Promotion by Prolactin of the Growth of Human Breast Neoplasms Cultured in Vitro in the Soft Agar Clonogenic Assay

Andrea Manni, Carol Wright, Glenn Davis, Jerry Glenn, Raymond Joehl, and Peter Feil

ABSTRACT

The role of prolactin (PRL) in supporting the growth of human breast cancer is still unclear. The ability to grow primary breast cancer specimens in the soft agar clonogenic assay in the absence of serum gave us the opportunity to evaluate the growth-promoting effect of PRL and to compare it to that of estradiol in the same tumor samples. PRL was tested both at physiological concentrations (20 ng/ml) as well as in pharmacological amounts (200 ng/ml) comparable to circulating blood levels in hyperprolactinemic states. Estradiol was simultaneously tested in physiological amounts (10^-8 M). In 17 infiltrating ductal carcinomas, the lower dose of PRL stimulated colony formation to 126 ± 5.2% (SE) of control, while the higher dose increased colony number to 159 ± 10.4% of control. This latter effect was comparable to that observed with estradiol (159 ± 8.5% of control). The effect of PRL was more pronounced in estrogen receptor-positive tumors. Nine of ten estrogen receptor-positive tumors were PRL sensitive, while three of seven estrogen receptor-negative tumors exhibited a clear response to PRL administration. PRL did not stimulate colony formation in a malignant cystosarcoma phylloides and in two benign lesions (fibroadenoma and fibrocystic disease). We conclude that, at least under the conditions of the soft agar clonogenic assay, PRL exerts a dose-dependent growth-promoting effect on human breast cancer. Such effect is comparable to that of estradiol when PRL is added in concentrations similar to circulating blood levels in hyperprolactinemic patients.

INTRODUCTION

While prolactin is a predominant hormone involved in the growth of experimental breast cancer (1), its role in supporting the growth of hormone-responsive human breast cancer is still unclear (2). The definition of the effect of prolactin on human breast cancer growth is of major importance, since hyperprolactinemia is frequently encountered in women, both under physiological conditions, such as pregnancy and oral contraceptive administration, and in pathological states, such as in the presence of pituitary tumors.

Recently we have extensively used the soft agar clonogenic assay to investigate the endocrine mechanisms affecting the growth of hormone-responsive breast cancer in rats (3–6). In addition, we have applied this technique to study the hormonal responsiveness of human breast neoplasms (7). In initial experiments we have evaluated the effect of estrogen deprivation and repletion on tumor colony formation in soft agar by individual human breast cancers (7). Thus, we thought that this technique might give us a unique opportunity to test the effect of prolactin administration on the growth of human mammary tumors and to compare its effect with that of estradiol. Furthermore, we had the opportunity to correlate the alterations in colony formation induced by such hormonal manipulations with the estrogen and progesterone receptor status of the tumor.

MATERIALS AND METHODS

ABSTRACT

The role of prolactin (PRL) in supporting the growth of human breast cancer is still unclear. The ability to grow primary breast cancer specimens in the soft agar clonogenic assay in the absence of serum gave us the opportunity to evaluate the growth-promoting effect of PRL and to compare it to that of estradiol in the same tumor samples. PRL was tested both at physiological concentrations (20 ng/ml) as well as in pharmacological amounts (200 ng/ml) comparable to circulating blood levels in hyperprolactinemic states. Estradiol was simultaneously tested in physiological amounts (10^-8 M). In 17 infiltrating ductal carcinomas, the lower dose of PRL stimulated colony formation to 126 ± 5.2% (SE) of control, while the higher dose increased colony number to 159 ± 10.4% of control. This latter effect was comparable to that observed with estradiol (159 ± 8.5% of control). The effect of PRL was more pronounced in estrogen receptor-positive tumors. Nine of ten estrogen receptor-positive tumors were PRL sensitive, while three of seven estrogen receptor-negative tumors exhibited a clear response to PRL administration. PRL did not stimulate colony formation in a malignant cystosarcoma phylloides and in two benign lesions (fibroadenoma and fibrocystic disease). We conclude that, at least under the conditions of the soft agar clonogenic assay, PRL exerts a dose-dependent growth-promoting effect on human breast cancer. Such effect is comparable to that of estradiol when PRL is added in concentrations similar to circulating blood levels in hyperprolactinemic patients.

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Received 9/17/85; revised 12/3/85; accepted 12/30/85.

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"This work was supported in part by National Cancer Institute Grant CA35944.

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RESULTS

All tumors plated successfully grew with an average colony formation under control conditions of 50.4 ± 11.3 (SE) (range, 3 The abbreviations used are: ER, estrogen receptor; PgR, progesterone receptor; PRL, prolactin.

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Table 1  Effect of ovine PRL and estradiol on the growth of human mammary neoplasms in the soft agar clonogenic assay

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<th>Patient No</th>
<th>Menopausal Status</th>
<th>ER Status</th>
<th>PgR Status</th>
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<th>% of Control</th>
<th>PRL (200 ng/ml) Control</th>
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<td>134,135,143 (137.7)</td>
<td>103</td>
<td>177,194,169 (180)</td>
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Malignant lesions ER(-) PgR(-)

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<tr>
<th>Patient No</th>
<th>Menopausal Status</th>
<th>ER Status</th>
<th>PgR Status</th>
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<th>% of Control</th>
<th>PRL (200 ng/ml) Control</th>
<th>% of Control</th>
<th>Estradiol (10^-8 M) Control</th>
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<td>25,25,27 (25.7)</td>
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Malignant lesions ER(-) PgR(-)

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<td>PgR(-)</td>
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Benign lesions

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<th>Menopausal Status</th>
<th>ER Status</th>
<th>PgR Status</th>
<th>PRL (20 ng/ml) Control</th>
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<th>% of Control</th>
<th>Estradiol (10^-8 M) Control</th>
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<tr>
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<td>18,14,13 (15)</td>
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Fig. 1. Effect of ovine PRL (oPRL) and estradiol (E2) on the growth of 17 human breast cancers in the soft agar clonogenic assay. The number of colonies formed under our experimental conditions is expressed as the percentage of control colonies formed in the absence of any treatment. Columns, mean; bars, SE. * P < 0.01 versus ovine PRL (200 ng/ml), 10^-8 M estradiol (Newman Keuls test), and versus control (one sample t test with Bonferroni adjustment for one-three-sided tests).

Fig. 2. Effect of ovine PRL (oPRL) and estradiol (E2) administration on colony formation by 17 human breast cancers according to the ER status of the tumor. The data are expressed as in Fig. 1. * P < 0.01 (unpaired t test) versus the ER-negative tumors.

9–196 (Table 1). As can be seen in Fig. 1, prolactin exerted a dose-dependent colony-stimulating effect in the 17 ductal carcinomas tested. The lower dose of ovine PRL (20 ng/ml) stimulated colony formation to 126 ± 5.2% of control (P < 0.01), while the higher dose increased colony number to 159 ± 10.4% of control (P < 0.01). The stimulation of colony formation observed with the larger amount of ovine PRL was identical to that observed with estradiol (159 ± 8.5% of control) (Fig. 1). As can be seen in Fig. 2, the effect of ovine PRL as well as that of estradiol was more pronounced in the ER-positive tumors. The stimulation of colony formation in ER-positive versus ER-negative tumors was 132 ± 6.4% versus 118 ± 7.9% (P = not significant) with ovine PRL (20 ng/ml), 179 ± 12.3% versus 132 ± 11.8% (P < 0.01) with ovine PRL (200 ng/ml), and 175 ± 14.9% versus 125 ± 9.1% (P < 0.01) with estradiol (10^-8 M). All ER-positive patients responded to estradiol, and all but one (Patient 7) responded to ovine PRL (Table 1).

Significant stimulation of colony formation was, however, also observed in some receptor-negative tumors. Among the seven ER-negative tumors, three (Patients 13, 14, and 15) and possibly four (Patient 12) clearly showed a response to both ovine PRL and estradiol (Table 1).

Ovine PRL did not stimulate colony formation in the case of the cystosarcoma phyllodes (Patient 18) and in the two benign lesions (Patients 19 and 20) (Table 1). The latter two, however, exhibited a moderate response to the administration of estradiol (Table 1).

DISCUSSION

While estradiol appears to be the predominant hormone involved in supporting the growth of human breast cancer (12,
Considerable evidence suggests that prolactin plays an important role in a variety of experimental breast cancers (1, 14, 15). In these experimental models, in fact, it has been consistently observed that suppression of prolactin secretion invariably results in tumor regression (1, 16). In contrast, administration of potent inhibitors of prolactin secretion to humans, such as lergotrile mesylate (17) and bromocriptine (18), has only rarely resulted in significant palliation. These observations raise serious doubts with regard to the possible mitogenic effect of prolactin in human breast cancer. This skepticism appeared to be corroborated by the preliminary observation that a variety of human breast cancer cell lines were not dependent on prolactin for growth in vitro (19). Subsequent reports, however, have provided some evidence in favor of a mitogenic effect of prolactin both in breast cancer cell lines as well as in a small fraction of primary cultures of human breast tumors (20-22). Furthermore, the presence of prolactin receptors has been detected in up to 50% of human breast cancers (23). The data presented here indicate that, at least under the conditions of the soft agar clonogenic assay, the vast majority of human breast cancers are prolactin sensitive.

It is worth emphasizing that significant, although modest, stimulation of colony formation was observed when prolactin was added at the dose of 20 ng/ml. Such a dose corresponds to normal circulating levels of prolactin in women. When the dose of prolactin was increased to 200 ng/ml, a superior colony-stimulating effect was obtained which was virtually identical to that observed with the administration of estradiol. Although this is clearly a pharmacological dose, it nevertheless corresponds to the observed circulating levels of prolactin in common hyperprolactinemic states, such as those secondary to prolactin-secreting tumors or administration of phenothiazine drugs. Thus, the growth-promoting effect of 200 ng of prolactin per ml may potentially have significant clinical relevance.

Our report for the first time correlates the prolactin responsiveness of the tumors in vitro with their estrogen receptor status. Our data clearly indicate that tumors that are positive for estrogen receptors exhibit a greater sensitivity to prolactin administration. However, also three estrogen receptor-negative tumors (Patients 13, 14, and 15; Table 1) exhibited a clear response to the administration of prolactin. In the same tumors as well as in an additional one (Patient 12), estradiol also exhibited a colony-stimulating effect. In these experiments the effect of estradiol on colony formation was also more pronounced in ER-positive than in ER-negative tumors (Fig. 2). This finding is somewhat at variance with our previous report (7), where the effect of estradiol was not influenced by the receptor status of the tumor. Nevertheless, both reports clearly indicate that at least some of the tumors that are defined as receptor negative on the basis of biochemical tests are influenced by alterations of the hormonal environment when grown in soft agar. These findings are consistent with the hypothesis that a significant fraction of ER-negative tumors contains clones of hormone-responsive cells, the growth of which may be facilitated in the soft agar clonogenic assay. Obviously, measurement of receptors in the colonies themselves by immunocytochemical methods would be required to fully test this hypothesis.

It would have been of interest to correlate the colony-stimulating effect of prolactin in our system with the prolactin receptor status of the tumor. Since discordance between the estrogen and prolactin receptor status has been described in human breast cancers (23), it is conceivable that the ER-negative tumors responding to prolactin might have been prolactin receptor positive. Unfortunately, however, this hypothesis remains speculative, since we did not measure prolactin receptors in our tumors.

Finally, it is worth noticing that we used ovine prolactin. Lack of sufficient tumor material prevented us from simultaneously testing the effect of ovine prolactin and human prolactin on tumor colony formation. This would have been of interest since some reports have shown that human prolactin but not ovine prolactin is able to stimulate breast cancer growth in culture (24), while others have shown a similar activity of the two forms of prolactin (25). In a recent study performed in a newly established mammary cancer cell line, Simon et al. (26) observed that human prolactin was more effective in binding to and promoting the growth of breast cancer cells than ovine prolactin or other lactogenic hormones. The reason for these discrepancies in the literature remains, at present, unclear.

ACKNOWLEDGMENTS

The authors wish to thank Sandra Wolfe for her secretarial assistance.

REFERENCES


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