J. Physiol. Biochem., 53 (4), 355-360, 1997
Revista española de Fisiología

Downmodulation of HLA class I expression by dexamethasone in MCF-7 cell line

F. Rodríguez [1], M. Redondo[2], M. L. Hortas- Nieto[2], T. Téllez-Santana[4], V. Pérez-Valero[1] and F. Ruiz-Cabello[3]

Servicios de Análisis Clínicos de los hospitales [1]General Básico, 16800 Motril/Granada, [2]Costa del Sol, Marbella/Málaga, [3]Virgen de las Nieves, Granada, and [4]Departamento de Bioquímica, Facultad de Medicina, Universidad de Málaga (Spain)

(Received on March 4, 1997)

F. RODRÍGUEZ, M. REDONDO, M. L. HORTAS-NIETO, T. TÉLLEZ-SANTANA, V. PÉREZ-VALERO and F. RUIZ-CABELLO. *Downmodulation of HLA class I expression by dexamethasone in MCF-7 cell line.* J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (4), 355-360, 1997.

Several reports have shown the importance of MHC class I antigens in enabling the host to regulate tumour growth *in vivo*. Glucocorticoid hormones have strong immunosuppressive effects and are known to be regulators of gene transcription. In this report the expression of Major Histocompatibility Complex (MHC) class I antigens in six breast carcinoma cell lines have been studied before and after treatment with the synthetic glucocorticoid dexamethasone. Hence, HLA class I expression in the MCF-7 cell line was down-regulated in the presence of dexamethasone . This down-modulation of expression appeared to be mediated by transcriptional mechanisms, as revealed by HLA-class I mRNA levels.

Key words: Dexamethasone, Breast cancer, MHC.

Eukaryotic gene transcription is normally regulated by interactions of cis-acting DNA elements which contain sequences that bind specifically to certain proteins or nuclear factors (3).

Hormone response elements (HREs) are DNA sequences found on the 5' gene sites which show binding affinity for either hormone receptors, activated by binding to the corresponding hormone, or other proteins induced by these receptors. Hormonal action derives from HREs activation (3).

The family of steroid hormone receptors has been the most widely used model for the study of these DNA sequences. Various hormones share consensus sequences, i.e. glucocorticoids, progesterone, androgens and mineralocorticoids

Correspondence to F. Rodríguez.

share the same GGTACAnnnTGTTCT sequence, while the receptors for oestrogen, thyroid hormones, retinoic acid and vitamin D share the AGGTCAnnTGACCT sequence (3).

Some proteins, induced by steroid hormones, can activate or inhibit the transcription of different genes which share the same regulator sequence (11). Thus, the H-2RIIBP protein activates the transcription of MHC Class I genes in response to several stimuli, which appears to occur in the MCF-7 breast tumor cell line when it is treated with oestrogens (19). Glucocorticoids can inhibit the transcription of certain genes, either through their protein receptors, or through the activation of other nuclear factors which bind specifically to negative regulatory DNA sequence elements (3).

The dexamethasone negative effect on the expression of HLA class I antigens in certain cell lines was shown by earlier studies (6). The mechanism by which some proteins contribute to the genetic transcription regulation is still largely unknown, and probably involves complex interactions (12, 15).

The present study examines the possible dexamethasone negative regulation on the expression of HLA class I antigens in breast tumour cell lines.

Materials and Methods

Cells and cell cultures.– Cell lines used in this study were: the hormone dependent MCF-7 and T47D, and the hormone independent MDA-MB-231, MDA-MB-435s, IMIN-MA-2 and IMIN-MA-3, derived from human breast carcinomas. They were obtained from the American Type Culture Collection (Rockville, MD), except IMIN-MA cell lines (IMIN, Barcelona), and maintained in RPMI 1640 medium (Gibcco, Paisley Scotland), supplemented with fetal calf serum (FCS), nonessential aminoacids and pyruvate. Cells were treated for six days with 10^{-7} M dexamethasone (Sigma).

The levels of the hormone receptors were analysed by Abbott ER-EIA Monoclonal (9842) and PgR-EIA Monoclonal (4012).

Monoclonal antibodies.– The following mAbs to test MHC class I expression in cell lines before and after dexamethasone treatment were used: W6/32, which recognizes assembled HLA-A, B and C locus products (2); GRH-1, which recognizes the ß-2 microglobulin (16) , and GRB-1, which does the same for the HLA-DR locus products (5).

CD44 (antiendothelium, Immunobiology, Paris) and HuTu (antiepithelium, Immunotech, Marseilles) were used to rule out the drug toxic effects.

Cytofluorometric studies.– A standard method of indirect immunofluorescence was used to determine surface HLA class I expression in the cell lines. Cells (10^5) were washed 3 times in phosphate buffered saline (PBS) and incubated at 4 °C with the appropriate class I specific mAb for 30 min. After three washes in ice-cold PBS, the cells were stained with 10 µl 0.1 mg/ml rabbit mouse-specific $F(ab')_2$ immunoglobulin-fluorescein isothiocyanate (Cappel, West Cheshire, PA). Fluorescence was analysed on a FACScan cytometer (Becton-Dickinson, Mountain View, CA); and 10^4 cells for each immunofluorescence profile were analysed. Cells incubated with the second antibody alone were used as negative control. To avoid contamination between samples, the flow cytometer was rinsed with a sheat buffer before each run.

RNA extraction and Northern blot analysis.– Total mRNA was obtained using guanidinium isothiocyanate with a cesium chloride gradient (7). RNA concentration was determined by UV absorbance at 260 nm. Twenty micrograms of total mRNA were denatured at 65 °C for 5 min, then electrophoresed on a 1.5 % agarose gel containing 6 % formaldehyde in 10 x 3-(N-Morpholine)propanesulfonic acid (MOPS) buffer (Sigma, M8899) and transferred to Gene Screen Plus (NEN, Boston, MA) membrane using 10 x SSC. Hybridization was performed as described previously (9). All Northern blots were normalized by comparison with the endogenous ß-actin mRNA using a densitometer (Molecular Dynamics 300S, Sunnivale, CA).

The probes used included HLA-A, B, C pDP-001 (21) and a β-actin probe as control (10).

Results

The influence of hormones on HLA antigens in 6 cell lines from breast tumours was studied. Only two of the lines showed estrogen (MCF-7) and progesterone receptors (T47D) (table I). The expression of HLA ABC antigens in the cell surface, levels of mRNA corresponding to HLA-A and HLA-B class I antigens were evaluated, both before and after treatment with dexamethasone.

To quantify the expression of Class I antigens, cell lines were analysed by flow cytometry incubating the cells with specific monoclonal antibodies following the standard technique of indirect immunofluorescence.

Under basal conditions all cells showed HLA class I expression in the cell surface (table II). No class II antigen expression was found in the cells studied, except to a limited extent in MCF-7 cell lines.

Results suggest that dexamethasone diminishes Class I HLA antigen expression in MCF-7 cells (which show high level of estrogen receptors) (table II, fig. 1), while in T47D cells (with high levels of progesterone receptors), dexametasone diminishes class I HLA expression to a lesser degree.

At 10^{-7} M dexamethasone in the culture medium, reduction of MHC class I expression reached its maximum extent (fig. 1).

The reduction of class I HLA antigen expression in MCF-7 cells treated with dexamethasone is specific because this effect was not observed in other antigens not belonging to the HLA system (CD 44, HüTü). In the cell lines showing low levels of hormone receptors, no significant modification of the expression of such antigens was found (table II).

The decrease in HLA antigen expression in the cell surface appears to relate to a decrease in the level of mRNA for HLA-A and B Class I antigens. Differences in the level of RNA between treated

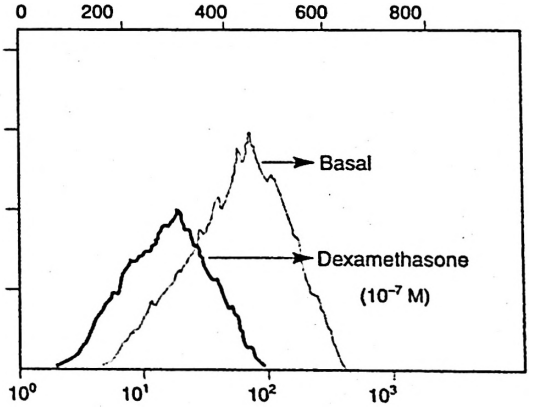

Fig. 1. *HLA class I antigen expression in MCF-7 cell lines after and before dexamethasone treatment, by indirect immunofluorescence.*

Table I. *Different cell lines used.*
Histopathology type, estrogen receptor (ER) and progesterone receptor (RP) levels from adenocarcinoma (ADENOCA.) and ductal carcinoma (DUCTAL CA.)

Cell lines	Histopath. Type	ER (fm/gp)	PR (fm/gp)
MCF-7	ADENOCA.	180	–
T47D	ADENOCA.	60	800
MDA-MB-231	ADENOCA.	–	–
MDA-MB435s	DUCTAL CA.	–	–
IMIN-MA-2	–	–	–
IMIN-MA-3	–	–	–

Table II. *Expression of HLA class I and class II antigens on the breast cancer cell lines, before and after dexamethasone treatment.*
Values represent fluorescence.

Cell lines	Class I		Class II	
	Basal	Dexame.	Basal	Dexame.
MCF-7	408	310	290	258
MDA-MB-231	467	465	173	171
MDA-MB-435$_S$	400	368	147	141
T47D	411	390	170	168
MIN-MA-2	362	360	159	165
IMIN-MA-3	390	392	161	163

and untreated cells with dexamethasone were not found in MDA-MB 231 cell line. As control, a β-actin probe was used (fig. 2).

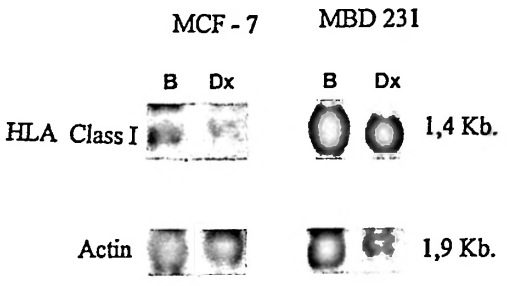

Fig. 2. *Northern blot analysis of HLA class I gene expression.*
Total RNA was isolated from MCF-7 and MDA MB 231 cell lines before and after dexamethasone treatment. RNA was fractionated in a 1.5 % agarose-formaldehyde gel, transferred and probed with (32P)-labeled probes to HLA-ABC and β-actin.

Discussion

MHC class I antigens are involved in the presentation of peptides to effector cells of the immune system, such as T lymphocytes. Earlier works have suggested that changes occur in the expression of such antigens during the tumour development (18).

Hormone regulation of the expression of MHC class I antigen of breast tumours has been shown to be dependent on the presence of the appropriate hormone receptor (22). The superfamily of steroid hormone receptors has been found to be involved in the transcription regulation of a large number of genes (3), such as class I genes (19).

In this work, possible regulation by dexamethasone on HLA class I antigen

has been evaluated in 6 cell lines from different types of breast tumours, which showed different levels of hormone receptor (table II).

The protein H-2RIIBP (11) appears to be involved in the oestrogen dependent increase of HLA class I antigen expression in MCF-7 cell lines (19).

Recent studies suggest that glucocorticoids can inhibit the expression of certain genes by repressing their transcription. This has been observed in the propiomelanocortin and prolactin genes and also in the alpha subunit of the glycoprotein gene (3).

CELADA *et al.* observed a decrease in the class II antigen expression by dexamethasone in the cell line WR19 M.1. None of the lines studied have expressed class II antigens in the cell surface under basal conditions, except for MCF-7 cell line, and to a limited extent. Treatment with dexamethasone did not modify class II antigen expression in any of the cell lines.

By using cytofluorimetry studies in this work, a decrease has been detected in HLA class I antigen expression on the cell surface of MCF-7 cell line when treated with dexamethasone.

The inhibitory effect of dexamethasone (10^{-7} M) on HLA class I antigen expression is not due to the drug toxicity, as this effect was not observed with other non HLA antigens.

The observed modifications of the cell surface antigen expression correlated with a decrease in the level of mRNA HLA-A and HLA-B antigens, studied by Northern Blot analysis. These expression modifications were not observed in the hormone independent MDA-MB-231 cell line, our findings suggesting that dexamethasone action may imply changes at the transcriptional level.

The activation or inhibition of gene transcription could depend on the protein complexes which bind to the appropriate consensus sequence (15, 17, 23). Various factors have been identified as upregulators (1, 4), and downregulators of HLA class I genes (14).

The glucocorticoid receptor shows binding affinity for the same consensus sequence as the progesterone receptor, thus a possible repressive effect of glucocorticoids on the progesterone gene may be related with the decreased effect observed on class I antigen expression in cells T47D (high levels of PR) treated with dexamethasone, in contrast to that occurring in MCF-7 cell line (high level of ER).

Summing up, our results strongly suggest that dexamethasone induces changes at the transcription level of HLA-class I genes in MCF-7 cell line, which presents decreased expression of the antigens in the cell surface.

Acknowledgement

This work was partially supported by the "Fondo de Investigaciones Sanitarias" (FIS 97/0414; FIS 96/1497) (Spain). We thank Dr. F. X. del Real for his donation of IMIN-MA cell lines, and Ms. C. Amezcua and A. Carrasco for their technical assistance.

F. RODRÍGUEZ, M. REDONDO, M. L. HORTAS-NIETO, T. TÉLLEZ-SANTANA, V. PÉREZ-VALERO y F. RUIZ-CABELLO. *Baja modulación de la HLA de clase I por dexametasona en la línea celular MCF-7.* J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (4), 355-360, 1997.

Diversos trabajos han puesto de manifiesto la importancia de los antígenos del Complejo Mayor de Histocompatibilidad (MHC) en la regulación del crecimento tumoral, así como que los glucocorticoides tienen acentuados efectos inmunosupresores y son conocidos reguladores de la transcripción génica. Se estudia la expresión de antígenos del sistema HLA en

6 líneas celulares derivadas de cáncer de mama, antes y después de ser tratadas con dexametasona. La expresión de antígenos HLA de clase I en la línea celular MCF-7 se modula negativamente en presencia de dexametasona. Este resultado parece estar mediado por mecanismos transcripcionales, ya que se evidencia una reducción en los niveles de ARNm correspondientes.

Pabras clave: Dexametasona, Cáncer de mama, HLA.

References

1. Baeuerle, P. A. (1991): *Biochim. Biophys. Acta*, 1972, 63-80.
2. Barnstable, C. J., Bodmer, W. F., Bronw, G., Glaffé, G., Milstein, C., Williams, A. F. and Ziegler, A. (1978): *Cell*, 14, 9-12.
3. Beato, M. (1989): *Cell*, 56, 335-344,
4. Blanchet, O., Bourge, J. F., Zinszner, H., Tatari, Z., Degos, L. and Paul, P. (1991): *J. Cancer*, 6, 138-145.
5. Cabrera, T., Ruíz-Cabello, F., López-Nevot, M. A., de la Higuera, B., Sánchez-Pérez, M. and Garrido, F. (1986): *Hybridoma*, 5, 191-197.
6. Celada, A., Mckecher, S. and Maki, R. A. (1992): *Inmunología*, 11, 42-43.
7. Chirgwin, J. J., Przbyla, A. E., MacDonald, R. J. and Rutter, W. J. (1979): *Biochemistry*, 18, 5294-5299.
8. Davi-Watine, B., Israel, A. and Kourilsky, P. (1990): *Immunol. Today*, 2, 286-292.
9. Feimberg, A. P. and Vogeltein, B. (1984): *Addendum. Anal. Biochem.*, 137, 266-267.
10. Fyrber, E. A., Kindle, K. L. and Davison, N. (1980). *Cell*, 19, 365-378.
11. Hamada, K., Gleaso, S. L., Levi, B. Z., Hirschfel, S., Appella, E. and Ozato, K. (1989): *Proc. Natl. Acad. Sci. USA*, 86, 8289-8293.
12. Hunter, T. and Karin, M. (1992): *Cells*, 70, 375-387.
13. Koller, B. H., Sidwell, D., De Mars, R. and Orr, H. T. (1984): *Proc. Natl. Acad. Sci. USA*, 81, 5175-5178.
14. Kuschner, D. B., Pereira, D. S., Liu, X., Graham, F. L. and Ricciardi, R. P. (1996): *Oncogene*, 12, 143-151.
15. Lewin, B. (1990): *Cells*, 61, 1161-1164.
16. López-Nevot, M. A., Cabrera, T., de la Higuera, B., Ruiz-Cabello, F. and Garrido, F. (1986): *Inmunología*, 5, 51-59.
17. Marks, M. S., Hallembeck, P. L., Nagata, T., Segars, J. H., Apella, E., Nikodem, V. M. and Ozato, K. (1992): *EMBO J.*, 2, 1419-1435.
18. Redondo, M., Concha, A., Oldiviela, R., Cueto, A., Gonzalez, A., Garrido, F. and Ruiz-Cabello, F. (1991): *Cancer Res.*, 51, 4948-4958.
19. Rodríguez, F., Perán, F., Garrido, F. and Ruiz-Cabello, F. (1994): *Immunogenetics*, 39, 161-167.
20. Singer, D. and Maguire, J. (1990): *Crit. Rev. Immunol.*, 10, 235-257.
21. Sood, A. K., Pereira, D. and Weissman, S. M. (1981): *Proc. Nact. Acad. Sci. USA*, 78, 616-621.
22. Teh, M. and Hui, K. M. J. (1989): J. *Immunogenet.*, 16, 397-405.
23. Ting, J. P. and Balwin, A. S. (1993): *Curr. Opin. Immunol.*, 5, 8-16.
24. Watson, A. J., Demars, R., Trowbridg, I. S. and Bach, S. H. (1986): *Nature* (Lond), 304, 358-361.
25. Ziegler, A., Uchanska-Ziegler, B., Zeuten, J. and Wernet, P. (1982): *Somatic Cell. Genet.*, 8, 775-789.