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Detection of an Antigen Related to Mason-Pfizer Virus in Malignant Human Breast Tumors

Abstract. *An antigen related to the major structural protein (p27) of Mason-Pfizer monkey virus has been found in malignant human breast tumors by radioimmunoassays. This antigen was not detected in normal placental tissues or in tumors that were not of breast origin.*

The Mason-Pfizer monkey virus (M-PMV) was originally isolated from a mammary carcinoma of a female rhesus monkey (*1*); it has biochemical and biophysical properties characteristic of the known RNA tumor viruses (*2*). Molecular hybridization experiments demonstrated that ^{3}H-labeled DNA complementary to the RNA of M-PMV hybridizes preferentially to the RNA of human malignant breast tumors (*3*).

The presence of antigens related to the major structural protein (p30) of mammalian type C viruses has been detected in tissues of animals (*4*) and man (*5*) by competition radioimmunoassay (RIA). Antigens related to the p30 protein of primate type C viruses were also found in peripheral white blood cells of patients suffering from acute leukemia (*6*). Recently, our laboratory has developed a specific RIA for the major structural protein (p27) of M-PMV (*7*). We used this assay to look for the presence of proteins related to M-PMV p27 in various types of human tissues. We now report the detection of a protein antigenically related to M-PMV p27 in human malignant breast tumors.

Frozen malignant breast tumors (Hackensack Hospital, New Jersey) were selected after gross and microscopic pathological examination. Frozen human malignant tumors that were not of breast origin were obtained through the Office of Program Resources and Logistics, National Cancer Institute. Normal human placentas were obtained from Hackensack Hospital. Tissues were homogenized and extracted with ether to remove the lipids and fats (*4*). The soluble proteins contained in the aqueous phase were further purified by diethylaminoethyl (DEAE)-cellulose ion exchange column chromatography as described for M-PMV p27 (*7*). The fractions of the column where M-PMV p27 would normally be eluted were collected, concentrated, and used as competing antigens in RIA for M-PMV p27 and simian sarcoma virus (SSV-1) p30 proteins (*8*).

Of 18 breast tumor specimens that were tested, 8 induced the release of > 25 percent of the maximum precipitable ^{125}I-labeled M-PMV p27 (Fig. 1). The range of ^{125}I release was from 27 to 78 percent. Figure 1A shows the competition curves obtained with four of the eight positive specimens. The other four positive specimens showed curves similar to those of the four presented. Each of these tumor specimens showed competition curves of similar slope to the competition curve developed with purified M-PMV p27 as the competing antigen, which suggests that the reacting proteins in the tumor tissues are similar to M-PMV p27. From the RIA curves, the amount of p27-like proteins detected in these eight breast tumors ranged from 0.3 to 5.7 ng per milligram of tissue extract. The remaining ten tumors each induced the release of less than 15 percent of the maximum precipitable ^{125}I-labeled M-PMV p27, and were considered negatives. No correlation between the age of the patient and the presence of reactive antigen was noticed.

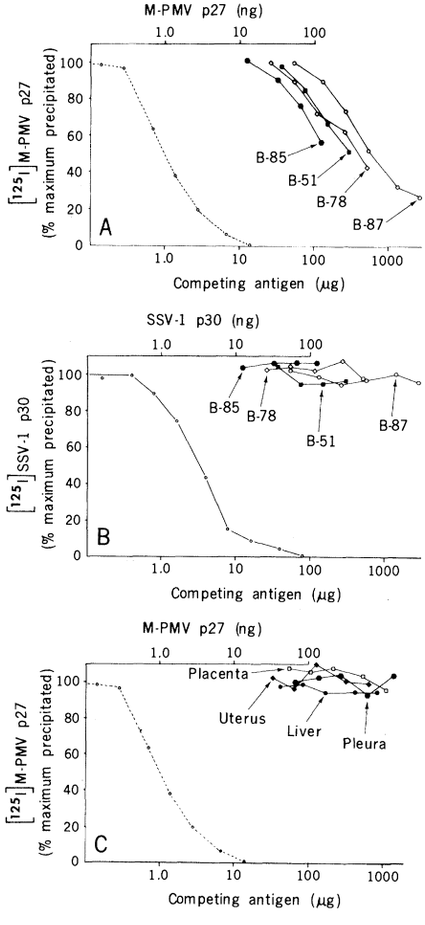

Fig. 1. Radioimmunoassay for M-PMV p27 and SSV p30. The conditions for competition RIA were as follows: A quantity of specific rabbit antiserum sufficient to precipitate 50 percent of the ^{125}I-labeled precipitable antigen was added to varying amounts of competing proteins and incubated for 1 hour at 37°C. Three nanograms of ^{125}I-labeled M-PMV p27 [8.6 × 10^4 count/min, 98 percent precipitable by trichloroacetic acid (TCA) and 85 percent precipitable with specific antiserum] or 5 ng of ^{125}I-labeled SSV p30 (6.2 × 10^4 count/min, 97 percent precipitable by TCA and 90 percent precipitable with specific antiserum) were added and incubated for 1 hour. Appropriate quantities of goat antiserum to rabbit serum was added, incubated for 1 hour at 37°C and 18 hours at 4°C. Precipitates were sedimented, washed with TNE (tris, sodium chloride, EDTA) buffer, and counted. Competition RIA of (A) M-PMV p27; (B) with SSV-1 p30, with four human breast tumors: B-85, B-78, B-51, and B-87; and (C) M-PMV p27, with nonbreast tissues: uterus, pleura, liver, and normal human placenta. Purified M-PMV p27 (o--o) and SSV p30 (o—o) were used as positive controls (top scale).

In order to determine if these M-PMV p27-related proteins found in malignant breast tissues can be found in other human tissues, 12 human tumors not of breast origin and two normal human placentas were extracted in the manner described above and used as competing proteins in RIA's. None of these tissues contained detectable antigen related to M-PMV p27. Four representative samples were plotted (Fig. 1C) for comparison with the results obtained with extracts derived from breast tumor tissues.

The possibility of nonspecific competition by the eight malignant breast tissue extracts was ruled out by allowing these extracts to compete with SSV-1 p30 in an RIA developed for that protein. None of the 18 breast tumors demonstrated competition against SSV-1 p30. The 14 nonbreast tissue extracts tested also showed no competition. Figure 1B illustrates the results obtained in RIA for SSV-1 p30 in assays conducted with four of the breast tumor extracts that were found to be positive for M-PMV p27.

As an additional control, to eliminate the possibility that some proteolytic enzyme from the malignant breast tumor extracts might have caused the release of ^{125}I from ^{125}I-labeled p27 and thus produced a

Table 1. Presence of antigen related to M-PMV p27 in human breast carcinomas as determined by competition radioimmunoassays. The detailed histopathology of human breast tumors is as follows: (B-34) poorly differentiated mammary duct cell carcinoma with osteoid metaplasia, left breast; (B-65) infiltrating poorly differentiated duct cell carcinoma, left breast; (B-44) mammary duct cell carcinoma; (B-51) poorly differentiated invasive duct cell carcinoma, right breast; (B-78) poorly differentiated invasive duct cell carcinoma, left breast; (B-84) poorly differentiated mammary duct cell carcinoma, right breast; (B-85) mammary duct cell carcinoma, left breast; (B-87) infiltrating mammary duct cell carcinoma; (B-70) not available; (B-89) not available; (B-91) invasive duct cell carcinoma, left breast with metastasies to lymph nodes; (B-92) invasive mammary duct cell carcinoma with marked desmoplasia, left breast; (B-83) poorly differentiated invasive adenocarcinoma, left breast; (B-63) not available; (B-79) not available; (B-77) moderately differentiated mammary duct cell carcinoma, right breast; (B-86) not available; (B-88) metastatic carcinoma. The 14 human nonbreast tissues that were examined included two normal human placentas, one hepatoma, one carcinoma of lung, two carcinomas of colon, one carcinoma of rectum, one carcinoma of gallbladder, two granulosa tumors of ovary, one adenocarcinoma of stomach, one leiomyosarcoma of uterus, one renal cell cancer of stomach, and one meothelioma of pleura. Ten to 15 g of each tissue was processed, resulting in 1 ml of protein extract containing 55 to 142 μg per milliliter of protein. None of these tissues competed with M-PMV p27 in our RIA's.

Patient	Age (years)	Tumor wet wt (g)	Final extract (ml)	Final protein (mg/ml)	Activity ratio (ng/mg)
B-34	52	8	0.5	27	0.4
B-65	65	8	0.5	31	0.3
B-44	48	5	0.5	33	1.2
B-51	62	8	0.4	15	2.9
B-78	56	13	0.8	55	2.3
B-84	60	10	0.5	63	1.9
B-85	45	4	0.4	13	5.6
B-87	40	17	1.0	260	1.7
B-70		18	1.0	230	*
B-89		5	0.5	36	*
B-91	58	6	0.5	26	*
B-92	71	7	0.5	29	*
B-83	55	8	0.7	18	*
B-63	48	13	1.0	116	*
B-79		16	1.0	52	*
B-77	74	17	1.0	155	*
B-86		8	0.5	27	*
B-88	29	7	0.5	20	*

*Not detectable.

false positive result, the RIA supernatants from the lowest dilutions of tumors B-87, B-51, and B-78 (these competed most effectively for ^{125}I-labeled M-PMV p27) were applied onto sodium dodecyl sulfate–polyacrylamide gels and the positions of the radioactive peaks compared to those of protein standards (*7*). There was no appreciable breakdown of the ^{125}I-labeled p27 since, in each case, more than 80 percent of the released radioactivity was found under the p27 peak (data not shown).

Among the group of eight positive breast tumor specimens (Table 1), the amount of antigens detected ranged from 0.3 to 5.7 ng per milligram of protein extract. Since the extraction and column chromatography procedure achieved from 200- to 1000-fold reduction of cellular material, the amount of p27-like protein in these breast tumors appears to be very small. Experiments were done to approximate the level of recovery of p27 from tumor material by adding 100 ng of purified p27 to 10 g of normal placental tissue before proceeding with the extraction and chromatography purification steps. The partially purified tissue material was quantitatively tested for p27 by RIA. Almost 50 percent of the input p27 was recovered at the end of the purification procedure. This value of 50 percent probably represents the maximum amount recoverable by this procedure because undoubtedly some intracellular antigens would have been trapped with the cell debris and therefore lost during the purification steps. Even assuming a 10 percent recovery of the M-PMV specific antigens from tumor tissues, the amount of antigens in breast tumor tissues is far less than the level of SSV-1 antigens found by Sherr and Todaro in peripheral white blood cells of patients with acute leukemia (*6*). This low level of viruslike antigens in malignant breast tissues may be a partial explanation for our inability to detect such antigens in the other ten breast tumors examined.

In summary, we detected the presence of an antigen similar to the M-PMV major structural protein in 8 out of 18 human malignant breast tumors. Such antigen was not detected in malignant or normal human tissues not of breast origin.

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Guanidinium-CsCl Density Gradients for Isopycnic Analysis of Nucleic Acids

Abstract. *The addition of guanidinium chloride to CsCl gradients lowers the apparent density of RNA, DNA, and hybrid polymers in such a way that all three can be banded, fractionated, and analyzed in one gradient with essentially no damage to their chemical integrity or to their biological activity.*

CsCl density gradients (*1*) are widely used for isopycnic analysis of DNA because of their simplicity and flexibility (*2*). However, CsCl, is not useful for the analysis of RNA. Several attempts (*3*) to design density gradients adequate for banding of RNA have met with incomplete success. It would be useful to have available a simple analytical procedure that would permit simultaneous isopycnic analysis of DNA, RNA, and DNA-RNA hybrids. In the course of some experiments with guanidinium chloride to dissociate nucleoproteins into their two moieties, it was noted that, in the presence of guanidinium chloride, DNA had a lower buoyant density in CsCl. Therefore we set out to determine whether this was also true for RNA so that it would band at a density within the range normally produced by CsCl gradients. After some