Tumor-Released Microvesicles as Vehicles of Immunosuppression

Roberta Valenti, Veronica Huber, Manuela Iero, Paola Filipazzi, Giorgio Parmiani, and Licia Rivoltini

Unit of Immunotherapy of Human Tumors, Fondazione IRCCS Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy

Abstract

Tumor-released microvesicles, or exosomes, which are abundant in the body fluids of patients with cancer, are likely to be involved in tumor progression. We recently showed that microvesicles released by human melanoma and colorectal carcinoma cells can promote the differentiation of monocytes to myeloid-derived suppressor cells which support tumoral growth and immune escape. These findings underscore an important role for these extracellular organelles in remodeling tumor-stromal interactions to promote malignancy.

Introduction

Tumor cells begin to mold their stromal environment starting at early phases of the neoplastic process. Although the immune system can initially restrict disease progression, its defenses are progressively blunted by the activation of suppressive pathways which deplete immune restraints on tumor spreading (1). Cancer cells are believed to promote this course mainly by pathways involving cell-to-cell contact and the release of immunosuppressive soluble factors. However, an alternative novel mechanism that is now emerging involves the active release by tumor cells of immuno-suppressive membrane microvesicles, also known as exosomes. These microvesicles are endosome-derived organelles of 50 to 100 nm size which are actively secreted through an exocytosis pathway that is used in cells under normal as well as pathologic conditions for receptor discharge and intercellular cross-talk (2). This mechanism is presently being linked to a series of functional alterations occurring in T cells of patients with cancer, ranging from induction of apoptosis (3–5) to defects in T cell receptor components and function (6). However, given the broad array of bioactive molecules incorporated in tumor-produced exosomes, it is expected that the harmful effects of these organelles might not be confined to effector T lymphocytes, but may target antigen-presenting cells as well, with a consequent upstream blockade in the development of antitumor immune responses.

Tumor Microvesicles Skew Monocyte Differentiation from Immunostimulatory to Immunosuppressive Cells

Since the pioneering observation of Taylor and Black regarding the inhibitory role of melanoma-derived microvesicles on the expression of costimulatory molecules in murine macrophages (7), the hypothesis that tumor-derived exosomes could also hamper the upstream phases of the immune responses by affecting antigen-presenting cells remained uninvestigated for a time. Meanwhile, evidence proving the occurrence of multiple myeloid cell dysfunctions in patients with cancer, emerging as defects in dendritic cell subsets (8), or expansion of suppressive cellular components, also known as “myeloid suppressive cells” (1, 9), has been accumulated over the past few years.

With the aim of bridging the phenomenon of microvesicle release to these alterations, we recently explored the effect of melanoma and colorectal carcinoma–derived exosomes on the differentiation of human monocyte precursors into dendritic cells (10). Unexpectedly, this led to the identification of a novel immunosuppressive circuit mediated by tumor-released microvesicles that is associated with skewing monocyte differentiation towards transforming growth factor β (TGFβ)–secreting myeloid suppressive cells. In fact, tumor-derived vesicles efficiently interact with monocytes by membrane fusion and profoundly alter the process of their differentiation into dendritic cells. Notably, on contact with tumor vesicles, monocytes acquire a peculiar phenotype that is characterized by the maintenance of HLA class II (HLA-DR) negativity, and a lack of costimulatory molecule up-regulation. This CD14+/HLA-DR– phenotype hallmark could be interpreted as the attainment of an immature status by monocytes encountering tumor-derived exosomes. Most importantly, these cells gain the ability to spontaneously secrete TGFβ, thereby exerting inhibitory effects on T lymphocyte proliferation and effector functions.

Interestingly, identical phenotypic and functional features can be observed upon treatment of monocytes with microvesicles isolated from the plasma of patients with advanced melanoma, derived at least in part from tumor cells (as suggested by the expression of tumor antigens such as gp100). Even if neoplastic cells are not clearly the only source of exosomes circulating in the peripheral blood of patients with cancer, the tumor dependence of this phenomenon is shown by the lack of detrimental effects on monocyte differentiation in the presence of microvesicles purified from healthy donors’ plasma, which resulted instead in a promaturative activity and a stimulatory effect on T cells.

A noteworthy remark of our study is represented by the observation that a significant expansion of cells with comparable phenotypic and immunosuppressive traits (meaning CD14+/HLA-DR– myeloid cells with TGFβ–mediated inhibitory activity on T cell proliferation and effector functions) can be detected in significantly higher frequency in the peripheral blood of patients with advanced melanoma, as compared with healthy donors. This evidence argues that a network involving these suppressive pathways may be operating in patients with cancer and possibly contributing to tumor progression.

Tumor Microvesicles May Bridge Immunosuppressive Mechanisms Which Operate in Patients with Cancer

It has now become widely recognized that, despite the expression of tumor-associated antigens and the existence of
tumor-specific T cells, growing tumors render anticancer immune responses progressively ineffective. Among the different pathways pointed out as responsible for this process, convincing data are emerging about the involvement of homeostatic immunomechanisms such as those mediated by regulatory T cells and myeloid suppressive cells (1). As for the latter, it should be underlined that this subset has been consistently reported to express the conserved Gr1^CD11b^ phenotype in murine models (9), but the human counterparts of this subset still lack a conclusive definition. Based on our findings, a potential candidate for this subpopulation could be represented in patients with melanoma by CD14^HLA-DR^ subpopulation could be represented in patients with melanoma by CD14^HLA-DR^ cells, which indeed exert consistent suppressive activity on antitumor T cell responses through TGF^β^ secretion. Although in contrast with data identifying arginase metabolism products as major suppression mediators of myeloid suppressive cells in some tumor models (9), our results are actually in line with studies proving the crucial role of TGF^β^ secretion in different experimental in vivo systems (11). In this setting, tumor cells could exploit exosome release as a possible mechanism to mediate the skewing of myeloid differentiation toward immunosuppressive subsets.

Tumor Microvesicles Can Be a Two-Edged Sword for Immune Responses

Our work adds a new piece of evidence to the emerging idea of tumor-derived exosomes as a type of messenger for homeostatic requirements of the originating cancer cell. Indeed, this vision is significantly distant from the initial concept of tumor-released exosomes as structures enriched in antigenic determinants, which inspired the first studies of vaccination conducted in animal models (12). However, the possibility that the same exosomes may also serve as a vehicle for suppressive signals and have negative effects on antitumor immune responses is currently becoming more than a speculative hypothesis.

Many tumor cells of different histologies have been shown to constitutively secrete microvesicles in abundant quantities. This common feature occurs both in vitro and in vivo, in plasma and other biological fluids obtained from patients with cancer, as we and several other groups have recently reported (4, 5, 10, 13).

Clear evidence has accumulated showing that these inconspicuous vesicles can indeed exert a vast array of unfavorable effects on different crucial components of the antitumor immune response, all theoretically contributing to a more favorable condition of in vivo tumor growth. In fact, we and others have shown that tumor-secreted microvesicles can: (a) induce apoptosis in activated T cells, through pathways used by the immune system itself to downsize immune reactions (such as the expression of the death ligands FasL and TRAIL); (b) impair dendritic cell differentiation from monocytes and simultaneously turn these cells into immunosuppressive players, as reported in our study; and (c) switch off natural cytotoxic responses, as those mediated by natural killer cells (14). However, considering that microvesicles carry a remarkably abundant repertoire of bioactive molecules (Fig. 1), one can readily hypothesize the existence of additional pathways turning other immune subsets into tumor-promoting players. In light of this evidence, the potential roles exerted by microvesicular organelles in the regulation of antitumor immune responses need to be extensively reconsidered.

Despite evidence that tumor exosomes might represent a font of tumor rejection antigens potentially able to stimulate antitumor responses in patients with cancer, such vesicles might turn out to be a sort of “Trojan horse,” capable of inducing tolerance or even active suppression instead of activation within the immune system.

Microvesicles Help Maintain Immunotolerance

Microvesicle or exosome release may represent a physiologic process used by virtually all normal cells for intercellular cross-talk. However, a majority of the attention has been given to exosomes released by immune cells, which have been shown to exert crucial immunologic functions (2). Exosomes produced by antigen-presenting cells such as dendritic cells and B lymphocytes can mediate antigen-specific T cell cross-priming comparably to whole originating cells, thus proving the ability of these organelles to deliver the optimal signal for T cell activation. This evidence has again led us to investigate the potential use of exosomes derived from dendritic cells, and pulsed with exogenous antigens, as a novel cancer vaccine strategy. However, the ability of these vesicles to surrogate the activity of professional antigen-presenting cells might strictly depend on the functional status of the originating cells and the in vivo immunologic context as well. In this view, exosomes released by antigen-presenting cells, and immune cells in general, may not exclusively exert an immunostimulatory effect, but they can also act as crucial players in the maintenance of peripheral tolerance by inducing anergy in specific T cells or activation-induced cell death for preventing autoimmune damage (15, 16). Similarly, exosomes, derived from donor bone marrow dendritic cells and given before transplantation, significantly prolong allograft survival (17). Not least, tolerogenic exosomes have been shown to contribute to maintaining pregnancy (18) as well as tolerance to food antigens in the gastrointestinal tract (19). These examples of exosome involvement in immunologic tolerance lead to the crucial question of what might happen under conditions of disease such as cancer, in which tumor-derived exosomes could be used by cancer cells to achieve immunotolerance and contribute to poor clinical outcomes.

Microvesicles Offer an Efficient Vehicle for Mediating Tumoral Immunosuppression

The ability of tumor cells to promote the accumulation of immunosuppressive factors in the host has been primarily linked to a large degree to the release of soluble factors which influence myeloid differentiation (1, 9). However, the secretion of cytokines/growth factors which may act locally or in the immediate vicinity of the releasing site, rapidly undergoing degradation by serum proteases upon reaching the blood circulation, may not be responsible for the totality of the generalized and multiple immune defects detectable in patients with cancer. In contrast, microvesicles could provide a rather resistant transporter of bioactive molecules that can promote a more effective propagation of tolerogenic signals from tumor site to distant compartments, such as draining lymph nodes.

Concluding Remarks

What is clearly emerging from recent studies is the concept that microvesicles and exosomes released by tumor cells constitute an active entity that highly reflects the functional properties of the originating cells. In this view, it is conceivable that their remodeling properties may not only be confined to the immune system, but could also involve a more pleiotropic effect on the host...
Immunosuppressive pathways mediated by tumor-released microvesicles. Tumor cells release microvesicular organelles (also known as exosomes) that express a large array of bioactive molecules. Through FasL and TRAIL, these vesicles can induce apoptosis in activated antitumor T cells, abrogating the potential of these effectors to kill tumor cells. By mechanisms still under investigation, the same vesicles can skew monocyte differentiation into myeloid suppressive cells, exerting inhibitory activity on T cell proliferation and functions through TGFβ secretion. Switching off T cell responses at upstream levels (i.e., by affecting cross-priming by antigen-presenting cells) as well as downstream levels (i.e., by eliminating antitumor effector cells) would be expected to efficiently abrogate antitumor immune potential.
Tumor-Released Microvesicles in Immunosuppression

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1. Rivoltini L, Canese P, Huber V, et al. Escape strategies produced by neoplastic cells should no longer be considered as a simple source of antigenic determinants for hypothetical cancer vaccines, but rather as an active entity with multiple and plastic abilities of remodeling host environment.

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