Pergamon

European Journal of Cancer Vol. 30A, No. 9, pp. 1348–1352, 1994

0959-8049/94 $7.00+0.00

0959-8049(94)E0175-4

# Timing of Surgery Influences Survival in Receptor-negative as Well as Receptor-positive Breast Cancer

Z. Saad, M. Vincent, V. Bramwell, L. Stitt, J. Duff, M. Girotti, T. Jory, G. Heathcote, I. Turnbull and B. Garcia

**Analysis of oestrogen and progesterone receptor (ER, PR) status was interpreted in relation to menstrual phase at the time of surgery and survival in 84 women diagnosed with breast cancer between 1975 and 1988. We showed previously (*Br J Surgery* 1994, 81, 217–220) that long-term survival was significantly poorer when surgery was performed during the follicular phase of the menstrual cycle compared to luteal phase; we now demonstrate that this effect on survival is at least as important in receptor-negative as receptor-positive patients. At 10 years, overall survival (OS) of ER-positive patients who had their biopsy during the follicular phase was significantly poorer than for those whose biopsy was performed during the luteal phase of their menstrual cycle (52 versus 88%, $P = 0.02$). OS for the ER-negative follicular phase group was also significantly poorer than that for the ER-negative luteal phase group (33 versus 76%, $P = 0.009$). The OS difference between the PR-positive follicular phase group and PR-positive luteal phase group was of borderline significance (60 versus 87%, $P = 0.06$), while the difference in OS between the PR-negative follicular phase group and that of the PR-negative luteal phase group was highly significant (13 versus 76%, $P = 0.001$). Disease-free survival for these groups followed a similar trend. The survival differences in receptor-negative women suggest that hormonal fluctuations at the time of surgery may have complex indirect effects on tumour growth and metastasis. The mechanism, if indeed independent of the tumour steroid receptors, could also apply in other cancers.**
*Eur J Cancer*, Vol. 30A, No. 9, pp. 1348–1352, 1994

## INTRODUCTION

OESTROGEN RECEPTOR (ER) protein assays were introduced into clinical practice by Jensen *et al.* in 1971 [1]. Since then, such assays have been widely used to predict survival, recurrence and response to therapy in breast cancer patients. The presence of ER and progesterone receptors (PR) in the breast cancer tissue may indicate the responsiveness to and thereby dependence of the cancer cells on complex interactions of the female sex hormones.

Recent studies [2–7] have suggested that the timing of surgery in relation to menstrual cycle influences disease-free survival (DFS) and overall survival (OS) of premenopausal women with operable breast cancer while others [8–11] have not found this difference. In a retrospective study [12] of all premenopausal women who were treated for early breast carcinoma in three teaching hospitals in London, (Ontario, Canada) between 1975–1988, we have shown that long-term survival was significantly poorer when surgery was performed during the follicular phase of the menstrual cycle when compared to luteal phase.

The outcome of the confirmatory studies [2–7, 12] emphasises the role of the hormonal milieu at the time of surgery in predicting survival in women with breast cancer. In an attempt to explore the possibility that this phenomenon may not be exclusive to breast cancer, we reviewed the relationship amongst ER and PR status, the hormonal milieu at the time of surgery and outcome.

## PATIENTS AND METHODS

The records of all premenopausal patients who were treated for operable breast cancer in London, Ontario, between 1975 and 1988 were initially reviewed in a previous study [12]. This current analysis is limited to a subgroup of those patients who had ER/PR status available in addition to the menstrual data and other conventional prognostic factors. These included tumour size, histological grading, nodal status, number of positive nodes, definitive surgical treatment and adjuvant systemic therapy. Dates of recurrence and last follow-up or death were obtained from the hospital records or through family doctors.

84 patients who had complete data were included in this analysis. 223 patients were excluded; 37 with no follow-up data, 12 in whom tumour ER/PR status was unknown and 174 with absent or incomplete menstrual data. This latter group

Correspondence to Z. Saad.
Z. Saad, M. Vincent and V. Bramwell are at the Dept. of Medical Oncology; L. Stitt is at the Dept. of Clinical Studies, The London Regional Cancer Centre, 790 Commissioners Road East, London, Ontario N6A 4L6; J. Duff and B. Garcia are at the Depts of Surgery and Pathology, University Hospital; M. Girotti and I. Turnbull are at the Depts of Surgery and Pathology, Victoria Hospital; and T. Jory and G. Heathcote are at the Depts of Surgery and Pathology, St. Joseph Hospital, London, Ontario, Canada.
Revised 1 Mar. 1994; accepted 28 Mar. 1994.

*Table 1. Distribution of prognostic factors between the menstrual phase groups*

| | ER+ | | ER− | | PR+ | | PR− | |
|---|---|---|---|---|---|---|---|---|
| Factors | Fol. | Lut. | Fol. | Lut. | Fol. | Lut. | Fol. | Lut. |
| No. of patients | 15 | 21 | 20 | 28 | 20 | 28 | 14 | 20 |
| Mean age (years) | 42 | 42 | 37 | 39 | 41 | 40 | 37 | 40 |
| Tumour size | | | | | | | | |
| < 2 cm | 7 | 7 | 5 | 6 | 8 | 9 | 3 | 4 |
| 2–4 cm | 8 | 12 | 12 | 18 | 12 | 17 | 9 | 12 |
| > 4 cm | 0 | 2 | 3 | 3 | 0 | 2 | 2 | 3 |
| Unknown | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| No. of positive nodes | | | | | | | | |
| None | 6 | 8 | 9 | 16 | 8 | 10 | 6 | 13 |
| 1–3 | 6 | 7 | 6 | 7 | 8 | 9 | 4 | 5 |
| 4–9 | 3 | 4 | 4 | 3 | 4 | 5 | 3 | 2 |
| > 10 | 0 | 2 | 1 | 2 | 1 | 4 | 1 | 0 |
| Grade | | | | | | | | |
| I | 4 | 4 | 2 | 2 | 3 | 4 | 2 | 2 |
| II | 5 | 9 | 5 | 5 | 7 | 8 | 3 | 6 |
| III | 5 | 5 | 12 | 21 | 9 | 13 | 8 | 12 |
| Unknown | 1 | 3 | 1 | 0 | 1 | 3 | 1 | 0 |
| ER+ | — | — | — | — | 14 | 19 | 2 | 2 |
| ER− | — | — | — | — | 6 | 9 | 12 | 18 |
| PR+ | 13 | 19 | 6 | 9 | — | — | — | — |
| PR− | 2 | 2 | 13 | 18 | — | — | — | — |
| PR unknown | — | — | 1 | 1 | — | — | — | — |
| Definitive surgery | | | | | | | | |
| Mastectomy | 11 | 16 | 13 | 20 | 14 | 21 | 9 | 15 |
| Breast conservation | 4 | 5 | 7 | 8 | 6 | 7 | 5 | 5 |
| Adjuvant therapy | | | | | | | | |
| None (node-negative) | 6 | 8 | 9 | 16 | 8 | 10 | 6 | 13 |
| None (node-positive) | 3 | 0 | 1 | 0 | 4 | 0 | 1 | 0 |
| Chemotherapy | 6 | 13 | 10 | 12 | 8 | 18 | 7 | 7 |

Fol., follicular phase groups; Lut., luteal phase groups; ER, oestrogen receptor; PR, progesterone receptor.

comprised women who had no recorded date of last menstrual period (LMP) before primary surgery, irregular or infrequent periods (intercycle time less than 21 days or greater than 36 days), previous hysterectomy, recently taken oral contraceptive or hormonal therapy, or were pregnant or lactating at the time of surgery.

Ovulation was estimated to have occurred on the 13th day of the cycle. In establishing the phase of menstruation, we used the start date of LMP prior to the initial surgical procedure (biopsy) which provided a histological diagnosis, i.e. true cut needle biopsy, incision or excision biopsy but not fine needle aspiration, with follicular phase determined as days 1–12 postLMP, and luteal phase > 12 days postLMP. The patients were divided into eight groups (Table 1)—ER positive (ER+): follicular phase 15 patients, luteal phase 21 patients; ER negative (ER−): follicular phase 20 patients, luteal phase 28 patients, PR+: follicular phase 20 patients, luteal phase 28 patients, PR−: follicular phase 14 patients, luteal phase 20 patients.

The menstrual phase classification used in this study is marginally different from that adopted by Badwe *et al.* [2] who divided the patients into two groups (days 3–12 postLMP and days 1, 2 and > 12 postLMP). Our classification permitted the inclusion of an additional 7 patients, who were documented to be menstruating at the time of surgery, in the follicular phase group (days 1–12 postLMP).

ER and PR status was assayed on the cytosol obtained from the tumour tissue using the dextrose-coated charcoal method [13]. Receptor levels were considered positive when the specific binding was equal to 10 femtomoles/mg protein or more. Values less than 10 were considered receptor negative.

DFS was defined as the period from the data of diagnosis to the date of first distant metastasis; regional nodal recurrence was included in this category. OS was defined as the period from date of diagnosis to last follow-up or death.

*Statistical methods*

OS and DFS were estimated using the Kaplan–Meier [14] technique. Survival curves were compared using the log rank statistic. Multivariate analysis was obtained by allowing for stepwise entry of potential prognostic factors to DFS and OS into a Cox proportional hazards model. These included age at diagnosis, tumour size, grade, nodal status, number of positive nodes, ER and PR status, definitive surgery (mastectomy/breast conservation) and systemic adjuvant therapy. There was no evidence to indicate that any factor failed to satisfy the assumption of proportional hazards.

## RESULTS

OS and DFS of the 84 study patients, with known LMP data and known ER/PR status who were included in this analysis,

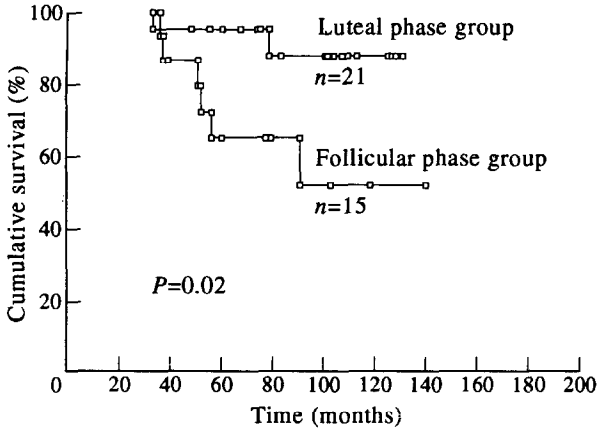


**Figure 1. Overall survival of ER+ patients comparing those with last menstrual period (LMP) 1–12 days before operation (follicular phase group) versus LMP > 12 days (luteal phase group).**

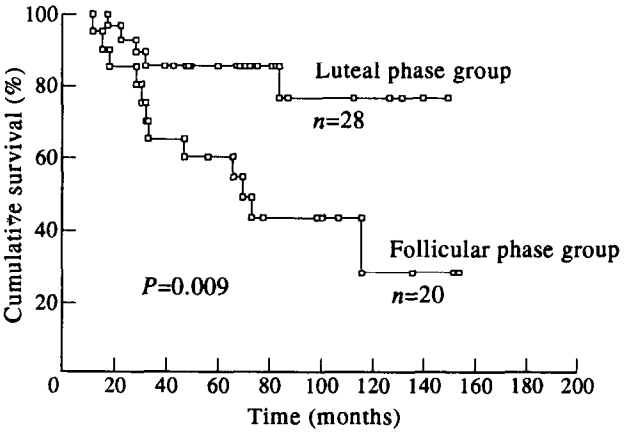


**Figure 2. Overall survival of ER− patients comparing those with last menstrual period (LMP) 1–12 days before operation (follicular phase group) versus LMP > 12 days (luteal phase group).**

did not differ significantly from the other 186 premenopausal patients who were excluded because of incomplete data.

There was no significant difference in the distribution of the conventional prognostic factors between the follicular and luteal menstrual phase groups, using Fisher's exact test (Table 1).

At 10 years, OS of ER+ patients who had their biopsy during the follicular phase was significantly poorer than for those whose biopsy was performed during the luteal phase of their menstrual cycle (52 versus 88%, $P = 0.02$) (Figure 1). OS for the ER− follicular phase group was also significantly poorer than that for the ER− luteal phase group (33 versus 76%, $P = 0.009$) (Figure 2). The OS difference between the PR+ follicular phase group and PR+ luteal phase group was of borderline significance (60 versus 87%, $P = 0.06$) (Figure 3), while the difference in OS between the PR− follicular phase group and that of the PR− luteal phase group was highly significant (13 versus 76%, $P = 0.001$) (Figure 4). DFS for the ER+ follicular phase group was not significantly different ($P = 0.12$) from the ER+ luteal phase group, while DFS for the ER− follicular phase group compared to the ER− luteal group was significantly worse ($P = 0.01$). Similarly, the DFS difference between menstrual phases for the PR+ patients was of borderline significance (0.09), and for the PR− patients, the difference in DFS between the two phases was highly significant ($P = 0.009$).

Multivariate analysis of the potential prognostic factors showed the follicular menstrual phase ($P = 0.0002$ for OS and 0.0003 for DFS) and PR− status ($P = 0.004$ for OS and 0.012 for DFS) to be significant adverse factors for OS and DFS. Nodal status ($P = 0.07$) and histological grading ($P = 0.08$) were borderline significant factors for OS. Age at diagnosis, tumour size, grade, number of positive nodes, ER status, definitive surgery and systemic adjuvant therapy were not significant prognostic factors.

## DISCUSSION

Our findings demonstrate that the influence of menstrual phase on survival is at least as important in patients with receptor-negative tumours as in those with receptor-positive tumours. These findings are in agreement with Badwe *et al.* [2], who found that the difference in both OS and DFS applied equally to those with ER+ and ER− tumours.

The dextrose-charcoal assay was used to determine ER and PR status. Since this conventional biochemical assay measures only unbound receptor present in the cytosol preparation, measurements performed in situations where there are high

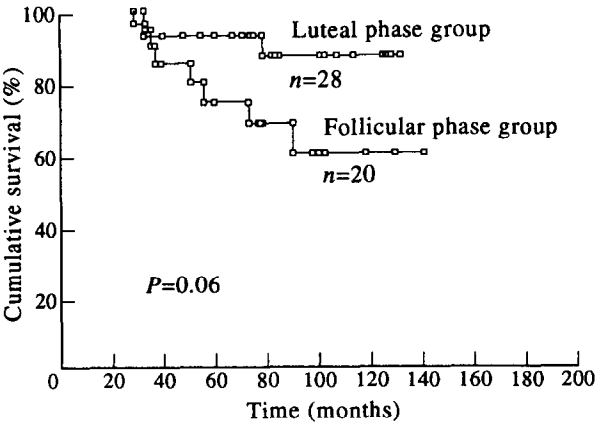


**Figure 3. Overall survival of PR+ patients comparing those with last menstrual period (LMP) 1–12 days before operation (follicular phase group) versus LMP > 12 days (luteal phase group).**

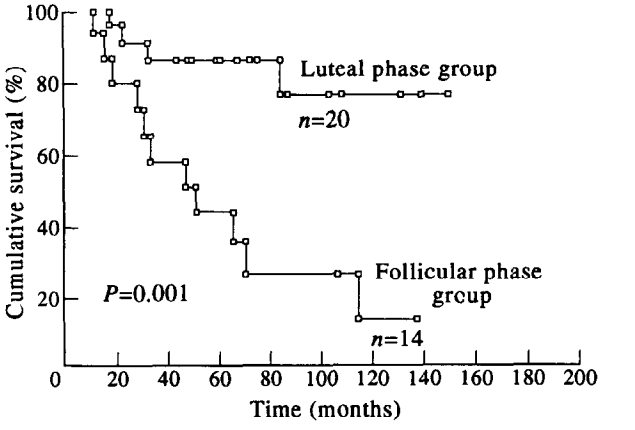


**Figure 4. Overall survival of PR− patients comparing those with last menstrual period (LMP) 1–12 days before operation (follicular phase group) versus LMP > 12 days (luteal phase group).**

circulating hormone levels may be falsely negative. Under these conditions, receptors may be occupied by hormone and hence unavailable to the ligand used in the routine assay.

We considered whether inappropriate assignment of receptors might explain our results, since PR status is an independent prognostic factor and ER status shows a similar trend. Endogenous progesterone, occupying receptors during luteal phase, might result in a false negative PR status. Survival of the PR− luteal (but not follicular) group could have been artefactually improved, if some PR+ luteal patients had been erroneously included. However, the distribution of ER− and PR− patients and values was similar in both menstrual phases. In addition, ER− and PR− patients operated on during the luteal phase tended to do better than ER+ and PR+ women who had their surgery during the follicular phase [76 versus 58% for ER, 76 versus 60% for PR ($P$ = ns)]. Furthermore, the menstrual phase at the time of surgery (LMP) had significantly more prognostic value ($P$ = 0.0002 for OS and 0.0003 for DFS) than PR status ($P$ = 0.004 for OS and 0.012 for DFS). Therefore, false assignment of receptor status is an unlikely confounding factor in our results.

All the patients who died developed metastatic disease before death. Breast cancer mortality is related to the capacity of tumour cells to invade and metastasise. In order to metastasise, cancer cells must successfully complete a number of distinct steps, including degradation of the basement membrane and local invasion of surrounding stroma. The tumour must also penetrate the vascular or lymphatic system, survive in the circulation, arrest in the capillary bed of a distant organ, extravasate and proliferate at the new site [15]. Extracellular matrix proteolysis is a highly complicated process involving abnormal expression of proteinases such as cathepsins, collagenases and plasminogen activators in both the tumour cells [16] and host cells [17–19]. The net proteolytic activity is determined by the balance between these enzymes and their inhibitors [20] also secreted by the tumour and normal host cells including fibroblasts, macrophages and monocytes [17]. It is, therefore, conceivable that host cells themselves play a significant role in the degradation of extracellular matrix during tumour invasion.

Surgical manipulation of cancerous tissues may disseminate cells, contributing further to those tumour cells already shed before surgery in some patients. In addition, surgery activates a physiological inflammatory response, associated with increased vascular permeability and reduction in interstitial pH with increased proteolytic enzyme activation [21]. Tumour cell invasion and metastasis may be facilitated further under these conditions.

One of the mechanisms through which the menstrual phase at the time of surgery influences survival may be related to the biological effects of oestrogen and progesterone on receptor-positive cells. Oestrogen stimulates the release of cathepsin D and plasminogen activators (uPA) from breast cancer cells [22–23]. Active uPA converts plasminogen to plasmin which both directly degrades certain matrix proteins and indirectly activates latent collagenases, which subsequently degrade the intercellular collagen matrix [16]. Zajchowski *et al.* [24] reported that cathepsin D expression in non-malignant ER-transfected human mammary epithelial cell line is enhanced by oestrogen. If this occurs in normal human cells, it may partly account for the poor survival of patients with receptor-negative tumour, when surgery is carried out under the influence of unopposed oestrogen. Progesterone, secreted during the luteal phase, may counteract the adverse effects of oestrogen by stimulating the release of β-hydroxy-steroid dehydrogenase [25] which inactivates oestrogen. Progesterone also down regulates ERs [26].

Proteolytic enzyme inhibitors are also under hormonal control. Oestrogen downregulates the natural inhibitors of proteolysis such as antithrombin III [27]. Casslen *et al.* [28] demonstrated that human stromal cells in culture secrete plasminogen activator inhibitor (PAI-1), and that its secretion is stimulated by progesterone. PAI-1 is synthesised by several cultured cell types, e.g. human fibroblasts and endothelium [29–30] and is also associated with the matrix. It may, therefore, serve to neutralise the PA released from adjacent cells or derived from the circulation. Marbaix *et al.* [31] in a study of cultured explants of human endometrium, reported that physiological concentrations of progesterone (10–200 nM, similar to luteal phase levels), almost abolished the release of collagenase and total gelatinase activity. These effects were inhibited by mifepristone, a progesterone antagonist.

The possible involvement of the host normal cells in the mechanism by which the hormonal milieu at the time of surgery influences the metastatic process and thereby long-term survival, may explain the differences in survival of women with receptor-negative tumours operated on during different phases of their menstrual cycle. These findings have profound clinical implications, as they suggest that the influence of the menstrual phase at the time of surgery may not be exclusive to breast cancer, but may also influence survival in other cancers. We recommend that menstrual data be documented prospectively for all premenopausal women undergoing surgery for any cancer.


1. Jensen EV, Block GE, Smith S, *et al.* National Cancer Institute, monograph 1971, **34**, 55–70.
2. Badwe RA, Gregory WM, Chaudary MA, *et al.* Timing of surgery during menstrual cycle and survival of premenopausal women with operable breast cancer. *Lancet* 1991, **337**, 1261–1264.
3. Senie RT, Rosen PP, Rhodes P, *et al.* Timing of breast cancer excision during the menstrual cycle influences duration of disease-free survival. *Ann Intern Med* 1991, **115**, 337–342.
4. Badwe RA, Fentiman IS, Saad Z, *et al.* Surgical procedures, menstrual cycle phase, and prognosis in operable breast cancer. *Lancet*, 1991, **338**, 815–816.
5. Ratajczak HV, Sothern RB, Hrushesky WJM. Estrous influence on surgical cure of mouse breast cancer. *J Exp Med* 1988, **168**, 88–96.
6. Hrushesky WJM, Bluming AZ, Gruber SA, *et al.* Menstrual influence on surgical cure of breast cancer. *Lancet* 1989, **ii**, 949–953.
7. Spratts J, Zirnhald, Yancy J. Breast detection demonstration project data can determine whether the prognosis of breast cancer is affected by the timing of surgery during the menstrual cycle. *J Surg Oncol* 1993, **53**, 4–9.
8. Rageth JC, Wyss P, Unger C, *et al.* Timing of breast cancer surgery within the menstrual cycle: influence of lymph-node involvement, receptor status, postoperative metastatic spread and local recurrence. *Ann Oncol* 1991, **2**, 269–272.
9. Powles TJ, Ashley SE, Nash AG. Timing of surgery for breast cancer and menstrual cycle. *Lancet* 1991, **337**, 1604.
10. Sainsbury R, Jones M, Parker D, *et al.* Timing of surgery for breast cancer and menstrual cycle. *Lancet* 1991, **338**, 391–392.
11. Goldhirsch A, Gelber R, Forbes J. Timing breast cancer surgery (letter). *Lancet* 1991, **338**, 691–692.
12. Saad Z, Bramwell V, Duff J, *et al.* Timing of surgery in relation to menstrual cycle in premenopausal women with operable breast cancer. *Br J Surgery* 1994, **81**, 217–220.
13. Menendez-Botet CJ, Nisselbaum JS, Fleisher M, *et al.* Correlation between estrogen receptor protein and carcinoembryonic antigen in normal and carcinomatous human breast tissue. *Clin Chem* 1976, **22**, 1366–1371.
14. Lee ET. *Statistical Methods for Survival Data Analysis.* Belmont, California, Life-time Learning Publications, 1980.

15. Nicolson GL. Molecular mechanisms of cancer metastasis: tumor and host properties and the role of oncogenes and suppressor genes. *Curr Opinion Oncol* 1991, **3**, 75–92.
16. Tryggvason K, Hoeyhtae M, Salo T, *et al.* Proteolytic degradation of extracellular matrix in tumor invasion. *Biochem Biophys Acta* 1987, **907**, 191–217.
17. Pepper MS, Vassalli J-D, Monterano R, *et al.* Urokinase-type plasminogen activator is induced in migrating capillary endothelial cells. *Cancer Res* 1988, **48**, 1270–1278.
18. Garcia M, Salazar-Retana G, Pages A, *et al.* Distribution of the Mr 52.000 estrogen-regulated protein in benign breast diseases and other tissues by immunohistochemistry. *Cancer Res* 1986, **46**, 3734–3738.
19. Werb Z, Mainardi C, Vater CA, *et al.* Endogenous activation of latent collagenase by rheumatoid synovial cells: evidence for a role of plasminogen activators. *N Engl J Med* 1977, **296**, 1017–1023.
20. Liotta LA, Steeg PS, Stetler-Stevenson WG. Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. *Cell* 1991, **64**, 327–336.
21. Madden JW, Arem AJ. Wound healing: biologic and clinical features. In DC Jr Sabiston, ed. *Textbook of Surgery*. Philadelphia, WB Saunders Co, 1991, 164–177.
22. Butler WB, Kirkland WL, Jorgensen TL. Induction of plasminogen activator in human breast cancer cell line (MCF-7). *Biochem Biophys Res Commun* 1979, **90**, 1228–1334.
23. Westley B, Rochefort H. Estradiol induced proteins in the MCF-7 human breast cancer cell line. *Biochem Biophys Res Commun* 1979, **90**, 410–416.
24. Zajchowski DA, Sager R. Induction of estrogen-regulated genes differs in immortal and tumorigenic human mammary epithelial cells expressing a recombinant estrogen receptor. *Mol Endocrinol* 1991, **5**, 1613–1623.
25. Jarvis PM, Kuttenn F, Gompi A. Estradiol and progesterone interaction in normal and pathological breast cells. Endocrinology of breast: basic and clinicl aspects. *Ann NY Acad Sci USA* 1980, **464**, 152–167.
26. Takeda A, Leavitt W. Progestin-induced down regulation of nuclear estrogen receptor in uterine decidual cells: analysis of receptor synthesis and turnover by the density-shift method. *Biochem Biophys Res Commun* 1986, **135**, 98–104.
27. Ball AP, McKee PA. Fibrin formation and dissolution in women receiving oral contraceptive drugs. *J Lab Clin Med* 1977, **89**, 751–762.
28. Casslen B, Urano S, Ny T. Progesterone regulation of plasminogen activator inhibitor (PAI-1) antigen and mRNA levels in human endometrial stromal cells. *Thrombosis Res* 1992, **66**, 75–87.
29. Sawa H, Fujii S, Sobel B. Augmented arterial wall expression of type-1 plasminogen activator inhibitor induced by thrombosis. *Arteriosclerosis and Thrombosis* 1992, **12**, 1507–1515.
30. Pollanen J, Saksela O, Salonen E, *et al.* Distinct localization of u-PA and its type-1 inhibitor in cultured human fibroblast and sarcoma. *J Cell Biol* 1986, **104**, 1085–1096.
31. Marbaix E, Donnez J, Courtoy PJ, *et al.* Progesterone regulates the activity of collagenase and related gelatinases A and B in human endometrial explants. *Proc Natl Acad Sci USA* 1992, **89**, 11789–11793.

**Acknowledgements**—We thank John Mackay from the Steroid Lab, Victoria Hospital for his excellent technical help and our librarian at the LRCC Emelia Kresk for her relentless efforts in the literature search.

Pergamon

*European Journal of Cancer* Vol. 30A, No. 9, pp. 1352–1359, 1994

0959-8049/94 $7.00+0.00

0959-8049(94)00230-4

# Evidence for a Growth Effect of Epidermal Growth Factor on MDA-MB-231 Breast Cancer Cells

N. Veber, G. Prévost, P. Planchon and A. Starzec

**MDA-MB-231 is a breast epithelial cell line which possesses large amounts of epidermal growth factor (EGF) receptor on its cell surface but does not respond to EGF under standard culture conditions. 8-bromo-cyclic AMP (8Br-cAMP) and cholera-toxin treatments inhibit its growth by increasing its intracellular cAMP level. However, when inhibited in this way, MDA-MB-231 remains unresponsive to EGF. Similar effects—cAMP accumulation and inhibition of cell growth—are produced by forskolin. In addition, this substance specifically blocks MDA-MB-231 cells in G1 phase of the cell cycle. EGF is able to reverse the effect of forskolin on cell proliferation and prevents accumulation of cells in G1 phase without any change of cAMP level. Thus, only when inhibiting cell growth with forskolin does a mitogenic effect of EGF become evident. As cAMP is increased to a similar degree by all three compounds, yet only the effect of forskolin is antagonised by EGF, we suggest that a non-cAMP-mediated effect of forskolin must be considered to explain this effect. In contrast, the mitogenic effect of EGF on the NPM14T4/9 breast epithelial cell line does not change in the presence of forskolin.**

**Key words: EGF receptor, forskolin, cAMP, breast carcinoma cell line**
*Eur J Cancer*, Vol. 30A, No. 9, pp. 1352–1359, 1994

## INTRODUCTION

EPIDERMAL GROWTH factor (EGF) is potentially mitogenic for a variety of cultured cells, including mammary epithelial cells. It exerts its activity through specific membrane receptors (EGFR) in both normal and tumour cells [1]. In breast tumours, EGFR are found in various amounts [2] and high levels are usually associated with a poor prognosis [3]. That is, primary breast tumours with larger amounts of EGFR are more likely to progress in malignancy than those with lower amounts. Indeed, large amounts of EGFR are more frequently found in lymph node metastases than in primary tumours suggesting that the presence of EGFR may be associated with the metastatic phenotype [4]. Further, tumour relapse appears even more frequently in cases with many EGFR and few oestrogen receptors [5, 6].