Influence of stroma-derived growth factors on the estradiol-stimulated proliferation of human breast cancer cells

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Summary

Purpose of investigation: Estradiol is the main proliferating substance for human epithelial breast cells. There is evidence that proliferation is also stimulated by growth factors mainly synthesized by the surrounding stroma. In the present in vitro study we have compared the proliferating activity of three growth factors alone and in combination with estradiol in human breast cancer cells.

Method: As a cell model the well-recognized human breast cancer cell line MCF-7 was used. The growth factors tested were basic fibroblast growth factor (bFGF), epithelial growth factor (EGF) and insulin-growth factor-1 (IGF-1) at the concentrations 10^{-10}, 10^{-9} and 10^{-8} M alone and in equimolar combination with 10^{-10} M estradiol. Cell proliferation was measured after seven days incubation by the ATP-assay.

Results: The growth factors alone significantly stimulated proliferation of MCF-7 cells in the tested concentration range. The increases were about 40% for EGF, between 40 and 60% for FGF and between 30 and 90 % for IGF-I. The strongest proliferating factor was estradiol alone with values between 70 and 130%. For all three growth factors an additive effect was seen in combination with estradiol, which was greatest for IGF-I.

Conclusion: These data indicate that estradiol is the strongest proliferating factor for human breast cancer cells. Therefore the predictive value of cell models using estradiol as a proliferative agent seems to be highly reliable. However, stromal influence by growth factors should not be disregarded, since proliferation of pre-existing malignant cells may be accelerated by the concomitant presence of estrogens and growth factors.

Key words: Estradiol; Growth factors; Proliferation; Human breast cancer cells.

Introduction

Human breast cancer cell lines such as MCF-7, derived from pleural effusion, are a well-recognized model for evaluating factors and/or mechanisms that modify breast cancer development [1-4]. However, these cell models often are under scrutiny since they are considered to neglect stromal/epithelial interactions which seem to be important for the in vivo situation [5].

Discussion is ongoing in as far as malignant breast cells are dependent on the influence of these growth factors in a paracrine or autocrine fashion or whether estradiol alone is the main stimulating factor. The most important stimulating growth factors seem to be epidermal growth factor (EGF), fibroblastic growth factor (FGF) and insulin like-growth factors (IGF) I and II [6].

In the present study we investigated the effect of EGF, FGF and IGF-I on the proliferation rate of the receptor-positive breast cancer cell line MCF-7 alone and in equimolar combinations with estradiol.

Methods

MCF-7, a human estrogen and progesterone receptor-positive breast cancer cell line, was purchased from European Collection of Cell Cultures (ECACC), UK. The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 5% (v/v) fetal calf serum supplemented with 0.3 mg/ml glutamine, 5 ng/ml bovine insulin and 100 U/ml penicillin plus 100 µg/ml streptomycin.

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Ninety-six well plates were seeded with approximately 1,000 cells per well in an assay kit medium. Subsequently, the cells were incubated for three days with phenol-red free medium containing charcoal/dextran treated serum. Subsequently the test substances were added and incubated for seven days. Cell proliferation was measured after seven days incubation by the ATP-assay. In brief, proliferation is quantified by measuring light which is emitted during the bioluminescence reaction of luciferine in the presence of ATP and luciferase.

Statistical analysis of the logarithmated values which followed normal distribution was first done by ANOVA and then by the Student's t-test of triplicates from two different experiments.

Results

In Figure 1 the effect of the various growth factors in comparison to estradiol at the concentrations 10^{-10}, 10^{-9} and 10^{-8} M is depicted. All growth factors significantly stimulated proliferation of MCF-7 cells in the tested concentration range. The increases were about 40% for EGF, between 40 and 60% for FGF and between 30 and 90% for IGF-I. The strongest proliferating factor was estradiol with values between 70 and 130%.

In Figure 2 the results are given for an equimolar combination of each growth factor with estradiol at a con-

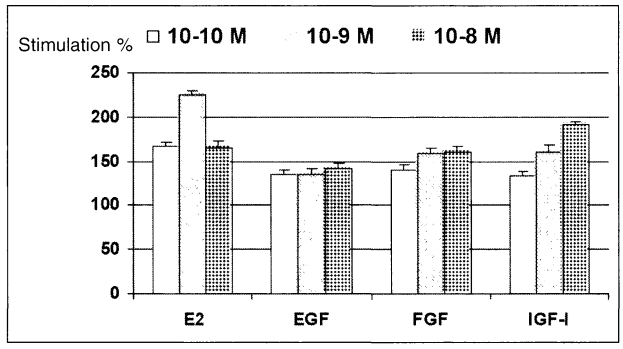

Figure 1. — Percentual increase of proliferation of MCF-7 cells after addition of various concentrations of estradiol (E2), epidermal growth factor (EGF), fibroblast growth factor (FGF) and insulin-like growth factor I (IGF-I) (means ± SD, n = 6).

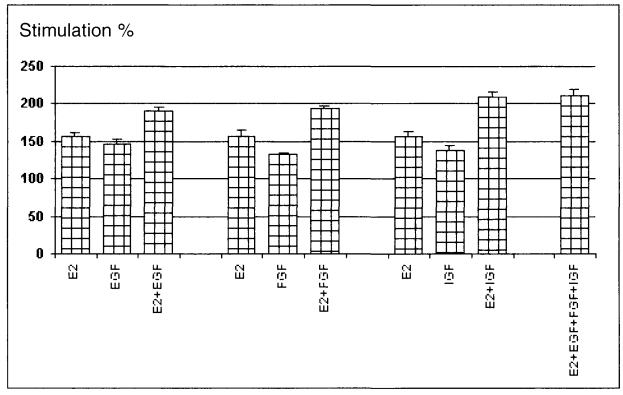

Figure 2. — Percentual increase of proliferation of MCF-7 cells after addition of an equimolar combination of estradiol (E2) with epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin-like growth factor I (IGF-I) and a combination of all test substances (means ± SD, n = 6).

centration of 10^{-10} M. In addition the effect of the mixture of all three growth factors with estradiol is illustrated. For each growth factor combined with E2 an additive effect was seen, which was greatest for IGF-I. The increases were in the range of 30 to 50% as compared to the effect of E2 alone. The mixture of all four substances elicited a proliferation rate which was comparable to the effect of E2 combined with IGF-I.

Discussion

The mammary gland comprises stromal and epithelial cells that communicate with each other through the extracellular matrix [7]. Breast cancer manifests itself in the epithelium, however, evidence is growing that the stroma may be important for cancerogenesis. The mammary stroma consists of multiple components: adipocytes, preadipocytes, fibroblasts, blood vessels, inflammatory cells, and extra cellular matrix, each subject to regulation throughout the developmental cycle [7].

There are multiple pathways, demonstrated and postulated, through which stromal/epithelial interaction can occur. Growth factors can be synthesized by the stroma and interact only on the epithelium. The contrary may also be true. Moreover, growth factors may be produced by both the epithelium and stroma and may affect both compartments.

As yet the understanding of hormone/growth factor interaction is far from being complete. This is of special interest with respect to tumor progression since it has been demonstrated that intensive changes in growth factor synthesis and/or receptor expression is observed in human breast cancer development [8].

However, our data indicate that estradiol seems to be the strongest proliferating factor at the physiological concentration range at least for receptor-positive human breast cancer cells. A biphasic reaction of E2 was found with a significant higher proliferation rate at 10^{-9} M as compared to 10^{-10} and 10^{-8} M. This pattern has already been observed in our previous work [3].

A significant increase in the estradiol-induced proliferation rate was found for the combination of estradiol with the growth factors EGF, FGF and IGF-I, the values being about 30 to 50%. The result of the combination of all growth factors with E2 revealed that IGF-I seems to have the greatest influence on the E2-induced proliferation, since no further increase was observed as compared to the combination of E2 with IGF-I. In as far as such combination may increase the in vivo breast cancer risk over the estradiol-induced risk currently remains unknown.

At least five families of growth factors seem to be relevant for breast carcinogenesis: transforming growth factors (TGFs), epidermal growth factors (EGFs), fibroblast growth factors (FGFs), platelet-derived growth factors (PDGFs), and insulin-like growth factors (IGFs) [6]. Each of these growth factor classes binds to its own multimember class of tyrosine kinase-encoding receptors. In vitro studies on human hormone-responsive breast cancer cell lines have revealed that growth stimulation by estradiol is accompanied by increased IGF-II production, induction of EGF and IGF-I receptor production and inhibition of TGF-β production [9-11]. These data suggest that growth control of breast cancer cells may depend on a complex steroid-growth factor interaction system.

Nevertheless, we have investigated probably the most important growth factors and according to our results the predictive value of cell models using estradiol as the single proliferative agent seems to be highly reliable. However, stromal influence by growth factors should not be disregarded, since proliferation of pre-existing malignant cells may be accelerated by the concomitant presence of estrogens and growth factors.

Usually limitations of in vitro studies are the application of too high, non-physiological concentrations. In the present work this does not hold true for the estradiol concentrations, but might be true for the growth factors of which the in vivo concentrations are not known. However, the effect of the growth factors might be overestimated, since lower concentrations can probably be found locally.

An in vitro model can never replace prospective clinical or epidemiological studies. However, the improvement and refinement of in vitro models can help in the elucidation of possible mechanisms.

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