REVISTA ESPAÑOLA DE FISIOLOGIA, 46 (1), 89-94, 1990

# Hormone-Dependence of Experimental Mammary Tumours

E. Escrich

Departamento de Biología Celular y Fisiología (Fisiología Médica).
Universidad Autónoma de Barcelona.
08193 Bellaterra/Barcelona (Spain)

(Received on December 19, 1989)

E. ESCRICH. *Hormone-Dependence of Experimental Mammary Tumours.* Rev esp Fisiol, 46 (1), 89-94, 1990.

The effect of hormones on mammary tumours induced experimentally in animals are revised. It is stated that the effect on tumoural induction and/or growth may depend on their concentration and on the moment, in relation to animal exposure to the carcinogen, in which they are administered. The presently known action mechanisms are also indicated, bringing out the importance of the increased level of differentiation which high estrogen doses induce, as well as some effects of progesterone and pregnancy in the protection against experimental cancer and its promoters. The efficacy of therapeutic ablative operations —ovariectomy, hypophysectomy and adrenalectomy— and additive ones —antihormones— in tumor regression, is shown. Finally the molecular bases of hormonedependence of experimental mammary tumours is established, while trying to provide an integrated concept between: hormones, receptor, growth factors, oncogens and experimental carcinogenesis.

Key words: Hormone-dependence, Breast cancer.

*Effect of hormones on experimental mammary tumours.* — Hormone dependence of carcinogenic processes depends on the stage on which they act. Some hormones may have a cocarcinogenic role at initiation; at that point gland differentiation state constitutes the basic factor rather than the variations in the basal hormonal levels, as it had previously been considered (6). After transformation has been initiated, some hormones exert a clear promoting effect on the tumours that possess cells with specific and functional receptors (hormone-dependent tumours).

The role played by various hormones on experimental mammary tumours has previously been discussed (10). The most widely accepted concepts are briefly treated here.

*Ovarian hormones and ovariectomy.* — Numerous studies have been carried out on experimental models of mammary cancer to determine the effects of ovarian hormones and/or ovariectomy, with the following conclusions: 1) suppression of the ovarian hormones makes most of the experimental mammary tumours regress. After ovariectomy a series of histological and biochemical changes in the tumours occur, so that towards the seventh day the enzymatic pattern of the epithelial tumour

cells mimics that of the normal mammary gland. 2) Strong estrogens stimulate tumoural mammary cell growth and provoke reappearance of tumours previously diminished by ovariectomy. Contrariwise, high estrogen doses induce an effect alike that of ovariectomy. Research shows that such doses provoke tumoural cell differentiation, so that the cellular metabolic potential derives towards terminal products, such as milk proteins and fatty acids of medium and short chains, which are useless for tumoural growth. Therefore, experimental tumours seem to respond to the excess of estrogens in the same way as the normal mammary gland during pregnancy and lactation, when it is less sensitive to neoplasic transformation by carcinogens. On the other hand, this inhibitory effect might also be produced through the interference that they exert on the peripheric action of prolactin and insulin or by altering the regulation of its own receptor. 3) In normal conditions, progesterone has an inhibitory effect on experimental mammary tumours. When it is administered early, it protects against carcinogenic action or its promotion by estrogens. This effect seems to be due also to the differentiation increase which provokes in the mammary gland. Once the tumour has been established, progesterone induces tumoural inhibition, probably due to its antiestrogenic properties, to be described later. In contrast to recently stated inhibitory properties, other papers attribute a stimulating role to progesterone, especially when it is found in high doses.

*Pregnancy.* — In principle pregnancy exerts a different role depending on whether it precedes or follows the carcinogen administration. In the first case it acts, through the differentiation which it induces, protecting the mammary gland against the carcinogen. On the contrary, if it is produced after carcinogenic induction, the tumoural process becomes stimulated. The exogenous administration of the chorionic gonadotrophic hormone also exerts a protecting effect which has been attributed to its capacity to inhibit the carcinogen binding to DNA and, again, to the differentiation induced by this hormone. These effects are not observed when the experiments are carried out with placental lactogen.

*Hypophysectomy and adrenalectomy.* — The effects of hypophysectomy or adrenalectomy on experimental mammary cancer have been seldom studied separately. Their conclusions determine the efficiency of both methods, especially of the former, to produce tumour regression.

*Prolactin.* — The stimulating factors of hypohyseal prolactin release are numerous, among them estrogens. In turn, prolactin stimulates gland and mammary tumour growth and estrogen receptor (ER) synthesis in those systems. The administration of high estrogen doses, nevertheless, exerts a concomitant inhibitory effect on the tumour and on prolactin binding to its receptor in the cellular membrane. On the other hand prolactin stimulating action on the ER is negatively regulated by progesterone.

Prolactin has constituted the main biochemical difference existing between human mammary tumours and the experimental ones. Whereas experimental tumours are highly prolactin-dependent, human tumours seen to be rather estrogen dependent. However, there is no general agreement on this subject and, besides, new determination methods of bioactive, instead of immunoreactive prolactin, as well as the evaluation of the fact that this hormone may act a long time before the clinical tumour appearance (5) being significantly diminished from the effect of early pregnancies (19), are factors that place this subject under revision at present.

*Insulin.* — Insulin and glucose, separately or mainly combined, stimulate

mammary tumoural growth. Glucose capacity to provoke insulin rate increases and that of the hormone to stimulate ER seems to be involved in regulating that stimulation. High concentrations of estrogens also inhibit insulin binding to its membrane receptor. In agreement with these results some authors have obtained mammary tumoural regression by inducing diabetes in animals. In diabetic rats non-regressing tumours show also their incapacity to respond to insulin in culture.

*Androgens.* — Androgens have experimentally been shown to participate in the growth of the normal mammary gland and to be able to be aromatized into estrogens.

High doses, when they are administered before exposure to the carcinogen, delay, but not hinder, mammary cancer formation in animals. Once a tumour has been established, they exert an estrogenic antagonism at two possible levels: inhibiting secretion of hypophyseal gonadotrophins and competing peripherally for ER.

*Antihormones.* — The hormone-dependent character of the experimental mammary tumours becomes also evident from the responses obtained with hormonal antagonists. Antiestrogens, prolactin inhibitors, androgenic derivatives (9), etc. have shown some degree of effectiveness in tumour growth reduction.

*Molecular basis of Hormone-dependence.* — The hormone-dependence confirmation of experimental tumours was produced when its molecular basis were established. First of all certain organs, called «target», were shown to capture radioactive estrogens. Subsequently, an attempt was made to observe its cellular distribution through autoradiographic methods, which became the object of dispute. Finally, analytical techniques were introduced to qualify and quantify the receptors (see revision in 10). Practically all the experimental tumours of the models commonly used have estrogen and progesterone (PR) receptors, and their content in fmols./mg. protein is variable just as in human tumours. Mean values oscillate, according to our results, from 30 to 93 for ER and from 286 to 395 for PR in function of the method used and the experimental design (9). A study of the receptor content as contrasted with the response to the hormones has shown that there exists a correspondence between both. Hormone-dependent tumours usually have many more receptors than hormone-independent ones, which in most cases lack them (16, 7). Nevertheless hormone-dependent and hormone-sensitive tumours have a similar content in both receptors, indicating that receptor determination is not sensitive enough to distinguish functional hormone-dependence (21). Another aspect that differentiates hormone-dependent tumours from the hormone-independent ones is the greater rate of phosphorylation of the mammary cell histones, which the former undergo. These changes are important because they modify chromatin structure and affect gene expression, replication and segregation, during cell division. Histone phosphorylation in tumours and in the mammary gland is produced in serine and threonine residues. Tyrosine residues are not affected (21).

The regulation of these receptors in experimental mammary tumours is quite well known at present. 17,β-estradiol stimulates the synthesis of its receptor and that of PR. ER is likewise stimulated by insulin and prolactin. Contrariwise progesterone has an antiestrogenic effect which is exerted through the inhibition of both receptors and prolactin peripheral action. This antiestrogenic character is reinforced by its stimulation of 17,β-dehydrogenase which transforms 17,β-estradiol into another weaker estrogen (see revision in 10).

In close relationship to the described system, are to be found the growth fac-

tors. In contrast to the precedent case, the growth factors have basically been studied in cultured cell lines. Certain evidences suggest that estrogens do not regulate cellular proliferation in a direct way. In this sense, these hormones seem to increase the factors of the transforming α group and the insulin-like growth factor (IGF-I) which stimulates tumoural growth, and inhibits the transforming factor β, which slows that growth (8, 18). On the other hand, antiestrogens behave at a molecular level in ways contrary to the estrogens and they might possibly act at the times independently of the ER system (4). Other interrelations have also been described: insulin inhibition by TGF β or the activation by the epidermal growth factor (EGF) of a kinase protein which phosphorylates the progesterone receptor (12). EGF is an important growth factor which seems to play an important role in te initiation and progression of mammary cancer. It has been observed experimentally that the removal of the submandibular gland —sialoadenectomy— in a strain of mice, especially susceptible of developping mammary tumours, reduces the incidence and tumoural growth rate. On the other hand, when EGF is administered to the sialoadenectomized animals a normal condition is restored to the entire animal (17).

*Steroid, oncogens and chemical carcinogenesis.* — Certain similarities exist between the steroid action mechanism and that of certain chemical carcinogens. Thus, the polycyclical aromatic hydrocarbons (PAH) in mouse skin cells have been described to bind specifically and covalently to proteins of the soluble fraction in direct proportion to its carcinogenic activity. Other receptor proteins have also been cited in skin, liver and the transformed cells which bind non-covalently to PAH. Some of these proteins have similar properties, although not identical, to corticosteroid receptors (13).

An important aspect to elucidate is the participation of oncogens in the cellular transformation due to chemical carcinogens. On one hand a possible cause and effect relationship should be considered to exist between the activation of oncogens and malignancy and, on the other, the relation of its expression with the phenotypic markers of the transformation.

The experimental induction of mammary or skin cancers with nitrosomethylurea (NMU) or with dimethylbenz(a)antracene (DMBA) respectively, has shown that it produces the specific activation of the *ras* oncongens (22). It has also been possible to identify transformer genes in other animal tumoural systems induced by different carcinogens (3). DMBA and NMU differ from each other in as much as the former requires a previous metabolization; but both systems, be it through the metabolite or directly, activate the specific oncogen in a similar way, although NMU is much more effective (2, 20). Diverse experimental proofs suggest that the transformation requires several stages for the mammary cancer to be produced in rat (2, 14, 20) as well as that in this transformation two or more oncogens acting in coordination (1, 2) could be involved. Chemical carcinogens and oncogens share some of their characteristics. Both require cellular proliferation to manifest their carcinogenic action. Such a proliferation could be the result of normal physiological processes of the mammary gland, as occurs during embryogenesis and puberty. It may be induced experimentally with forbol esters. On the other hand, oncogens may also play a role in tumour promotion and/or growth if they are activated in cells which have been initiated by other mechanisms, such as chemical carcinogens or other types of oncogens (20). In this sense, the homologies between the steroid and thyroid receptors and that of EGF with the proteins expressed, respectively, by the erb-A and erb-B oncogens of the avian

erythroblastosis virus, should be kept in mind (11). The last two situations enclose the possibility that the protooncongens may express in the same way, in normal physiological situations or during mammary carcinogenesis. This fact has been brought out in mouse with two cellular oncogens distinct from *ras*, int-1 and int-2 (15). These oncogens correspond to the integration sites of exogen virus in the 15 and 7 chromosomes respectively of some experimental mammary tumours in mouse. The int-2 gene is related to fibroblastic growth factors (21).

Up to recently the study of mammary cancer at the molecular level was viewed from different independent approaches. At present, thanks to the great advances of Molecular Biology, hormones, receptors, growth factors and oncogens constitute an integrated concept, as well as an integrator of the physiology of the mammary gland and the physiopathology of breast cancer, which improves the theoretical knowledge and, therefore, opens the way to resolve that disease.

## Resumen

Se revisa el efecto de las hormonas sobre los tumores mamarios inducidos experimentalmente en animales. Se subraya que el efecto sobre la inducción y/o el crecimiento tumoral puede depender de su concentración y del momento, en relación a la exposición de los animales al carcinógeno, en que se administren. Se indican los mecanismos de acción conocidos, destacando la importancia del aumento de la diferenciación que inducen las altas dosis de estrógenos y algunos efectos de la progesterona y el embarazo en la protección contra el cáncer experimental y sus promotores. Asimismo, se muestra la eficacia de las maniobras terapéuticas ablativas —ovariectomía, hipofisectomía y adrenalectomía— y aditivas —antihormonas— en la regresión de tales tumores. Finalmente, se establecen las bases moleculares de la hormonodependencia de los tumores mamarios experimentales intentando proporcionar un concepto integrado entre hormonas, receptores, factores de crecimiento, oncogenes y carcinogénesis experimental.

Palabras clave: Hormonodependencia, Cáncer de mama.

## References

1. Balmain, A.: *Br. J. Cancer.*, 51, 1-7, 1985.
2. Barbacid, M.: *Carcinogenesis*, 7, 1037-1042, 1986.
3. Barbacid, M.: In «Virus, oncogenes y cáncer», (J. Oró, C. M. Cuchillo, E. Querol, R. Segura and P. Suau, eds.), Universidad Autónoma de Barcelona, Bellaterra, 1988. pp. 101-113.
4. Baum, M.: *Vth. Int. Congress of Breast Diseases*, Buenos Aires, 1988. C.P. 2.
5. Bruning, P. F.: In «Hormonal manipulation of cancer. Peptides, growth factors, and new (anti) steroidal agents» (J. G. M. Klijn *et al.*, eds.). Raven Press, New York, 1987. pp. 167-173.
6. Ciocca, D. R., Parente, A. and Russo, J.: *Am. J. Pathol.*, 109, 47-56, 1982.
7. Desombre, E. R., Kledzik, G., Marshall, S. and Meites, J.: *Cancer Res.*, 36, 354-358, 1976.
8. Dickson, R. B. and Lippman M. E.: *Trends in Pharmacol. Sci.*, 7, 294-296, 1986.
9. Escrich, E.: Efecto de un derivado androgénico sobre la inducción y el crecimiento de tumores mamarios experimentales. Universidad Autónoma de Barcelona. Bellaterra, 1985.
10. Escrich, E.: *Int. J. Biol. Markers*, 2, 197-206, 1987.
11. Green, S., Gronemeyer, H. and Chambon, P.: In «Growth factors and oncogenes in breast cancer», (M. Sluyser, ed.), VCH, Chichester-Horwood, 1987. pp. 9-28.
12. Harris, A. L. and Neal, D. E.: In «Growth factors and oncogenes in breast cancer» (M. Sluyser, ed.), VCH, Chichester-Horwood, 1987. pp. 60-90.
13. Heidelberger, C.: *Ann. Rev. Biochem.*, 44, 79-121, 1975.
14. Isaacs, J. T.: *Cancer Res.*, 45, 4827-4832, 1985.
15. Jakobovits, A., Shackleford, G. M., Varmus, H. E. and Martin, G. R.: *Proc. Natl. Acad. Sci. U.S.A.*, 83, 7806-7810, 1986.
16. Koenders, A. J. M., Geurts-Moespot, A., Zolingen, S. J. and Benraad, Th. J.: In «Progesterone receptors in normal and neoplasic tis-

sues», (W. L. McGuire, J. P. Raynaud and E. E. Baulieu, eds.), Raven Press, New York, 1977. pp. 71-83.
17. Kurachi, H., Okamoto, S. and Oka, T.: *Proc. Natl. Acad. Sci. U.S.A.*, 82, 5940-5943, 1985.
18. Lippman, M. E., Dickson, R. B., Kasid, A., Gelmann, E., Davidson, N., McManaway, M. *et al.*: *J. Steroid. Biochem.*, 24, 147-154, 1986.
19. Musey, V. C., Collins, D. C., Musey, P. I., Martino-Saltzman, D. and Preedy, J. R. K.: *N. Engl. J. Med.*, 316, 229-234, 1987.
20. Russo, J. and Russo, I. H.: *Lab. Invest.*, 57, 112-137, 1987.
21. Sluyser, M.: In «Growth factors and oncogenes in breast cancer», (M. Sluyser, ed.), VCH, Chichester-Horwood, 1987. pp. 123-141.
22. Sukumar, S., Notario, V., Martin-Zanca, D. and Barbacid, M.: *Nature*, 306, 658-661, 1983.