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Tumor-derived exosomes
A message delivery system for tumor progression

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Intercellular communication is a key process in the development and progression of cancer. The dynamic and reciprocal interplays between the tumor and its microenvironment orchestrate events critical to the establishment of primary and metastatic niches and maintenance of a permissive environment at the tumor-stroma interface. Atay and colleagues found that gastrointestinal stromal tumor cells secrete vesicles known as exosomes. These exosomes contain oncogenic KIT and their transfer and uptake by surrounding smooth muscle cells lead to enhanced AKT and MAPK signaling and phenotypic modulation of several cellular processes, including morphological changes, expression of tumor-associated markers, secretion of matrix metalloproteinases, and enhanced tumor cell invasion. This provocative study emphasizes that exosome-mediated signaling within the tumor microenvironment acts as a positive feedback loop that contributes to invasiveness and that interfering with this message delivery system may represent promising therapeutic approaches, not only for GIST, but for other types of cancer.

Normal and Tumor Cells Secrete Nanosized Vesicles Called Exosomes

In response to physiological and/or pathological cues all cells in the body communicate with each other via secretion of a heterogeneous mixture of vesicles differing in size and composition, including apoptotic bodies, microparticles, shed microvilli, ectosomes and exosomes.[1] Exosomes, the focus of our studies, are small membrane vesicles of endocytic origin with a size range of 30 to 150 nm.[2] Exosomes are released by a variety of "normal" cells including mast cells (MC),[3] dendritic cells[4,5] reticulocytes,[6] epithelial cells,[7] B-cells,[8] trophoblastic cells,[9,10] and neural cells,[11] as well as a variety of tumor cells.[12–14] In addition, exosomes are found in various biological fluids including bronchoalveolar lavage,[15] blood,[16] ascites,[17,18] urine,[19] pregnancy associated sera,[20] breast milk,[21] saliva,[22] and malignant effusions.[17,23] Because of their endosomal origin, exosomes contain several proteins involved in the Endosomal Soritng Complexes Required for Transport (ESCRT) complex (e.g, TSG101, Alix) and in transport and fusion (e.g., Rab11, Rab7, Rab2 and various annexins). Further markers expressed in or on exosomes include tetraspanins (CD81, CD63, CD9), heat shock proteins (HSC70 and HSP90), and cytoskeletal proteins (actin, tubulin and moesin).[24–26] In addition, the molecular characterization of various healthy cell type-derived and tumor-derived exosomes revealed enhanced expression of cell-specific and tumor-associated antigens on the exosomal surface. In fact, exosomes isolated from antigen presenting cells harbor MHCII on their surface,[8] those from urine possess surface aquaporin-2,[19] from reticulocytes contain the transferrin receptor,[6] and from T-cells carry the TCR/CD3/zeta complex.[27] These cell-specific proteins are thought to represent a means by which exosomes can specifically target various recipient cells by either interaction with cell surface adhesion molecules or through interaction with cell-surface heparan sulfate proteoglycans. Alternatively, exosomes can enter another cell via lipid-dependent endocytosis, in which a high content of sphingomyelin/ganglioside GM3 in the exosomal membranes enhances the fusion efficiency with the plasma membrane of target cells.[28] Therefore, exosome internalization by recipient cells appears to be a cell type dependent process, and the extent of exosome internalization likely depends upon the phagocytic abilities of the recipient cell.[29] As such, we and others are exploiting this information to isolate specific populations of exosomes from heterogeneous biological fluids for use in early detection and disease monitoring. Proteomic analysis of malignant effusion-derived exosomes from various sources has increased our knowledge of exosome protein composition and likewise, our understanding of the role of exosomes in biological processes.[30–33] Proteome analyses have been conducted in a number of cancer-derived exosomes including, mesothelioma,[34] melanoma,[14] gastric carcinoma,[35] breast carcinoma,[36] ovarian,[18,37] prostate,[38] malignant pleural effusions,[17,23] brain,[24] and colorectal.[26,39] These isolated exosomal proteins constitute a "cancer signature" which may help in improving the diagnosis and treatment of cancer patients.

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Tumor-Derived Exosomes as Mediators of Intercellular Communication During Tumor Progression

Cellular communication is key to the regulation of physiological and pathological processes.[40] During the development and progression of cancer, the cellular composition of the tumor microenvironment is influenced by the activity of the tumor cell[41] which recruit and educate host stromal cells into tumor supportive cells that actively participate in tumor progression.[42] One way that tumor cells can communicate and alter the microenvironment is by the constitutive release of exosomes.[13,43] Recent studies have shown that exosomes produced by tumor cells can interact with target cells by a number of mechanisms, including i) direct stimulation of the target by surface-expressed ligands;[44] ii) receptor transfer between the tumor cell and the target;[44] iii) horizontal transfer of genetic information to the target,[44] and iv) direct stimulation of the target cell by endocytic-expressed surface receptors.[45] Growing evidence supports the view that tumors constitutively shed exosomes with pleiotropic immunosuppressive effects[46,47] that are protective and supportive of the tumor with effects that range from regulation of tumor growth, to invasion, and to angiogenesis and metastasis.[41,46,48] Recently, Al-Nedawi and colleagues demonstrated that exosome mediated transfer of an oncogenic epidermal growth factor variant 3 (EGFRvIII) from human glioma cells to glioma cells lacking the mutant receptor induced expression of EGFRvIII-regulated genes (such as VEGF, Bcl-x_L, p27).[49] In a subsequent study, oncogenic EGFR from human squamous cell carcinoma taken up by tumor-associated endothelial cells activated MAPK and AKT cell signaling pathways and promoted endothelial VEGF expression.[50] Therefore, the regulatory properties attributed to tumor-derived exosomes are essential in shaping the tumor microenvironment and promoting tumor growth.[32] Collectively, these studies support a role for exosomes in remodeling the tumor microenvironment into a tumor supportive milieu and thereby contribute to tumor progression via enhanced angiogenesis and metastasis.[51,52] In fact, melanoma-associated exosomes have recently been shown to promote metastasis through the preparation of the metastatic niche via crosstalk between the released exosomes and bone marrow progenitor cells.[53]

GIST Tumor Microenvironment and Tumor-Derived Exosomes

GISTs are the most common mesenchymal tumor of the gastrointestinal tract and are thought to arise from Interstitial Cells of Cajal (ICC),[54] named after Santiago Ramón y Cajal, a Spanish pathologist and Nobel laureate. ICCs are found in specific locations within the tunica muscularis of the gastrointestinal tract, and serve as electrical pacemakers and mediators of enteric neurotransmission. Alternatively, GISTs may also arise from interstitial mesenchymal precursor stem cells.[55] The majority of GISTs develop in the sub-mucosal layer of the stomach surrounded by smooth muscle cells and interstitial extracellular matrix (ECM) rich in collagen and invade the mucosa in a regulated fashion.[56] Nearly 90% of GISTs have a mutation in a tyrosine kinase receptors encoded either by c-*KIT* or *PDGFRA*.[57] Imatinib mesylate (Gleevec™) is a specific molecular inhibitor of KIT/PDGFRA and is used as the first-line therapy in the treatments of GIST patients.[58–62] Although the use of imatinib has drastically changed the outcome of patients with metastatic GIST, additional therapeutic strategies are needed since the vast majority of patients eventually develop resistance to imatinib treatment, leading to disease progression and posing a significant challenge in the clinical management of these tumors.[63] Importantly, once a GIST becomes metastatic, the median disease-specific survival of patients is only ~19 months with second- and third-line therapies. Although many studies focused on molecularly defining these tumors,[64–66] the importance of the stromal microenvironment during metastasis remains an understudied area of research and clearly needed to be better defined in order to design novel targeted therapeutics.

It is becoming apparent that the tumor microenvironment – non-malignant (stroma) cells, soluble molecules, extracellular matrix components, and exosomes – plays an important role in modulating metastatic properties and sensitivity of tumor cells to therapy. Several studies have shown that ascites-derived exosomes from ovarian cancer patients carry extracellular matrix-remodeling enzymes such as metalloproteinases 2 and 9 (MMP-2, MMP-9),[67,68] and urokinase plasminogen activator[69,70] leading to an increase in extracellular matrix degradation, which has been shown to increase the invasive phenotype of tumor cells and promote metastasis.[71] We believe that by better understanding the myriad of interactions that exist between tumor cells and host cells present in the tumor microenvironment, new insights into the pathogenesis of cancer will be uncovered that will ultimately have profound therapeutic implications.

Epithelial cells require mesenchymal transition (EMT) to metastasize; during this process tumor cells dissociate from each other and the ECM and become more motile and able to invade the surrounding stroma.[72] In contrast, mesenchymal cells are generally more motile than epithelial cells,[73] thus GIST cells have been reported to grow in an endophytic manner parallel to the organ lumen, between the muscularis mucosa and muscularis propria. This finding suggests that a tight regulation exists between the growing tumor and the surrounding stroma.[74,75] Matrix metalloproteinases, particularly MMP1, actively shape the stromal microenvironment during sarcoma development.[76–78] In fact, a recent study reported that chondrosarcoma cell invasion correlates with MMP1 expression in tumor cells and that a transient downregulation of MMP1 expression decreases invasion in vitro.[79,80] In our recent study, we found that GIST cells not only constitutively released low levels of MMP1, but that challenging myometrial smooth muscle cells with GIST patient-derived exosomes (but not exosomes from healthy donors) significantly increased MMP1 production, which in turn enhanced GIST cell invasion.[81] We assessed direct/indirect exosome-mediated MMP1 induction using siRNA and inhibitory drug strategies to reduce MMP1 production by myometrial cells and were able to mimic, in vitro,

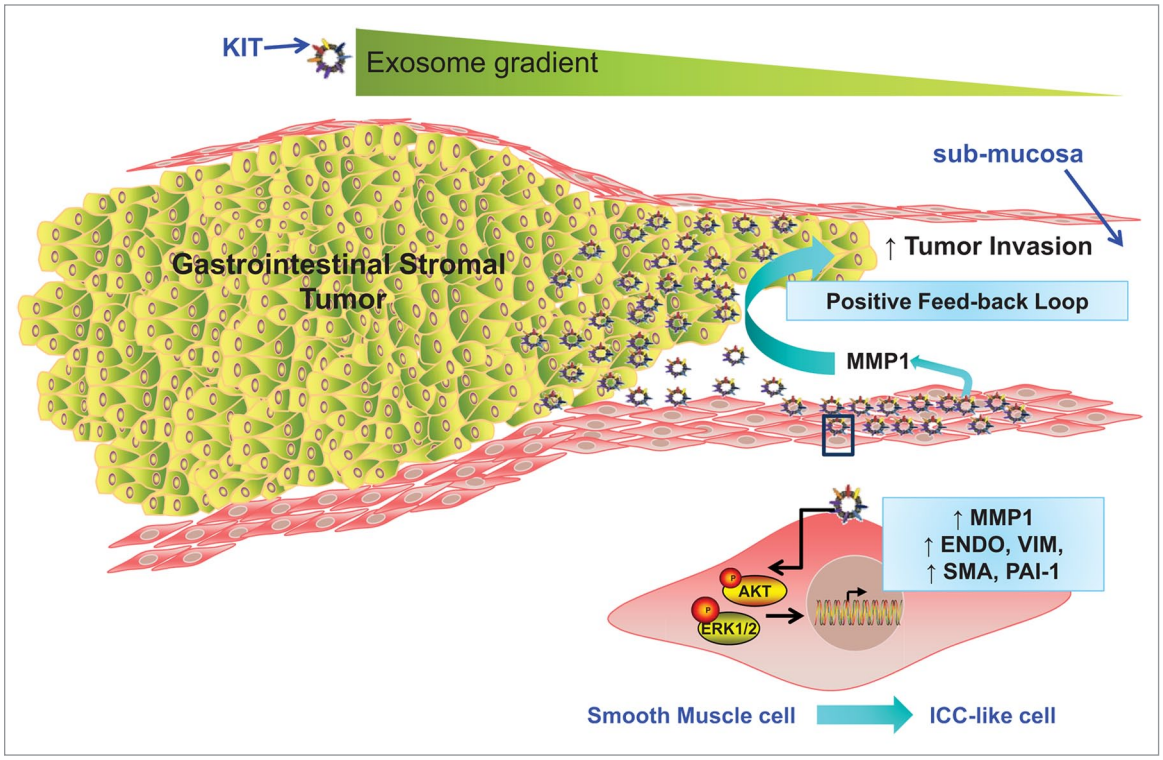

Figure 1. Proposed model of tumor-stromal positive feed-back loop mediated by oncogenic KIT-bearing tumor exosomes in the regulation of tumor invasion. GIST cells secrete a gradient of exosomes carrying mutant KIT, which after internalization by surrounding smooth muscle cells activate downstream signaling pathways of KIT (e.g., AKT and MAPK pathways) and induce an enhanced expression of endoglin, vimentin (VIM), smooth muscle actin (SMA), and plasminogen activator inhibitor-1 (PAI-1) in the recipient cells, resembling an ICC-like phenotype. This tumor-stromal interaction creates a positive feed-back loop in which tumor-derived exosome-mediated signaling in stromal cells increases MMP1 secretion. In turn, tumor cells utilized MMP1 to invade the submucosa. This model describes a previously unreported mechanism by which tumor-derived exosomes can modulate their host microenvironment and promote local invasion and potentially distant metastasis.

the exosome–MMP1 expression feedback loop (**Fig. 1**). In particular, in vivo-derived exosomes appeared to be a potent exogenous source of MMP induction in stromal cells, which in turn acted as a pro-invasion factor for GIST cells. It is known that tumor cells acquire some of the required properties for growth and invasion by the specific modification of the tumor microenvironment.[79] However, due to the complex nature of these interactions, it is only by altering specific components of this network that it will be possible to identify molecules with pro-tumorigenic and anti-tumorigenic functions. Our study reveals a complex interplay between tumor-derived exosomes and factors produced in response to their internalization by tumor-associated stromal cells. The release of tumor-derived exosomes appears to represent a novel pathway enabling GIST cells to modulate the host microenvironment and thereby promote their ability to invade and spread (**Fig. 1**). Further studies aiming to elucidate the exact mechanism leading to MMP1 production by smooth muscle cells in response to exosome uptake are ongoing. In fact, a more complete understanding of the mechanisms used by tumor-derived exosomes in the induction of MMPs might permit the development of a successful clinical strategy for novel MMP inhibitors.[82,83]

Another important aspect of tumorigenesis is the epigenetic regulation of gene transcription that mediates cell proliferation, differentiation, and survival which represent additional targets in tumor progression,[84] resulting in genomic instability.[85] Skog et al. (2008) have demonstrated that glioblastoma-derived microvesicles tRNA to endothelial cells, resulting in the production of pro-angiogenic proteins which promote tumor progression.[86] Studies of lung and adenocarcinoma–derived microvesicles (pancreatic and colorectal) indicated a transfer of growth factor encoding mRNA (VEGF, HGF, IL-8, CD44H) to tumor-associated monocytes which enhanced their anti-apoptotic effect and activated the AKT signaling pathway.[87] In our recent study, we reported that mutant KIT carrying exosomes modified the transcriptomic, proteomic and secretomic profile of smooth muscle cells via induction of new transcripts within the recipient cells. In addition, we provide the first evidence that large numbers of oncogenic KIT-bearing exosomes are released into the circulation of GIST patients and that these extracellular vesicles represent potent phenotypic modifiers of the tumor microenvironment. Our results indicated that recipient cells that take up these vesicles assume many of the characteristics of ICC cells which in turn secrete significant amounts of interstitial collagenase MMP-1, which is important to enhance invasion of tumor cells in the interstitial stroma. Furthermore, our results confirmed that the resulting cells displayed activation of downstream signaling pathways of KIT,

namely AKT and MAPK pathways, and enhanced adhesion to fibronectin and type I collagen. Hence, these data suggest that the release of exosomes may represent a novel pathway enabling tumor cells to modulate the host microenvironment to support tumor invasion.

In summary, although several tumor-stromal communication based on an exosome mediated exchange has been reported,[88–91] our recent study provides the first evidence of tumor-stromal communication based on an exosome mediated exchange in GIST. This tumor-stromal feedback loop in which tumor-derived exosome-mediated signaling in host stromal cells increases MMP1 secretion, which in turn enhances tumor cell invasion and further suggests that exosomes released by primary GISTs progressively remodel the host environment, which in turn aids in the tumor's survival (**Fig. 1**). In addition, our study demonstrated a positive feedback loop that enable the creation of "space" for growing tumors: this is achieved via active release of exosomes, leading to a continual release of exogenous matrix metalloproteinases, such as MMP-1. Although further studies are needed to identify the receptors involved in uptake of tumor-derived exosomes and the molecular mechanisms involved in the production of MMP-1, our study offers novel insights into the pathogenesis of GIST and provide new therapeutic goals aimed to help improve the survival of patients by reducing the development of metastatic disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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