Inhibition of Human Platelet Glycoprotein IIb/IIIa Binding to Fibrinogen by Tumor Cell Membrane Proteins

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Abstract

Immunoechemical and functional characteristics of tumor cell membrane proteins and human platelet glycoproteins were studied. Immunoblotting revealed that membrane proteins of a cultured breast tumor cell line (BT-20) had three protein bands, which were each recognized by monoclonal antibodies to human platelet glycoprotein Ib, IIb, and IIIa, suggesting some immunoechemical similarities between the tumor cell membrane proteins and platelet glycoproteins. The monoclonal antibodies failed to bind to an extract of a lung tumor cell line (A549). Neither tumor extract induced platelet aggregation. However, tumor-associated antigens isolated from the breast tumor cells markedly inhibited platelet glycoprotein IIb/IIIa binding to fibrinogen. In contrast, tumor-associated antigens from the lung tumor cells had no effect. These results suggest that tumor cell membrane glycoproteins and platelet glycoproteins were studied. Immunochemical and functional characteristics of tumor cell membrane proteins and platelet glycoproteins (1-6). Tumor cell-induced platelet aggregation (6-13) and tumor cell adhesion to plateslets (14-17) have also been reported. These reports indicate that GPIIb/IIIa is involved in tumor cell adhesion. Monoclonal antibodies directed against platelet GPIIb/IIIa complex and peptides that inhibit cellular binding to fibronectin were found to inhibit tumor cell growth and colony formation (12, 18). Pearlstein et al. (8) reported that the isolation of PAM from tumor cells. To investigate immunoechemical and functional similarities between tumor cell membrane proteins and human platelet glycoproteins, we used two in vitro cultured tumor cell lines, a breast adenocarcinoma cell line (BT-20) and a lung adenocarcinoma cell line (A549). The immunological properties of BT-20 had previously been well characterized by one of the authors (19). In this paper we report on (a) the platelet-aggregating activity of the tumor cell membrane proteins, (b) the immunological similarity between the tumor cell membrane proteins and platelet glycoproteins as determined by monoclonal antibodies to human platelet glycoproteins, and (c) TAA isolated from the tumor cell lines on in vitro binding of GPIIb/IIIa to fibrinogen.

Materials and Methods

Materials. NP-40 was purchased from Bethesda Research Laboratories (Bethesda, MD). Human fibrinogen (Fraction I, type I) was obtained from Sigma Chemical (St. Louis, MO). Monoclonal antibodies against human platelet GPIIb (CD242b), GPIIIa (CD241b), GPIIIa (CD61), and GPIIb/IIIa complex (CD41a) were obtained from AMAC, Inc. (Westbrook, ME). Microtiter plates for enzyme-linked immunosorbent assay (96-well flat-bottomed microtiter plates; Nunc-Immu No. MaxiSorp, Nunc, Inc.) were obtained from InterMed (Roskilde, Denmark). Horse serum (conjugated and alkaline phosphatase-conjugated anti-mouse-IgG antibodies were obtained from Accurate Chemicals and Scientific Co. (Westbury, NY).

Tumor Cell Lines. Human breast adenocarcinoma (ATCC HTB 19, BT-20) and lung adenocarcinoma (ATCC CCL185, A549) were obtained from the American Type Culture Collection (Rockville, MD).

PAM and TAA. PAM was extracted from the cultured cell lines with 1 M urea in veronal buffer, dialyzed, and concentrated as described by Pearlstein et al. (8). TAA were isolated from the cell lines and processed as described earlier (19). To summarize the isolation procedures, membrane-bound TAA were solubilized with 0.5% Nonidet P-40 in PBS at room temperature. The extracts were then filtered on a Bio-Gel A-50 column. The elution pattern showed two well-separated peaks. The first peak contained the TAA. The proteins in this peak were concentrated by pressure filtration and lyophilized.

Aggregometry. Platelet aggregation was determined by a platelet aggregometer from Chrono-Log Co. (Haverstown, PA) using PRP.

Platelet Extraction. Fresh platelets were obtained from healthy donors. Blood was drawn into a 10 ml plastic tube containing 0.9 ml of 3.8% sodium citrate. PRP was harvested by centrifugation at 200 x g for 15 min, and the platelet number was counted. Platelets were pelleted by centrifuging the PRP at 1000 x g for 15 min. The platelets were washed 3 times with platelet washing buffer consisting of 10 mM Tris-HCl (pH 7.0), 0.6 mM EDTA, 150 mM NaCl, 30 mM N-ethylmaleimide, and 1 mM phenylmethylsulfonyl fluoride. The platelet concentration was adjusted to 2 x 10^9/ml with 0.6% sodium citrate. Platelet-rich plasma was allowed to stand for 30 min at room temperature with occasional stirring. The mixture was centrifuged at 27,000 x g for 30 min, and the supernatant was then divided into smaller portions and kept frozen at -20°C until used.

Immunoblotting. The harvested tumor cells were washed with PBS and extracted with 0.5% NP-40, 2 M KCl, or 8 M urea and reduced with 2% β-mercaptoethanol and 1% SDS at 95°C for 5 min prior to SDS-PAGE. SDS-PAGE was performed at 20 mA/gel for 1 h, and membrane glycoproteins were transferred to a nitrocellulose membrane in a transfilter chamber for 3 h at 20 mA/gel. Glycoproteins on nitrocellulose membranes were blocked with 3% gelatin in 0.02 M Tris-buffered saline and incubated with 500-fold diluted monoclonal antibodies to GPIIb, GPIIIa, or GPIIIa in Tris-buffered saline for 3 h or overnight at room temperature. The nitrocellulose strips were further incubated with the 1:1000 dilution of alkaline phosphatase-conjugated Fab, antibodies against mouse IgG for 2 h at room temperature, and subsequently color was developed with 5-bromo-4-chloro-3-indolyl phosphate and p-nitro blue tetrazolium chloride.
TUMOR CELL MEMBRANE GLYCOPROTEINS

Results

Aggregation of Human Platelets. Platelet aggregations were measured in an aggregometer using fresh PRP within 2 h after preparation. Aggregability of the platelets was confirmed by testing with ADP or collagen. Neither breast nor lung tumor cell fractions, either (a) whole cells, (b) crude extracts with NP-40 (dialyzed to ensure removal of detergent or salts), (c) purified TAA, or (d) the PAM fraction (8) induced platelet aggregation.

Detection of Tumor Glycoproteins by Monoclonal Antibodies to Human Platelet Glycoprotein. The breast tumor cell membrane proteins transferred to nitrocellulose membranes after SDS-PAGE were incubated separately with monoclonal antibodies against GPIb, GPIIb, and GPIIIa, and then color was developed with the alkaline phosphatase-conjugated second antibodies. Fig. 1 shows that the breast adenocarcinoma cells had three main proteins with approximate molecular weights of 125,000, 94,000, and 68,000, which were detectable by monoclonal antibodies against GPIb, GPIIb, and GPIIIa. In contrast, the lung cancer proteins were not recognized by these monoclonal antibodies, even though both tumor cell membranes contained several protein bands as revealed by SDS-PAGE (data not shown). When the strips containing the breast tumor cell membrane proteins were incubated with 20-fold dilution of sera from a patient with systemic lupus erythematosus and a patient with immune thrombocytopenic purpura who were known to have anti-platelet antibodies, serum anti-platelet antibodies were noted to bind to the tumor cell proteins (data not shown).

Inhibition of Platelet GPIIb/IIIa Binding to Fibrinogen by Tumor Cell Membrane Extracts. The binding of platelet GPIIb/IIIa to immobilized fibrinogen was inhibited by TAA isolated from the breast tumor cells. As shown in Fig. 2, a mixture containing equal amounts of the breast TAA and platelet extract showed approximately 40% inhibition of GPIIb/IIIa binding. The lung TAA had almost no effect on the binding.

Discussion

In the past few years, several reports have been published which demonstrate (a) the presence of glycoproteins on tumor cell membranes which are detectable with antibodies against platelet glycoproteins (1-5, 14-17) and (b) platelet aggregation induced by tumor cells or PAM isolated from tumor cells (6, 8-13). Glycoproteins on some tumor cells have been found to be immunologically related to platelet GPIIb/IIIa complex (IR-GPIIb-IIIa) (4, 14, 16). To study immunochemical and functional similarities between tumor cell membrane proteins and human platelet glycoproteins, we used a lung tumor cell line and a breast tumor cell line which had been characterized earlier by one of the authors. Tested at various dilutions, neither the breast nor the lung tumor cell materials induced platelet aggregation.

Tumor cell membrane glycoproteins immunologically related to platelet GPIIb/IIIa may mediate the interaction among tumor cells, platelets, and endothelial cells. Monoclonal antibodies to GPIIb/IIIa have been shown to inhibit human melanoma cell adhesion to fibronectin (15) and tumor cell-induced platelet aggregation (14). There have been reports showing that monoclonal antibodies to GPIIIa reduced carcinoma metastasis (12) and inhibited the growth of human melanoma cells implanted in nude mice (18).

In our experiment, three proteins in the BT-20 cell extract were recognized by monoclonal antibodies against human platelet GPIb, GPIIb, and GPIIIa. In addition, anti-platelet antibodies in sera from patients with systemic lupus erythematosus and immune thrombocytopenic purpura bound to tumor cell membrane proteins on nitrocellulose strips. These data suggest that there is a certain structural similarity between the membrane proteins of the breast tumor cells.
and human platelet glycoproteins. The presence of Ib/Illa-like glycoproteins on the tumor cell membranes may be interpreted as the aberrant expression of antigens which occurs during the process of malignancy (2), although the exact nature of these Ib/Illa-like glycoproteins remains to be determined. The TAA isolated from BT-20 cells markedly inhibited the GPIIb/IIIa binding to fibrinogen, whereas the lung TAA had no effect on the binding. These results suggest that tumor cells carrying glycoproteins immunologically related to human platelet glycoproteins may affect hemostasis and coagulation in vivo.

References


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