Very low prevalence of epidermal growth factor receptor (EGFR) protein expression and gene amplification in Saudi breast cancer patients

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Abstract

Background: Breast cancers which demonstrate EGFR protein expression, gene amplification and/or gene mutations may benefit therapeutically from tyrosine kinase inhibitors. In Western studies, EGFR protein expression has been demonstrated in 7-36% of breast cancer patients, while gene amplification has been found in around 6% of cases and mutations were either absent or extremely rare. Studies addressing EGFR protein expression and gene amplification in Saudi breast cancer patients are extremely scanty and the results reported have been mostly non-conclusive. Herein we report the prevalence of EGFR protein expression and gene amplification in a cohort of Saudi breast cancer patients.

Findings: We noticed a remarkably low incidence of EGFR protein expression (1.3%) while analyzing the spectrum of molecular subtypes of breast cancer in a Saudi population by immunohistochemistry. Also, EGFR gene amplification could not be demonstrated in any of 231 cases studied using silver enhanced in situ hybridization.

Conclusions: The extremely low incidence of EGFR protein expression and gene amplification in Saudi breast cancer patients as compared to Western populations is most probably ethnically related as supported by our previous finding in the same cohort of a spectrum of molecular breast cancer types that is unique to the Saudi population and in stark contrast with Western and other regionally based studies. Further support to this view is provided by earlier studies from Saudi Arabia that have similarly shown variability in molecular breast cancer subtype distribution between Saudi and Caucasian populations as well as a predominance of the high-grade pathway in breast cancer development in Middle East women. More studies on EGFR in breast cancer are needed from different regions of Saudi Arabia before our assumption can be confirmed, however.

Findings

Background and research hypothesis

EGFR is a tyrosine kinase receptor in the HER family which is widely expressed in a number of epithelial tumors and is believed to play a key role in cell proliferation. It is now well established that non-small-cell lung cancers which demonstrate EGFR protein expression, gene amplification and/or gene mutations at exons 18 - 21 show a dramatic therapeutic response to tyrosine kinase inhibitors such as gefitinib and erlotinib [1-3]. Although the same may be true for other cancers including breast cancer, data regarding the presence or absence of EGFR abnormalities in tumors other than lung cancer and the response of such tumors to anti EGFR therapy are still limited and rather conflicting. EGFR protein expression as assessed by immunohistochemistry has been demonstrated in 7-36% of breast cancer patients, while gene amplification as assessed by CISH or FISH has been found in around 6% of cases [4-8]. Mutations in exons 18 - 21 of the EGFR gene investigated by PCR were either absent [1,7] or present in only rare breast cancer patients [9], such mutations being much frequent in lung cancer [10]. Differences in the prevalence of EGFR over-expression reported by different studies have been attributed to probable variations in techniques and type of antibodies used, criteria for determining over-expression and inter-observer variability [7].
In a recent study that analyzed the spectrum of molecular subtypes of breast cancer in a Saudi population [11], we noticed (but have not reported) a remarkably low incidence of EGFR protein expression in our patients. Also, EGFR gene amplification could not be demonstrated in any of 231 cases studied using silver enhanced in situ hybridization (assessed after the study was published). In this article we aim to explore whether this extremely low incidence of protein expression and gene amplification reflects a truly low prevalence of EGFR gene abnormalities in the Saudi population which may be ethnically related or is, alternatively, due to possible suboptimal sensitivity of the immunohistochemistry technique/antibodies or the in situ hybridization method used.

Patients, methods and results

We have recently published a study that analyzed the spectrum of molecular subtypes of breast cancer in 231 Saudi patients [11]. The age of the patients ranged between 25 and 97 years with a mean of 49.5 years (SD ± 11). Representative cancerous tissues obtained from paraffin blocks of mastectomy and lumpectomy specimens were incorporated into 5 tissue microarray reception blocks, from which 4 micron thick sections were cut for immunohistochemical and in situ hybridization studies. For tru-cut biopsies, conventional paraffin blocks were utilized. The cases were randomly selected from the archives of our pathology department based on the availability of representative blocks and sufficient tissue material to perform the required procedures. An immunohistochemical panel including ER, PR, HER2, Ck5/6 and EGFR antibodies was used as a surrogate for gene expression profiling to classify the 231 breast cancer specimens. Moreover, each class was correlated with its Ki-67 proliferation index and p53 gene over-expression, as revealed by IHC, and also with the histologic type and grade of the tumor. The histopathological and molecular characteristics of breast cancer in these patients are shown in table 1.

The anti EGFR antibody was used solely as an indicator of the basal molecular subtype (together with CK5/6) and we have not reported or commented on the prevalence of EGFR protein expression among the studied cohort. A revisit to the study revealed that only three out of 231 cases were positive for EGFR (1.3%). Positivity was defined as membrane staining (Figure 1A) and was scored according to the criteria originally developed.

Table 1 Histologic and molecular characteristics of cancer in a cohort of 231 Saudi breast cancer patients

| Histologic type | LUMA No. (%) | LUMB No. (%) | HER2 No. (%) | Basal No. (%) | Hybrid No. (%) | UC No. (%) | Total No. (%) |
|-----------------|--------------|--------------|--------------|--------------|---------------|------------|--------------|
| IDC             | 6 (3.3)      | 27 (14.8)    | 29 (15.6)    | 20 (10.9)    | 16 (8.7)      | 85 (46.4)  | 183 (79.2)   |
| ILC             | 3 (3.3)      | 5 (55.6)     | 0 (0)        | 0 (0)        | 0 (0)         | 1 (11.1)   | 9 (3.89)     |
| ISC             | 0 (0)        | 3 (20)       | 7 (46.7)     | 0 (0)        | 3 (20)        | 2 (13.3)   | 15 (6.49)    |
| other*          | 0 (0)        | 2 (8.3)      | 4 (16.7)     | 3 (12.5)     | 4 (16.7)      | 11 (45.8)  | 24 (10.38)   |

IDC = Invasive ductal carcinoma-NOS, ILC = Invasive lobular carcinoma, ISC = In situ carcinoma, LUMA = luminal A, LUMB = luminal B, HER2 = human epidermal growth factor receptor 2, UC = unclassified

*Include medullary carcinoma, mixed ductal and lobular carcinoma, metaplastic carcinoma, apocrine carcinoma and juvenile secretory carcinoma

Figure 1 EGFR protein expression by immunohistochemistry and gene amplification by SISH in a case of metaplastic breast carcinoma. A) Membrane positivity by immunohistochemistry, × 100 B) No gene amplification (less than 5 gene copies per nucleus) by SISH, × 400.
Table 3 Histologic type in relation to patient age, tumor grade and tumor stage in EGFR positive breast cancer cases

| Case No | Histologic type                      | Patient age (years) | Tumor grade | Tumor stage |
|---------|--------------------------------------|---------------------|-------------|-------------|
| 1       | Metaplastic carcinoma                 | 35                  | III         | pT2N0M0 (IIA) |
| 2       | Invasive ductal carcinoma, NOS        | 61                  | III         | pT2N0M0 (IIA) |
| 3       | Metaplastic carcinoma                 | 78                  | III         | pT3N0M1 (IV)  |
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