Review Article

Exosomes in lymph and cancer

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Abstract: A primary tumor can spread locally through direct extension, regionally through lymphatic vessels, and distantly through lymphatic and blood vessels. The lymphatic and circulatory systems are interconnected at sites such as the thoracic and right lymphatic ducts, where they empty into the left and right subclavian veins, respectively. In this article we review how normal and tumor exosomes influence the lymph system, with special emphasis on tumor related exosomes.

Keywords: Lymph nodes; lymph; exosomes

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Exosome physiology and use to treat disease

Using *in vivo* near-infrared imaging it has been demonstrated that exosomes are transported within minutes by lymphatics from the periphery to their draining lymph node. Once in the lymph node, macrophages and B-cells are key players in exosome uptake. Lymphatic endothelial cells actively enhance lymphatic uptake and transport of exosomes to the luminal side of the vessel. There is a differential distribution of exosomes in the draining lymph nodes that is dependent on the lymphatic flow. The findings suggest that exosome delivery by lymphatic flow from the periphery to a lymph node is a mechanism for the rapid exchange of information, priming the receiving lymph node for an effective immune response (1).

The immune system plays an important role in protecting an individual from anything that the body sees as foreign. Within lymph nodes exosomes associate with dendritic cells (DCs), subcapsular sinus macrophages, B lymphocytes and stromal cells (2). Interactions with endothelial cells are also likely. The functional significance of these associations depends on exosome type. Both normal cell and tumor derived exosomes often suppress the immune response. Immunosuppressive exosomes have been found in healthy individuals in the blood, in breast milk and in colostrum. In normal cells, the immunosuppressive activity of exosomes is thought to prevent self and foreign antigens from causing chronic inflammation or autoimmunity (3).

Exosomes from non-cancer cells are able to inform the lymphatic immune system. Exosomes isolated from murine MODE-K intestinal epithelial cells express major histocompatibility complex (MHC) I and II antigens, tetraspanins, A33 and milk fat globule (MFG) E8. MFGE8 binds DC integrins. A33 was found in murine lymph nodes which drain the intestinal tract but not in the blood (4). In a mouse model of arthritis, DC exosomes were modified to express Fas ligand and injected into the foot pad of mice that had arthritis related swelling. The exosomes were found after 48 h both in the foot pad and in the draining popliteal lymph node (5). Swelling in the foot pad decreased.

Exosomes in lymph nodes induce an immune response

DCs play a central role in the initiation and regulation of antitumor immunity (6). DC take in malignant cells, they respond to foreign antigen(s) and travel to regional lymph nodes to present tumor antigens to T cells (6). Subcutaneous and intradermal routes of DC vaccination have been found superior to IV injection in inducing lymphatic T cell responses to antigens (2). A separate study demonstrated that intradermal injection of DC exosomes was four-fold as effective in delivery to lymph nodes as subcutaneous injection (7). It has also been demonstrated that DC exosome antigen presentation in lymph nodes does not require the presence of DC cells, and can continue after

DCs leave a lymph node (8). Exosomes that express CD169 travel to the subcapsular sinus of lymph nodes (2) whereas exosomes from CD169-/- mice localize deeper in the node paracortex. This suggests that the localization of exosomes within lymph nodes depends on antigens expressed by the exosome.

Toll-like receptors (TLRs) are an integral part of the innate immune system. They are expressed on macrophages and DCs and protect the body against foreign antigens, as are found on microbes and cancer cells (9). B lymphocytes are part of the adaptive immune system. They secrete antibodies in response to foreign antigens. T cells are activated in response to foreign antigens and secrete cytokines to increase a person's immune response to the antigen. Exosomes play a role in immunity, but to date their exact contribution to the dissemination of the TLR response is unknown. Ovarian cancer exosomes from TLR stimulated cells were able to largely reproduce TLR activation *in vitro*. The activation by exosomes was interrupted by ultraviolet light suggesting that exosomal RNA is needed for their activation function (10).

Exosomes induce an *in vivo* macrophage M1-like polarization within murine lymph nodes and they recruit neutrophils to the node. M1 exosomes from proinflammatory macrophages were observed to track to lymph nodes after subcutaneous injection. Within lymph nodes they were mostly taken up by the local macrophages and DCs, which induced the release of T helper cell cytokines. The immune stimulation was greater than with M2 exosomes, inducing a stronger antigen specific cytotoxic T cell response (11). Mast cell exosomes transport miRNA and mRNA to target cells (12), activate B and T lymphocytes (13) and aid DC maturation (14).

Tumor derived exosomes prepare the nodal pre-metastatic niche

There is both cell culture and *in vivo* evidence for the ability of tumor derived exosomes to influence the local microenvironment. The concept of a pre-metastatic niche was first proposed by Kaplan and collaborators (15). The idea posits that factors provided by tumor cells optimize the local microenvironment in a lymph node, bone marrow or a solid organ to receive tumor cells. The conditioning of the microenvironment is thought to include the delivery of growth factors and cytokines that support metastasis, as well as remodeling of the extracellular matrix (ECM) (16). The idea of a pre-metastatic niche has perhaps been most extensively studied in lymph nodes.

Evidence in cell culture supports the influence of tumor derived exosomes on other cells. Lymphatic endothelial cells transport ovarian cancer exosomes from the basal to the apical side of *in vitro* membranes (1). Exosomes from seven melanoma cell lines, classified as nontumorigenic, tumorigenic or metastatic, were investigated to determine if the contents of exosomes from the different lines had a differing influence on the aggressiveness of the tumor cells (17). Exosomes from aggressive tumor cells contained a higher concentration of proteins involved in cell motility, angiogenesis, and immune response. When less aggressive tumor cells were exposed to exosomes from aggressive cells, the less aggressive cells developed increased migratory capacity (17).

Lymph from primary tumors generally drains to one or a few so-called sentinel lymph node(s) in the lymphatic drainage basin (18). Tumor cells initially collect in the nodal subcapsular sinus (19). Within the lymph node, the tumor cells may be destroyed, may pass through the node after entering the efferent lymphatic channel, may remain dormant within the node or may proliferate within the node, forming a nodal metastasis (20). The fact that many tumor types spread to lymph nodes, while others do not (or do so only rarely, such as renal cell carcinoma and most sarcomas) (19), suggests that conditioning of the nodal microenvironment is important. Tumor draining lymph nodes may enlarge by increasing their number of lymphocytes and sinus histiocytes (19), or they shrink as a result of a decrease in the number of lymphocytes. Histocyte hyperplasia has been associated with improved patient survival from cancer (21), whereas lymphocyte depletion is associated with a poor cancer prognosis (19).

Tumor cells that spread through the lymphatics and bloodstream often end upon preferentially in a given organ. To address why this might be true, investigators determined the biodistribution of tumor secreted exosomes (22). They observed that exosomal integrins direct site-specific metastasis by joining with specific target tissue cells. Tumor derived exosomes were able to direct the spread of tumor cells that normally lack the ability to metastasize to a given organ.

The impact of cancer cell derived exosomes on host cells was investigated in a rat pancreatic carcinoma cell (ASML) based system (23). Wild-type ASML cells metastasize through the lymphatics to the lung (but not locally) whereas ASML knockdown cells (in which CD44v4-v7 has been knocked down) poorly metastasize. The model was studied

to determine the role CD44v in creating a metastasis supporting environment (23). Exosomes derived from the wild-type ASML cell line, in combination with CD44v, degraded the ECM of rat host cells such as lung fibroblasts, lymph node stromal cells and aorta endothelial cells. A significantly increased number of ASML knockdown cells were found in the draining lymph nodes and lungs of the rodents.

The ability of exosomes to influence the spread of SGC-L gastric cancer to lymph nodes in cells lacking CD97 (which supports tumor growth in lymph nodes) was determined (24). Exosomes from SGC-L cells increased SGC-L knockdown cell recruitment to lymph nodes by 60–85%, whereas exosomes from cells lacking CD97 increased recruitment of the same cells to lymph nodes only minimally (24).

Exosomes from head and neck cancers often spread through the lymphatic system, and tumor derived exosomes appear to influence this spread. It has been reported that decreased expression of CD9, a tetraspanin expressed on exosomes, has been observed in oral cancers that spread to lymph nodes compared to cancers that did not (25). A similar trend was found in exosomes from patents with oral cavity cancers *vs.* healthy individuals (26). Exosomes were found to promote the progression of nasopharyngeal cancer (27), in part by delivering matrix metalloproteinase 13 to stromal cells in the tumor microenvironment to increase tumor cell migration and invasion.

Whether a tumor draining lymph node prevents or supports tumor growth is an ongoing area of investigation. Findings thus far suggest that tumor antigens in lymph nodes can induce an antitumor response and, at least initially, restrict metastasis formation (19). This antitumor response often includes the recruitment of natural killer (NK) cells to lymph nodes (28). However, as tumor related influence increases, the predominant effect on the lymph node is one of immunosuppression, supporting metastatic tumor growth (19). Additionally, tumors within lymph nodes produce growth factors and cytokines which support the attraction of further tumor cells and tumor growth.

Exosomes from melanoma cells can condition tumor draining lymph nodes for metastasis (29). Melanoma derived exosomes injected into the murine footpad hone to the sentinel lymph node(s). Subsequent injection of melanoma cells in the same footpad led to the formation of a tumor in the footpad and melanoma spread to the sentinel node in the popliteal fossa (29), with the tumor cells in the subcapsular sinus of the lymph node where the exosomes were concentrated. This honing occurs because of changes in the nodal microenvironment, including the initiation of angiogenesis and the induction of lymphocyte anergy. Genes involved in cell recruitment, ECM formation and angiogenesis are upregulated (29). The next lymphatic drainage site, the inguinal nodes, were also found after injection of melanoma derived exosomes to have upregulated expression of tumor necrosis factor, vascular endothelial growth factor β and hypoxia inducible factor 1α. The spread of melanoma exosomes containing superparamagnetic iron oxide nanoparticles, which can be detected by magnetic resonance imaging (MRI), from the footpad to the popliteal lymph nodes was also demonstrated to accumulate in the subcapsular sinus of the nodes (30).

Breast cancer cells secrete miR105, which influences tumor cell migration by targeting tight junction protein ZO-1 (31). miR105 can be transferred to endothelial cells by tumor derived exosomes, destroying cell barriers and increasing blood vessel permeability (32).

Tumor derived exosomes suppress the immune response

Tumor cells suppress the immune response to enhance their survival. Some tumor derived exosomes express death ligands such as FasL and TRAIL, which induce death in activated T cells. Tumor derived exosomes may also contain PD-L1 and CD40L, which suppress the T cell response (3). It has been proposed that melanoma derived exosomes induce tumor tolerance in lymph nodes (33). Injection of pancreatic cancer-derived exosomes carrying the tumor antigen OVA results in suppression of a delayed-type hypersensitivity (DTH) response to OVA (34). NK cell and CD8+ T cell cytotoxicity was impaired by exosomes from breast cancer cells containing the C-type lectin-like receptor NKG2D (3). Mammary carcinoma exosomes are also able to suppress NK cell function (3).

Summary

Exosomes from both benign and tumor cells influence the lymphatic system, either to increase or to suppress the immune response. There is evidence that DC derived exosomes within lymph nodes can increase the anti-tumor response, but that as tumor burden increases, tumor derived exosomes create an environment within the draining lymph node favoring tumor growth. Both tissue culture and *in vivo* studies have demonstrated how tumor derived exosomes

influence the local microenvironment, in lymph nodes and in other organ systems. For example, tumor derived exosomes inhibited the differentiation of myeloid precursor cells in the bone marrow, and the cells switched toward a myeloid derived suppressor cell phenotype (35). Continued investigations into the relationship between exosomes and lymph nodes will further our understanding of how exosomes regulate immune cell subsets and will hopefully lead to new exosome based therapies for cancer.

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