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Regulation of Fatty Acid Synthetase by Progesterone in Normal and Tumoral Human Mammary Glands

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Progesterone and estrogens play an important role in the control of growth, differentiation and function of mammary epithelial cells. Their mechanism of action can be studied in human metastatic breast cancer cell lines (MCF7, T47D, ZR75-1...) that contain progesterone and estrogen receptors. We used this system to try to define progestin-regulated human genes which would permit to study progestin-regulation of gene expression in cell culture and to develop clinical markers of progestin-responsiveness. This paper summarizes our investigation of the progestin-regulated 250K protein, recently identified as human fatty acid synthetase (FAS).

Key words: Progestins, Fatty acid synthetase, Breast cancer.

Progestins inhibit estradiol-induced proliferation of human breast cancer cells (14) and have often been used in the treatment of advanced breast cancer (1). However, their effect on the growth of normal mammary glands and on mammary cancer incidence remains controversial. It is thus important to define progestin regulated human genes for developing clinical markers of progestin-responsiveness. This present paper summarizes our investigations on progestin-regulated fatty acid synthetase (FAS).

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FAS IS INDUCED BY PROGESTINS IN BREAST CANCER CELL LINES

Incubation of human breast cancer cells (MCF7, T47D, ZR75) with synthetic progestin R5020 results in the accumulation of an abundant, molecular weight 250.000, cellular protein (5). This protein has recently been identified as human fatty

acid synthetase: i. with R5020, there is a parallel increase in FAS activity and 250K protein synthesis; ii. 250K protein is immunoprecipitated by antibodies into FAS and iii. 250K protein antibodies immunoprecipitate FAS convalently labeled by pantothenate (3). We have developed rabbit polyclonal antibodies directed against human FAS from MCF7. These antibodies are monospecific as determined by protein A sepharose immunoprecipitation (6), western immunoblots and staining extinction by purified human FAS (10).

By differential screening of a cDNA library constructed from R5020-treated MCF7 cells, a cDNA clone (2kb insert), corresponding to the 3' end of FAS mRNA (8kb), has been isolated (6). FAS protein and mRNA are induced following incubation with progestin or higher concentrations of dihydrotesterone, both hormones acting via interaction with the progesterone receptor (5, 6). Their constitutive level is low in PR-negative breast cancer cell lines (BT20, MDA-MB231) (5, 11). The 5-fold increase in FAS mRNA level is identical to the increase in FAS protein synthesis, suggesting a lack of regulation at the translational level. By contrast, the mechanism of accumulation of FAS mRNA appears to be more complex. Protein synthesis inhibitors do not decrease progestin induction of FAS, suggesting a primary effect of the hormone on FAS gene expression. However, FAS mRNA accumulation with progestin results from both an increased transcription rate of the FAS gene and from FAS mRNA stabilization (11).

Initial clinical studies of FAS expression in progestin-responsive tissues

Along with the progesterone receptor which is down regulated by progesterone (13), FAS is one of the first well-characterized progestin-regulated proteins with available antibodies and cDNA. In an attempt to specify the potential importance of this marker, several breast cancer, benign mastopathy, endometrium and mammary gland clinical studies have been initiated. FAS gene expression in human biopsies was measured using two techniques: *in situ* hybridization with antisense FAS mRNA and immunohistochemistry with rabbit polyclonal antibodies. We used computerized image analysers (IMSTAR and SAMBA) to quantify FAS mRNA and protein levels in tissue sections.

a) *FAS expression in human breast diseases.* — In mammary glands, the FAS gene appears to be expressed in epithelial cells, whereas no labeling has been detected in connective tissues. In benign mastopathies, a high level of FAS RNA was found in some lobules and cysts. In lobular structures, the FAS RNA level was correlated with the degree of proliferation quantified by *in situ* hybridization with the H4 histone probe, and estimated by histological examination (4). In contrast to estrogen-induced 52K-cathepsin D, whose level is high in ductal hyperplasia (8), the progestin-induced FAS marker could be useful for detecting lobular hyperplasia. Extensive studies and a clinical follow-up of the patients are required before a conclusion may be reached concerning the clinical interest of FAS as a marker in detecting high risk mastopathies and as a possible means of prevention or early detection of breast cancer.

In primary breast cancer, no correlation was found between FAS RNA level and progesterone and estrogen receptor concentration or status in the 27 patients studied. However, the FAS RNA level was significantly higher in pre-menopausal patients than in post-menopausal patients who do not secrete progesterone. In addition, FAS RNA concentration in pre-menopausal patients was correlated with the degree of differentiation of the tumor;

the highest levels being found in well-differentiated cancers (4).

b) In vivo *progestin regulation of FAS.* — Since FAS expression varies depending on breast pathologies, we have chosen to study FAS regulation in «normal» tissues. In «normal» epithelial cells (adjacent to non-proliferative benign breast lesions collected by biopsy), FAS expression is clearly regulated by progestin: **i.** it increases from the follicular to the luteal phase of the menstrual cycle; **ii.** it increases during progestin treatment (10). Progesterone receptor, measured in serial sections, appears to be regulated during the menstrual cycle, as reported for the endometrium (9, 12). There are two advantages of FAS as a marker of progesterone responsiveness, compared to PR: **i.** it is positively regulated and **ii.** it is more homogeneously expressed in one given sample which enables it to be quantified in a few number of cells.

In endometrium, highest FAS RNA levels are found in the luteal phase of the menstrual cycle, except in patients with low plasma estradiol and progesterone concentrations, indicative of dysovulation, which also suggests FAS regulation by progestin (7). However, FAS expression is always very low, as compared to FAS expression in mammary glands which limits its clinical interest as a marker of progestin responsiveness in this tissue.

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 quantifying 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. The fact that FAS expression seems to be associated to less agressive breast cancers and to proliferative mastopathies which are known to increase breast cancer risk in benign mastopathies is still unclear. However, these results could reflect the fact that while progestins appear to be efficient in treating breast cancers, their effect on the proliferation of normal mammary cells is still controversial (2, 13). These pilot studies should lead to other more extensive clinical studies to specify the clinical interest of FAS, as a new marker.

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Resumen

La progesterona y los estrógenos juegan un papel importante en el control del crecimiento, diferenciación y función de las células epiteliales mamarias. Se puede estudiar su mecanismo de acción en las lineas celulares de cáncer de mama metabólico humano (MCF7, T47D, ZR75-1...) que contienen receptores de progesterona y estrógeno. Utilizamos este sistema para intentar definir los genes humanos regulados por la progestina que permitiría estudiar la regulación por la progestina de la expresión de los genes en el cultivo celular y desarrollar marcadores clínicos sensibles a la progestina. Este trabajo resume nuestra investigación de la proteína 250K regulada por la progestina, recientemente identificada como sintetasa de ácido graso humano (FAS).

Palabras clave: Progestinas, Sintetasa de ácidos grasos, Cáncer de mama.

References

1. Alexiena-Figusch, J. Van Gilse, H., Hop, W., Phoa, C., Bonde, V., Wijst, J. and Treurniet, R.: *Cancer*, 46, 2.369-2.372, 1980.
2. Anderson, T.: *Breast Cancer Res. Treat.*, 10, 65-66, 1987.
3. Chalbos, D., Chambon, M., Ailhaud, G. and Rochefort, H.: *J. Biol. Chem.*, 262, 9.923-9.926, 1987.
4. Chalbos, D., Escot, C., Joyeux, C., Carayon, M., Pages, A., Rochefort, H.: *J. Natl. Cancer Inst.* in press, 1990.
5. Chalbos, D. and Rochefort, H.: *Biochem. Biophys. Res. Commun.*, 121, 421-427, 1984.
6. Chalbos, D., Westley, B., May, F., Alibert, C. and Rochefort, H.: *Nucleic Acids. Res.*, 14, 965-982, 1986.
7. Escot, C., Joyeux, C., Mathieu, M., Maudelonde, T., Pages, A., Rochefort, H. and Chalbos, D.: *J. Clin. Endocrin. Metab.*, in press, 1990.
8. Garcia, M., Salazar-Retana, G., Pages, A., Richer, G., Domergue, J., Pages, A. M., Cavaliᴰe, G., Martin, J. M., Lamarque, J. L., Pau, B., Pujol, H. and Rochefort, H.: *Cancer Res.*, 46, 3.734-3.738, 1986.
9. García, E., Bouchard, P., Debrux, J., Berdah, J., Frydman, R., Schaison, G., Milgrom, E. and Perrot-Applanat, M.: *J. Clin. Endocrin. Metab.*, 67, 80-87, 1988.
10. Joyeux, C., Chalbos, D. and Rochefort, H.: *J. Clin. Endocrin. Metab.*, in press, 1990.
11. Joyeux, C., Rochefort, H., Chalbos, D.: *Mol. Endocrinol.*, 4, 681-686, 1989.
12. Lessey, B., Killam, A., Metzger, D., Maney, A., Greene, G. and McCarty, K.: *J. Clin. Endocrin. Metab.*, 67, 334-340, 1988.
13. Mauvais-Jarvis, P., Kutten, F., Gompel, A.: *Breast Cancer Res. Treat.*, 8, 179-187, 1986.
14. Vignon, F., Bardon, S., Chalbos, D. and Rochefort, H.: *J. Clin. Endocrin. Metab.*, 56, 1.124-1.129, 1983.
15. Wei, L., Krett, N., Francis, M., Gordon D., Wood, W., O'Malley, B. and Horwitz, K.: *Mol. Endocrinol.*, 2, 62-72, 1988.