REVIEW

Epidermal Growth Factor Receptors in Breast Cancer: from Experiment to Clinical Practice

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The role of auto-paracrine regulation of cell proliferation in breast cancer is analyzed. Experimental data indicate that the epidermal growth factor receptor is the key transmitter of mitogenic signals from the polypeptide growth factors and participates in the formation of sensitivity and/or resistance of breast cancer cells to steroid hormones. Data of representative clinical studies indicate that the expression of the epidermal growth factor receptor is prognostically unfavorable in breast cancer. Screening of patients with breast tumors for this receptor will detect the high-risk group at early stages of the disease. A scheme of the screening is proposed.

Key Words: *epidermal growth factor receptors; breast cancer; proliferation regulation; steroid hormone sensitivity; prognosis*

Epidermal growth factor receptor (EGFR) is a large transmembrane glycoprotein with a molecular weight of 170 kD. It is a product of an *erb-c-erb*B1 oncogene and belongs to so-called tyrosine kinases that are important regulators of cell proliferation and malignant transformation [17,32,46]. Transmembrane localization and need in relevant polypeptide ligand for realization of the kinase activity differs the receptor tyrosine kinases from the nonreceptor ones, which are also products of some important oncogenes, for example, *v-src*, *v-abl*, and *bcr-abl*.

In addition to the epidermal growth factor (EGF), α-transforming growth factor (α-TGR), *Vaccinia* virus growth factor, and the peptides amphiregulin, cripto and β-cellulin binding heparin EGF-like factor act as specific EGFR ligands [12,23,53,75]. All these ligands are polypeptides consisting of about 40 amino acids with a molecular weight of about 6 kD. They are 24-40% homologous by primary structure and similar by spatial configuration due to conservative sequences in 3 disulfide bridges determining and stabilizing the molecule conformation. These peptides exert similar biological effects on sensitive cells and cross-induce the production of each other.

Like molecules of all receptor tyrokinases, a EGRF molecule contains 3 major domains [17,75]: extracellular N-terminal glycosylated ligand-binding site constituting about 50% of the molecule (621 of 1173 amino acid residues) and ensuring specificity of the signal perception, transmembrane α-spiral site consisting of only 23 hydrophobic amino acids, and intracellular tyrosine kinase domain (542 amino acids) similar in all receptor tyrokinases.

Schematically, all main events in which EGFR is involved as a result of its reaction with the ligand are as follows [17,54,61,62,69,75]: immediately after the ligand binding, the receptor is dimerized, which leads to tyrosine kinase activation and autophosphorylation of tyrosine residues of the C-terminal domain and alteration of its conformation, thus promoting binding and phosphorylation of exogenous substrates and subsequent competitive inhibition of the receptor autophosphorylation. After autophos-

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phorylation, the ligand-receptor dimers aggregate on cell surface into larger complexes, form vesicles covered with a membrane fragment, and are then internalized. Among the most important intracellular signal systems regulated by EGFR are the phosphatidyl inositol metabolism and the *ras* gene system [17,46], although the effects of EGFR are of course not confined to these two systems.

The regulatory role of EGFR system in breast cancer has been investigated in detail in cell culture. The majority of cultured breast cancer cells produce α-TGF and have EGFR on their membranes, which suggests autocrine regulation of their proliferation by this factor [20,21,68,89]. Artificial amplification of α-TGF gene transforms cultured mammary epithelial cells [21,22]. In addition, increased production of α-TGF is apparently an important factor in epitheliocyte transformation by *Ha-ras* but negligible for transformation by *neu* oncogene [22]. The expression of α-TGF and EGFR is increased in many cultured breast cancer cells as well as the amounts of the adapter protein GPB2 and the corresponding mRNA [29]. Recent studies showed that activated EGFR phosphorylates and activates nonreceptor tyrosine kinase *c-src* in cultured breast cancer cells [57].

Exogenous α-TGF stimulates the growth of cultured breast cancer cells and this effect is suppressed by antibodies to the growth factor and EGFR [9, 21,33]. Amphiregulin seems to act similarly [53]. These two factors, primarily α-TGF, are now regarded as auto/para/juxtacrine regulators of breast cancer cells through the EGFR signal system. EGF exerts different effects on breast cancer cells, depending on its concentration and cell strain. It stimulates the growth of hormone-dependent cells and exerts no mitogenic effect on hormone-independent cells, particularly if used in high (at least 10^{-8} M) concentrations, and can even inhibit the proliferation [59] and induce apoptosis in MDA-MB cells [10].

Comparison of EGF effects on MDA-MB-231 cells and hormone-dependent MCF-7 cells showed differences at the level of EGFR redistribution (the number of receptors on the membrane increased after ligand binding in MCF-7 and decreased in MDA-MB) and in the spectrum of phosphorylated proteins: mainly EGFR was phosphorylated in MDA-MB and several different substrates in MCF-7 [59]. Another study demonstrated different regulation patterns for the expression of *ras*-like genes *rho*A, *rho*B, and *rho*C in hormone-independent and hormone-dependent breast cancer cells [27]. In hormone-dependent cells *rho*-genes were expressed at a high level which did not depend on EGF, while in hormone-independent cells basal expression of *rho* genes was extremely low but markedly increased under the effect of EGF. Both basal expression of *rho*B gene and its induction by EGF were observed in cultured normal mammary epitheliocytes. Therefore, hormone-dependent and hormone independent breast tumors differ by regulation with the *ras* signal system.

The production of endogenous α-TGF is induced by estrogens and suppressed by antiestrogens in estrogen-sensitive cells MCF-7, ZR-75.1, and T47D [11,13,90]. Antibodies to EGFR and α-RGF block the growth-stimulating effect of estradiol on sensitive cells and inhibit their basal proliferation [11]. Changes in the expression of α-TGF are observed at the level of mRNA and at the level of the immunoreactive protein amount in the cells and culture media [13]. α-TGF gene contains estrogen-sensitive sites [72], i.e., the production of α-TGF is regulated at the level of transcription. Other researchers claim that the induction of α-TGF production is an important but not the only mechanism of the mitogenic effect of estrogens. B. S. Leung *et al.* [55] showed that CAMA-1 cell proliferation is stimulated by estrogens under different experimental conditions but is not sensitive to EGF and α-TGF. Moreover, these cells contain no functionally active EGFR.

The data on the estradiol effect on the content of EGFR in estrogen-sensitive cells are contradictory; however, there is general agreement that after incubation no longer than 24-48 h estradiol decreases the concentration of EGRF by affecting the production of its mRNA [73]. By contrast, during longer incubation estradiol increases the content of EGFR [15]. An opposite effect of EGF on the content of estrogen receptors (ER) and, to a lesser degree, on progesterone receptors (PR) was noted [25,60]. The effect may be induction and suppression, depending on the experiment conditions. EGFR activated by its ligand binding affects the ER function as a transcription factor even in the absence of estradiol due to phosphorylation of N-terminal transcription-activating ER fragment (AF-1), a mitogen-activated protein kinase.

In estrogen-resistant breast cancer cells containing no ER (MDA-MB-231, Hs578T, EVSA-T, BT-20, etc.) the production of α-TGF is rather high and is not modified by estrogens or antiestrogens [30,59,78]. The number of EGFR on the membranes of hormone-resistant cells is several times higher than on the membranes of hormone-sensitive cells. Hybridization analysis showed that the increase in the expression of the EGFR gene in hormone-resistant breast cancer cells is not caused by gene amplification. On the other hand, artificial amplification of EGFR gene in hormone-sensitive

cells ZR-75.1 [86] led first to the loss of cell sensitivity to the growth-inhibitory effect of tamoxiphene at high ER level and then to their rapid progress toward the ER-negative phenotype. Addition of exogenous growth factors to the medium and increased expression of endogenous factors leads to a decrease or loss of hormonal sensitivity in cells containing steroid receptors [8,43,60]. On the other hand, ER-positive K3 cells (a variant of MCF-7), which lost estradiol sensitivity, are characterized by a higher production of α-TGF than the initial estrogen-sensitive strain [63]. Therefore, breast cancer cells containing many functionally active EGFR and actively producing the respective ligands need no exogenous hormones to maintain their growth.

Growth factors modify tumor cell proliferation via the autocrine and paracrine pathways. Intratumor fibroblasts play an important role as a source of paracrine growth factors. Culture medium conditioned by fibroblasts isolated from diseased and normal mammary gland stimulates the proliferation of various breast cancer cells [87]. However, the expression of mRNA for insulin-like growth factors I and II and β-TGF, but not for α-TGF or EGF, was demonstrated in the mammary tissue fibroblasts [28]. Hence, the autocrine regulation is the predominant mechanism of the α-TGF/EGFR system operating in breast cancer.

Experimental data point to important role of EGFR in the regulation of breast cancer proliferation and in the formation of cell sensitivity or resistance to hormones.

The clinical significance of EGFR expression and ligands in breast cancer was discussed in many studies on detection of the so-called molecular markers of tumors which helped develop individual approaches to postoperative therapy. Molecular markers will be helpful for the detection of high-risk patients requiring extra treatment or more through clinical and laboratory examination of patients at early stages not treated by adjuvant therapy and assessment of sensitivity to other therapies, for example, endocrine therapy, or development of individual treatment protocols. Preliminary studies of each marker are carried out with these aims in view. We shall stick to such an approach in future, when discussing the role of EGFR and their ligands.

The development of new antitumor drugs aimed at blocking the processes regulated by molecular markers is the most promising approach when the studied marker is directly involved in the regulation of cell proliferation and/or differentiation, as is the case with EGFR [4,5,7,17,83].

Many attempts to evaluate the role of EGFR in breast cancer have been made. However, many factors, among which is the lack of a universal method, have hampered the agreement on the clinical significance of this parameter in different patients.

In 1984 EGFR was detected in bioptates from patients with primary breast cancer [34]. Since then more than 50 research groups at different centers have been involved in this study. More than 9,000 patients have been examined and about 200 reports published. The results reported up to the year 1993 and included in Medline/ERSO computer database are reviewed by J. G. Klijin *et al.* [48,49], and the most interesting of these are discussed in detail in our review [1]. Despite the great number of publications, many aspects in the prognostic significance of EGFR and correlation of their expression with other prognostic factors in breast cancer are still under discussion.

A great variety of methods (radioligand, immunohistochemical, autoradiographic, enzyme immunoassay, and assessment of mRNA expression) and the controversy over the threshold receptor-positive values are one of the main causes of the differences between the available data. In the overwhelming majority of studies EGFR has been detected in 30-50% samples (n=291) from breast cancer patients. Our data obtained by the standard radioligand method [14] indicate that EGFR are expressed in 38% of patients with breast cancer [2,5,42].

Assessment of the relationship between the expression of EGFR and other well-known or actively studied factors of the breast cancer prognosis indicates an inverse relationship between EGFR and one or both steroid receptor types [1,48,49]. Double immunohistochemical staining for EGFR and ER [76] showed that tumor cell populations are heterogeneous and contain cells with three phenotypes: $EGFR^+/ER^-$, $EGFR^-/ER^+$, or $EGFR^-/ER^-$; no cells containing both ER and EGFR were detected. Therefore, the sensitivity and resistance of tumor cells to endocrine therapy depend on the composition of tumor cell population.

$EGFR^+/ER^+$ cells were detected in some cultured breast cancer cells [77] and in normal mammary gland and carcinoma *in situ*. This agrees with a possible correlation between EGFR and ER expression in normal mammary tissue [31]. A hypothesis was put forward about the principal modification of the mechanisms responsible for cooperative effects and interactions between growth factors, steroids, and their receptors in tumor transformation of the mammary epithelium cells [20].

A positive correlation was established between the occurrence of EGFR and the breast cancer malignancy [1,48,49,2,6,41]. Concerming the clinical factors of breast cancer (age and menstrual function of

patients, disease stage judging from the tumor size and the degree of lymph node involvement), only the status of the lymph node can be regarded as a parameter more or less associated with EGFR. One-third of reports dealing with this problem note increased occurrence of EGFR in patients with metastases to the lymph nodes in comparison with patients with early breast cancer. We failed to detect this relationship [2,42].

As for other modern cell markers, the most frequent association with EGFR expression is the modified product of protein p53 suppressor gene and nuclear antigen of proliferating cells *Ki*-67, and a high proliferative index [48,49,65,84,88]. The relationship between EGFR expression and the products of homologous oncogenes *c-erb*B2/HER-2/*neu* is hardly probable [45,48,49,65,80,88].

In 1990, the Department of Clinical Biochemistry of the Oncology Research Center joined a cooperative study of the clinical significance of EGFR and its major ligands EGF and α-TGF in breast cancer. The use of a universal radioligand method has provided the possibility of correct comparison of results. It was expected that clinical application of EGFR and its combinations with steroid receptors will be extended and the strategy used at the Oncology Research Center of the Russian Academy of Medical Sciences will be substantiated.

Statistical analysis of the occurrence and mean levels of EGFR in the membrane fraction of breast tumors in patients with different characteristics showed a higher occurrence of EGFR expression which correlated with the degree of ductal and lobular invasive tumor malignancy [2,6,41]. Analysis of tumor samples from 8 patients with benign mammary diseases and 13 specimens of intact mammary tissue from patients with breast cancer detected EGFR in 50% benign tumors and in 31% intact tissue samples [40]. These results confirm different roles of the EGFR system in the regulation of proliferation of normal and malignant mammary tissue.

In 25 specimens of breast cancer the status of EGFR was comparable to the activity of cathepsin D measured by spectrophotometry by the rate of hemoglobin cleavage [3]. The enzyme activity was significantly higher in EGFR-positive specimens (n=6) than in EGFR-negative specimens (57.2±8.7 and 36.7±4.1 U, respectively, p<0.05), which agrees with immunohistochemical data [19] and the hypothesis that high activity of cathepsin D and EGFR expression are typical of more aggressive breast cancers.

Despite certain controversy over the findings of different research groups, it can be postulated that EGFR expression on breast cancer cells is associated with unfavorable prognostic factors (tumor malignancy, process dissemination, and proliferative activity) and hormone resistance (the absence of the steroid hormone receptors), and it is probable that the presence of EGFR is a sign of unfavorable course of disease.

The results of statistical analysis of relapse-free survival of patients with breast cancer with different tumor EGFR status have been published by 20 research groups (Table 1), ten of which analyzed the relationship between EGFR and total survival. The number of patients in the groups varied from 55 to 459, EGFR was detected in 14-67% tumors, and the maximum duration of follow-up was 12-180 months. Patients differed considerably by the disease stages and postoperative treatment. However, 13 research groups revealed by unifactorial analysis a significant inverse relationship between tumor EGFR and prediction of survival without relapses, and only 5 out of 10 groups detected a similar effect of EGFR status on the general survival of breast cancer patients. The prognostic significance of EGFR was confirmed in several multifactorial studies [38, 39,56,70].

There is no agreement on the predominant prognostic significance of EGFR for different groups of patients. Some researches who studied breast cancer with and without lymph node involvement came to conclusion that EGFR are significant for predicting general and/or relapse-free survival mainly in patients with intact lymph nodes [18,38,45,70,81]. Others [36,71,84] claim that EGFR are prognostically significant only in patients with metastases to the lymph nodes. Similar controversy occurs regarding the patients with ER-positive and ER-negative tumors. There are data on greater significance of EGFR in ER-negative than in ER-positive tumors [45,56,70], and that there is an inverse relationship [36,71]. Some researchers failed to note the significance of EGFR for any specific group of patients with breast cancer, others have reported that the significance of previously studied prognostic factors increases if taken into consideration together with EGFR content in mammary tumors; this regularity is particularly strong for ER [38,39,45]; the lowest survival rate without relapses has been observed in patients with ER^-EGFR^+ tumors and the highest in patients with ER^+EGFR^- tumors.

The role of EGFR in the assessment of the sensitivity of breast cancer patients to endocrine therapy is of special interest. Postmenopausal patients (n=61) were treated by hormones (tamoxiphene) for various causes [66,67]. Twelve patients were operated because of complete failure of tamoxiphene therapy, and EGFR were detected in the tumors of 10 patients. Eight other patients were operated because of progressing tumor after its ini-

TABLE 1. Relationship between EGFR Status and Relapse-Free and Total Survival of Patients with Breast Cancer

Authors	Year	Reference No.	Number of patients	Method*	EGFR, %	Follow-up, months	Correlation with survival	
							without relapses	total
M.A.Rios *et al.*	1988	[71]	179	RL	43	30	None	Not assessed
S.Costa *et al.*	1988	[26]	376	RL	50	12	Negative	Not assessed
M.Grimaux *et al.*	1989	[44]	55	RL	37	92	None	Yes/no
F.Spyratos *et al.*	1990	[81]	109	RL	34	96	None	Not assessed
S.Lewis *et al.*	1990	[56]	90	IHC	14	36	Negative	Not assessed
R.C.Coombes *et al.*	1990	[24]	107	mRNA	55	72	None	None
A.L.Harris *et al.*	1992	[45]	231	RL	35	72	Negative	Negative
R.Sauer *et al.*	1992	[74]	425	IHC	35	41	Negative	Not assessed
H.Iwase *et al.*	1993	[47]	118	IHC	33	Not specified	None	Not assessed
			116	RL	26	Not specified	None	Not assessed
			80	EIA	39	Not specified	Negative	Not assessed
P.G.Koenders *et al.*	1993	[51]	459	RL	22	50	Negative	Negative
R.Zeillinger *et al.*	1993	[91]	326	RL	19	Not specified	None	Not assessed
J.G.Klijn *et al.*	1994	[50]	214	RL	67	180	None	None
M.Toi *et al.*	1994	[84]	115	RL	41	96	Negative	Not assessed
G.Gasparini *et al.*	1994	[39]	164	IHC	56	60	Negative	None
M.Bolla *et al.*	1994	[16]	229	RL	51	34	None	None
R.Castellani *et al.*	1994	[18]	73	IHC	Not specified	52	Negative	Negative
R.I.Nicholson *et al.*	1994	[64]	106	IHC	59	Not specified	Negative	Not assessed
F.Spyratos *et al.*	1994	[82]	319	EIA	35	Not specified	Negative	Negative
S.B.Fox *et al.*	1994	[38]	370	RL	57	18	Negative	None
	1994	[37]	109	RL	57	25	Negative	Negative
M.J.Railo *et al.*	1994	[70]	149	RL	15	50	Negative	Not assessed

Note. *RL: radioligand; IHC: immunohistochemical; EIA: enzyme immunoassay.

tial regression due to tamoxiphene therapy; EGFR were detected in none of the tumors in this group. These groups did not differ by the occurrence of ER.

The sensitivity of relapsing breast cancer to tamoxiphene depends on the content of EGFR in the primary tumor [67]. Objective response to tamoxiphene (tumor regression) was observed in 8.5% patients with EGFR-positive tumors and in 30% patients with tumors containing no EGFR. The difference was more pronounced for ER-positive and ER-negative tumors (37.5 and 5% regressions, respectively). On the other hand, if patients with at least 6-month stabilization of the process were included in the "responsive" group, EGFR turned out to be a more reliable test for the efficacy of treatment than ER. Thus, there is preliminary evidence that the presence of EGFR on breast cancer cells indicates the resistance to tamoxiphene.

R. I. Nicholson *et al.* [64,65] analyzed the results of endocrine therapy in 106 patients with breast cancer relapses in whom EGFR, ER, *Ki*-67 antigen, and *c-erb*B2 gene product were immunohistochemically detected before treatment. The expression of EGFR is significantly related to the loss of sensitivity to endocrine therapy in patients with both ER^- and ER^+ tumors. Further division of patients into groups with different expression of *Ki*-67 antigen increased the accuracy of prediction of sensitivity to treatment, while the presence of protein *c-erb*B2 did not affect hormone sensitivity of the tumor.

Thus, analysis of reports investigating the relationship between EGFR expression and prognosis of relapse-free course of breast cancer and sensitivity of patients to endocrine therapy in general shows that the prognosis is worse and that hormone resistance develops in patients with EGFR-positive tumors; this regularity is confirmed by general statistical analysis. Clinical application of this parameter will be determined by the results of further cooperative studies on large groups of patients comparable by clinical characteristics.

Retrospective statistical analysis of the significance of EGFR for predicting the survival of breast cancer patients without relapses according to the

LIFE-TABLES method included two large stages [6]: at the first stage we assessed the EGFR parameter individually, comparing it with ER and PR, biologically the most close routine biochemical criteria of hormone sensitivity and prediction of breast cancer course; at the second stage we assessed the prognostic significance of combinations of EGFR and steroid hormone receptors and the possible advantages of adding EGFR to the spectrum of studies.

Two hundred and twenty-eight patients with breast cancer were observed for 3-49 months (median 15 months). Thirty-nine patients developed relapses and metastases in various periods after surgery. Six patients died because of disease progress during the follow-up period.

While assessing the significance of EGFR expression for the prediction of survival without relapses for patients with breast cancer in general without consideration for specific features of tumor process and the protocol of postoperative treatment, we failed to detect statistically significant differences in the survival curves for patients with EGFR-positive and EGFR-negative tumors. On the other hand, patients with ER^+ and PR^+ tumors had a better prognosis for the first 4 years of follow-up than patients with tumors containing none of these receptors ($p<0.05$ in both cases). These differences leveled in a longer period of observation, which is in line with some published reports [50].

Analysis of local metastases showed a strong tendency to deterioration of survival without relapses for breast cancer patients without metastases to the lymph nodes with EGFR in the tumor ($p=0.06$). By contrast, for the steroid hormone receptors the prognostic significance was observed only in the group with metastases to the lymph nodes ($p<0.05$).

Thus, analysis of curves representing the survival of breast cancer patients without relapses irrespective of the postoperative treatment showed no significant prognostic value of EGFR, although a pronounced tendency to a worse survival rate without relapses in patients with $EGFR^+$ tumors with intact lymph nodes suggested that EGFR can be used for forming groups at increased risk of the disease relapse in these relatively favorable patients. After singling out a group administered no postoperative treatment in this cohort, we confirmed this hypothesis: a clear-cut tendency to deterioration of survival without relapses in EGFR-positive tumors ($p=0.07$) and negligible opposite tendencies for ER and PR.

Another situation was observed with assessing the significance of EGFR and steroid receptors for predicting the effectiveness of hormone therapy, specifically, for including the endocrine component (as a rule, tamoxiphene) in the protocol of postoperative treatment. PR was the most informative parameter in these cases: the curves of relapse-free survival of patients treated by hormones after the intervention differed by 20-28%, depending on the RP status of their tumors ($p<0.01$). EGFR ranks second: relapse-free survival in the presence of hormone therapy, particularly during the first two years postoperation, was much worse in the patients with EGFR-positive tumors than in those with EGFR-negative tumors, and the difference in the curves approximated the reliability level ($p=0.05$). We should like to emphasize almost equal occurrence of ER and PR in $EGFR^+$ and $EGFR^-$ tumors. Consequently, the above differences in the curves determined by the EGFR status cannot be due to different presentation of the tumors with steroid receptors in the groups compared. We detected no statistically significant differences in the curves demonstrating survival without relapses in relation to the presence of EGFR or steroid receptors in other types of adjuvant therapy (chemo-, radio, and chemoradiotherapy) [6].

There are no published reports about the prognostic significance of EGFR with various schemes of postoperative treatment. The relationship between the effectiveness of endocrine therapy and of the EGFR content was evaluated either by the sensitivity of relapsing cancer in the patients in whom receptors were detected at primary examination [64-66] or by EGFR content in tumors of patients treated by tamoxiphene before surgery [67]. There are no data on the significance and potentialities of clinical application of the combinations of receptor parameters, including steroid hormone receptors and EGFR.

Analysis of these combinations showed significantly worse rate of relapse-free survival in the patients with tumors with receptor composition corresponding mainly to local regulation ($EGFR^+ER^-PR^-$) in comparison with the patients whose tumors were expected to be sensitive mainly to the endocrine factors ($EGFR^-ER^+PR^+$). This difference was the greatest (up to 19%) within 2-3 years after surgery and leveled later. Still more obvious differences in the curve demonstrating survival without relapses were observed in patients administered no treatment after the intervention (Fig. 1, *a*) and in patients treated by hormones (Fig. 1, *b*). In the former case the survival of patients with the "endocrine" receptor phenotype of breast cancer (favorable combination of signs) was at the level of 100% throughout the observation, while the majority of patients with the "auto/paracrine" receptor phenotype (unfavorable combination of signs) had relapses or metastases, the survival median being 26 months.

The differences are not so great in patients treated by hormones, although half of the patients with

unfavorable combinations of receptor parameters developed relapses and metastases during the second year postoperation, while almost 90% patients with favorable combinations of signs lived without disease relapse during the entire observation period. However, it seems that the key factor for the favorable prognosis of endocrine therapy is not the absence of EGFR but the presence of both types of steroid hormone receptors, because patients with $EGFR^+$ ER^+PR^+ tumors had 100% survival without relapses in the presence of hormone therapy.

In patients administered no postoperative treatment, EGFR seem to be the decisive factor of prognosis: there were no significant differences in the relapse-free survival curves of patients with ER^+PR^+ and ER^-PR^- tumors administered no specific therapy. In general, survival without relapses in patients administered no therapy after radical treatment of breast cancer with different receptor status of tumors deteriorates in the following order: $EGFR^-ER^+PR^+ > EGFR^+ER^+PR^+ > EGFR^-ER^-PR^- > EGFR^+ER^-PR^-$, but the difference is significant only for the first and the last combinations.

Thus, simultaneous account of the status of EGFR, ER, and PR in breast cancer more reliably than assessment of EGFR alone or steroid hormone receptors without EGFR helps distinguish at the early stages a group with the most probable favorable prognosis ($EGFR^-ER^+PR^+$ tumors) among the patients not liable to specific therapy, to whom common check-ups should be recommended, and a group at the highest risk of tumor relapse ($EGFR^+$ ER^- PR^-) in need of thorough follow-up and, probably, adjuvant therapy without the endocrine component.

Statistical analysis of the value of complex detection of EGFR, ER, and PR in breast tumors helped us devise the following simplified scheme for evaluating the results of detection for choosing the strategy of postoperative treatment (Fig. 2).

1. At early stages special attention should be paid to patients with EGFR-positive tumors without steroid hormone receptors, among all patients whose treatment can be confined to surgery. Tumors with such a receptor phenotype are characterized by an extremely unfavorable course, and the patients are in need of thorough check-ups and/or adjuvant chemotherapy. Patients with $EGFR^-ER^+PR^+$ tumors at the early stages may not need postoperative treatment.

2. The highest efficacy of postoperative endocrine therapy may be expected in patients with tumors of the $EGFR^-ER^+PR^+$ or $EGFR^-PR^+$ phenotype. Addition of endocrine preparations to postoperative protocols of such patients is recommended. If the combination of receptors is dubious, RP status is the most significant for evaluating hormone sensitivity.

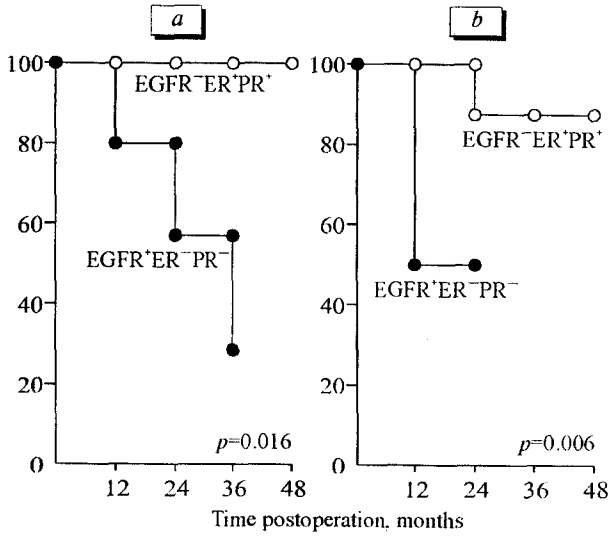

Fig. 1. Relapse-free survival of breast cancer patients receiving no postoperative treatment (*a*) and treated after surgery by hormones or by hormones and chemotherapy (*b*) and the content of epidermal growth factor receptors (EGFR) and steroid hormone receptors in the tumors. Ordinate: survival without relapses, %.

For patients with an opposite combination of receptors intensive chemotherapy or chemoradiotherapy can be recommended.

We tried to extend the spectrum of parameters of breast cancer regulation by measuring EGFR ligands in tumor tissue: EGF and α-TGF [2]. The fact that receptor expression and the presence of ligands activating the receptors in sufficiently high concentrations are obligatory conditions for effective function of the auto-paracrine mechanism prompted us such an investigation. Growth factors were measured by specific radioimmunoassays in ethanol-ethylacetate extracts of tumor cytosols of 108 primary patients with breast cancer [2].

The reports about the clinical significance of EGF and α-TGF expression are scarce and contradictory; the studies were carried out mainly by immunohistochemical methods [18,85]; mRNA expression was evaluated [24], and EGF-like activity was detected by the radioreceptor method [35], i.e., virtually total concentration of both EGFR ligands. No results of separate radioimmunoassays of EGF and/or α-TGF in breast tumor extracts are reported up to date.

Investigation of tissue fragments by biochemical methods does not allow the identification of cell origin of detected proteins. By specific radioimmunoassays of cytosol extracts we measured EGF or α-TGF which could be autocrine (produced by tumor cells) and paracrine (produced by stromal cells) regulators toward breast cancer cells.

Analysis of the results of EGF and α-TGF detection in breast cancer cytosol extracts showed their

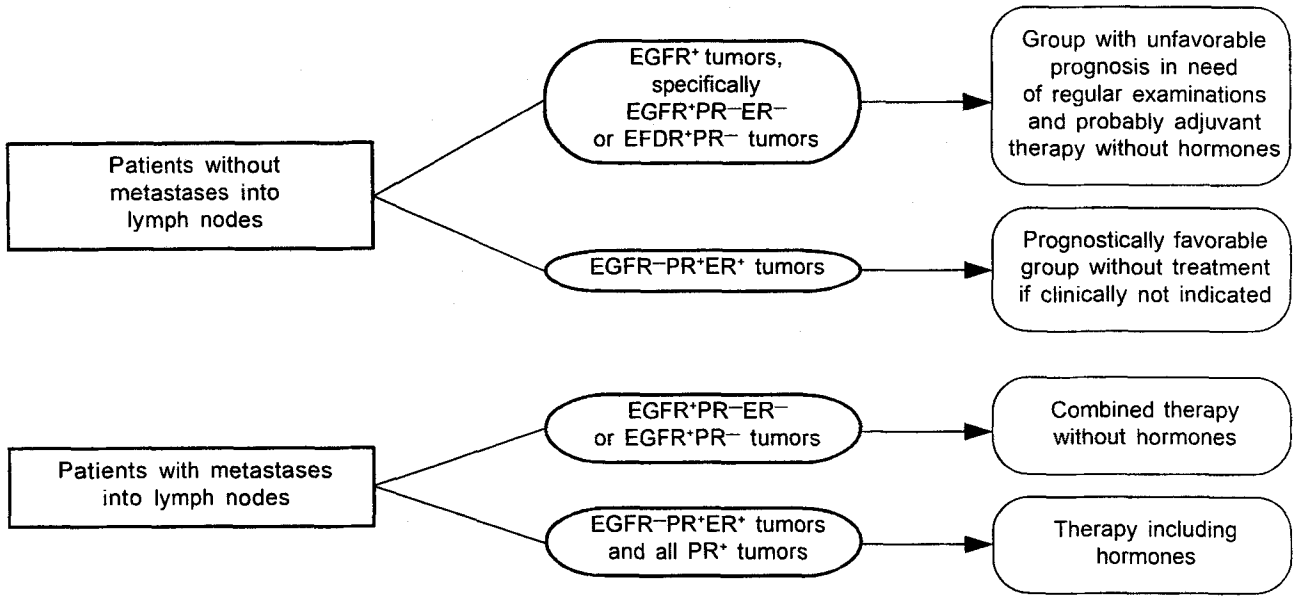

Fig. 2. Use of the results of simultaneous detection of epidermal growth receptors (EGFR), estrogen and progesterone receptors (ER and PR) for choosing postoperative treatment of patients with breast cancer.

much higher occurrence than that of EGFR: immunoreactive EGF was detected in 69% tumors and α-TGF in 76% tumors. Only 8% tumors contained none of EGFR ligands [2]. Their incidence and mean concentrations did not significantly depend on any common clinical morphological prognostic factor or steroid receptor and EGFR status. Only 2% of all tumors (6% of the total number of EGFR-positive samples) had receptors but no ligands. Such a ratio between the expression of EGFR and their ligands suggests that the production of growth factors by tumor or adjacent cells is not the limiting component of autoparacrine regulation of breast cancer cell proliferation, in which EGFR takes part. The presence of functionally active receptors may determine the tumor ability to autoregulated growth.

This hypothesis was indirectly confirmed by analysis of the curves of relapse-free survival of breast cancer patients in relation to the expression of EGF and/or α-TGF and their combination with the expression of EGFR and steroid hormone receptors. The only significant difference was detected in the total group of patients with ER^+ tumors in which the expression of α-TGF significantly improved the survival without relapses, i.e., its effect was opposite to that of EGFR.

Thus, analysis of published data and our findings on the prognostic significance of EGF and α-TGF expression in malignant breast tumors indicate that the expression of ligands is less significant than the expression of receptors. EGFR may occupy special place in the examination of breast cancer patients as an indicator that helps detect patients at a high risk of disease relapse at the early stages of disease. The ligand binding is the first stage in the realization of the EGFR regulatory effect; more information on the clinical significance of this auto-paracrine system in breast cancer can be obtained in studies of the activity of some EGFR-regulated processes in clinical material; specifically, phospholipid metabolism and receptor autophosphorylation rates, expression and activity of EGFR-regulated protein kinases.

REFERENCES

1. E. S. Gershtein, L. S. Bassalyk, and V. P. Letyagin, *Vopr. Onkol.*, **40**, Nos. 7-12, 266-272 (1994).
2. E. S. Gershtein, V. D. Ermilova, Z. V. Kuz'mina, *et al.*, *Vestn. Rossiisk. Akad. Med. Nauk.*, No. 3, 15-19 (1996).
3. E. S. Gershtein, E. A. Kozyreva, Z. V. Kuz'mina, *et al.*, *Vestn. Onkol. Nauch. Tsentra Rossisk. Akad. Med. Nauk.*, No. 1, 22-26 (1994).
4. E. S. Gershtein, O. I. Kostyleva, and N. E. Kushlinskii, *Ibid.*, No. 2, 51-59 (1995).
5. N. E. Kushlinskii and E. S. Gershtein, *Klin. Lab. Diagn.*, No. 1, 9-12 (1996).
6. N. E. Kushlinskii and E. S. Gershtein, *Mammologiya*, No. 3, 15-25 (1996).
7. N. E. Kushlinskii and E. S. Gershtein, *Eksp. Klin. Farmakol.*, No. 1, 74-80 (1996).
8. N. E. Kushlinskii, E. S. Gershtein, V. A. Shatskaya, *et al.*, *Vestn. Onkol. Nauch. Tsentra Rossisk. Akad. Med. Nauk.*, No. 1, 17-22 (1994).
9. S. R. Ahmed, B. Badger, C. Wright, *et al.*, *J. Steroid Biochem. Mol. Biol.*, **38**, 687-693 (1991).
10. D. K. Armstrong, S. H. Kaufmann, Y. L. Ottaviano, *et al.*, *Cancer Res.*, **54**, 5280-5283 (1994).
11. C. L. Arteaga and C. K. Osborne, *Cancer Treat. Res.*, **53**, 289-304 (1991).

12. J. A. Barnard, D. R. Graves, M. R. Pittelkow, *et al.*, *J. Biol. Chem.*, **269**, 22827-22822 (1994).
13. S. E. Bates, N. E. Davidson, E. M. Valverius, *et al.*, *Mol. Endocrinol.*, **2**, 543-555 (1988).
14. T. J. Benraad and J. A. Foekens, *Ann. Clin. Biochem.*, **27**, 272-273 (1990).
15. Y. Berthois, X. F. Dong, and P. M. Martin, *Biochem. Biophys. Res. Commun.*, **159**, 126-131 (1989).
16. M. Bolla, M. Chedin, M. Colonna, *et al.*, *Breast Cancer Res. Treat.*, **29**, 265-270 (1994).
17. A. W. Burgess and C. M. Thumwood, *Pathology*, **26**, 453-463 (1994).
18. R. Castellani, D. W. Visscher, S. Wykes, *et al.*, *Cancer*, **73**, 344-349 (1994).
19. C. Charpin, B. Devictor, P. Bonnier, *et al.*, *Breast Cancer Res. Treat.*, **25**, 203-210 (1993).
20. S. A. Chrysogelos and R. B. Dickson, *Ibid.*, **29**, 29-40 (1994).
21. F. Ciardiello, N. Kim, M. L. McGeady, *et al.*, *Ann. Oncol.*, **2**, 169-182 (1991).
22. F. Ciardiello, M. L. McGeady, N. Kim, *et al.*, *Cell Growth Differ.*, **1**, 407-420 (1990).
23. A. Ciccodicolo, R. Dono, S. Obici, *et al.*, *EMBO J.*, **8**, 1987-1991 (1989).
24. R. C. Coombes, P. Barrett-Lee, and Y. Luqmani, *J. Steroid Biochem. Mol. Biol.*, **37**, 833-837 (1990).
25. E. M. Cormier, M. F. Wolf, and V. C. Jordan, *Cancer Res.*, **49**, 576-580 (1989).
26. S. Costa, H. Stamm, A. Almendral, *et al.*, *Lancet*, **2**, 1258-1260 (1988).
27. P. de Cremoux, C. Gauville, and V. Closson, *Int. J. Cancer*, **59**, 408-415 (1994).
28. K. J. Cullen, H. S. Smith, S. Hill, *et al.*, *Cancer Res.*, **51**, 4978-4985 (1991).
29. R. J. Daly, M. D. Binder, and R. L. Sutherland, *Oncogene*, **9**, 2723-2727 (1994).
30. N. E. Davidson, M. E. Gelmann, M. E. Lippman, *et al.*, *Mol. Endocrinol.*, **3**, 372-380 (1989).
31. R. Dittadi, P. M. Donisi, A. Brazzale, *et al.*, *Brit. J. Cancer*, **67**, 7-9 (1993).
32. W. C. Dougall, X. Qian, N. C. Peterson, *et al.*, *Oncogene*, **9**, 2109-2123 (1994).
33. B. W. Ennis, E. M. Valverius, S. E. Bates, *et al.*, *Mol. Endocrinol.*, **3**, 1830-1838 (1989).
34. S. L. Fitzpatrick, J. Brightwell, and J. L. Wittliff, *Cancer Res.*, **44**, 3448-3453 (1984).
35. J. A. Foekens, H. Portengen, M. Jansen, *et al.*, *Cancer*, **63**, 2139-2147 (1989).
36. J. A. Foekens, H. Portengen, W. L. J. van Putten, *et al.*, *Cancer Res.*, **49**, 7002-7009 (1989).
37. S. B. Fox, R. D. Leek, K. Smith, *et al.*, *Breast Cancer Res. Treat.*, **29**, 109-116 (1994).
38. S. B. Fox, K. Smith, J. Hollyer, *et al.*, *Ibid.*, 41-49.
38. G. Gasparini, P. Boracchi, P. Bevilacqua, *et al.*, *Ibid.*, 59-61.
40. E. Gershtein, N. Kushlinsky, V. Ermilova, *et al.*, *Scand. J. Clin. Lab. Invest.* Suppl., **55**, No. 233, 406 (1995).
41. E. S. Gershtein V. D.Ermilova, Z. V. Kuzmina, *et al.*, *Int. J. Oncol.*, **5**, Suppl., 370 (1994).
42. E. S. Gershtein, R. E. Leake, N. E. Kushlinsky, *et al.*, *Eur. J. Cancer*, **30A**, Suppl. 2, S43 (1994).
43. E. S. Geshtein, V. A. Shatskaya, O. I. Kostyleva, *et al.*, *Cancer*, **76**, 2524-2529 (1995).
44. M. Grimaux, S. Romain, Y. Remvikos, *et al.*, *Breast Cancer Res. Treat.*, **14**, 77-90 (1989).
45. A. L. Harris, S. Nicholson, R. Sainsbury, *et al.*, *J. Natl. Cancer Inst. Monogr.*, **11**, 181-187 (1992).
46. J. J. Hsuan, *Anticancer Res.*, **13**, 2521-2532 (1993).
47. H. Iwase, S. Kobayashi, Y. Itoh, *et al.*, *Breast Cancer Res. Treat.*, **28**, 215-221 (1993).
48. J. G. Klijn, P. M. Berns, P. I. Schmitz, *et al.*, *Endocr. Rev.*, **14**, 3-6 (1993).
49. J. G. Klijn, P. M. Berns, P. I. Schmitz, *et al.*, *Ibid.*, **13**, 3-17 (1992).
50. J. G. Klijn, M. P. Look, H. Portengen, *et al.*, *Breast Cancer Res. Treat.*, **29**, 73-83 (1994).
51. P. G. Koenders, L. V. Beex, C. B. Kienhuis, *et al.*, *Ibid.*, **25**, 21-27 (1993).
52. R. D. Leek, L. Kaklamanis, F. Pezzella, *et al.*, *Brit. J. Cancer.*, **69**, 135-139 (1994).
53. S. LeJeune, R. Leek, E. Horak, *et al.*, *Cancer Res.*, **53**, 3597-3602 (1993).
54. M. A. Lemmon and J. Schlessinger, *Trends Biochem. Sci.*, **19**, 459-463 (1994).
55. B. S. Leung, L. Stout, L. Zhou, *et al.*, *J. Cell. Biochem.*, **46**, 125-133 (1991).
56. S. Lewis, A. Locker, J. H. Todd, *et al.*, *J. Clin. Pathol.*, **43**, 358-390 (1990).
57. D. K. Lutrell, A. Lee, T. J. Lansing, *et al.*, *Proc. Natl. Acad. Sci. USA*, **91**, 83-87 (1994).
58. M. Martinazzi, F. Crivelli, C. Zampatti, *et al.*, *Pathologica*, **85**, 637-644 (1993).
59. H. Mueller, P. Loop, K. Wosikowski, *et al.*, *Eur. J. Biochem.*, **22**, 631-637 (1994).
60. L. C. Murphy and H. Dotzlaw, *Cancer Res.*, **49**, 599-604 (1989).
61. A. Nesterov, R. C. Kurten, and G. N. Gill, *J. Biol. Chem.*, **270**, 6320-6327 (1995).
62. A. Nesterov, S. Lysan, I. Vdovina, *et al.*, *Arch. Biochem. Biophys.*, **313**, 351-359 (1994).
63. R. I. Nicholson, J. M. W. Gee, A. B. Francis, *et al.*, *Endocrine-Related Cancer*, **2**, 115-121 (1995).
64. R. I. Nicholson, R. A. McClelland, J. M. Gee, *et al.*, *Breast Cancer Res. Treat.*, **29**, 117-125 (1994).
65. R. I. Nicholson, R. A. McClelland, P. Finlay, *et al.*, *Eur. J. Cancer*, **29A**, 1018-1023 (1993).
66. R. I. Nicholson, P. Halcrow, J. R. Sainsbury, *et al.*, *Brit. J. Cancer*, **58**, 810-814 (1988).
67. R. I. Nicholson, J. R. Sainsbury, P. Halcrow, *et al.*, *Lancet*, **1**, No. 8631, 182-185 (1989).
68. N. Normanno, F. Ciardiello, R. Brandt, *et al.*, *Breast Cancer Res. Treat.*, **29**, 11-27 (1994).
69. L. K. Opresko, C. Chang, B. H. Will, *et al.*, *J. Biol. Chem.*, **270**, 4325-4333 (1995).
70. M. J. Railo, K. V. Smitten, and F. Pekonen, *Acta Oncol.*, **33**, 13-17 (1994).
71. M. A. Rios, A. Macias, R. Perez, *et al.*, *Anticancer Res.*, **8**, 173-176 (1988).
72. T. Saeki, A. Cristiano, M. J. Lynch, *et al.*, *Mol. Endocrinol.*, **5**, 1955-1963 (1991).
73. D. S. Salomon, W. R. Kidwell, N. Kim, *et al.*, *Recent Results Cancer Res.*, **113**, 57-69 (1989).
74. R. Sauer, A. Schauer, H. F. Rauschecker, *et al.*, *Strahlenther. Onkol.*, **168**, 191-202 (1992).
75. J. Schlessinger, *Biochemistry*, **27**, 3119-3123 (1988).
76. A. K. Sharma, K. Horgan, A. Douglas-Jones, *et al.*, *Brit. J. Cancer*, **69**, 1032-1037 (1994).
77. A. K. Sharma, K. Horgan, R. A. McClelland, *et al.*, *Histochem. J.*, **26**, 306-310 (1994).
78. M. S. Sheikh, Z. M. Shao, J. C. Chen, *et al.*, *J. Cell. Biochem.*, **54**, 289-298 (1994).
79. E. Skasko, Z. Paszko, B. Bielinska, *et al.*, *Tumori*, **80**, 56-60 (1994).
80. R. Spaventi, E. Kamenjicki, N. Pecina, *et al.*, *In Vivo*, **8**, 183-189 (1994).
81. F. Spyratos, J. -C. Delarue, C. Andrieu, *et al.*, *Breast Cancer Res. Treat.*, **17**, 83-88 (1990).
82. F. Spyratos, P. M. Martin, K. Hacene, *et al.*, *Ibid.*, **29**, 85-95 (1994).
83. R. K. Sweeb and J. H. Beinjen, *Pharm. World. Sci.*, **15**, 233-242 (1993).

84. M. Toi, T. Tominaga, A. Osaki, *et al.*, *Breast Cancer Res. Treat.*, **29**, 51-58 (1994).
85. Y. Umekita, N. Enokizono, Y. Sagara, *et al.*, *Virchows Arch.*, **420**, 345-351 (1992).
86. T. van Agthoven, Th. L. van Agthoven, H. Portengen, *et al.*, *Cancer Res.*, **52**, 5082-5088 (1992).
87. C. P. van Roozendaal, B. van Ooijen, J. G. M. Klijn, *et al.*, *Brit. J. Cancer.*, **65**, 77-81 (1992).
88. D. W. Vischer, R. Castellani, S. M. Wykes, *et al.*, *Breast Cancer Res. Treat.*, **28**, 261-266 (1993).
89. R. A. Walker and J. M. Varley, *Cancer Surv.*, **16**, 31-57 (1993).
90. K. Wosikowski, W. Kung, M. Hasmann, *et al.*, *Int. J. Cancer*, **53**, 290-297 (1993).
91. R. Zeillinger, F. Kury, P. Speiser, *et al.*, *Clin. Biochem.*, **26**, 221-227 (1993).