samples heterozygous for either a 15 bp deletion mutation in exon 19 or L858R missense mutation in exon 21. These DNA samples were kindly provided by Daphne Bell (14).

Statistics. Statistical analysis was performed using the SPSS (SPSS Inc., Chicago, IL, USA) statistical software package (13-0/2005). The focus was on descriptive analysis. Differences in the mutation in

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<th>Parameters</th>
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<tr>
<td>Age at first diagnosis (years)</td>
<td>58.59 (39-76)</td>
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<tr>
<td>Median (range)</td>
<td>17.84 (2-43)</td>
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<tr>
<td>Follow-up time (months)</td>
<td>20 (0-39)</td>
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<td>Disease-free survival (months)</td>
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<tr>
<td>Median (range)</td>
<td>14.3 (2-43)</td>
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Table I. Patient characteristics.
c-erbB-1 were analysed using the non-parametric Mann-Whitney test for independent samples. Two-tailed \( p<0.05 \) was considered statistically significant. The Kaplan-Meier method was used to calculate overall survival time or progression-free survival time. Survival curves were compared by the log-rank test.

**Results**

The median age at first diagnosis of the OC patients was 58.5 years (range, 39-76 years) and 81.3% were diagnosed in FIGO stage III-IV. In 46.9% of all the patients, optimal tumor resection was achieved with no postoperative residuals. The median follow-up was 17.8 months (range 2-43 months) (Table I). Within this period 14 deaths occurred. The median postoperative survival was not reached (Figure 1).

The median disease free survival was 20 months (95% CI 6.8-33.2) (Figure 2).

Neither deletions in exon 19 nor L858R mutations in exon 21 were detected in the ovarian cancer specimens. No distribution differences in EGFR mutation status were found between platinum-sensitive and platinum-refractory ovarian carcinoma patients. Similarly for the human ovarian cancer cell lines, neither deletions in exon 19 nor L858R mutations in exon 21 were present.

**Discussion**

The EGFR is a new target for various solid tumors (12). Recently, gefitinib was approved by the FDA as single-drug therapy for refractory lung cancer (19).

More than 28 mutations have been identified in the tyrosine kinase domain of the EGFR to date, but only the L858R missense mutation in exon 21 and deletions in exon 19 have been proven to be activating mutations (20).

Several studies in non-small cell lung cancer suggest that the presence of a clinical response to tyrosine kinase inhibitors (such as ZD 1839) correlates with somatic mutations in the kinase domain of EGFR, exons 18-21 (14). These two common mutation types are associated with an increase in the amount and duration of ligand-dependent activation, which explains the much greater sensitivity of cells bearing these mutations to EGFR kinase inhibitors (14). The coding region of EGFR has been investigated in tumors from patients with a response to gefitinib and in tumors from those without a response. Among nine patients with a response to gefitinib, eight had tumor specimens with heterozygous mutations within the tyrosine kinase domain of the EGFR (14).

Various working groups have been exploring the role of EGFR inhibitor in ovarian cancer treatment (21).

A phase II study in ovarian cancer patients has been reported in the past (21) in which 34 patients with advanced, heavily pre-treated, ovarian cancer received oral erlotinib, 150 mg daily. There were two confirmed (and two unconfirmed) partial responses, and 16 patients (41%) experienced disease stabilization. The median survival for the patients in this study was 242 days.

In the study from the Arbeitsgemeinschaft Gynäkologische Onkologie e.V. (AGO), 56 heavily pretreated patients with refractory ovarian cancer were treated with tamoxifen and gefitinib. There were no complete or partial responders but 28.6% of the patients achieved stable disease, while 58.9% had progressive disease (13).

It seems that a clinical response to kinase inhibitor treatment can be achieved only in those patients with a mutation in the EGFR gene.

For example, Pao et al. supported the theory that the kinase domain of the EGFR gene was often mutated in lung cancer patients who showed a clinical response to kinase inhibitor treatment, and was more common in female adenocarcinoma patients with low exposures to cigarette smoking (16).
Since no mutation in the kinase domain of EGFR exons 19 and 21 was found in the present study, the hypothesis of Lynch and Pao et al. that EGFR gene mutations may have demographic, ethnic or geographic features (14, 16) could not be confirmed.

Some studies have shown that the frequency of the mutation in the EGFR may have demographic features (22). Paez et al. (15) reported that the prevalence of this mutation seems to differ between populations; for example the Japanese population has a much higher rate of EGFR gene mutation (15 out of 58, 26%) compared with a US cohort (1 out of 61, 2%).

High EGFR mutation profiles have also been reported from South Korea, Taiwan and China (23-25). We have to conclude that the frequency of mutation seems to be very low, despite the limited number of patients in our study.

Currently, EORTC is conducting a study with erlotinib, an EGFR inhibitor as a maintenance therapy in patients with ovarian cancer. In this study, the presence of mutations and polymorphisms in the EGFR gene will be explored and correlated with clinical outcome.

Generally, the EGFR coding region mutation points to the need for a global view of the genome of ovarian cancer. The possibility that somatic mutations in the EGFR gene vary with ethnicity indicates the investigation of possible differences in the response to anticancer treatment of different ethnic groups.

References