Volume 285, number 1, 28–30 FEBS 09865 July 1991

ADONIS 0014579391005715

Radiation inactivation of estrogen receptor in intact human breast cancer cells (MCF-7)

Lack of energy transfer to the estradiol binding domain

Christine Toulas[1], Guy Beauregard[2], Felicité Delassus[1], Louise Thauvette[3], Michel Potier[3], Francis Bayard[1] and Jean-Charles Faye[1]

[1]*Laboratoire d'Endocrinologie Expérimentale, CHU Rangueil, Chemin du vallon, 31054, Toulouse cedex, France,* [2]*Endocrinologie, Hôpital Maisonneuve Rosemont, Université de Montréal, Montréal, Quebec, Canada* and [3]*Service de Génétique Médicale, Hôpital Sainte-Justine, Université de Montréal, Montréal, Quebec, Canada*

Received 25 April 1991

Whole MCF-7 human breast-cancer cells were irradiated at −78°C in a calibrated Gammacel ^{60}Co irradiator. Freezing or storing conditions induce neither an alteration of the viability of cells nor a change in estradiol binding activity. Hexosaminidase was used as internal marker, and we measured the radiation inactivation size (RIS) of the estrogen receptor in whole cells. After various cell treatments, the estradiol binding unit always presents a molecular mass of 25 kDa. This value, which corresponds to the size of the defined hormone binding domain of the estrogen receptor, suggests that the energy delivered to the protein by the radiation is efficient to inactivate estradiol binding only when the hit occurs directly in the smaller hormone binding domein.

Estrogen receptor; Radiation inactivation size

1. INTRODUCTION

Gene regulation by steroid hormones such as estradiol, is a traditional field of research in biochemical endocrinology. Characterization, purification and cloning of receptor genes have been achieved in the last 10 years. The human breast cancer cell line MCF-7, which possesses the estrogen receptor, is proving to be an excellent model for the study of estrogen action. Estradiol is able to stimulate cell proliferation otherwise specificaly inhibited by the antiestrogen tamoxifen [1]. However, in certain conditions we have reported that estradiol remains ineffective for growth stimulation [2]. In this work our focus has been to study the molecular size of the estradiol binding polypeptide structure in whole cells. Previous results obtained by radiation fragmentation have shown that the estrogen receptor covalently bound with the antiestrogen tamoxifen aziridine is part of a protein complex of about 265 kDa when cells are at subconfluency or of a 360 kDa species in superconfluent cells [3]. It is reported here that in whole cells the radiation inactivation size (RIS) of estradiol binding is 25 kDa in all the conditions used: cells were untreated or submitted to 10^{-8} M estradiol or 10^{-7} M tamoxifen, moreover, unlike the target size [3] this value is independent of the MCF-7 cellular density.

Correspondence address: J-Ch. Faye, Department d'Endocrinologie, CHU Rangueil, Chemin du vallon, 31054 Toulouse cedex, France.

2. MATERIALS AND METHODS

The MCF-7 human breast-cancer cell line has been adapted to grow in serum-free medium and in absence of phenol red as described previously [4]. Cells were treated 3 days before scrapping and collection; they were frozen at −170°C, stored in conditions which do not decrease their viability and irradiated at −78°C. The irradiation procedure was conducted on frozen samples (10^7 cells) in a Gammacell model 220 instrument (Atomic Energy of Canada, Ottawa, Canada) at a dose rate of about 2 Mrad/hour in a special tube rack allowing isodose exposure of 2 sets of samples [5]. The data were analyzed with the single hit model of radiation inactivation [6] and apparent molecular weights were calculated using the empirical formula: $\log M_r = 5.89 - \log D_{37,t} - 0.0028t$.

Where $D_{37,t}$ is the radiation dose (in Mrad) necessary to inactivate the estradiol binding or the hexosaminidase activity to 37% of its initial value, at t, the irradiating temperature in °C [7]. After irradiation, cells were quickly thawed at 37°C, put on ice, washed with saline phosphate buffer and pelleted at 800 × *g*. [^{3}H]Estradiol binding assay was performed using the ligand exchange procedure described by Anderson et al. [8]. Hexosaminidase assay was realized on 20 μl samples of total homogenate as previously described [9].

3. RESULTS AND DISCUSSION

The inactivation of estradiol binding and hexosaminidase activity occurs as a simple exponential function of the radiation dose (Fig. 1) in agreement with the target theory of Lea [6]. The calculation of the RIS of the estradiol binding site and of the hexosaminidase activity was performed on the same irradiated samples, the hexosaminidase being used as an internal control. For hexosaminidase a RIS of 50 000 ± 6000 (mean ±

Published by Elsevier Science Publishers B.V.

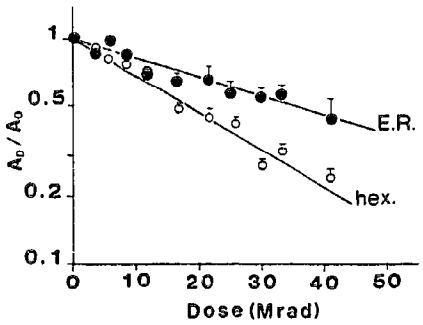

Fig. 1. Effect of ^{60}Co Ω radiations at −78°C on intact MCF-7 samples. Fractional surviving of estrogen receptor binding capacity (•), and hexosaminidase enzymatic activity (○), are plotted as a function of radiation exposure. Each point is the mean of 2 independent experiments realized in triplicate.

SD; $n = 15$) was obtained (Fig. 1) in good agreement with previous reports [10]. From Fig. 1 we found that the RIS of estradiol binding on intact untreated cells is 25 000 ± 3000. About the same value, was obtained after either estradiol or tamoxifen cell treatments and independently of the cell density (Fig. 2). This molecular size does not correspond to the whole hetero-oligomer [3] nor to the M_r = 67 000 estrogen receptor polypeptide [11], but it fits with the size of the estradiol binding domain [12]. To insure that the decrease of binding was not due to a variability of the affinity of the receptor for its ligand following radiations, the dissociation constant at equilibrium (K_d) was determined at 10 different radiation doses and 6 different ligand concentrations. Both the RIS and K_d were determined as not significantly different (Fig. 3). However, another possibility, to explain the low RIS analysis could have been an artefactual release of masked receptors induced by irradiating intact cells. To test this possibility another set of radiation experiments has been conducted on cytosolic extracts. In these conditions, the same RIS value is found again for all cell culture conditions and treatments (data not shown). This is com-

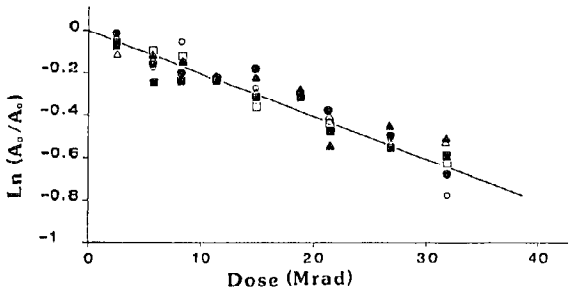

Fig. 2. Radiation inactivation analysis of estrogen receptor after different MCF-7 cell treatments. MCF-7 cells in log phase growth (•, ▲, ■), or at confluency (○, △, □), were submitted to: vehicle (•,○), 10^{-8} M estradiol (▲, △), 10^{-7} M tamoxifen (■, □). The slope ($\mu_{-78°C}$) of the line was used to calculate RIS = $\mu_{-78°C} \times 1.2836 \times 10^6$.

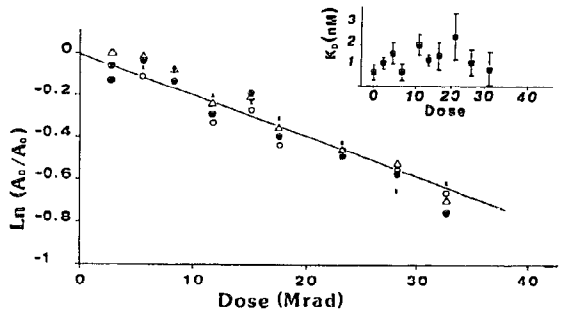

Fig. 3. Influence of irradiation on the K_d of estradiol binding. MCF-7 cells in log phase growth were irradiated at −78°C. The natural logarithm of fractional surviving binding activity [ln (A_D/A_0)] as a function of radiation exposure was assessed by measuring specific estradiol binding over the concentration range 0.1–10 nM. In a view of clarity only 4 concentrations are plotted here (10 nM, •; 2.5 nM, ○; 1 nM, △; 0.1 nM, X). The independence of the K_D at various radiation doses shown on the insert was checked by a Student's t-test. The coefficient of correlation for 10 points is $r = 0.138$. This is not significant (at 5%) $t = 0.39$, evidencing that K_D is not influenced by radiation doses.

pletely different from the highly homologous androgen receptor whose RIS corresponds to the whole receptor polypeptide [13]. The reason for such a discrepancy is not clear. We thus conclude that the RIS for estradiol binding (25 000 ± 3000) determined by radiation analysis corresponds to the hormone binding domain previously described [12]. RIS usually relates to the monomer or the oligomer. However, occasionally, the RIS is much smaller than the known subunit size and has been attributed to a putative domain [7]. Moreover it is shown here that a single radiation hit on the M_r 67 000 protein structure of the estrogen receptor is not fully efficient in disrupting its whole polypeptide chain, suggesting that the energy deposited by a hit on the protein is not necessarily transferred to the hormone binding domain. Only direct hits of this domain would destroy the estradiol binding capacity. This result integrates into a larger mechanism suggesting that under radiation: (i) the fragmentation of a polypeptide could preferentially occur in loops or β-turns of the chain [14]; and (ii) each domain exerting a function independently of the others, in a multidomain protein, could be inactivated independently by a direct hit [14]. Finally the fact that in the whole cell, a same RIS value was obtained in absence or in presence of antiestrogenic agents indicates that the important biological differences induced by agonist and antagonist ligands do not proceed from the intervention of other receptor domains or proteins. Thus, the loops and/or β-turns of the polypeptide chain close to the binding domain are not drastically modified by the binding of some ligand on the estrogen receptor.

Acknowledgements: This work was supported in part by grants from Association pour la Recherche contre le Cancer and INSERM. C. Toulas is supported by a fellowship from the Ligue Française contre le cancer.

REFERENCES

[1] Rochefort, H. (1987) Trends Pharmacol. Sci. 8, 126–128.
[2] Faye, J-C., Toulas, C. and Bayard, F. (1989) J. Recept. Res. 9, 203–219.
[3] Toulas, C., Beauregard, G., Delassus, F., Thauvette, L., Potier, M., Bayard, F. and Faye, J-C. (1990) Mol. Cell. Endocrinol. 72, 89–94.
[4] Jozan, S., Faye, J-C., Tournier, J-F., Tauber, J-P., David, J-F. and Bayard, F. (1985) Biochem. Biophys. Res. Commun. 133, 105–112.
[5] Beauregard, G., Giroux, S. and Potier, M. (1983) Anal. Biochem. 132, 362–364.
[6] Lea, D.E. (1955) Actions of Radiations on living cells, 2nd edn., pp. 69–99, Cambridge University Press, Cambridge.
[7] Beauregard, G., Maret, A., Salvayre, R. and Potier, M. (1987) Methods in Biochemical Analysis, vol. 32, pp. 313–343, (Glick Ed.) John Wiley and sons, New York.
[8] Anderson, J.M., Clark, J.H. and Peck, E.J. (1972) Biochem. J. 126, 561–567.
[9] Okada, S. and O'Brien, J.S. (1960) Science 165, 698–700.
[10] Levade, T., Salvayre, R., Potier, M. and Douste-Blazy, L. (1986) Neurochem. Res. 11, 1131–1138.
[11] Walter, P., Green, S., Green, G., Krust, A., Bornet, J., Jeltsch, J., Staub, A., Jensen, E., Scrace, G., Waterfield, M. and Chambon, P. (1985) Proc. Natl. Acad. Sci. USA 82, 7889–7893.
[12] Kumar, V., Green, S., Staub, A. and Chambon, P. (1986) EMBO J. 5, 2231–2236.
[13] Turcotte, G., Beauregard, G., Potier, M. and Chevalier, S. (1991) Biochem. J. (in press).
[14] le Maire, M., Thauvette, L., de Foresta, B., Viel, A., Beauregard, G. and Potier, M. (1990) Biochem. J. 267, 431–439.
[15] Potier, M., Huot, C., Koch, C., Hamet, P. and Tremblay, J., Methods in Enzymology (in press).