Acta virol. **33** : 137—142, 1989

EXPRESSION OF PROTEINS IMMUNOLOGICALLY RELATED TO MURINE MAMMARY TUMOUR VIRUS (MMTV) CORE PROTEINS IN THE CELLS OF BREAST CANCER CONTINUOUS LINES MCF-7, T47D, MDA-231 AND CELLS FROM HUMAN MILK

S. V. LITVINOV, T. V. GOLOVKINA

All-Union Oncological Centre, U.S.S.R. Academy of Medical Sciences, 115478 Moscow, U.S.S.R.

Received August 10, 1987

Summary. — Expression of antigens immunologically related to the *gag* gene products of murine mammary tumour virus (MMTV) in continuous cell lines MCF-7, T47D and MDA-231 was followed by indirect enzyme immunoassay (EIA). Some cells of the 2 clonal lines derived from MCF-7 and T47D cells contained MMTV antigens, but these were not detected in MDA-231 cells and in epithelial cells from the milk of healthy women. The expression of antigens related to MMTV *gag* proteins correlated with the expression of proteins immunologically related to MMTV gp52. Cells positive for antigens related to p27 were also found when healthy women sera containing antibodies against MMTV p27 were examined. Before testing, the donor sera containing antibodies against MMTV protein p27 were absorbed to the negative MCF-7 cell clone and their antibody activity to MMTV p27 was also tested by immunoblotting.

Key words: *human breast cancer cells*; *murine mammary tumour virus*; *virus core antigens*

Introduction

Proteins immunologically related to the major protein of murine mammary tumour virus (MMTV) envelope were detected in human breast carcinomas and cell lines derived from these tumours (Mesa-Tejada, Spiegelman, 1983). Proteins related to MMTV polypeptide p27, major virus core protein, however, were found neither in cell lines T47D or MCF-7 (Yang *et al.*, 1977, 1978; Callis and Retzi, 1981; Keydar *et al.*, 1984) nor they were present within virus particles produced by T47D cells (Keydar *et al.*, 1984). In contrast, such proteins were present in breast tumour tissue extracts (Calafat *et al.*, 1976; Hendrick *et al.*, 1978; Kryukova *et al.*, 1981), in the human milk (Hageman *et al.*, 1978; Zotter *et al.*, 1980; Litvinov *et al.*, 1987) and within 734B particles (Rich *et al.*, 1976). The presence of p27-related proteins in humans was

confirmed by formation of p27-reactive antibodies in some subjects (Zotter *et al.*, 1983; Tomana *et al.*, 1981; Litvinov *et al.*, 1986). Controversal data do not allow one to understand whether human breast tumour cells are a source of antigens immunologically related to MMTV *gag* gene products and whether the expression of such antigens correlates with production of antigens related to gp52 (*env* gene product). Searching the answer to these questions we have studied human breast carcinoma cell lines for the expression of proteins related to MMTV p27 using sera against MMTV proteins and human sera reacting with MMTV p27.

Materials and Methods

Continuous human mammary tumour cell lines: MCF-7 (Soule *et al.*, 1973), T47D (Keydar *et al.*, 1979) and MDA-231 (Cailleau *et al.*, 1974) were the generous gift of Dr. Kovařek (Institute of Experimental Oncology, Brno, Czechoslovakia). The MCF-7 clones A6, B6 and C6 were prepared in our laboratory (Litvinov and Golovkina, 1985). The clones A6 and B6 contained a high percentage of cells reacting with the serum to MMTV proteins; clone C6 was not found to contain reactive cells.

Human milk epithelial cells were sedimented from the milk of healthy women collected under sterile conditions on day 2 of lactation. The cells were washed 3 times with warm Earle's solution (37 °C) and grown in plastic Petri dishes (Falcon) in the medium HAM'S'F-12 with 15 % foetal serum and hormones: 10^{-6} mol/l estradiol and 10^{-8} mol/l progesteron; the cells were fixed on days 2 or 3 of cultivation.

Sera. Sera against MMTV of C3H mice protein prepared by the authors, sera against proteins p27 and p14, sera against gp52 and gp36 prepared by Parcks (goat) were used. Sera of healthy women (Nos. 206 and 313) reacting with MMTV p27 and not reacting with other MMTV proteins were selected previously and their specificity was characterized by immunoblotting. The following sera were used as controls: serum against milk proteins of C57BL mouse (rabbit), serum of normal nonimmune male rabbit, goat sera against MPMV p27, RaLV gp70, RaLV p30 and SSAV p27. All sera against type C and D retroviruses were supplied by National Cancer Institute (Bethesda, U.S.A.) according to the U.S.S.R.—U.S.A. agreement.

Rabbit IgG against goat IgG and against human IgG conjugated with peroxidase according to the procedure of Nakane and Kawaoi (1974) were also used. Commercial conjugate against rabbit IgG (Dako) was employed.

Indirect enzyme immunoassay on fixed cells. The cells were grown on DMEM medium with addition of 10 % calf foetal serum and 10^{-6} mol/l estradiol and 10^{-8} mol/l progesterone (Koch-Light). They were then rinsed twice with cool (8 °C) phosphate buffered saline (PBS), fixed by 4 % formaldehyde solution in PBS for 1 hr, and treated with 1 % Triton X-100 in PBS (15 min) and washed at 8 °C for 18 hr to remove the fixative. The first antibody solution prepared in PBS (1 : 150) was added to the preparation of cells grown in small Petri dishes. The cells were incubated at room temperature for 3 hr, rinsed with PBS and further incubated for 2 hr with the conjugate. The volume of antibody solution was always adjusted so that the liquid layer was about 5 mm. After final washing the substrate was added: diaminobenzidinetetrahydrochloride (0.5 mg/ml in 0.05 mol/l Tris-HCl, pH 7.5) solution with addition of 2 µl/ml of 33 % H_2O_2.

Immunoblotting. The proteins were analysed according to Laemmli (1974) in 15 % polyacrylamide gel and transferred to nitrocellulose filter BA85 (Schleicher, Schull) according to the method of Towbin and Gordon (1984). Treatment with antibodies was performed as described elsewhere (Litvinov *et al.*, 1986).

Results

The sera used in the present paper were characterized using the immunoblotting technique. It has been shown that none of the anti-MMTV sera reacted with proteins of retroviruses C or D, and specific sera against gp52 and p27 did not react with major proteins of mouse milk or serum against

human milk. The serum against p27 revealed polypeptides p27 and p14 and that against gp52 — proteins gp52 and gp36. By means of the MMTV antiserum 4 major virion proteins could be detected: gp52, gp36, p27 and gp68 — a nonviral mouse milk protein incorporated into the virion. Human sera Nos. 206 and 313 reacted only with MMTV p27 (Fig. 1).

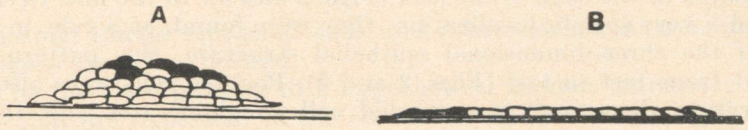

Fig. 2.
Position of cells containing MMTV- related antigens in the epithelial layer of MCF-7 cells (A) and T47D (B)
Positive cells are marked with black colour.

Clone C6 cells were used for abolishing the background reaction of human sera with human cell antigens and to achieve a totally negative response with the cells of this clone.

Rabbit serum against MMTV proteins and goat serum against gp52 allowed the identification of positive cells expressing MMTV-related antigens in clones A6 and B6 of line T47D. However, under these conditions they failed to react with the cells of lines MDA-231 and with the clone C6. Nor did they react with ductal epithelial cells, macrophages or fibroblast-like cells present in the human milk cell sediment.

Table 1. Expression of proteins related to structural MMTV proteins in human mammary epithelial cells in vitro

Serum	Cell line				Cells from
	A6	B6	T47D	MDA-231	women milk
Anti-MMTV	+	+	+	—	—
Anti-gp52 MMTV	+	+	+	—	—
Anti-p27 MMTV	+	+	+	—	—
Anti-gp70 RaLV	—	—	—	n.t.	n.t.
Anti-p30 RaLV	—	—	—	n.t.	n.t.
Anti-p27 SSAV	—	—	—	n.t.	n.t.
Anti-p27 MPMV	—	—	—	n.t.	n.t.
No. 206 (donor)	+	+	+	—	—
No. 313 (donor)	+	+	+	—	—
Donor, negative	—	—	—	n.t.	n.t.

Note. (+) — presence of cells detectable by given serum;
(—) — no positive cells observed; n.t. — the cells of given line were not tested with given serum

Noteworthy was the localization of positive cells of the clones of MCR-7 and T47D lines. Not each cell of the clones appeared positive, and next to the positive ones morphologically identical negative cells were situated. The expression of antigens related to MMTV *env* gene products was observed both in cells of clones A6 and B6 within the epithelial layer and in separated cells. Among cells growing in foci of epithelial monolayers, positive cells accumulated at the edge of the islet (Figs. 2 and 3). In the line T47D positive cells had a very specific localization: they were found, as a rule, in the upper layer of the three-dimensional epithelial structure, this pattern regularly recurred from test to test (Figs. 2 and 3). Positive cells were also detected after trypsin dispersion of epithelial cell conglomerates and their single cell plating.

When using control sera — normal rabbit serum and goat serum against RaLV gp70 — no positive cells were found in any of the cell lines.

Employing sera against p27 and donor sera reacting with p27 allowed to detect positive cells in the lines A6, B6 and T47D, but not in the line MDA-231 or in the human milk cells. Pretreatment of cells with sera against milk proteins from C57BL mice failed to inhibit the reaction of sera against MMTV core proteins. When sera against major core proteins of retroviruses C and D were used instead of specific serum against MMTV p27, no positive cells were found in any of the lines. The same result was observed upon replacing in the reaction sera Nos 206 and 313 by a negative donor serum.

Cells containing p27-related antigens occurred among the cells of clones A6 and B6 at the same frequency as cells detected with the sera against gp52 and T47D. We believe that localization in the upper layer suggests that p27-related and gp52 like antigens were present in the same cells and that their expression was coupled one to another (Figs 2 and 3). Table 1 summarizes the results obtained with sera against MMTV proteins and control sera reacted with line MCF-7, T47D and MDA-231 cells as well as with human milk cells.

Discussion

We have described the expression of antigens immunologically related to MMTV *gag* gene products in some cells of lines MCF-7 and T47D. We have found that no such antigens are present in the cells of line MDA-231 or among the cells from women milk. The clonal line C6 derived from cells MCF-7 appeared to have no such antigens either. Sera of healthy women reacting with MMTV protein p27 were absorbed by the cells of this negative clone. So they could be used in the reaction with cells of all other lines helping to detect positive cells in lines A6, B6 and T47D. Simultaneously, we have studied the expression of antigens immunologically related to MMTV *env* gene products in the cells of all these lines.

The following conclusions can be drawn on the basis of the data obtained:

— cells expressing antigens immunologically related to MMTV *gag* gene products occur in continuous lines MCF-7 and T47D. Even in clonal line these antigens are expressed in a certain percentage of cells only. Their

expression is to some extent dependent on the position of the cell in the epithelium layer and hence, on its proliferation and differentiation;

— these antigens can be recognized by healthy women's sera reacting with MMTV protein p27 and may induce the formation of antibodies against MMTV core proteins;

— the expression of MMTV *env* protein-related and *gag* protein-related antigens is coupled with each other and in the line T47D it occurs in the same cells;

— in normal ductal epithelial cells and other normal human milk cells these antigens are not expressed.

It may be there are proteins of a hypothetical human mammary tumour retrovirus that are expressed in the cells of lines T47D and MCF-7 for it was for these lines in which isolates 734 B (Rich *et al.*, 1976) and HuMTV (Keydar *et al.*, 1984) were described. Human genome was found to contain sequences that are homologous to a certain extent to the sequences of MMTV *gag* and *pol* genes, and although cloned fragments of such sequences are inactive in transcription because of a termination signal in the reading frame, they may be suggested to have active homologs (Westley and May, 1984; Deen and Sweet, 1986).

In fact, the milk of certain women has been found to contain antigens immunologically related to MMTV core proteins and we have detected a protein with molecular weight 27-28 kD in a particulate fraction of human milk, the density of the particles being characteristic of retroviruses (1.16—1.18 g/ml) (Litvinov *et al.*, 1987). It should be noted, however, that milk is secreted by alveolar cells, whereas the lines under investigation were derived from ductal mammary carcinoma. It is also noteworthy that no such antigens related to MMTV protein p27 were found in ductal epithelial cells that sloughed off into milk. Therefore, we do not believe that the problem of the source and spectrum of human antigens related to MMTV internal proteins has been clarified as yet.

References

Cailleau, R., Young, R., Olive, M., and Reeves, W. J. (1974): Breast tumor cell lines from pleural effusions. *J. natn. Canc. Inst.* **53**, 661—670.

Calafat, J., Daams, J., Hageman, Ph., Swen-Wierda, S., and Verstraeten, A. (1976): A possible viral etiology of human mammary tumors. *Ann. Rep. Netherland Canc. Inst.* **1976**, 47—48.

Callis, A., and Ritzi, E. M. (1981): Protein A assay for mouse mammary tumour virus gp52 determinants on murine and human mammary tumour cells. *Virology* **111**, 656—661.

Deen, K., and Sweet, R. (1986): Murine mammary tumour virus pol-related sequences in human DNA: characterization and sequence comparison with the complete murine mammary tumour virus pol gene. *J. Virol.* **57**, 422—432.

Hageman, Ph. V., Calafat, J., Hilgers, J., and Timmermans, A. (1978): The biology of mammary tumour viruses. *Ann. Rep. Netherl. Cancer Inst.* **1978**, 79—86.

Hendrick, J. C., Francois, S., Calberg-Bacc, C., Colin, C., Franchimont, P., Gosselin, L., Kozma, S., and Osterrieth, P. M. (1978): Radioimmunoassay for the protein p28 of murine mammary tumour virus in organs and serum of mice and search for related antigens in human sera and breast cancer extracts. *Cancer. Res.* **38**, 1826—1831.

Keydar, I., Chen, L., and Karby, S. (1979): Establishment and charaeterization of a cell line of human breast carcinoma origin. *Eur. J. Cancer* **15**, 659—661.

Keydar, I., Ohno, T., Nayak, R., Sweet, R., Simori, F., Weiss, F., Karby, S., Mesa-Tejada, R., and Spiegelman, S. (1984): Properties of retrovirus-like particles produced by a human breast carcinoma cell line: immunological relationship with mouse mammary tumour virus proteins. *Proc. natn. Acad. Sci. U.S.A.* **81**, 4188—4192.

Kryukova, I., Komarova, E., Zotter, St., and Grossmann, H. (1981): Use of an electroimmunodiffusion method on cellulose acetate films for the detection of antigenicity in human breast cancers that is related to the major core antigen of mouse mammary tumour virus. *Arch. Geschwulstforsch.* **51**, 517—521.

Laemmli, U. K. (1974): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680—684.

Litvinov, S., and Golovkina, T. (1985): The expression of MMTV-related antigens in breast cancer cells (in Russian). *Vopr. Onkol.* **31**, 53—61.

Litvinov, S., Kryukova, I., Hint, E., and Purde, M. (1986): Antibodies to MuMTV proteins in the sera of mammary carcinoma patients healthy daughters. *Arch. Geschwulstforsch.* **56**, 407—412.

Litvinov, S., Golovkina, T., Kryukova, I., and Vasilevskaya, L. (1987): A protein related to major core protein of murine mammary tumour virus from virus-density fraction of human milk. (in Russian). *Byull. eksp. Biol. Med.* **103**, 338—340.

Mesa-Tejada, R., Spiegelman, S.(1983): Retroviruses and human breast cancer, pp. 473—500. In L. A. Phillips (Ed.): *Viruses Associated with Human Cancers.* M. Dekker, New York.

Nakane, P., and Kawaoi, A. (1974): Peroxidase- labelled antibody. A new method of conjugation. *J. Histochem. Cytochem.* **22**, 1084—1091.

Rich, M., Furmanski, Ph., McGrath, Ch., McCormick, J., Russo, J., and Soule, H. (1976): The etiology of breast cancer, pp. 15—27. In *Steroid Hormone Action and Cancer.* New York—London.

Soule, H. D., Vasques, J., Long, A. (1973): A human cell line from a pleural effusion derived from a breast carcinoma. *J. natn. Cancer Inst.* **51**, 1409—1413.

Tomana, M., Kajdos, A., Niedermeier, W., Durkin, W., and Mestecky, J. (1981): Antibodies to mouse mammary tumor virus-related antigen in sera of patients with breast carcinoma. *Cancer* **47**, 2696—2703.

Towbin, H., and Gordon, J. (1984): Immunoblotting and dot immunoblotting — current status and outlook. *J. immun. Meth.* **72**, 313—325.

Westley, B., and May, F. (1984): The human genome contains multiple sequences of varying homology to MMTV DNA. *Gene* **28**, 221—227.

Yang, N. S., Soule, H. D., and McGrath, C. M. (1977): Expression of murine mammary tumour virus-related antigens in human breast carcinoma MCF-7 cells. *J. natn. Cancer Inst.* **59**, 1357—1367.

Yang, N. S., McGrath, C. M., and Furmanski, P. (1978): Presence of a mouse mammary tumour virus-related antigen in human breast cells and its absence from normal mammary epithelial cells. *J. natn. Cancer Inst.* **61**, 1205—1208.

Zotter, St., Kemmer, Ch., Lossnitzer, A., Grossmann, H., and Johannsen, B. A. (1980): Mouse mammary tumour virus-related antigens in corelike density fractions from large samples of women's milk. *Eur. J. Cancer* **16**, 180—186.

Zotter, St., and Grossmann, H. (1983): Among the human antibodies reacting with intracytoplasmic A particles of mouse mammary tumour virus, some react with p14, the nucleic-acid binding proteins and others with MMTV p28, the main core protein. *Int. J. Cancer* **32**, 27—35.

Legend to Figures (Plate XXI—XXII):

Fig. 1. Reaction of MMTV proteins with sera against MMTV (1). gp52 (2), p27 (3) and normal women's sera No. 206 (4) and No. 313 (5). Immunoblotting technique.

Fig. 3. Indirect peroxidase immunoassay with fixed cells of lines MCF-7 (a, b, c) and T47D (d, e, f, g, h) of sera against MMTV proteins: anti-MMTV (a, e), anti-gp52 (b, f,), anti-p27 (c, g) and No. 206 (d, h).