Thrombosis Research (2008) **123**, 324–330

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THROMBOSIS RESEARCH

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REGULAR ARTICLE

Platelet-induced inhibition of tumor cell growth

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Received 19 February 2008; received in revised form 3 June 2008; accepted 30 June 2008
Available online 9 August 2008

KEYWORDS
Platelet;
Tumor;
Cell cycle;
Proliferation

Abstract

Background: Previous studies have suggested that platelets play a role in hematogenous metastasis of cancer cells by enhancing their survival in and extravasation from the bloodstream. We initiated studies to determine the effect of platelets on the proliferation of tumor cells.
Methods: Intact platelets or platelet subfractions were prepared and used for co-culture with various tumor cell lines of different major histocompatibility complex (MHC) backgrounds. Proliferation of tumor cells was monitored using a colorigenic method; flow cytometry was used to measure apoptosis or the cell cycle in L1210 cells.
Results: Co-culture of platelets with tumor cells inhibited proliferation of tumor cells in an MHC-independent manner; soluble factors released from platelets as well as physical contact between platelets and tumor cells were involved. Cell-cycle analysis showed that platelets inhibited proliferation mainly through arrest of the cell cycle and inhibition of DNA synthesis. Neither cytotoxicity nor apoptosis mechanisms dominate in the platelet-induced inhibition of tumor cell proliferation.
Conclusion: We observed that murine platelets inhibit growth of tumor cells in vitro in an MHC-I-independent way, and this inhibition is not limited to specific tumor types, nor is it dependent on cytotoxicity or apoptotic pathways; rather it relies on impairment of the cell cycle.

Introduction

The presence of tumor cells in thrombi of cancer patients was first recognized over a century ago [1], but the potential biological significance of tumor-cell–platelet aggregates was first identified in the 1960s when Gasic et al. [2] established a correlation

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doi:10.1016/j.thromres.2008.06.021

between neuraminidase-induced reduction in metastasis and reduced platelet counts. Further studies revealed that most tumor cells – whether from cell lines or from primary tumor tissue – produce soluble factors (e.g. thrombin and ADP) that can activate platelets and lead to the formation of platelet–platelet and platelet–tumor-cell aggregates [3]. Tumor cells also aggregate with and activate platelets through direct contact via surface molecules on platelets (e.g. GPIIb/IIIa) and tumor cells (e.g. α3 integrins) [4]. Platelets on the surface of tumor cells form a shield that protects them from attack by immune cells such as natural killer (NK) cells [5], which are known to provide effective and innate antitumor activity in the hematogenous phase of metastasis. Furthermore, formation of a platelet–tumor-cell complex retards movement of tumor cells in the blood and facilitates their adherence to endothelial cells [1,6]. Platelets in the tumor-cell–platelet aggregates release such mediators as heparitinase [7] that cause endothelial cell retraction or subendothelial membrane matrix degradation. Other molecules also appear to mediate or affect the interaction between platelets and tumor cells [8]. These structural changes in the blood vessel enhance tumor cell extravasation. It is well documented that, in experimental animals and clinical patients, manipulations that block platelet–tumor-cell aggregation – such as depleting platelets, blocking platelet activation, or removing binding sites on the tumor cell surface – diminish or eliminate tumor metastasis [4,9,10]. Considering the abundance of molecules that are expressed or released by platelets [11], we hypothesize that platelets may directly affect the biological properties of the tumor cells that they bind. This hypothesis is supported by a recent report that platelets produce lysophosphatidic acid, which in turn promotes tumor growth [12]. Here we used an in-vitro model to show that platelets inhibit tumor cell growth by blocking cell cycle progression.

Materials and methods

Preparation of platelets and platelet subfractions

C57BL/6 (H-2^b) mice were used at around 8 months of age for platelet preparation as described previously [13]. To prepare subfractions of activated platelets, the washed platelets were resuspended to around 5×10^9/mL in Dulbecco's modified Eagle's medium (DMEM) without serum, and thrombin was added to 0.5 U/mL for 10 minutes. After spinning at 1300 ×*g* for 10 minutes, the supernatant (sup-), which contained microparticles, exosomes and soluble factors, was removed. The pellet, designated 1.3T, was resuspended in the same volume of medium and vortexed to maximize resuspension. To prepare fixed unactivated platelets, platelets in Tyrode's buffer were mixed with an equal volume of 4% neutral buffered formaldehyde for 10 minutes, followed by three washes with phosphate-buffered saline (PBS) and resuspension in DMEM medium. For preparation of fixed activated platelets, platelets were treated with 0.5 U/mL thrombin for 10 seconds before addition of an equal volume of 4% formaldehyde buffer and washes as above.

Cell lines

Murine lymphoma cell lines EG7 (H-2^b), L1210 (H-2^d), YAC-1 (H-2^a), melanoma cell line B16 (H-2^b) and prostate cancer cell line RM1 (H-2^b) were cultured in optimal complete medium (RPMI-1640 for L1210, YAC-1, or DMEM for EG7, B16 and RM1) supplemented with 10% heat-inactivated fetal calf serum and antibiotics.

Assays of tumor cell proliferation and death

Tumor cells at the exponential growth phase were harvested and seeded into 96-well plates at different concentrations. Platelets or platelet fractions were added and mixed well with tumor cells. In some experiments the platelets were substituted with the same concentration of Polybead® Polystyrene Microsphere 2 μm (Polysciences, Warrington, PA), which are about the same size as platelets. In other experiments, 8 well strip inserts with 0.2-μm pore size Anapore™ membranes (Nunc, Denmark) were used to separate tumor cells from platelets to establish whether direct contact was necessary for potential effects of platelets on tumor cells. In all circumstances, the cells were incubated at 37 °C for different times before 20 μL of solution from the CellTiter96® AQueous One Solution Cell Proliferation Assay kit (Promega, Madison, WI) were added to each well. The plates were incubated in the dark for 1 hour, and 25 μL 10% SDS were added to each well. The plate was left at room temperature for 2 hours, and then centrifuged at 1000 ×*g* for 10 minutes; 150 μL of supernatant were removed from each well and transferred to a new plate, and the plate was read at a wavelength of 490 nm with a microplate reader. In some cases the co-culture of platelets with tumor cells was done in 24-well plates, and the numbers of tumor cells at the