

EGFR-Activating Mutations Are Not Present in Breast Tumors of Japanese Patients

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Abstract. *Background: Despite advances in treatment, recurrent breast cancer remains a lethal disease. The epidermal growth factor receptor family has been suggested to play a role in breast cancer. However, no investigations of the frequency of EGFR mutation and the potential role of EGFR tyrosine kinase inhibitor (TKI) in Japanese patients with breast cancer. Materials and Methods: Tumor specimens were collected from 84 breast cancer patients who underwent surgery. Polymerase chain reaction-based methods were used to examine EGFR-activating mutations (exon 19 and 21). Results: Activating mutations in the tyrosine kinase domain of EGFR were not identified in any of the tumors. Conclusion: EGFR-activating mutations were not present in the Japanese breast cancer series studied here. Therefore, unlike lung cancer, EGFR tyrosine kinase inhibitors are unlikely to provide any benefit for Japanese breast cancer patients.*

Breast cancer (BC) is one of the leading causes of death in women and recurrent BC is still lethal in most patients (1). However, systemic therapy has been reported to contribute to the prolongation of patient survival (1). In particular, molecular targeted drugs against HER2, which is a member of the EGFR family, have emerged as standard therapy for BC with overexpression of HER2 (2).

Molecular-targeted therapies have provided a new approach to fight malignancies such as chronic myeloid leukemia (3) and lung adenocarcinoma (4). However, while such strategies can provide beneficial effects, they also are known to induce various harmful adverse events. Therefore, it is necessary to individualize therapy and identify patients

who will not only respond to the treatment, but who are also less likely to suffer from its adverse events. We previously reported that somatic mutations in the tyrosine kinase (TK) domain of the epidermal growth factor receptor (*EGFR*) gene have been identified in lung adenocarcinoma patients who showed an increased response to an EGFR-TK inhibitor, thus suggesting that screening for such mutations may have clinical utility in predicting response (5). However, differences in the expression of specific genes related to antineoplastic drug disposition based on ethnic differences are anticipated (6). In fact, the incidence of the *EGFR* mutation in non-small cell cancer (NSCLC) was reported to be higher in Asians than in Caucasians (7). Therefore, we hypothesized that the incidence of *EGFR* mutations would be higher in Japanese patients with BC, since that in Caucasians was reported to be remarkably rare in breast cancer samples (8-10). If so, this subgroup of BC patients may benefit from EGFR-TK inhibitor treatment. However, to our knowledge, there are no reports investigating the frequency of *EGFR* mutation in Asian patients with BC.

Materials and Methods

Patients and clinical features. The Institutional Review Board approved this study, and informed consent for the use of the tumor specimens was obtained from all of the patients or from their legal guardians. Tumor samples were obtained from 108 patients with primary BC who had undergone a surgical resection between February of 2000 and November of 2004 in our Department. The tumor samples from 24 patients were too small to analyze. Finally, a total of 84 patients were included in the analysis. The clinical data including the preoperative examinations, details of the surgical resection, histopathological findings, and TNM stages of all patients were also retrieved. All patients underwent a physical examination, ultrasonography for the contralateral breast, and mammography during the preoperative evaluation. The assessments for distant metastasis included chest roentgenography, computed tomography (CT) of the chest and upper abdomen, and bone scintigraphy. For evaluation of the extent of the disease, magnetic resonance imaging (MRI) was performed if required. All of the resected specimens were examined for tumor histology and the extent of tumor invasion. The histopathological findings were classified according to the General Rules for Clinical and Pathological Recording of Breast Cancer (11).

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The expression of the estrogen receptor (ER) and progesterone receptor (PgR) in the cancer tissues were measured by an enzyme immunoassay, or immunohistochemical (IHC) staining of sections taken from formalin-fixed, paraffin-embedded blocks of the surgical specimens. All of the tumor samples (100%) and samples from 41 patients (48.8%) were available for the evaluation of hormone receptors and HER2 status, respectively.

EGFR mutation analysis. Genomic DNA was extracted and purified from either fresh frozen tumors or tumors embedded in paraffin blocks. The *EGFR* mutations were examined by previously described methods (12). Briefly, the exon 19 deletion of *EGFR* was detected by a simple screening method, which was the detection of a band shorter than the 147 bp PCR product expected from the wild-type allele on agarose gel electrophoresis. The substitution of Leu for Arg at codon 858 in exon 21 (L858R) as the point mutation was detected by mutant-allele specific amplification (12). The positive controls for in-frame deletion in exon 19 and L858R point mutation of *EGFR* were the B901L and G603 lung cancer cell lines, respectively (13). The negative control for wild-type *EGFR* was the A904L lung cancer cell line (13). Furthermore, positive and negative controls for *EGFR* mutations were also confirmed by a direct sequencing method (13).

Results

All of the patients in this series were Japanese females, with a mean age of 56.6 years (range 27 to 97 years). They consisted of 25 premenopausal and 59 postmenopausal women. The location of the tumor was on the right side in 38 patients, the left side in 45, and on both sides in one patient. The surgical procedures included a mastectomy (Bt) with dissection of the axillary lymph nodes (Ax) (Auchincloss procedure) in 38 patients, a partial resection (Bp) with Ax in 18, a radical mastectomy in 17, a quadrantectomy (Bq) with Ax in 5, tumorectomy with Ax in 3, Bp in 2, and a radical mastectomy plus a flap of the rectus abdominis muscle in one patient. The histological types included 32 papillotubular carcinomas, 30 scirrhous carcinomas, 7 solid-tubular carcinomas, 6 mucinous carcinomas, 4 invasive lobular carcinomas, 3 non-invasive ductal carcinomas, one spindle cell carcinoma, and one apocrine carcinoma. The tumor size was classified as T1 in 33 patients, T2 in 40, T3 in 7 and T4 in 4. Seventy-nine (94.0%) of the 84 patients had undergone dissection of the lymph nodes. The lymph node metastasis of the patients was diagnosed pathologically as N0 in 52 patients, N1 in 25 and N2 in 2 patients. The pathological stages were determined to be stage IA in 32 patients, II in 42, III in 8, and stage IV in 2 patients. Of all cases, 55 (65.5%) and 55 (65.5%) patients were positive for ER and PgR, respectively. The score of IHC staining for HER2 was 0 in 14, 1+ in 3, 2+ in 17, and 3+ in 7 patients.

No activating *EGFR* mutations were identified, suggesting that such mutations are unlikely to be common in Japanese women with BC.

Discussion

The present study is the first to demonstrate the frequency of *EGFR* mutations in Japanese breast cancer patients. At present, no activating mutations in *EGFR* have been reported in primary BC nor BC cell lines from Western countries (8-10, 14). Our results, in addition to those of previous studies, confirm the presence of activating mutations in the *EGFR* exon is uncommon in primary BC. On the other hand, Weber *et al.* detected a higher rate of *EGFR* missense mutations in BRCA1/2-positive tumors (11/24) compared with sporadic BC (7/48), indicating that *EGFR* mutations are more likely to be elevated in hereditary BC (15). However, the majority of missense mutations in this and another report were within exon 20 (15, 16). This is in contrast to lung adenocarcinoma in which threonine-to-methionine mutation at codon 790 (T790M) in exon 20 of *EGFR* have been reported to correlate with resistance to EGFR-TK inhibitors (4, 5, 17, 18).

In this study, 55 (65.5%) of the 84 tumors were ER positive. Recently, we reported that *EGFR* mutations occur more frequently in lung adenocarcinoma with strong expression of ER β , and a strong expression of ER β predicts a good clinical outcome for patients with adenocarcinoma of the lung after treatment with EGFR-TK inhibitors (19, 20). Furthermore, an *in vitro* study showed that the combination of tamoxifen (ER antagonist) and gefitinib (EGFR-TK inhibitor) in NSCLC enhanced the antiproliferative effects of treatment, possibly providing a rationale for combining an EGFR-TK inhibitor with anti-estrogen therapy (21). In addition, in a pilot study of combination therapy with gefitinib and fulvestrant (an ER antagonist) for NSCLC, the overall survival of the patients whose tumors exhibited strong expression of ER β was 65.6 weeks, while that of patients with weak expression was 21 weeks (22). The exploitation of EGFR interactions with ER as a new therapeutic strategy might therefore warrant further investigation (23).

The human monoclonal antibody against HER2, trastuzumab, has improved the outcome of patients with HER2-positive BC (24). Furthermore, HER2 overexpression in human BC cell lines or tumor xenografts also increases the cytotoxicity and/or the antitumor effects of gefitinib (25, 26). However, clinical studies of EGFR TK inhibitors have resulted in few clinical responses, and in a disease control rate of only approximately 10% (14, 27). These observations clearly indicate that intrinsic resistance to gefitinib is a common phenomenon in BC (27). This discrepancy might correlate with the lack of activating mutations of *EGFR* in BC.

In summary, we have herein shown that *EGFR* activating mutations in Japanese patients with BC are very rare, and that treatment with an *EGFR*-TK inhibitor is unlikely to result in any clinical benefit. A firm conclusion cannot be drawn from the results of this study because of its

retrospective nature of the study and the fact that it was carried out at a single institution; nonetheless, we believe that EGFR-TK inhibitors will be ineffective for BC therapy.

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