Molecular Pathways

Exosomes and cancer: a newly described pathway of immune suppression

Huang-Ge Zhang and William E. Grizzle

Department of Microbiology and Immunology, University of Louisville, Louisville, KY

Department of Pathology, University of Alabama at Birmingham, Birmingham, AL

Corresponding Author:
William E. Grizzle, M.D., Ph.D.
Professor of Pathology
University of Alabama at Birmingham
Zeigler Research Building, ZRB 408
703 South 19th Street
Birmingham, AL 35294-0007
(205) 934-4214
(205) 975-7218 Fax
wgrizzle@uab.edu
Abstract
Exosomes are small (30 to 100 nm) membrane bound particles that are released from normal, diseased and neoplastic cells and are present in blood and other bodily fluids. Exosomes contain a variety of molecules including signal peptides, mRNA, microRNA, and lipids. Exosomes can function to export from cells unneeded endogenous molecules and therapeutic drugs. When exosomes are taken up by specific cells, they may act locally to provide autocrine or paracrine signals or at a distance as a newly described nano-particle-based endocrine system. Specifically, mRNA transferred to cells by exosomes can result in the production of new proteins. In cancer, signals via exosomes affect the immune system via inhibition of the functions of T cells and normal killer (NK) cells and by inhibiting the differentiation of precursors to mature antigen presenting cells. Also, exosomes increase the number and/or activity of immune suppressor cells including myeloid derived suppressor cells, T regulatory cells, and CD116+, HLA-DR−/low cells. The effects of exosomes on the development and progression of cancers with an emphasis on suppression of immune surveillance is described. Also discussed are potential uses of exosomes clinically, in the development of vaccines, in targeting tumors, and in diagnosis/early detection.
Background

Exosomes are membrane bound nanoparticles that are ovoid to cup-shaped and develop from exophytic budding of the cellular membrane after the fusion of multivesicular bodies or mature endosomes with the cellular membrane (1-5). After release from diseased or normal cells, some exosomes are transported by the blood to distant sites (6). Thus, normal individuals have exosomes present in blood and other bodily fluids.

In studies of exosomes, exosomes usually are separated using ultra-centrifugation or by immunoprecipitation using antibodies to typical surface antigens of exosomes. Analysis of isolated exosomes has demonstrated their morphology using electron microscopy and the localization of molecules in exosomes using either immunoelectron microscopy and/or fluorescent techniques. For biochemical analysis, exosomes isolated from bodily fluids are washed to remove contaminating bodily fluids. Also controls frequently include the associated bodily fluids from which the exosomes have been removed.

Exosomes contain signal proteins/peptides, microRNAs, mRNAs and lipids (7, 8). In exosomes from mast cells, mRNAs for over 1300 genes were detected and over 100 microRNAs (7). Some of the mRNAs from exosomes have been demonstrated to be functional and murine exosomes that were “taken up” by human cells resulted in the synthesis of mouse proteins (7). How a molecule is determined to be excluded or included in exosomes and which molecules are functional after their cellular uptake are questions whose answers are still elusive; however, JAB1/CSN5, a component of the COP9 signalosome regulatory complex, may play a role in sorting ubiquitinated proteins into exosomes (9).

Exosomes aid in the removal of unneeded/harmful molecules from cells (2, 10, 11) including proteins without sequences for secretion. Of importance, exosomes can remove specific therapeutic drugs from cells and cells releasing exosomes containing therapeutic drugs have been reported to be resistant to these drugs (11, 12). Specifically, Shedden et al. (12) correlated genes associated with the shedding of exosomes into a “vesicle shedding index” which in the “NCI 60-cell-line panel” positively correlated with the 50% growth inhibition (G150)
for most of the 171 compounds of the NCI “Standard Anticancer Agent Database”. Also, when the actual shedding of exosomes from 6 cell lines were measured, the shedding rate correlated positively with resistance to doxorubicin which is exported in exosomes; however, there was not a correlation with resistance to 5-fluorouracil which is not extensively exported in exosomes.

Other functions of exosomes are not adequately characterized including their functions in autocrine and paracrine signaling (7). Because exosomes provide signals to distant cells, exosomes act as a newly described nanoparticle-based endocrine system (7, 13). For cells affected by exosomes, cellular membranes and the surface molecules of exosomes interact in uptake of exosomes probably via Class I and Class II MHC molecules, ICAM-1, integrins, and tetraspanins on exosomal surfaces (13, 14).

Exosomes are present in biological fluids including ascites, pleural fluid, urine and semen (15-18). Exosomes from different environments may have varying molecular and biophysical properties; protasomes, were described as round to egg-shaped with internal vesicles and as ranging in size from 50 to 500nm (16), but ascitic exosomes were similar to those in matching blood which typically measure 30 to 100nm (17). Exosomes obtained from media from some cultured cells are biphasic in size 100-200nm and 400 – 1000nm.

**NEOPLASIA AND EXOSOMES:** Tumor derived (TD) exosomes can be isolated from tumors and bodily fluids from patients with tumors. Tumors reported to release exosomes include cancers of the breast, oral cavity, colorectum, brain, ovary, bladder, prostate and melanomas (4, 18, 19). Exosomes in bodily fluids from patients with tumors are the same exosomes found in normal individuals plus TD-exosomes. Exosomes contain molecules of the neoplastic cells of origin, e.g., urinary exosomes have molecular features of associated urological malignancies (15) and exosomes from patients with melanomas contain Melan A/Mart 1 (4). TD-exosomes in the blood of patients with gliomas, which were assumed to be behind an intact blood-brain
barrier, contained L1-NCAM/CD171 which was present in exosomes isolated from the brain tumors, but not in exosomes in blood from controls (6,18); however, heat shock protein (HSP) 70 was detected in exosomes in blood from individuals with or without brain tumors (6). TD-exosomes variably contain EGFR, EGFRvIII, HSPs 27, 60, 70, 72, 73, 80 and 90 (6, 18, 20) and TNFα, FasL, and TGFβ (6, 7). How these heterogenic exosomes from different cells and the signal molecules they contain can preferentially target and modulate specific cells is yet to be determined.

**IMMUNE REGULATION BY EXOSOMES:** Exosomal regulation of immunity is exemplified by autoimmunity. Treatment of dendritic cells by interleukin 10 increased secretions of exosomes which inhibited inflammation as well as arthritis induced in animals by collagen injections (21). Exosomes from the salivary gland contain autoantigens that may be involved in autoimmunity (22). Some types of exosomes efficiently provide antigens to antigen presenting cells (APCs) which present these to T lymphocytes and NK cells (23, 24).

Fibroblasts from patients with rheumatoid arthritis (RA) produce exosomes containing TNFα which can kill cells that are sensitive to TNFα (25) and induce in T cells the phosphorylation of Akt and increase NF-KB potentially affecting the severity of RA. Exosomes also are involved in other diseases including liver inflammation caused by fatty diets and type 2 diabetes (26, 27).

**EFFECTS OF EXOSOMES IN IMMUNE SURVEILLANCE OF NEOPLASIA:** The immune system should be activated as immune cells infiltrate and are activated by their contact with tumors and as neoplastic cells continuously die releasing antigens which cause production of acute phase reactants, activation of T and NK cells and production of antibodies (28-31). Also, hypoxia causes increased cellular death and tissue damage activating the immune system and releasing cytokines, e.g., interleukin 6 and 8 (29-30).
In contrast, tumors typically cause suppression of the immune system which facilitates their growth and progression (31). Thus, tumors must have mechanisms that overcome their activation of the immune system. The involvement of TD-exosomes in the progression of neoplasia is supported by our observation that tumors transplanted into mice increased in size compared to controls after injection into the mice of exosomes from the tumors. The mechanisms associated with the decrease in the size of tumors are postulated to be decreased cytotoxicity and reduced interleukin 2 (il-2) mediated proliferation of both T and NK cells. The changes in NK cells involved decreased perforin release and expression of cyclin D3 and inhibition of Jak-3 (32). Thus, immune suppression has been hypothesized to be mediated by exosomes (17,33,34).

Some tumors release exosomes which express Fas ligand (FasL) and/or TRAIL (17, 22, 35-37) and can cause apoptosis in activated T cells (17, 35-38). Specifically, exosomes from ovarian carcinomas which express FasL, suppress CD-3ζ and Jak-3 in T lymphocytes leading to apoptosis (33). Neither FasL nor TRAIL were expressed on the exosomes in our prior study (34), suggesting alternative mechanisms of immune suppression. For example, TD-exosomes containing TGFβ1 cause a down regulation of the NKG2D receptor which is an activating receptor for NK and CD8+T cells (23). Thus the exosomes from tumors can partially suppress immune reactions by multiple mechanisms.

Immune suppression is facilitated via an increase in CD11b+ Gr-1+, myeloid-derived suppressor cells (MDSCs) in the spleens, blood and tumors of mice with syngenic tumors; this increase can be reversed by removal of the tumors (39-41). MDSCs in humans with tumors correlate with poor survival and tumor progression (39,41,42), probably via suppression of CD4+ and CD8+ lymphocytes and NK cells. MDSC-NK contact inhibits interleukin-2 mediated NK activation and expression of perforin via inhibiting the phosphorylation of Stat-5 (40). Increases in MDSCs may
be secondary to release of both exosomes and soluble factors, e.g., GM-CSF (34,39,41,43-45). Exosomes also increase MDSCs via TGFβ and regulation of prostaglandin E2 (46). In addition, HSP 72 on exosomes may interact with Toll-Like receptor2 and MyD88 of MDSCs, causing activation of MDSCs via increased expression of IL-6 and phosphorylation of Stat 3 (20,47). However, the biological effects of TD-exosomes via TLR pathways should be evaluated carefully because tumor cells undergoing many passages frequently have different phenotypes than cells isolated in vivo from tumors.

Because exosomes from dendritic cells (DC) can potentiate presentation of antigens by APCs so that they attack tumors and thus inhibit tumor growth (4, 48-53), TD-exosomes could inhibit immune responses by reducing APCs. In support of this, TD-exosomes cause time dependent inhibition of the maturation of immature DCs via a dose dependent, increased expression of interleukin 6 (IL-6) and phosphorylation of Stat 3 (32,50). Similarly, TD-exosomes impair the differentiation of CD14+ monocytes to APCs and generate a CD14+ HLD-DR^low subset of cells which can secrete TGFβ inhibiting T cells (51).

Exosomal suppression of immunity also may be caused by a transition of CD4^+, CD25^- T cells to CD4^+, CD25^+, Foxp3^+ T regulatory cells (Treg) via phosphorylation of SMAD2/3 and Stat3 (54). Such interactions in peripheral tissues mediated by TD-exosomes may participate in the maintenance of immune tolerance (55,56).

Thus, exosomes from DCs would be expected to cause tumor regression; however, this response is decreased by signals mediated by TD-exosomes which inhibit the maturation of DCs, cause increases in MDSCs, Tregs, and CD14^+, HLA-DR^low cells and reduce numbers and cytotoxic capabilities of T and NK cells (see murine model, Figure 1).

Clinical-Translational Advances
Because TD-exosomes contain many molecules characteristic of their matched tumors, exosomes have been proposed as a vehicle via which cytotoxic T-lymphocyte responses (CTL) could be increased via the interaction of TD-exosomes and DCs (2). The production of exosomes derived from the DCs could be carried out in vitro to avoid the immunosuppressive effects of TD-exosomes. Such approaches have been shown to be relatively safe and have resulted in limited responses in patients with tumors (57,58).

In contrast, because TD-exosomes are involved in the suppression of the immune system in patients with cancers, TD-exosomes could be targeted to permit the immune system to suppress tumors more effectively. Specifically, developing strategies to prevent selectively the sorting into TD-exosomes of molecules which could suppress the immune system could lead to exosomes which can activate immune cells and target tumors in an antigen-specific manner. Also, because exosomes act in the selective removal of some drugs (e.g., doxorubicin and cis-platinum) from cells (11, 12), targeting exosomes might have a dual effect by making specific drugs more effective. Approaches to target TD-exosomes, also could rely on drugs that affect the secretion and/or release of exosomes. An alternative approach could be to remove selectively TD-exosomes from the circulation using purification of blood, e.g., use of a “hemopurifier” adapted to remove TD-exosomes (54). Specifically, this might be accomplished via bound antibodies similar to those currently used to precipitate exosomes.

Because exosomes may be taken up selectively by the cells of tumors, exosomes may serve as a specific delivery system of molecules and/or drugs targeted to specific tumors. The polyphenol, curcumin, has been reported to have anti-inflammatory as well as anti-tumor activities (59,60). Curcumin also reduces the inhibitory effects of exosomes on NK cytotoxicity (61). The poor solubility of curcumin has limited its usefulness, although its bioavailability and activity can be improved by its encapsulation in liposomes (62). However, the delivery of curcumin by exosomes was found to be much more effective than delivery by liposomes in preventing septic
shock (63). This demonstrates how drugs could be delivered selectively by exosomes to target specific cellular populations.

TD-exosomes contain some molecular features of tumors at an enriched concentration. Thus, the detection of the biomarkers in exosomes might be useful in early detection, diagnosis, and risk assessment, and for determining prognosis of tumors (29,30). For example, exosomes collected from the blood of women with ovarian carcinoma contained the biomarker, claudin-4 (64) and from patients with lung carcinomas, microRNAs that could aid in the diagnosis of lung cancer (65). In addition, δ-catenin, a potential biomarker of prostate cancer, has been found in urinary exosomes from patients with prostatic adenocarcinomas (66).

In summary, exosomes function as a newly described pathway of intercellular communication which may be a major factor in suppression of immune surveillance in patients with specific tumors. In addition, exosomes likely are involved in resistance of some tumors to various chemotherapeutic agents. Thus, the release of exosomes from tumors or circulating exosomes might be novel targets for therapy. Also, exosomes could represent a vehicle for selective delivery to tumors of drugs, therapeutic small molecules, or agents for gene therapy. In addition, molecules in exosomes might serve as biomarkers for the early detection and diagnosis of diseases, for determining prognosis, and for prediction of therapeutic efficacy via a molecular pattern of exosomes which differs from that of the exosomes found either in associated different diseased controls or in normal controls.

Supported in part by the Early Detection Research Network (EDRN), grant number 5U24CA086359-10, and Susan G. Komen for the Cure, grant number BCTR0600484.
References:


64. Li J, Sherman-Baust CA, Tsai-Turton M, Bristow RE, Roden RB, Morin P. Claudin-containing exosomes in the peripheral circulation of women with ovarian cancer. BMC Cancer 2009;9:244.

Figure 1. demonstrates a murine model of the multiple actions to suppress immune surveillance of exosomes released from neoplastic cells. Exosomes reduce antigen presenting cells (i.e., mature dendritic cells), and the activity and/or proliferation of NK and T cells. In addition, cells which suppress the immune system, including Treg, MDSCs and CD14+, HLA-DR−/low are increased in number and/or activity via the actions of exosomes. Overall these actions reduce the cytotoxicity against tumor cells of the immune system.

The following abbreviations are used: Myeloid derived suppressor cells (MDSCs), natural killer (NK), T regulatory (Treg), heat shock protein (HSP), prostaglandin (PG), interleukin (IL), transforming growth factor (TGF).
Clinical Cancer Research

Exosomes and cancer: A newly described pathway of immune suppression

Huang-Ge Zhang and William E Grizzle

Clin Cancer Res Published OnlineFirst January 11, 2011.

Updated version  Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-10-1489

Author Manuscript  Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.