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High level of EGF-R expression in carcinomatous skin invasion: Does it reflect the tissue characteristics or the breast carcinoma aggressiveness?

KEYWORDS: Breast Neoplasms; Receptor, Epidermal Growth Factor

ABSTRACT

Background: *The normal function and distribution of EGF-R and its role in breast cancer aggressiveness, prognosis and prediction, have become extremely important in the light of the recently developed methods of EGF-R targeting. In the aim to investigate the relationship between EGF-R and the aggressive tumor behavior, the EGF-R content was analyzed as related to the presence of inflammatory breast skin involvement.*

Methods: *EGF-R, ER and PR content was determined at diagnosis, using the biochemical methods, in the group of 103 unselected breast cancer patients, either in primary tumors (TU), lymph nodes (LN) or skin tissue samples (65, 27 and 11 cases, respectively). In 10 patients with inflammatory breast cancers, TU/LN tissue was sampled from 3, and skin from 7 patients.*

Results: *ER and PR content was significantly higher in tumor and LN tissue, compared to the invaded skin; the EGF-R content was, on the contrary, significantly higher in skin than in TU or LN tissue. However, no difference was found between TU and LN in all three receptors' content. When the receptor content was analyzed in 10 patients with inflammatory breast cancer, higher levels of both ER and PR were found in tumor biopsies than in skin biopsies, while for the EGF-R the result was opposite. Significantly lower ER content and a trend towards higher EGF-R content was found in the inflammatory breast cancers in comparison to the non-inflammatory ones.*

Conclusion: *Although we examined a small number of patients, our results suggest that the EGF-R could be a marker of breast cancer aggressiveness. However, the influence of the normal skin cells contaminating the biopsied tumor tissue cannot be ruled out. The predictive role of EGF-R deserves to be further investigated, especially in locally advanced inflammatory breast cancer patients.*

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INTRODUCTION

EGF-R has a key role in promoting cancer spread, driving tumor invasion, metastasizing and angiogenesis (1). It is one of the most interesting breast cancer biomarkers, not only because of its prognostic and predictive role, but also due to the recently developed methods of its inhibition (2, 3).

Growth factor receptors as well as steroid receptors in breast cancer patients are commonly determined in primary breast cancer tissue, or other involved tissues such as lymph nodes, skin lesions, local recurrences and rarely in other involved organs. It is still controversial whether the receptor content depends on the site of biopsy or tissue cellularity, and whether the more advanced disease is followed by the decrease in steroid receptor content and increase in GF receptors. Moreover, the biopsy specimens could be "contaminated" with normal cells or tissue. The reports on the receptor content in normal breast and other tissues are rare. However, it was shown that the steroid receptor content is low in normal breast and other tissue (4), while the EGF-R content in normal skin is higher than in other tissue (5). The normal breast tissue in breast cancer patients was shown to express lower level of SRs, and higher level of EGF-R than the corresponding breast cancer tissue (6). Pekonen et al. found in 13 patients similar EGF-R content in local recurrences and skin metastases, compared to primary tumors (7), while Sainsbury et al. found that breast cancer metastases have in general higher level of EGF-R expression (8).

The relation of EGF-R content in lymph node metastases and primary tumors is also controversial. In a study of Grimaux et al. (9) slightly higher levels of EGF-R were found in lymph nodes, compared to primary tumors, while Macias et al. (10) found higher levels of EGF-R both in lymph nodes and other distant metastases. Other authors showed the almost unchanged ER and EGF-R content between primary tumors and involved lymph nodes (11), but the decrease in ER and PR content in very advanced metastatic stage of the disease (12). We have found a trend towards higher EGF-R level in clinical stage IV compared to stage I of the disease, but relatively stable EGF-R content during the progression trough intermediary stages (13).

It was reported that the EGF-R status inversely correlated to the ER and PR status, suggesting that the expression of EGF-R could be the marker of the autocrine tumor growth regulation, and thus the marker of more aggressive tumor behavior. In the same line is the finding of Guerin et al. (14) of more transcripts of EGF-R gene in inflammatory primary tumors than in non- inflammatory ones.

In this study, the content of steroid receptors and EGF-R, determined in primary breast carcinomas, lymph nodes and involved skin were compared. In particular, the inflammatory breast cancers were compared to all other, and to those with skin lesions without carcinomatous inflammatory skin involvement.

PATIENTS AND METHODS

Patients. Receptors for estrogen, progesterone and epidermal growth factor were determined in breast cancer tissue samples, taken at diagnosis of 103 female patients. In case of 58 patients diagnosis was made in operable clinical stages (I or II) and initial sampling was done on the primary tumor during the radical surgical procedure. The remaining patients were submitted either to the primary breast cancer biopsy, or to the biopsy of the lymph nodes or skin lesions. The patients' characteristics are presented in Table 1.

The site of BC biopsy and the corresponding initial clinical stage are presented in Table 2. Tissue of primary tumor was sampled in 65 patients, lymph nodes in 27 patients (22 ipsilateral and in 5 distant lymph nodes), and the skin biopsy was done in 11 patients (7 with inflammatory breast cancer and 4 with non-inflammatory lesions). In the group of 10 inflammatory breast cancers, the tumor was biopsied in 3/10 patients. None of the patients were previously treated with either systemic or any other anti-tumor treatment.

Table 1. Patients characteristics

Age (yrs)	Range	27-79
	Median	51.5
Menopausal status (No of patients)	pre	49
	peri	9
	post	45
Histology type	IDC	47
	ILC	19
	Others	37
Histology grade	I	8
	II	58
	III	12
	X	25

Table 2. Relation between clinical stage and tumor tissue biopsied (No. of patients)

Stage \ Biopsy	Tu	LN	Skin	Total
I	18			18
II	40			40
III	5	20	5	30
IV	2	7	6	15
Total	65	27	11	103

Steroid receptors and EGF-R determination. Steroid receptors (SR) and EGF-R were determined using biochemical methods (15-17).

Statistics. For comparison of the nonparametric values Mann-Whitney U test was used. Significant differences were determined at $p<0.05$ level, for all statistical tests.

RESULTS

The ER and PR content were similar in primary tumor and lymph nodes. Both were significantly lower in skin biopsies, compared to either primary tumor or lymph nodes, and when taken together ($z=3.35$, $p<0.05$ for ER in primary tumors and lymph nodes *vs*. ER in skin biopsies; $z=3.49$, $p<0.05$ for PR in primary tumors and lymph nodes *vs*. PR in skin biopsies, Figures 1 and 2).

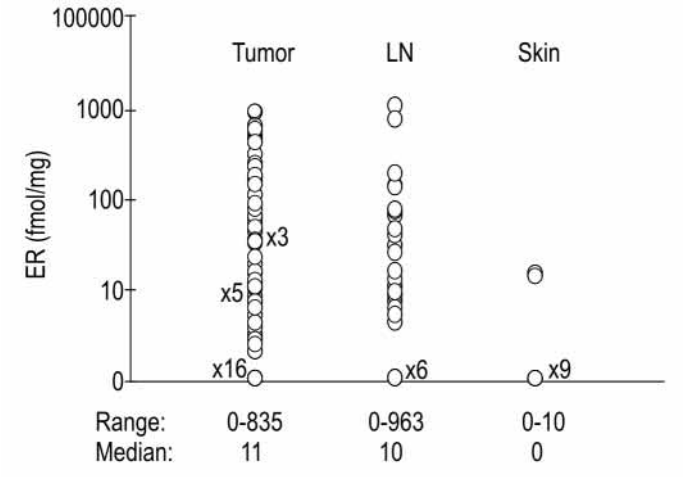

Figure 1. ER content in relation to the biopsied tissue

EGF-R content was similar in primary tumors and lymph nodes, but it was significantly higher in skin biopsies *vs*. primary tumors and lymph nodes together ($z=3.038$, $p<0.05$, Figure 3). The content of all three receptors was comparable in operable *vs*. inoperable tumors, as well as in ipsilateral vs. distant lymph nodes (not shown). The receptor content was comparable in all clinical stages for all three receptors, respectively, except for the EGF-R content in stage I *vs*. stage IV of the disease (13). It was not influenced by the tumor size, grade and histological type, or by nodal involvement (18).

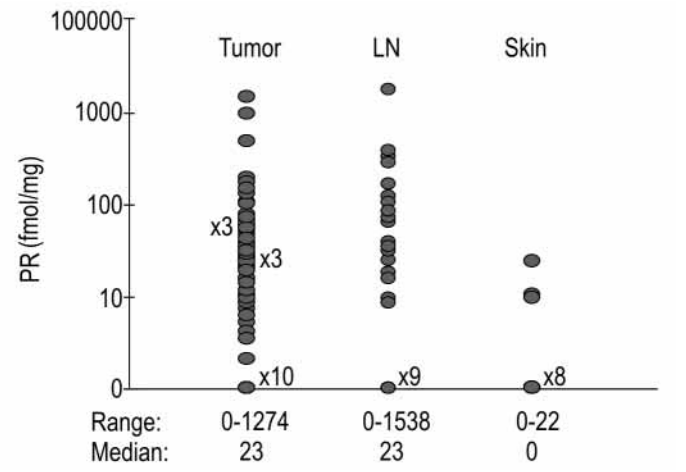

Figure 2. PR content in relation to the biopsied tissue

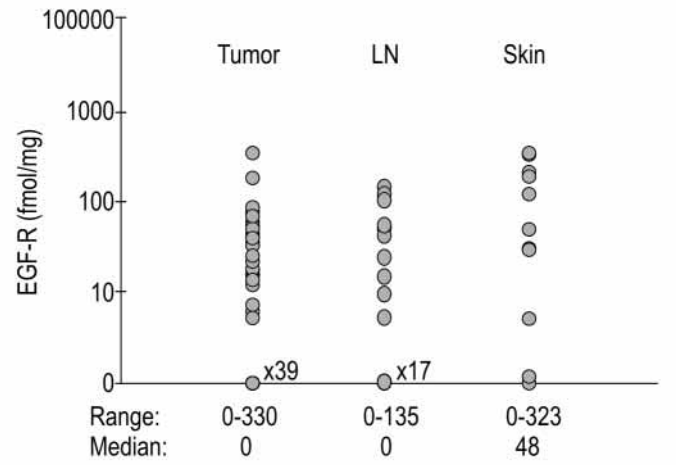

Figure 3. EGF-R content in relation to the biopsied tissue

Next the receptors content was analyzed in 10 inflammatory breast carcinomas, determined in tumor tissue in 3, and in skin biopsies in 7 consecutive cases. When we compared the receptor contents it appeared that both ER and PR contents were lower in skin biopsies than in primary tumor tissue (range 0-13 *vs*. 4-36, median 0 *vs*. 10 fmol/mg, for ER, and 0-22 *vs*. 8-338, median 0 *vs*. 33 fmol/mg, for PR, respectively). On the contrary, EGF-R content was significantly higher in skin than in tumor tissue (range 0-319 *vs*. 0-40, median 115 *vs*. 0 fmol/mg, Figure 4).

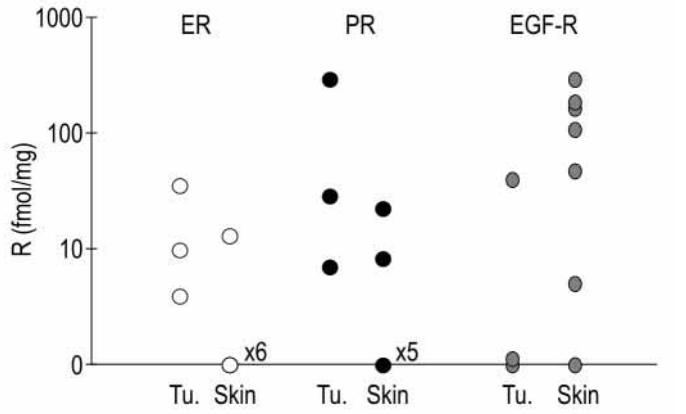

Figure 4. ER, PR and EGF-R content in inflammatory cancers, in relation to the biopsied tissue

Since all inflammatory carcinomas belonged to the group of locally advanced tumors (n=40), the comparison of the receptors content was made between inflammatory and non-inflammatory locally advanced breast carci-

nomas, irrespective to the site of biopsy. A trend towards higher EGF-R content and significantly lower ER content was found in inflammatory, than in non-inflammatory locally advanced carcinomas (Figure 5).

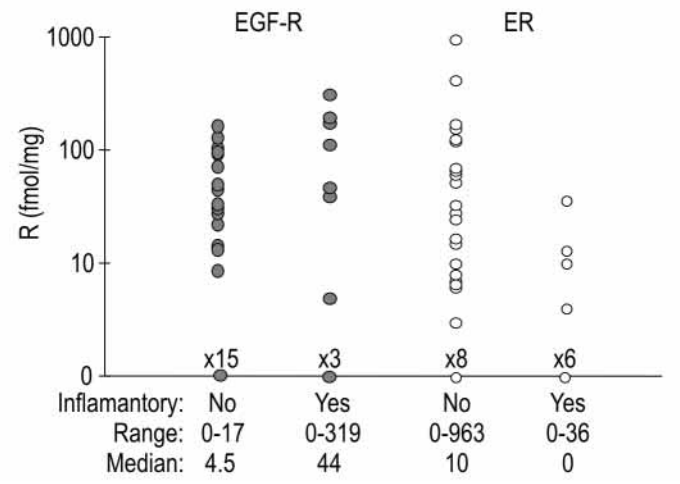

Figure 5. ER and EGF-R content in inflammatory and non-inflammatory locally advanced breast carcinomas

The same magnitude of difference was found when all breast cancers, irrespective to clinical stage, were compared to the inflammatory ones. The PR content was similar in inflammatory and non-inflammatory carcinomas (not shown).

DISCUSSION

Our results confirmed our previous observation that the steroid receptor status determined in lymph node tissue might well represent the steroid receptor status of the primary tumor (19). This conclusion now can be generalized to include the EGF-R status. This is clinically very important, because of the cases of breast cancer patients in which the regional or distant lymph nodes need to be sampled for histological diagnosis and receptor determination. In other tumor tissue sampling the receptor status of the most recent tumor lesions represents the actual endocrine responsiveness of the disease better, when compared to the receptor status of the primaries, determined at diagnosis (20). It is well known that the steroid receptor content is slightly decreased in distant metastases, compared to the primary breast cancer (19). Fewer data exist about the receptor content in distant metastases, relevant to the content of metachronous primary tumor. Some localizations of tumor invasion have been found as most inconvenient for receptor determination. For example, steroid receptors determined by ligand binding method in pleural fluid cells have been shown as unreliable because of low cellularity of the samples and a possible contamination with the normal epithelial cells that require additional procedure for malignant cell separation (21). Similar problems may arise when steroid receptors are determined in skin lesions.

In fact, two possible explanations exist for the difference between primary tumors and skin lesions in the steroid receptors and EGF-R content, as found in our study. One could suppose that the normal skin cells contain higher levels of EGF-R that may contaminate the biopsy sample, giving the false increased values. On the contrary, skin cells probably contain lower levels of steroid receptors, and, if contaminate the tumor sample, they would give false lower values of SRs. However, the possibility exists that steroid receptor content is truly lower, and EGF-R content truly higher in distant metastases, compared to primary tumors, as a sign of tumor progression, as it was found in previous studies (8, 12, 19).

Our results suggest the third possible explanation. Most of the cases, in which the receptor content was determined in skin lesions, represented the locally advanced breast carcinomas with histological and/or clinical signs of inflammatory skin involvement. Carcinomatous skin invasion was histologically confirmed in 7/10 patients. In 3 patients the primary tumor biopsy was done, inflammatory breast cancer was clinically evident, although histologically unconfirmed. Since it is well known that the inflammatory breast carcinoma is the most aggressive clinical type of the disease presentation, it is likely that the highest EGF-R content in skin biopsies simply reflects the aggressiveness of the disease. In other words, clinically most unfavorable case of the disease - the inflammatory breast cancer, expresses lower SR content, and higher EGF-R content, than other non-inflammatory breast cancers. Harvey et al. found the lower ER levels in a small group of inflammatory breast cancer (22). It could be supposed that breast carcinomas with the poorest prognosis express the highest level of EGF-R as the biological marker of an autonomous growth control. However, a small number of cases were presented and the conclusions have to be confirmed in larger patients' group. On the other hand, the immunohistochemistry should probably be the useful method to check the cellularity, and exclude the contamination with normal skin cells, in skin lesion biopsies. Finally, it could be clinically of interest to find out whether the patients whose inflammatory breast carcinomas express the steroid receptors and have lower levels of EGF-R could be endocrine responsive, and could have better outcome. And, on the contrary, whether the patients with the highest EGF-R level in their inflammatory breast cancers could respond better to non-endocrine treatments, i.e. chemo- and radiotherapy.

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