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Reversible Inhibitory Activity of Sera from Breast Cancer Patients on Oestrogen

Effect on Cell Proliferation and Protein Synthesis of the Human Breast Cancer Cell Line MCF-7

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Abstract—*Sera from foetal calves, newborn calves, athymic mice, and healthy postmenopausal women exert a growth inhibitory effect on the oestrogen receptor positive human breast cancer cell line MCF-7. This inhibitory effect of serum can be abrogated by oestradiol. Serum samples from 22 breast cancer patients were analysed for the amount of inhibitory activity in order to clarify whether regulation of cell proliferation of human breast cancer may occur via a modulation of the inhibitory activity in the patient's serum. Twenty of the 22 serum samples showed inhibitory activity and no difference was found in the degree of inhibition. These results do not support the hypothesis that breast cancer cells grow* in vivo *solely as a function of a reduced level of a serum-borne inhibitory activity; other mechanisms must be involved in the regulation of growth. We have found that MCF-7 cells, the growth of which is inhibited by serum from breast cancer patients, exhibit a reduced synthesis of three secreted proteins, and an increased amount of one protein, a 46K protein. Oestradiol induced cell proliferation is concomitant with stimulation of the synthesis of these three proteins and inhibition of the 46K protein. Regulation of growth of breast cancer may therefore occur via changes in the synthesis of secreted proteins, which exert a regulatory function on cell proliferation.*

INTRODUCTION

THE MECHANISM by which oestradiol stimulates the growth of human breast cancer cells has been studied intensively since oestrogen responsive human breast cancer cell lines have been established in long-term tissue culture [1, 2]. Oestradiol has been demonstrated to stimulate the synthesis of autocrine or paracrine growth factors in breast cancer cells [3–5], and synthesis of an inhibitory factor has recently been suggested to be down regulated consequent to oestradiol treatment [6–8]. We have found that the growth of the human breast cancer cell lines MCF-7 and T47D can be inhibited by serum from different sources [9, 10]. Moreover, this growth inhibition can be abrogated by oestradiol. We have speculated whether growth of breast epithelial cells is regulated by an inhibitory factor present in the environment of the cells, and whether the growth of breast cancer cells can be affected via a down regulation of the synthesis or the activity of such an inhibitory factor. Inhibitory activity is present in serum from normal healthy postmenopausal women, and Soto and Sonnenschein have shown that also sera from normal men and premenopausal women contain growth inhibitory activity towards MCF-7 cells [11]. They detected no significant variability as to the inhibitory activity of different normal sera [11], indicating that the presence of inhibitory activity towards the cell proliferation of oestrogen receptor positive breast carcinoma cells is a normal phenomenon. In this paper we have studied whether sera from breast cancer patients contain a similar growth inhibitory activity, and whether the amount of inhibitor is the same in all patient sera. Finally we have investigated the synthesis of secreted proteins in growth inhibited and in oestradiol stimulated human breast cancer cells.

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PATIENTS AND METHODS

Blood was taken from 22 postmenopausal women, who had undergone modified radical mastectomy for primary breast cancer and were suspected to have recurrent disease. The clinical work up included physical examination, blood tests, bone scan, bone biopsy, X-ray of the chest and axial skeleton, and ultrasound of the liver. Based upon these examinations, local, regional, or distant metastases were found in five, 10 and two patients, respectively, whereas five patients were without sign of relapse. None of the patients received adjuvant therapy at the time of blood sampling. Serum was isolated 2 h after clotting and stored at −80°C until use. Oestrogen receptor determinations were performed according to the EORTC recommendation [12], with the minor modifications previously described [13]. The cut-off level used to define receptor positivity was 10 fmol/mg cytosol protein.

The method of tissue culture has been described in detail previously [9]. The MCF-7 cells were grown in the presence of [^{35}S]methionine for protein labelling; the labelling conditions, polyacrylamide gel electrophoresis (PAGE) and autoradiography were performed as described in Ref. [14]. Agfa Structurix D7P films were used for the autoradiograms.

RESULTS

We have previously demonstrated that serum contains growth inhibitory activity towards two oestrogen receptor positive human breast cancer cell lines, MCF-7 and T47D [9, 10]. The inhibitory activity was first described for newborn calf serum (NCS) [9], but, as shown in Fig. 1, foetal calf serum (FCS), athymic mice serum (AMS), and human serum (HS) from a healthy postmenopausal woman also exert growth inhibitory activity, which can be abolished by oestradiol. In Fig. 2 we show the result from a representative growth experiment in which we have added serum from three different breast cancer patients to MCF-7 cells growing in DME/F12 medium supplemented with insulin (6 ng/ml) and 1% FCS. It is evident that all three human sera inhibit cell proliferation uniformly, and that this inhibition can be abolished, if oestradiol is added together with the patient serum. Figure 3 shows the result of a similar growth experiment in which phenol red, which recently has been shown to exert oestrogenic activity [15], was omitted from the conventional growth medium. As expected, the only effect of omission of phenol red is that the oestradiol stimulation is much more pronounced, whereas the growth inhibition is at about the same level as that obtained in the phenol red containing medium. We have tested 22 sera from postmenopausal patients with a diagnosis of primary breast cancer and varying degree of metastatic disease. Two of the 22 sera contained no growth inhibitory

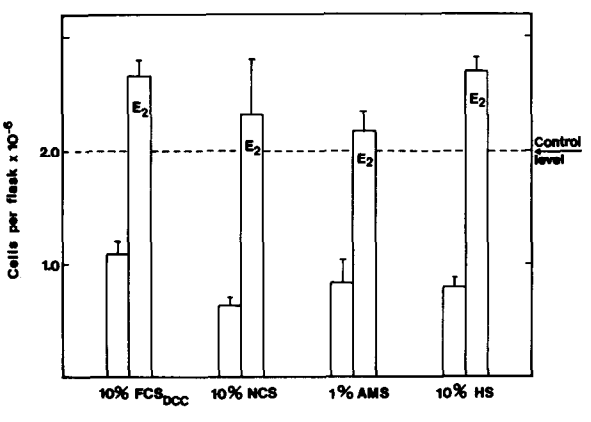

Fig. 1. MCF-7 cells were seeded with 125,000 cells per T-25 flask. Two days after seeding medium was shifted to the indicated concentration of the different sera and oestradiol (10^{-8}M) Medium was renewed three times weekly. Cell number in triplicate cultures were determined at day 6 after change to experimental medium. The control level is the average number of cells in a culture grown with 0.5% FCS.

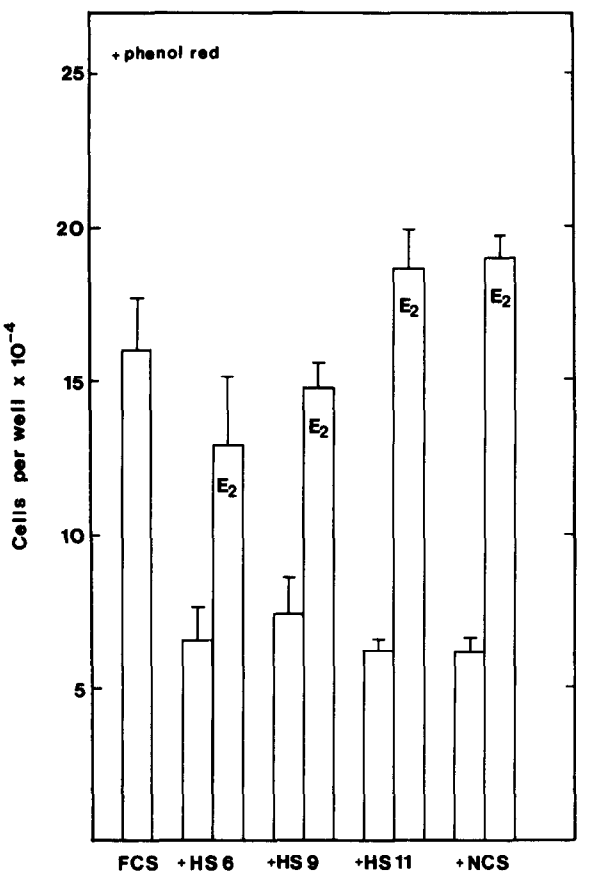

Fig. 2. MCF-7 cells were seeded with 10,000 cells per well in a multidish plate with growth medium containing 1% FCS. 10% patient sera or 10% NCS with or without oestradiol (10^{-8} M) were added to the growth medium with 1% FCS, and the cells were cultivated for 6 days with the indicated media. The cell number is the average cell number in four wells.

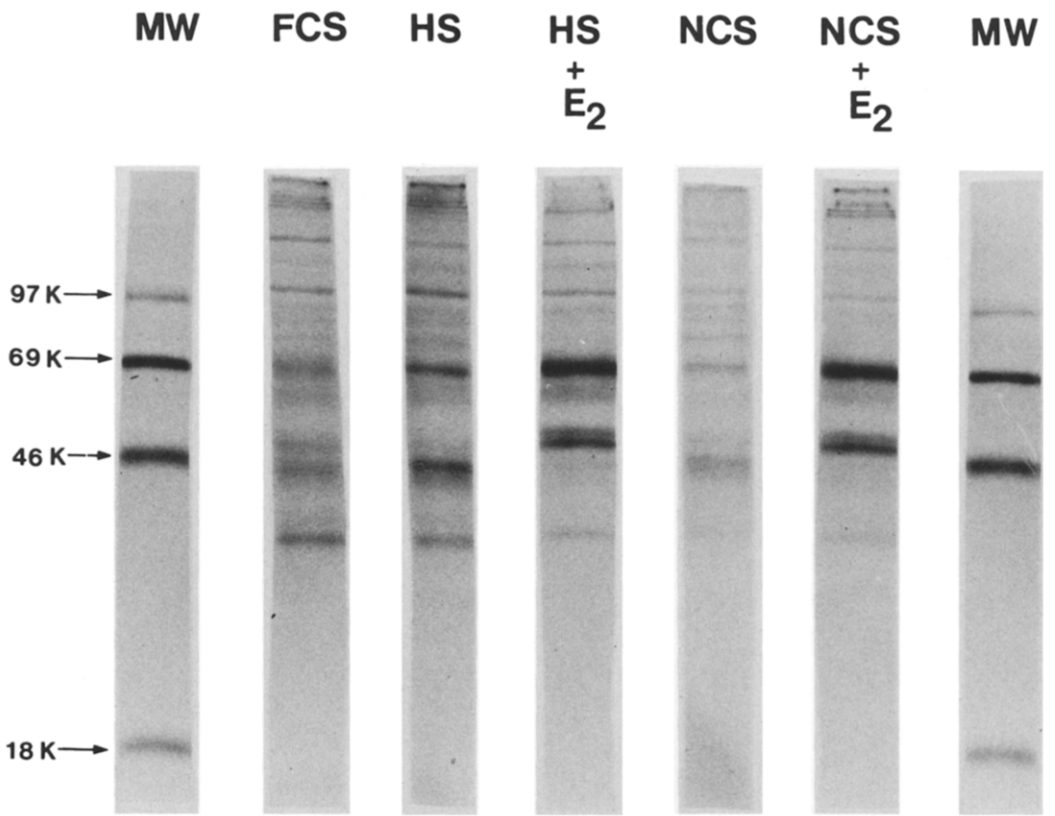

Fig. 4. MCF-7 cells were grown in multidish cultures for 6 *days in experimental medium. All cultures contained* 1% *FCS. Where indicated HS and NCS is present at a concentration of* 10% *and E2 is present at* 10^{-8} M. *The cultures were washed with PBS before change to DME/F*12 *medium with* [^{35}S]*methionine. Conditioned medium was collected* 6 h *after addition of* [^{3}S]*methionine. PAGE analysis and autoradiography were performed as described in Patients and Methods.*

activity, whereas the remaining 20 sera exerted growth inhibition. Seventeen of these analyses were performed in medium containing phenol red, and, as seen from Table 1, the average inhibition was 49%, which was the same as the inhibition obtained with NCS. In phenol red free medium the inhibition was more pronounced; the average inhibition was 32% with the patient sera, and 31% with NCS. The same batch of NCS was used as a control of the inter assay variation, and it is notable that the standard deviation (S.D.) for the inhibition with different sera from breast cancer patients is not significantly higher than the S.D. for the control cultures with NCS.

Oestrogen receptor data on the primary tumour were available for 11 of the 22 patients (Table 2). All but one of the sera exerting growth inhibitory activity came from patients with oestrogen receptor positive tumours. The sera containing no growth inhibitory activity came from one patient with an oestrogen receptor negative tumour and one patient from whom no receptor data were available.

NCS and a serum pool from the 20 sera in which growth inhibitory activity had been found were used in an experiment for analysis of the synthesis of secreted proteins from MCF-7 cells by PAGE. The autoradiogram from these experiments is shown in Fig. 4. It can be seen that cultures growing with 10% NCS or 10% HS have a pattern of secreted proteins very similar to that obtained in the control culture (1% FCS) with a tendency to a reduced amount of a 52K, a 65K, and a 70K protein, and

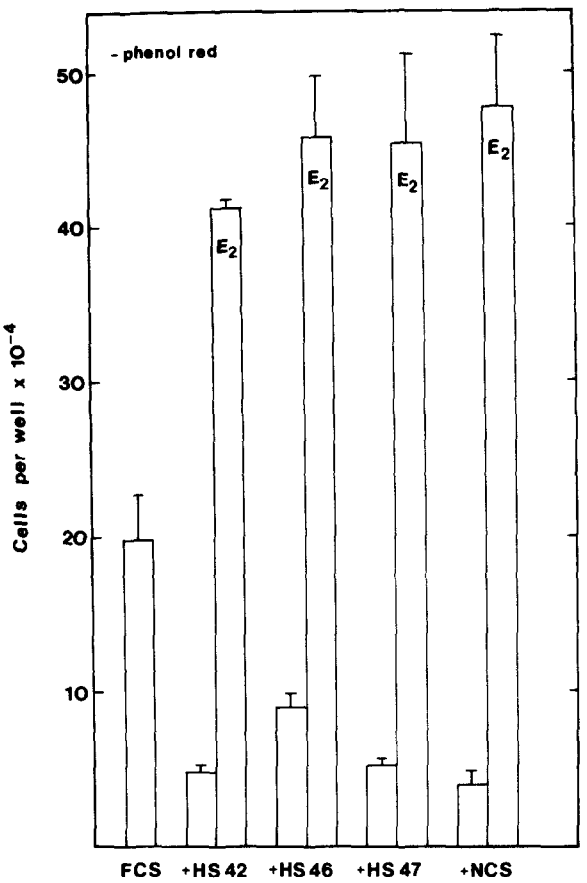

Fig. 3. The cultures were grown as described in Fig. 2, except that phenol red was omitted from the growth medium.

Table 1. Effect of serum with and without oestradiol on proliferation of MCF-7 cells grown in medium with 1% FCS ± phenol red

Medium	Serum	*N*	Mean cell number (% of control)	S.D.
+ phenol red	HS	17	49	11
	NCS	6	48	9
+ phenol red	HS + E2	16	124	28
	NCS + E2	6	120	17
− phenol red	HS	3	32	12
	NCS	4	31	9
− phenol red	HS + E2	3	222	12
	NCS + E2	4	210	48

MCF-1 cells were grown as described in the Legend to Fig. 2. Human serum (HS) or newborn calf serum (NCS) with and without oestradiol (E2) were added to the cells growing with 1% FCS. N indicates the number of different patient sera or the number of experiments with NCS. Only patient sera which exerted a statistically significant growth inhibition are included in this table. Mean cell number is calculated from the cell number in the individual experiments and expressed as a percentage of the cell number in the control cultures. S.D. is the standard deviation of the mean cell number.

Table 2. Inhibitory activity of patient sera in relation to the oestrogen receptor status of the primary tumour

	ER+	ER−	Unknown
Sera with inhibitory activity	9	1	10
Sera without inhibitory activity	0	1	1

Oestrogen receptor determination on the primary tumour was performed as described in Patients and Methods.

an increased amount of a 46K protein. In return these three protein bands are very prominent in the cultures with 10% serum and oestradiol, whereas a 46K protein band is reduced in the cultures with oestradiol, i.e. lower amounts of the 46K protein are secreted.

DISCUSSION

We have shown that sera from foetal calves, newborn calves, athymic mice and normal postmenopausal women contain growth inhibitory activity towards the human breast cancer cell line MCF-7. This growth inhibition can be abolished by oestradiol. Similar results have been obtained with human sera from normal men, normal premenopausal and normal postmenopausal women [11], whereas this is the first paper presenting an analysis of serum from breast cancer patients. We found that 20 out of 22 patient sera contained an oestrogen reversible growth inhibitory activity towards estrogen receptor positive human breast cancer cells, and the degree of growth inhibition was quite similar in all patient sera, irrespective of the degree of dissemination and the receptor status. We assume, therefore, that a serum-borne factor exerts a negative regulatory function on the breast cancer cells, and the results indicate that the regulation of growth of the breast cancer cells *in vivo* is not achieved via down regulation of the production of the inhibitory factor in serum. The fact that two out of 22 sera exerted no growth inhibition suggests that a minority of the patients may lack the inhibitory activity or have a reduced level. Since the inhibitory activity is associated to the protein fraction of serum (unpublished results), inactivation during the preparation and storage cannot be excluded.

We have shown that oestradiol stimulates the synthesis of several proteins which are secreted to the surrounding growth medium. The 65K protein and the 70K proteins have not previously been described by others, and we find that the synthesis of these two proteins are correlated to the growth rate ([16], unpublished results), indicating that they may act as autocrine or paracrine growth factors. The 52K protein, which is shown in the presented autoradiogram (Fig. 4), is probably identical to the 52K protein first described by Westley and Rochefort [14, 17], and recent papers from Rochefort's group have demonstrated that the purified 52K protein stimulates growth of oestrogen deprived MCF-7 cells [5, 18], i.e. acts as a growth factor. The mechanism by which serum exerts the observed growth inhibition is unknown. The reduced level of secreted 70K, 65K and 52K protein and the increased level of 46K protein secreted from cells grown with 10% human serum or 10% newborn calf serum could indicate that serum acts by reducing or somehow masking the oestradiol content in the medium (supplied via the addition of 1% FCS). However, this is not the only effect of high serum concentration, since MCF-7 cells growing in chemically defined growth medium without oestrogen can also be growth inhibited by 10% NCS [19]. The results in Ref. [19] were obtained with MCF-7 cells growing in medium containing phenol red; however, a similar growth inhibition is also obtained by 10% NCS added to MCF-7 cells growing in medium without phenol red (unpublished).

In all the presented experiments with patient sera, cell proliferation increased considerably by the addition of oestradiol, supporting the well known fact that sera from postmenopausal women contain insufficient amounts of active oestrogen components. How a sufficient oestrogen level is achieved to give rise to oestrogen stimulated growth in breast tumours in postmenopausal women is not yet fully clarified [20, 21].

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