ONCOLOGY

A New Hypothesis on the Role of c-erbB2 Oncogene in the Progress of Breast Cancer

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Proliferation and the state of adhesion molecules (E-cadherin, galectin-3) and estrogen and progesterone receptors were immunohistochemically analyzed in breast cancer biopsy specimens under conditions of c-erbB2 overexpression with and without gene amplification. It was hypothesized that c-erbB2 overexpression without gene amplification led to suppression of proliferation and "conservation" of tumor cell.

Key Words: *breast cancer; c-erbB2; proliferation; immunohistochemistry*

Participation of c-erbB2 oncogene in the pathogenesis of breast cancer (BC) is amply studied. It was demonstrated that c-erbB2 overexpression is associated with intensive proliferation and aggressive development of BC [2].

We evaluated the role of c-erbB2 oncogene in the progress of BC by immunohistochemical (IHC) methods.

MATERIALS AND METHODS

A total of 414 cases of BC were analyzed by IHC methods. The expression of c-erbB2 was evaluated by Hercept-Test (DakoCytomation). Amplification of c-erbB2 was evaluated by FISH reaction. Proliferation was estimated using Leica CTR5000 morphometric station and Leica Qwin Plus software by IHC staining of products of Ki-67 (336 cases) and PCNA (78 cases) gene expression. The expression of progesterone and estrogen receptors, p53 protein, E-cadherin, galectin-3 was evaluated in cases with c-erbB2 gene overexpression (Novocastra and Pharmingen antibodies).

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RESULTS

Positive reaction to c-erbB2 was detected in 24% cases. Of these, intensive proliferation was detected in 31% cases, moderate in 23%, and low in 46% cases. Thus, proliferation of tumor cells was not high in the majority of cases (Table 1). We hypothesized that suppressor function of p53 under conditions of c-erbB2 overexpression was not realized.

Cells immunohistochemically positive for c-erbB2 ("++" without amplification) exhibited low E-cadherin expression and were positive for galectin-3. However, proliferation in these cases was also very low (the content of Ki-67$^+$ cells <5%). In cases with c-erbB2 overexpression with amplification some cells were also E-cadherin-negative, galectin-3-positive, and exhibited a low proliferation level.

E-cadherin inhibits cell proliferation as a result of suppression of mitogen-activating cascade with epidermal growth factor. The suppressor function of E-cadherin is realized due to pRb hypophosphorylation, decreased level of D1-cyclin, and increased expression of p27 [11]. Intracellular domain of E-cadherin is bound to β-catenin, through which E-cadherin interaction with actin cytoskeleton is realized [1]. The loss of E-cadherin by cells

also leads to sequestration of β-catenin into the cytoplasm. In the cytoplasm β-catenin binds to Tcf4, which leads to activation of D1-cyclin and Myc-kinase [12]. The interaction of galectin-3 with stromal elastin should induce proliferation of BC cells. Thus, cells lacking E-cadherin and expressing galectin-3 should demonstrate high proliferation level and intensive IHC reaction to Ki-67. However, we observed an opposite picture: negative reaction to Ki-67. This phenomenon was detected in tumors with high expression of c-erbB2 (without gene amplification). Moreover, the level of proliferation was low in 69% BC cases with c-erbB2 overexpression (Fig. 1).

One of the mechanisms of c-erbB2 overexpression in malignant cells is similar to the physiological pathway [6]. The interaction of progesterone receptor with its ligand (progesterone) leads to an increase in c-erbB2 expression. This cell starts interacting with epidermal growth factor, which leads to disappearance of progesterone receptors. The cell continues interacting with progesterone, as a result of which c-erbB2 expression increases [6]. This is in line with negative reaction of progesterone receptors under conditions of c-erbB2 overexpression and positive reaction of c-erbB2 (++) in histologically normal mammary gland cells adjacent to the tumor. In some cases IHC reaction to c-erbB2 was weaker in positively stained (++) peripheral zone of *in situ* cancer and sharply reduced in the invasive component.

Increased expression of c-erbB2 leads to reduction of E-cadherin and α_2-integrin levels, suppression of focal adhesion kinase (FAK) physically bound to integrins. The absence of FAK activity arrests the kinase cascade starting from c-erbB2 heterodimers (manifesting under conditions of overexpression) at the intermediate Mek1 MAP-kinase, which does not phosphorylate its targets Erk1/2 [4] (Fig. 1).

Overexpression of c-erbB2 also increases expression of p21 (waf1/cip1) [14]. This interaction is well known, but, we think, is neglected by the majority of authors. Under conditions of c-erbB2 overexpression p21 is simultaneously detected in the cytoplasm and nucleus. Some scientists believe that under conditions of c-erbB2 overexpression p21 is predominantly located in the cytoplasm, and as the function of p21 is realized in the nucleus, it means that p21 does not work under conditions of c-erbB2 overexpression [13]. Overexpression of c-erbB2 increases the level of p21, which inhibits cdc2 and leads to taxol-induced apoptosis [15]. Taxol targets are the microtubules; the presence of taxol leads to the formation of abnormally stable and non-functioning microtubules [9]. The main

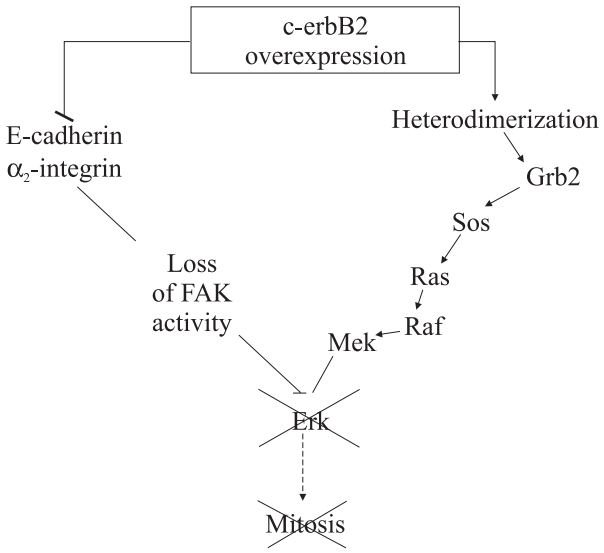

Fig. 1. Arrest of kinase cascade starting from c-erbB2 heterodimers as a result of loss of α_2-integrins by the cell and inactivation of FAK.

function of microtubules in the cell cycle is realized during mitosis prometaphase, when M-checkpoint inhibits mitosis until all microtubules attach to the kinetochores [10]. The main target of p21 is cdc2-cyclin B complex inhibited by p21, which is paralleled by the arrest of cell progression from G2 phase into mitosis [3]. Hence, the cell does not reach the mitotic prometaphase and taxol cannot induce M-checkpoint. Moreover, cyclin-dependent kinase inhibitors (p21) can be sequestrated from the nucleus into the cytoplasm only in cdc-cyclin complex [7]. This means that only a higher level of cdc-cyclin complex can cause blocking of p21 function. However, high level of cdc-cyclin complex is expected to induce proliferation, while in our studies it was low. These two above phenomena suggest that p21 function is realized under conditions of c-erbB2 overexpression (Fig. 2).

All this suggests that c-erbB2 overexpression without gene amplification leads to suppression of cell proliferation. This hypothesis was confirmed in previous studies carried out by radioimmunochemical methods. High level of c-erbB2 was detected by these methods in 85% BC cases, very high level in 25% of these, which was due to gene amplification.

TABLE 1. Distribution of BC Cases by p53 Status and Proliferation Level

Proliferation level	High	Moderate	Low
$p53^+$	8	4	9
$p53^-$	15	8	18

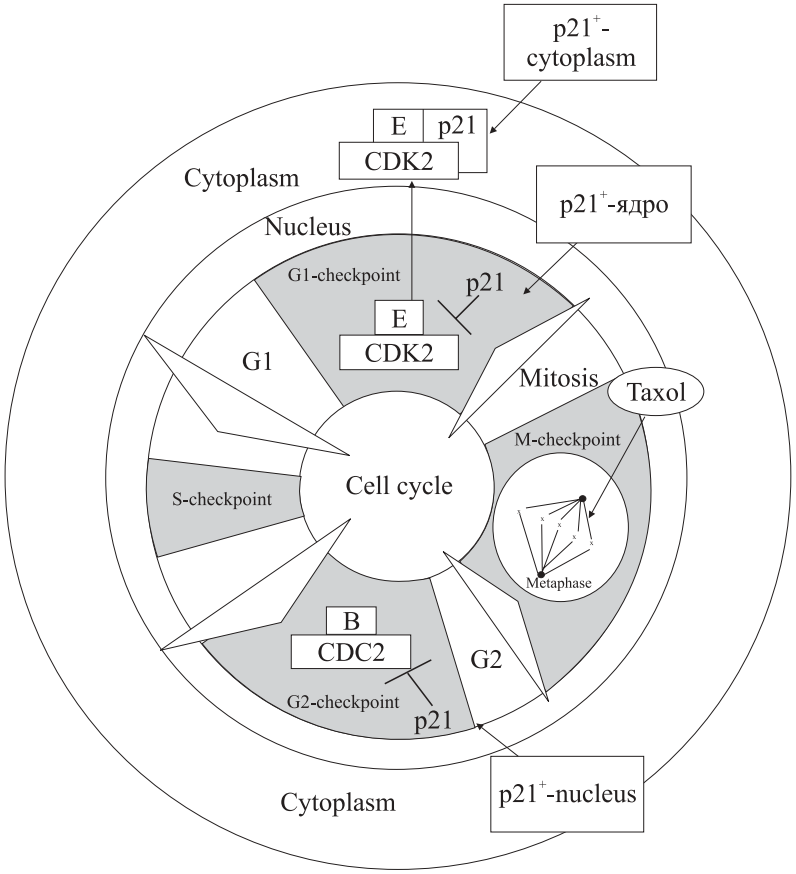

Fig. 2. Functioning of p21 (waf1, cip1) under conditions of c-erbB2 overexpression.

No amplification was detected in the rest 60% tumors, but c-erbB2 protein overexpression was by one order of magnitude lower than in cells with gene amplification. No c-erbB2 was detected in 15% tumors. Clinical studies showed that the prognosis was the worst for tumors with c-erbB2 gene amplification and tumors without c-erbB2 protein in comparison with 60% tumors with only overexpression of c-erbB2 oncogene [8].

However, overexpression of c-erbB2 also leads to activation of PI3K/Akt route, which leads to apoptosis suppression [2].

Amplification of c-erbB2 often causes co-amplification of the adjacent gene groups: topoisomerase-2-α (TI-2-α), retinoic acid receptor, v-erbA/thyroid hormone receptor, and MLNs 50, 51, 62, 64. Combined TI-2-α amplification is observed in 50% BC tumors with c-erbB2 amplification. As a result, the content of TI-2-α increases many-fold, this leading to intensification of proliferation. In addition, the content of c-erbB2 protein on the surface of cancer cell increases significantly as a result of c-erbB2 amplification. This creates prerequisites for the formation of c-erbB2 homodimers activating Src-kinase irrespective of FAK [2], thus overcoming the block of intact MEK1; these processes also activate cell proliferation. However, TI-2-α gene is deleted in 40% BC tumors with c-erbB2 amplification, which leads to reduction of TI-2-α level [5]. This phenomenon explains low cell proliferation in some tumors with c-erbB2 amplification and negative reaction to E-cadherin. It is obvious that only coarse gene disorder (TI-2-α deletion) can arrest potent activation cascade of c-erbB2 homodimers when contact inhibition is completely absent. We think that competition between the activation cascade of c-erbB2 homodimers and the decrease in TI-2-α level resulting from TI-2-α gene deletion develop in these tumors. This phenomenon deserves further study.

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