Abstract. In vitro studies concerning the growth-stimulating effect of hormones, especially of estradiol and its metabolites, have mainly been performed using pure substances and breast cancer cell lines. In order to take into account the metabolism of inactive into active hormones or drugs and vice versa which occurs in several tissues, the influence of individual patients’ sera on the growth of breast cancer cells in vitro was tested. Besides measuring the growth promoting action of several hormone replacement therapies, the antiestrogenic effect was determined by measuring the effect of $10^{-10}$ M estradiol added to the culture medium (E2-sensitivity). Influence on proliferation and stimulatability was similar in MCF-7 and T47-D cells. Growth-promoting potential correlated significantly with patient age, being higher in young ladies than in older ones. The converse was true for E2 sensitivity. From the different steroid hormones tested, only higher estradiol levels were associated with increased growth stimulation and diminished E2 sensitivity. Hormone replacement therapy (HRT) of different types did not significantly increase growth potential of serum, however these results are preliminary. Treatment with tamoxifen of breast cancer patients led to a decrease of E2 sensitivity, whereas growth potential was not affected significantly. For the aromatase inhibitor Arimidex, a tendency towards growth inhibition and increased E2 sensitivity was observed. Our in vitro system allows identifying differences between individual persons and groups of women of different age or treatment with respect to stimulation of growth or influence on estrogen sensitivity of breast cancer cells by serum. It is speculated that results might reflect the personal risk or the risk under treatment to develop breast cancer.

Material and Methods

Cell culture. Cells (MCF-7 and T47D breast cancer cells) were cultured for six days in modified Eagle's medium (MEM) without phenol red, with the addition of 10% individual patient serum. Culture was carried out in microtiter plates, proliferation was measured by total protein determination with the Micro BCA Protein Assay (Pierce Biotechnology, Rockford, USA).
Results were related to cell number observed under the addition of control serum or FCS (fetal calf serum). The control serum was obtained from a postmenopausal blood donor and was stored in aliquots at –30˚C. To detect any estrogen antagonism of administered hormones or antihormones, the ratio of cell numbers obtained with estradiol substituted serum (10–10 M) and serum alone were determined.

**Hormone analyses.** Levels of estradiol, progesterone and dehydroepiandrosterone-sulfate (DHEAS) were determined by ELISA technology using the Immulite 2000 (DPC, Biermann, Switzerland).

**Patients.** Serum samples of 87 women who received either no treatment (n=44), hormone replacement therapy with various substances (n=9), antihormonal (n=26), or aromatase inhibitor (n=8) treatment for breast cancer were tested.

**Statistical analysis.** Data were analyzed using the Mann-Whitney test and the Pearson test, respectively, using SPSS statistical software (SPSS Inc., Chicago, USA).

**Results**

**Comparison of MCF-7 and T-47D growth rate under the influence of patient serum.** MCF-7 and T-47D tumor cells were cultured in culture medium with the addition of 10% patients’ sera; in a parallel set, estradiol 1x10–10 M was added to the culture medium. When cells were incubated for six days, a significant correlation of growth rate as well as growth stimulation rate under estradiol (1x10–10 M) was observed between the two cell lines (Figure 1). However, there was a nonsignificant correlation between growth rate and the growth stimulation rate by estradiol in T-47D cells (Table I).

**Table I. Correlation, indicated as correlation coefficient (r), between MCF-7 and T-47D cell growth in medium containing 10% patient serum and cell growth and the effect of E2 stimulation (ratio between stimulated and unstimulated growth). P-values are shown in brackets.**

<table>
<thead>
<tr>
<th></th>
<th>MCF-7 growth stimulation by estradiol</th>
<th>T-47D growth stimulation by estradiol</th>
<th>MCF-7 growth stimulation by estradiol</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-47D growth</td>
<td>0.72 (&lt;0.001)</td>
<td>–0.63 (0.001)</td>
<td>–0.22 (0.31)</td>
</tr>
<tr>
<td>MCF-7 growth</td>
<td>–0.80 (&lt;0.001)</td>
<td>–0.34 (0.11)</td>
<td>0.69 (&lt;0.001)</td>
</tr>
</tbody>
</table>

Figure 1. Correlation of MCF-7 and T-47D growth in medium supplemented with 10% individual patient’s serum (cell number, left side), or with serum plus E2 (10–10 M) (relation of E2 treated/untreated growth, right side).
Dependence of MCF-7 cell growth and MCF-7 cell growth stimulation by estradiol on patient's age. After six days of incubation in the presence of patient serum, MCF-7 cell growth correlated negatively with the patient's age ($R^2=0.26, p<0.01$), whereas growth stimulation by estradiol showed a positive correlation ($R^2=0.50, p<0.01$) (Figure 2). To further investigate this observation we evaluated individual serum hormone levels.

Correlation between serum steroid hormone levels in blood and growth rate under serum alone and serum with the addition of $1 \times 10^{-10} \text{ M estradiol}$. A significant correlation was found between estradiol blood levels and MCF-7 growth in test serum substituted culture medium as well as MCF-7 growth stimulation by the addition of estradiol ($1 \times 10^{-10} \text{ M}$). Progesterone and DHEAS serum levels showed no association with the growth behavior of MCF-7 cells (Table II).

Comparison of sera from premenopausal and postmenopausal women. Growth stimulation was highest for premenopausal women and lowest for postmenopausal women without any HRT. The opposite was true for the...
stimulation by estradiol. Sera from women under HRT were in-between, however the effect of HRT was not statistically significant (Figure 3).

Influence on growth rates by exogenous estrogenic and anti-estrogenic agents. MCF-7 cells were cultured as described earlier with the serum of patients who received either no treatment (n=18), HRT with tibolone (n=17), adjuvant treatment for breast cancer with the aromatase inhibitor Arimidex (n=8), or tamoxifen (n=26). There were no significant effects on cell growth by any treatment (Table III). Growth stimulation by the addition of 1×10⁻¹⁰ M estradiol was significantly reduced (p<0.001) for the tamoxifen treated group. In the Arimidex-treated group, stimulation by 1×10⁻¹⁰ M estradiol was significantly enhanced compared to the tamoxifen group, whereas cell growth stimulation by the patient’s serum itself tended to be reduced. Tibolone had no significant effect on growth nor stimulation by estradiol.

Discussion

We have established a test system using patient serum instead of FCS to investigate the influence of exogenous hormones and other drugs on breast cancer cell proliferation. Using this in vitro system we aimed to approximate an in vivo situation. As the test persons were of different age and menopausal status, different endogenous hormone levels could therefore be expected. For exogenously administered hormones or antihormones, one could expect various amounts of metabolites in individual sera. Our assay is assumed to take the anabolic and catabolic metabolism that occurs in various body organs into consideration. Results obtained when using two different cell lines (MCF-7 and T-47D) were comparable, however growth rate did not correlate with E2-stimulation in T-47D. This led us to the decision of using MCF-7 cells in the final version of our bioassay. The fact that the growth-promoting effect on MCF-7 cells of serum from younger patients was significantly higher than that of older women could be explained by higher levels of estrogens or their precursors but it may also have yet unknown causes. Circulating levels of sex steroid

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Serum/Control serum</th>
<th>Serum + E2/ Serum</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>p-value</td>
</tr>
<tr>
<td>No therapy (n=18)</td>
<td>1.02</td>
<td>0.610</td>
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<tr>
<td>Tibolone (n=17)</td>
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<td>Tamoxifen (n=26)</td>
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<td>Arimidex (n=8)</td>
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<td>0.190</td>
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<tr>
<td>Arimidex vs. tamoxifen</td>
<td>0.90</td>
<td>0.190</td>
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</tbody>
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Table III. Growth of MCF-7 cells in medium containing serum from either control persons or patients treated with the drugs indicated (in relation to control serum). The relation of cell growth in serum substituted with E2 (1×10⁻¹⁰ M) and in serum only is also shown.
hormones in postmenopausal women are thought to be associated with the risk of ER+/PR+ breast tumors (5-7), although the nature of this association is not known. Our results agree with this observation as there was a correlation between serum estradiol levels and growth stimulation. On the other hand, this was not the case with progesterone and DHEAS. Serum from postmenopausal women showed lower growth-promoting activity in T-47D cells than serum from premenopausal women, but growth stimulation by the addition of estradiol was significantly higher in cells treated with serum from postmenopausal women. The relatively high levels of estradiol or other growth factors in premenopausal sera may prevent any relevant additional stimulation by estradiol. Different kinds of hormone replacement as well as antiestrogen therapies had been applied in some of the women whose sera were used for this investigation. As expected, tamoxifen treatment significantly inhibited the effect of 1×10^{-10} M estradiol added to the culture medium, but it did not reduce cell growth activity compared to the serum of untreated patients. This indicates that tamoxifen is able to protect cells against estrogen action but does not inhibit cell growth per se (8). Arimidex (anastrozole), an aromatase inhibitor, depletes breast tissue and serum of estradiol but cannot protect cells against estrogenic action at the receptor level (9). This concept is supported by our results (Table III). Tibolone is assumed to have no negative influence on breast cancer growth in breast cancer patients. Our preliminary results support this opinion. In summary, this system of testing the general growth promoting action of an individual woman’s serum and the influence of endogenous and exogenous hormones and drugs needs further validation. However, the presented results are in agreement with current concepts of the in vivo influence of estradiol and various antiestrogen treatments.

References


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