Involvement of a cathepsin B-like cysteine proteinase in platelet aggregation induced by tumor cells and their shed membrane vesicles

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Murine 15091A mammary adenocarcinoma cells and membrane vesicles spontaneously shed from these tumor cells in culture can induce aggregation of washed human platelets. A spectrum of proteinase inhibitors was tested for their ability to inhibit 15091A induced platelet aggregation. Of the inhibitors tested the most effective were those selective for cysteine proteinases. The effect of the spectrum of proteinase inhibitors on 15091A induced platelet aggregation was compared to the effect on cathepsin B-like cysteine proteinase activity in homogenates of 15091A tumor cells and their spontaneously shed vesicles. The results suggest that there is a correlation between activity of a cathepsin B-like proteinase in 15091A cells and vesicles and the ability of these cells and vesicles to induce aggregation of washed human platelets.

Introduction

Patients with malignant neoplasms often have abnormalities in hemostatic function which can include alterations of both platelet aggregation and coagulation [35]. The relationship among these is not yet completely understood; however, the interaction between host platelets and circulating tumor cells is believed to facilitate metastasis [20]. In in vivo studies Gasic et al. [12] have shown that thrombocytopenia reduces the number of lung tumor colonies formed after i.v. injection and that this reduction can be prevented by infusion of platelets. Aggregation of platelets in vitro can be induced by tumor cells, by membrane vesicles spontaneously shed from tumor cells in culture and by a membrane-derived fraction of the tumor cells [5, 8, 11, 15, 20]. Platelet aggregation can also be induced in vitro by ADP, collagen, thrombin, trypsin, papain, platelet activating factor, etc.

We had previously shown that the tumor cell activities of a cathepsin B-like cysteine proteinase correlate with the lung colonization potential (tail vein metastasis) of murine B16 melanoma variants [30] and that B16 tumor cells in culture release a cathepsin B-like proteinase [31]. Since there is 100 per cent sequence homology at the active site and active site groove between papain and cathepsin B [33] and since papain has been shown to induce platelet aggregation [1], we speculated that one tumor cell principle which might be responsible for inducing platelet aggregation is a cathepsin B-like proteinase. Recently we demonstrated that
the activity of a cathepsin B-like proteinase in B16 amelanotic melanoma cells correlates with the ability of those cells to induce platelet aggregation in vitro [18]. In the present study we compare the effects of a spectrum of proteinase inhibitors on the induction of platelet aggregation by 15091A mammary adenocarcinoma cells, and by membrane vesicles spontaneously shed from these cells, with the effects of these inhibitors on cathepsin B-like activity in homogenates of these cells or vesicles. The results indicate that a correlation exists between the cathepsin B-like proteinase activity in these cells and vesicles and their ability to aggregate platelets.

Materials and methods

Tumor; tumor cell isolation

Line 15091A, which originated as a spontaneous mammary adenocarcinoma [9], was passaged as an ascites tumor by intraperitoneal injection into CAF2/J mice (Jackson Laboratories, Bar Harbor, Maine). For the experiments described below, 15091A ascites tumor cells were harvested from peritoneal fluid at 160 g for 10 min. Cells were washed twice with Eagle’s minimal essential medium (MEM) containing Hank’s balanced salt solution (HBSS). Washed cells were separated from contaminant red blood cells, macrophages, etc. by centrifugal elutriation [30] and counted with a model ZBI Coulter counter.

Isolation of spontaneously shed membrane vesicles

Spontaneously shed membrane vesicles (SSMV) were isolated by a modification of the procedure of Gasic et al. [8]. Suspension cultures of 15091 cells were grown on Bio Carrier beads (BioRad, Richmond, CA) in a chemically defined medium (Hamm’s F12: Dulbecco’s MEM (1:1) supplemented with insulin, transferrin and selenium) at 37°C. For 24 hours prior to vesicle harvesting the cells were cultured in fresh medium. The medium was decanted and centrifuged at 160 g for 10 min. The resulting supernatant was centrifuged at 10 000 g for 10 min. Na2EDTA was added to the 10 000 g supernatant to a final concentration of 2 mM. This solution was centrifuged at 100 000 g for 60 min. The resulting pellet was resuspended in Ca2+, Mg2+-free HBSS, sonicated and overlaid on a 40 per cent (w/v) sucrose solution and centrifuged at 100 000 g for 180 min. The band of material at the interface was aspirated, resuspended in 10 ml of Ca2+, Mg2+-free HBSS and recentrifuged at 100 000 g for 30 min. The pellet was resuspended in a small amount of Ca2+, Mg2+-free HBSS to yield a suspension containing 500–1000 µg/ml of protein. The final pellet has been shown to consist of plasma membrane-derived vesicles [8].

Platelet aggregometry

Washed human platelets (WHP) were obtained from platelet-rich plasma according to the method of Mustard et al. [24]. Platelets were counted with a model ZBI Coulter counter and adjusted to 50 ± 0.5 × 108 platelets/ml. Aggregometry studies were performed with Sienco DP247E dual channel aggregometer. Previous investigators [9, 21, 23] have found that tumor cell-induced platelet aggregation requires Ca2+ and platelet-poor plasma (PPP). For these studies WHP were recalcified (final concentration 2 mM) and PPP added to a final concentration of 2 per cent. Aggregation of WHP by 15091A tumor cells (2 × 10⁵) or SSMV (10 µg protein) was investigated in the absence and the presence of proteinase inhibitors. Only