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PROGESTIN-INDUCED FATTY ACID SYNTHETASE IN HUMAN MAMMARY TUMORS: FROM MOLECULAR TO CLINICAL STUDIES

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Summary—Fatty acid synthetase (FAS) is one of the first well-characterized progestin-induced proteins with available antibodies and cDNA. This paper reviews basic studies on FAS regulation in human breast cancer cell lines and recent data on the possible clinical significance of this new marker of hormone responsiveness in mammary cancer and benign breast diseases.

INTRODUCTION

Progesterone and estrogen play an important role in the control of growth, differentiation and function of mammary epithelial cells [1]. In carcinogenesis however, while the role of estrogen is clear, that of progesterone and progestin is more debated, mostly in human [2]. While progestin appears to be efficient in treating breast cancers, its effect on the proliferation of normal mammary cells and on mammary cancer incidence remains controversial since both a protective effect of progesterone in inhibiting the stimulatory effect of estrogen and a synergistic effect have been proposed. Moreover, there is a clinical need for markers of progestin responsiveness since, at present, the major progestin regulated human gene which has been studied, the progesterone receptor, is down regulated by progestin and up regulated by estradiol. In order to define markers positively regulated by progestin in human, using a differential approach, we have searched proteins and mRNA whose accumulation is increased by progestin in progesterone receptor positive human metastatic breast cancer cell lines (MCF7, T47D, ZR75-1).

FATTY ACID SYNTHETASE (FAS): A NEW PROGESTIN-RESPONSIVE GENE

FAS appears to be the most abundant cellular progestin-induced protein in human breast cancer cell lines. In fact, the two approaches we used to define human progestin-regulated responses (analysis of proteins and differential screening of a cDNA library) have pointed to the same progestin-induced gene corresponding to a protein of 250 kDa [3, 4]. This protein is now defined as human FAS [5]. In vertebrates, FAS contains two identical subunits, each with M_w 250,000. Both of them are multienzyme proteins containing domains for the acyl-carrier-peptide and the seven different catalytic activities required for the conversion of acetyl-CoA and malonyl-CoA into fatty acid [6].

FAS is responsible in all tissues for *de novo* long chain fatty acid synthesis. In MCF7 cells incubated with the synthetic progestin R5020, enhanced FAS activity is followed by an increased synthesis of fatty acids especially those contained in the triacylglycerols storage lipids (8-fold increase) [7]. Phosphoglyceride membrane components, phosphatidylcholine, phosphatidylserine, phosphatidylinositol and phosphatidylethanolamine are also increased around 2-fold. Under these culture conditions, however, progestins by themselves have no effect on cell proliferation, but inhibit estradiol-induced cell growth [8]. In T47D cells, increased lipid synthesis anticipates the accumulation of lipid droplets [7] in agreement with previous work [9].

We have developed rabbit polyclonal antibodies against human FAS. These antibodies are monospecific based on Western immunoblots, staining extinction by purified FAS and protein A-Sepharose immunoprecipitation of [^{35}S]methionine labeled cell extracts [4, 10]. They have been specially useful in identifying FAS and have allowed us, with the 2kb Pg8 cDNA clone corresponding to the 3′ end of the 8kb FAS mRNA [4], to study FAS regulation and to

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quantify its expression in biopsies of human progestin-responsive tissues.

REGULATION OF HUMAN FAS IN BREAST CANCER CELLS IS COMPLEX

The FAS protein and mRNA are specifically accumulated in breast cancer cells following incubation with progestin or higher concentrations of dihydrotestosterone (DHT) [3, 4]. Both hormones appear to induce FAS via interaction with the progesterone receptor (PR) since the effect of R5020 and DHT is not inhibited by the antiandrogen flutamide, but inhibited by antiprogestin RU486. Glucocorticoids have no effect on FAS expression in these cells. FAS was induced by R5020 in all the PR-positive breast cancer cell lines studies. Its constitutive level was variable in PR-negative breast cancer cell lines. It was low in BT20 and MDA-MB231 cells but identical to the induced level in the HBL100 cell line derived from cells collected in human milk [11] and in SKBR-3 cells where FAS constitutes up to 28% by weight of cytosolic proteins [12]. In the liver, FAS was shown to be induced by insulin and thyroid hormone and inhibited by cAMP [13, 14]. In breast cancer cell lines, FAS mRNA level was slightly increased (2-fold) by insulin growth factor-1 (IGF-1) and insulin (Fig. 1). This last result is in agreement

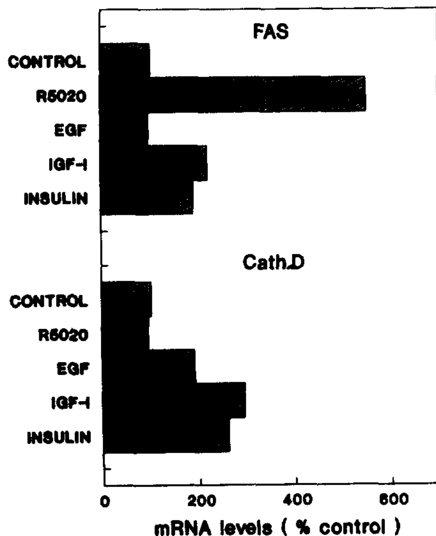

Fig. 1. Effect of growth factors and insulin on FAS mRNA accumulation. Steroid-stripped MCF7 cells [3] were incubated in the presence of 1 nM R5020, 8 nM EGF, 5 nM IGF-1, 50 nM insulin or vehicle (control) for 24 h. Total RNA was analyzed as described previously [4] and hybridized with FAS cDNA, cathepsin D cDNA (as a positive control) and 36B4 cDNA probes. Densitometric scanning of FAS and cathepsin D mRNA levels was corrected for slight variations in the levels of constant 36B4 RNA.

with previous work demonstrating an increased synthesis of fatty acid in MCF7 cells [15]. Epidermal growth factor (EGF) had no effect in the EGF-responsive MCF7 cell line. We did not detect any effect of 3,5,3′-triiodohyronine (T3) on FAS expression in these cells. Activation of protein kinase C (TPA) or protein kinase A (cAMP, forskolin) was also unable to modulate FAS concentration (D. Chalbos, unpublished).

Progestin-induced FAS protein synthesis (5 to 10-fold) appears to be secondary to the accumulation of FAS mRNA (5 to 10-fold) [4]. FAS mRNA accumulation results from a dual transcriptional and post-transcriptional mechanism [11]. Progestin-induced FAS gene transcription appears to be a primary effect of the hormone–receptor complex since it is still detectable in the presence of protein synthesis inhibitors. Two potential progesterone-responsive elements have been described recently by Amy *et al.* [16] in the first intron of the rat FAS gene supporting this result. However, FAS mRNA accumulation does not only result from increased gene transcription but also from increased mRNA stabilization by progestin [11]. In fact, FAS mRNA half-life was increased approx. 3 to 4-fold in R5020-treated cells as compared to control cells.

The antiprogestin RU486 has a dissociated effect on these two levels of regulation [17]. It inhibits R5020-induced FAS transcription and when added alone causes decreased FAS transcription as compared to the control untreated cells. This negative effect of the antagonist receptor complex could suggest that the PR was still occupied by an agonist ligand resistant to the withdrawal step of the cells. This hypothesis is however unlikely because of the rapid progesterone metabolism in cell culture. Another hypothesis would be that the PR, in the absence of ligand, would have some stimulatory activity on gene transcription as recently described for the estrogen receptor [18]. Finally, the RU486–PR complex might interfere with other transcription factors also involved in stimulating FAS transcription. Surprisingly, RU486 was as efficient as R5020 in increasing the half-life of FAS mRNA. FAS mRNA stabilization, the mechanism of which is unknown, is rapid. The addition of RU486 during the chase by RNA synthesis inhibitors of after short cell labeling with [^{3}H]uridine, methods used to measure mRNA half-life, was sufficient to stabilize the mRNA. This suggests that *de novo* synthesis of additional regulatory factors is not

required. The opposite effects of RU486 on FAS gene transcription and FAS mRNA stability suggest that either different thresholds of receptor activation or different domains of the PR protein would be required in these two regulatory steps. As the balance of these 2 effects allows delayed accumulation of FAS mRNA, these results show that some agonist effects of RU486 following long term treatment could result from mRNA stabilization.

FAS IS REGULATED BY PROGESTIN *IN VIVO* IN NORMAL CELLS

In order to know whether FAS is regulated by progestin in normal cells as it is in breast cancer cell lines, we studied its expression in normal human mammary gland and endometrium [10, 19]. FAS gene expression in human biopsy specimens was measured using two different techniques: *in situ* hybridization with antisense FAS mRNA and immunohistochemistry with rabbit polyclonal antibodies. Semi-automatic quantification of FAS mRNA [20] and protein levels [10] in tissue sections was performed using computerized image analyzers (IMSTAR and SAMBA).

In normal mammary tissue (adjacent to non-proliferative benign breast lesions collected by biopsy) FAS immunostaining was restricted to epithelial cell cytoplasm and no labeling was detected in surrounding connective tissue. It was approx. 2-fold more elevated in lobular than in ductal structures. In the 2 structures FAS expression was regulated by progestin since it increased in the luteal phase of the menstrual cycle and during progestin treatment [10]. PR, measured in serial sections, appeared to be down-regulated during the menstrual cycle, as reported for the endometrium [21, 22]. We do not know the physiological meaning of the FAS induction by progesterone which therefore appears to occur in normal mammary glands as in breast cancer. In mammary glands, high lipid synthesis occurs at the onset of lactation. It is consistent that increased FAS expression was detected in lobules rather than in ducts since milk fat is mainly synthesized in lobulo-alveolar structures. In the lactating rat, FAS mRNA level, which is low during the first days after parturition, increases until day 8 and finally returns to basal levels at the end of feeding [23]. This rise in FAS mRNA levels cannot be due to progesterone since it corresponds to a marked decrease in circulating progesterone levels at parturition. Increased FAS during lactation appears rather to be due to the synergistic effect of insulin, glucagon and glucocorticoids and is classically considered to be inhibited by progesterone [24]. In several animal species progesterone treatment prevents the initiation of milk secretion [25]. It has also been reported to decrease FAS in pseudopregnant rabbit mammary alveolar explants [25]. We have not evaluated whether progestin-induced FAS expression in normal non-lactating mammary gland is, as in breast cancer cells, followed by increased lipid synthesis.

In the endometrium, FAS mRNA levels were found to be much lower than in mammary glands. It was detected in epithelial cells and to a lower extent in stromal cells [20]. However FAS mRNA levels were also increased in the luteal phase of the menstrual cycle which suggests FAS induction by progestin. The low expression of FAS in the endometrium limits its clinical interest as a marker of progestin responsiveness in this tissue.

FAS: A MARKER IN BREAST CANCERS AND BENIGN BREAST DISEASES?

While several markers of response to estrogen, such as the PR [26], cathepsin-D [27] and pS2 [28], are being used as prognostic markers in breast cancer, the major marker of response to progestin that has been studied is the PR, which is down-regulated by progesterone and induced by estrogen [26]. One advantage of FAS as a marker of progesterone responsiveness, compared to the PR, is that it is positively regulated. FAS is a well-characterized progestin-induced protein with available probes (antibodies and cDNA) that can be used in clinical studies to evaluate its prognostic value in hormone dependent tumors. In an attempt to specify the potential interest of this marker we have initiated clinical studies in breast cancer and benign mastopathies [29]. The FAS gene expression was measured in frozen sections from human biopsies by *in situ* hybridization with antisense FAS mRNA.

In tumoral tissues, as in normal mammary cells, FAS mRNA and protein were mostly expressed in epithelial cells and not in connective tissue. The overexpression of this gene previously described in hormone dependent breast cancer cell lines was confirmed in the 27 breast cancers we studied, most of them being ductal adenocarcinomas (Fig. 2). An

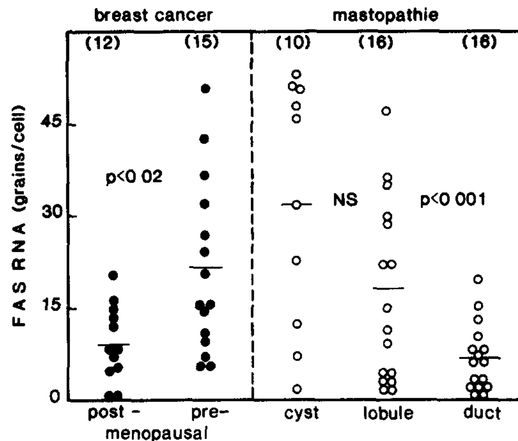

Fig. 2. FAS RNA levels in human epithelial mammary cells. FAS RNA levels were quantified as described previously [29]. P according to non-parametric Wilcoxon rank sum test (two-tailed). Menopausal status was arbitrarily defined according to the age of the patients (< 50 years).

up to 20-fold overexpression on average was measured in cancer cells when compared to endometrium epithelial cells. FAS expression, however, greatly varied from one breast cancer biopsy to another from 1 to 51 silver grains per cell. We found no correlation with classical prognostic parameters such as node invasiveness and estrogen receptor PR level or status (Table 1). High levels of FAS mRNA were also measured in some estrogen receptor-negative tumors suggesting that factors other than progesterone might induce FAS in breast cancer. The absence of correlation with the PR level or status is not surprising since the two markers regulated in opposite direction by progesterone, are unlikely to be present at the same time. When progesterone acts at luteal phase, one marker (FAS) is expected to be increased, while the other (RP) is decreased. A correlation was found between FAS expression and patients' age (Table 1 and Fig. 2) which is consistent with its induction by progesterone since FAS RNA levels were significantly higher in premenopausal patients than in post-menopausal patients who no longer secrete progesterone. In addition, FAS RNA concentrations in premenopausal patients correlated with the degree of differentiation of the tumor; the highest levels were found in well-differentiated cancers. Independently, Wysocki *et al.* [30] have studied FAS mRNA levels by Northern blotting in 87 breast cancers. They found, in agreement with our study, no correlation between increased levels of FAS mRNA in tumors and estrogen receptor and PR status, but a significant increase of FAS expression in breast cancers from premenopausal compared to post-menopausal patients.

We conclude, from these 2 pilot studies, that FAS appears to be a marker of differentiation and of progestin responsiveness in ductal invasive breast cancer rather than a marker of cell proliferation. The fact that FAS mRNA concentrations increased proportionally to the density of MCF7 cells and was maximal in non-dividing cells [11] also supports this idea. FAS might therefore be useful as a marker of good prognosis or of efficiency of progestin therapy, however no data are yet available on the clinical evolution of the patients in which FAS was assayed. Clearly more clinical studies should be performed.

FAS expression has also been quantified in benign breast diseases [29] in an attempt to evaluate its predictive value for detection of high risk mastopathies. Higher levels of FAS mRNA were found in some cysts and lobules as compared to ductal structures (Fig. 2). These results have since been confirmed by immunohistochemistry (Joyeux *et al.*, unpublished results). In lobular structures, FAS levels correlated with the degree of proliferation quantified by *in situ* hybridization with the H4 histone probe, and estimated by histological examination. This correlation might support the findings of Anderson [31] and others [32] indicating that progestin might be mitogenic, since the highest thymidine labeling are found in the luteal phase. Possible mechanisms could be that increased FAS catalyzes increased synthesis of either membrane phospholipids or mitogenic lipids [33]. It may also be in agreement with the significance of FAS in breast cancer if one assumes that FAS is a marker of lobular proliferation and that adeno-carcinoma are mostly derived from ductal structures.

To conclude, in contrast to the estrogen-induced cathepsin D, whose level is high in

Table 1. FAS RNA expression in breast cancer correlation with receptor and menopause status

	FAS RNA in 27 breast cancers		
	Number of patients	FAS RNA (grains/cell) Mean ± SD	
ER+ PR+	14	15 ± 14	Pre-menopausal
ER− PR−	8	17 ± 12	21 ± 14
ER+ PR−	3	19 ± 12	Post-menopausal
ER− PR+	2	11 ± 14	9 ± 6
			$P < 0.02$

FAS RNA levels were quantified by *in situ* hybridization as described previously [29] P according to non-parametric Wilcoxon rank sum test (two-tailed) Menopausal status was arbitrarily defined according to the age of the patients (< 50 years)

ductal hyperplasia [34], the progestin-induced FAS marker might be useful in the detection of lobular hyperplasia. Extensive studies and a clinical follow-up of the patients are however required before a conclusion may be reached concerning the clinical interest of FAS as a marker for detection of high risk breast diseases at early steps of carcinogenesis.

CONCLUSIONS

Basic cellular and molecular level studies in human breast cancer cell lines have demonstrated the progestin regulation of FAS and enabled the isolation of specific molecular probes to quantify FAS expression in human biopsies. Initial clinical studies show that FAS is regulated by progestin *in vivo* in mammary glands and endometrium. In addition to its potential interest as a marker of progestin responsiveness, FAS may be useful as a differentiation marker in breast cancer and as a proliferation marker in lobular mastopathies. These encouraging pilot studies should however lead to more extensive studies with a follow up of the patients to confirm the clinical interest of FAS, as a tissue marker.

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REFERENCES

1. Haslam S. Z.: Role of sex steroid hormones in normal mammary gland function. In *The Mammary Gland: Development, Regulation and Function* (Edited by M. C. Neville and C. W. Daniel). Plenum Press, New York (1987) pp. 355–373.
2. Key T. J. A. and Pike M. C.: The role of oestrogens and progestagens in the epidemiology and prevention of breast cancer. *Eur. J. Clin. Oncol.* **24** (1988) 29–43.
3. Chalbos D. and Rochefort H.: A 250-Kilodalton cellular protein is induced by progestins in two human breast cancer cell lines MCF7 and T47D. *Biochem. Biophys. Res. Commun.* **121** (1984) 421–427.
4. Chalbos D., Westley B., May F., Alibert C. and Rochefort H.: Cloning of cDNA sequences of a progestin-regulated mRNA from MCF7 human breast cancer cells. *Nucleic Acids Res.* **14** (1986) 965–982.
5. Chalbos D., Chambon M., Ailhaud G. and Rochefort H.: Fatty acid synthetase and its mRNA are induced by progestins in breast cancer cells. *J. Biol. Chem.* **262** (1987) 9923–9926.
6. McCarthy A. D. and Hardie G.: Fatty acid synthetase an example of protein evolution by gene fusion. *Trends Biochem. Sci.* **9** (1984) 60–63.
7. Chambon M., Rochefort H., Vial H. and Chalbos D.: Progestins and androgens stimulated lipid accumulation in T47D breast cancer cells via their own receptors. *J. Steroid Biochem.* **33** (1989) 915–922.
8. Vignon F., Bardon S., Chalbos D. and Rochefort H.: Antiestrogenic effect of R5020, a synthetic progestin in human breast cancer cells in culture. *J. Clin. Endocr. Metab.* **56** (1983) 1124–1130.
9. Judge S. M. and Chatterton R. T. Jr: Progesterone-specific stimulation of triglyceride biosynthesis in a breast cancer cell line (T47D). *Cancer Res.* **43** (1983) 4407–4412.
10. Joyeux C., Chalbos D. and Rochefort H.: Effect of progestins and menstrual cycle on fatty acid synthetase and progesterone receptor in human mammary glands. *J. Clin. Endocr. Metab.* **70** (1990) 1438–1444.
11. Joyeux C., Rochefort H. and Chalbos D.: Progestin increases gene transcription and mRNA stability of fatty acid synthetase in breast cancer cells. *Molec. Endocr.* **4** (1989) 681–686.
12. Thompson B. J., Stern A. and Smith S.: Purification and properties of fatty acid synthetase from a human breast cell. *Biochim. Biophys. Acta* **662** (1981) 125–130.
13. Stapleton S. R, Mitchell D. A., Salati L. M. and Goodridge A. G.: Triiodothyronine stimulates transcription of the fatty acid synthetase gene in chick embryo hepatocytes in culture. *J. Biol. Chem.* **265** (1990) 18442–18446.
14. Paulauskis J. D. and Sul H. S.: Hormonal regulation of mouse fatty acid synthetase gene transcription in liver. *J. Biol. Chem.* **264** (1989) 574–577.
15. Monaco M. E. and Lippman M. E.: Insulin stimulation of fatty acid synthesis in human breast cancer in long term tissue culture. *Endocrinology* **101** (1977) 1238–1246.
16. Amy C. M., Williams-Ahlf B., Naggert J. and Smith S.: Molecular cloning of the mammalian fatty acid synthetase gene and identification of the promotor region. *Biochem. J.* **271** (1990) 675–679.
17. Chalbos D., Galtier F., Emiliani S. and Rochefort H.: The anti-progestin RU486 stabilizes the progestin-induced fatty acid synthetase mRNA but does not stimulate its transcription. *J. Biol. Chem.* **266** (1991) 8220–8224.
18. Tzuckerman M., Zhang X., Hermann T. and Wills K. N., Graupner G. and Pfahl M.: The human estrogen receptor has transcriptional activator and repressor functions in the absence of ligand. *New Biologist* **2** (1990) 613–620.
19. Escot C., Joyeux C., Mathieu M., Maudelonde T., Pages A., Rochefort H. and Chalbos D.: Regulation of fatty acid synthetase ribonucleic acid in the human endometrium during the menstrual cycle. *J. Clin. Endocr. Metab.* **70** (1990) 1319–1324.
20. Escot C., Le Roy X., Chalbos D., Joyeux C., Simonsen E., Daures J. P., Soussaline F. and Rochefort H.: Computer-aided quantification of RNA levels detected by *in situ*-hybridization of tissue sections. *Analyt. Cell. Path.* **3** (1991) 215–224
21. Garcia E., Bouchard P., Debrux J., Berdah J., Frydman R, Schaison G., Milgrom E. and Perrot-Applanat M.: Use of immunocytochemistry of progesterone and estrogen receptors for endometrial dating. *J. Clin. Endocr. Metab.* **67** (1988) 80–87.
22. Lessey B. A., Killam A. P., Metzger D. A., Maney A. F., Greene G. L. and McCarty K. S.: Immunohistochemical analysis of human uterine estrogen and progesterone receptors throughout the menstrual cycle. *J. Clin. Endocr. Metab.* **67** (1988) 334–340.

23. Braddock M. and Hardie D. G.: Cloning of cDNA to rat mammary gland fatty acid synthetase mRNA: evidence for the expression of two mRNA species during lactation. *Biochem J.* **249** (1988) 603–607.
24. Kuhn N. J.: The biochemistry of lactogenesis. In *Biochemistry of Lactation* (Edited by T. B. Mepham). Elsevier, The Netherlands (1983) pp. 351–370.
25. Martyn P. and Falconer I. R.: The effect of progesterone on prolactin stimulation of fatty acid synthesis, glycerolipid synthesis and lipogenic-enzyme activities in mammary glands of pseudopregnant rabbits, after explant culture or intraductal injection. *Biochem. J.* **231** (1985) 321–328.
26 Perrot-Applanat M., Groyer-Picard M. T., Lorenzo F., Jolivet A., Thu Vu Hai M., Spyratos F. and Milgrom E.: Immunocytochemical study with monoclonal antibodies to progesterone receptor in human breast tumors. *Cancer Res.* **47** (1987) 2652–2661.
27. Rochefort H., Capony F. and Garcia M.: Cathepsin D in breast cancer: from molecular and cellular biology to clinical application. *Cancer Cells* **2** (1990) 383–388.
28. Rio M. C., Bellocq J. P., Gairard B., Rasmussen U. B., Krust A., Koehl C., Calderoli H., Schiff V., Renaud R. and Chambon P.: Specific expression of the pS2 gene in subclasses of breast cancers in comparison with expression of the estrogen and progesterone receptors and the oncogene ERB2 *Proc. Natn. Acad. Sci. U.S.A.* **84** (1987) 9243–9247.
29. Chalbos D., Escot C., Joyeux C., Tissot-Carayon M. J., Pages A. and Rochefort H.: Expression of the progestin-induced fatty acid synthetase in benign mastopathies and breast cancer as measured by RNA *in situ* hybridization. *J. Natn. Cancer Inst.* **82** (1990) 602–606.
30. Wysocki S. J., Hahnel E., Wilkinson S. P., Smith V. and Hahnel R.: Hormone-sensitive gene expression in breast tumors. *Anticancer Res.* **10** (1990) 1549–1552.
31. Anderson T. J., Ferguson D. J. P. and Raab G. M.: Cell turnover in the "resting" human breast: influence of parity, contraceptive pill, age and laterality. *Br. J. Cancer* **46** (1982) 376–382.
32. Potten C. S., Watson R. J., Williams G. T., Tickles S., Roberts S. A., Harris M. and Howell A.: The effect of age and menstrual cycle upon proliferative activity of the normal human breast. *Br. J. Cancer* **8** (1988) 163–170.
33. Garcia M., Salazar-Retana G., Pages A., Richer G., Domergue J., Pages A. M., Cavalie G., Martin J. M., Lamarque J. L., Pau B., Pujol H. and Rochefort H.: Distribution of the Mr 52,000 estrogen-regulated protein in benign breast diseases and other tissues by immunohistochemistry. *Cancer Res.* **46** (1986) 3734–3738.
34. Imagawa W., Bandyopadhyay G. K., Wallage D. and Nandi S.: Phospholipids containing polyunsaturated fatty acyl groups are mitogenic for normal mouse mammary epithelial cells in serum-free primary cell culture. *Proc. Natn. Acad. Sci. U.S.A.* **86** (1989) 4122–4126.