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Comparison of Tumor and Microenvironment Secretomes in Plasma and in Platelets during Prostate Cancer Growth in a Xenograft Model[1,2]

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Abstract

To survive and metastasize, tumors interact with surrounding tissues by secreting growth factors and cytokines. In return, surrounding host tissues respond by changing their secretome. Numerous factors theoretically function as therapeutic targets or biomarkers of cancer growth and metastatic risk. However, it is unclear if these factors are tumor-derived or actually represent the host defense. To analyze the concentrations of tumor- and microenvironment-derived factors associated with neoplastic growth, we used ELISA-based arrays specific for murine or human proteins to establish a profile of tumor- or host-derived factors circulating in the plasma or within the platelets upon human tumor implantation into mice. Many factors characterized as tumor-derived were actually secreted by host tissues. This study uncovered the origin of various cytokines and revealed their circulation methods. We found that tumor-produced cytokines are predominantly sequestered in platelets. Sequestered proteins are protected from degradation and, thus, may be functional at metastatic sites. These findings identify tumor-specific targets for the detection and prevention of tumor growth and metastasis. As predicted by our model, monocyte chemotactic protein 1 and tumor necrosis factor α may be biomarkers for human cancers. Thus, our study identified several potential biomarkers that might be predictive of prostate cancer.

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Introduction

The mechanisms of tumor growth and metastasis have been studied for decades, and yet, in 2008, more people died of cancer than from cardiovascular diseases, thus making cancer the number one cause of death in the United States. Many aspects of tumor development remain enigmatic, precluding development of efficient diagnostic tests and treatments. The intricate interactions of a growing tumor with its microenvironment and macroenvironment make cancerous tissue the most elusive part of an organism. It seems that tumor functions as an ultimate parasite and uses an organism's resources to promote its own growth and to invade into distant locations. The growing tumor secretes a number of growth factors, cytokines, and proteases, which are transported by the host vascular system, reaching multiple organs and tissues. Many factors seem to be secreted by the tumor secretomes of various cancers, such as vascular endothelial growth factors (VEGFs) to promote tumor vascularization [1,2], matrix metalloproteinases (MMPs) to modify the extracellular matrix [1,3], cytokines to attract hematopoietic cells from bone

Abbreviations: G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; HGF, hepatocyte growth factor; IL, interleukin; MCP-1, monocyte chemotactic protein 1; MMP, matrix metalloproteinase; OPG, osteoprotegrin; OPN, osteopontin; RANK, receptor activator of NF-κB; RANKL, RANK ligand; SDF-1α, stromal-derived factor-1α; TGF-β_1, transforming growth factor-β_1; TIMP, tissue inhibitor of metalloproteinase; TNFα, tumor necrosis factor α; TPO, thrombopoietin; uPAR, urokinase-type plasminogen activator receptor; VEGF, vascular endothelial growth factor

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marrow [4,5], and growth factors involved in bone turnover to prepare future metastatic sites. Tumor activity triggers diverse reactions in host tissues, including angiogenic processes, recruitment of inflammatory cells, and changes in hemostasis. As a result, the host organism changes its own secretome, possibly as a defensive measure. Yet, many factors produced by surrounding tissues might promote tumor growth and its invasion rather than inhibit it. Although many factors circulating in the blood of a tumor-bearing organism have been identified and even proposed as diagnostic markers [1–3,6,7], it is unclear whether they are part of the tumor or host secretome.

In many instances, the tumor secretome is aimed at communication with distant organs, and therefore, many components should be "hidden" and protected while being transported to their target. Indeed, it was recently shown that whereas some factors circulate freely within the plasma, others are sequestered within platelets and might be selectively released on platelet activation [8]. Depleting platelets in tumor-bearing mice triggers intratumor hemorrhaging and stimulates tumor cell apoptosis within the hemorrhagic area [9]. In addition to the effects on tumor stability, thrombocytopenia diminished tumor cell proliferation. Thus, platelets seem to be required for continued tumor growth. In addition, platelets can directly bind to cells within the tumor, which, in turn, may permit the loading of platelets with tumor-derived factors [10] and promote tumor cell migration and invasiveness. Platelets also bind tumor cells in the circulation, which may assist tumor cells in evading the immune system [11]. Thus, it is not surprising that inhibition of platelet–tumor cell interactions diminishes the formation of metastases [10,11].

In this study, we compared the tumor secretome with the host response to cancer growth by measuring not only freely circulating growth factors but also the ones stored and released by platelets. Further, on the basis of our animal model data, we predicted that monocyte chemotactic protein-1/CCL2 (MCP-1) and tumor necrosis factor α (TNFα) might serve as markers of tumor presence. Indeed, this was confirmed in patients with prostate cancer.

Materials and Methods

Mouse Injection

Eight-week-old male NOD.CB17-*Prkdc*^{scid}/J (Jackson Laboratory, Bar Harbor, ME) mice were injected subcutaneously with PBS (control; $n = 5$) or 4×10^5 LNCaP-C4-2 human prostate cancer cells in PBS per side ($n = 5$). LNCaP-C4-2 cells were provided by Dr Lloyd A. Culp (Case Western Reserve University, Cleveland, OH). Tumors were permitted to grow for 28 days before mice were euthanized.

Platelet Isolation and Activation

While mice were anesthetized, the vena cava of age- and sex-matched mice was exposed, and blood was collected from the vein into acid-citrate-dextrose buffer containing 1 μg/ml prostaglandin E_1 (Sigma, St Louis, MO). Platelets and plasma were separated from the platelet-rich plasma of blood pooled from four to five mice by gel filtration, as previously described [12]. The activated supernatant was collected by centrifugation after platelets were treated with 100 nM phorbol 12-myristate 13-acetate (PMA; Sigma), 1 mM protease inhibitors (complete Mini; Roche, Indianapolis, IN), and 500 μM mouse PAR-4-amide (Bachem, Torrance, CA) at 37°C for 45 minutes to stimulate release of granular contents.

Protein Arrays

The plasma and activated platelet supernatants were assayed with custom-designed Quantibody protein arrays from RayBiotech (Norcross, GA). A human-specific array was used to detect circulating proteins derived from the tumor. A mouse-specific array was used to detect circulating proteins derived from the host. Arrays were assayed according to the manufacturer's protocol. Fluorescently labeled arrays (green fluorescence, Cy3 channel; 555 nm excitation and 565 nm emission) were analyzed using an Axon 4000B (Molecular Devices, Sunnyvale, CA) scanner in the LRI Genomics Core. Values of four replicates for each protein were extracted using the Axon Gene Pix Pro 4.1 software and analyzed using the RayBiotech Custom Raybio Q-Analyzer software, which uses standardized dilutions of each protein to create standard curves used in determining the concentration (pg/ml) of each protein in the samples.

Clinical Samples

An approval from the Cleveland Clinic Institutional Review Board was obtained before the initiation of blood sample collection from patients undergoing radical prostatectomy at the Cleveland Clinic Glickman Urological and Kidney Institute. A patient consent form was specifically created for the study with clearly stated goals and for describing the purpose of our research. Whole blood (3-4 ml) was collected by venipuncture in Na_2EDTA tubes (BD Biosciences, San Jose, CA) from patients before surgery and 3 months postoperatively. Plasma and platelets were isolated from whole blood by centrifugation and gel filtration, as previously described [12]. Platelets were activated with 50 μM human TRAP-6-amide (Bachem), 1 mM protease inhibitors, and 100 nM PMA to stimulate granule release.

ELISAs

Isolated plasma and platelet releasates from six patients were assayed using the RayBiotech Human MCP-1 ELISA or Human TNFα ELISA according to the manufacturer's instructions. Two separate samples from each patient at each time point were analyzed and compared with a standard curve to obtain the concentration (pg/ml) of each protein in the samples.

Statistical Analysis

Student's *t* test analysis or one-way ANOVA with Newman-Keuls posttest was used to determine statistical significance using GraphPad Prism 4.03 software (La Jolla, CA). $^*P < .05$, $^{**}P < .01$, and $^{***}P < .005$.

Results

To compare the tumor secretome to the host tissue defense, we injected human prostate cancer cells into immunocompromised mice (mice without tumors served as controls) and performed a protein array analysis of human or murine proteins in two compartments: plasma and platelet releasates. Candidate proteins were chosen on the basis of their potential roles in cancer progression, invasion, and bone metastasis. Our species-specific system allowed for the detection and quantitative analysis of tumor and host secretomes separately. On the basis of antibody specificity, we measured 15 and 22 proteins of host and tumor origin, respectively. No cross-reaction of antibodies was observed because control samples from mice without tumors were negative for human proteins. A complete list of assayed growth factors and cytokines secreted by host or tumor and their respective values in plasma and platelets is presented in Table 1.