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The role of tumor-derived exosomes in epithelial mesenchymal transition (EMT)

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Exosomes are membrane-bound small vesicles (30–150 nm) produced by all cell types and present in all body fluids (1). They are a part of the intercellular communication system that is evolutionarily conserved and operates in bacteria as well as all multicellular organisms (2). Tumor cells produce and release masses of exosomes into the extracellular space. These exosomes carry information in the form of molecular signals and/or genetic materials (mRNA, miRNA, DNA) from the parent tumor cell to locally- or distantly-located recipient cells. Exosome-mediated transfer of information results in re-programming of the recipient cell genome and proteome and ultimately leads to the acquisition of new cellular functions (3). In the tumor microenvironment (TME), where tumor orchestrates cellular interactions, tumor-derived exosomes (called TEX) carry messages from the tumor to host cells, to other tumor cells or via autocrine signaling back to the parent tumor cell (4). For this reason, and also because their content in part resembles that of the parent cell, TEX have been of special interest as potential "tumor surrogates" or as biomarkers of the tumor behavior, including its growth, differentiation, progression or the potential for metastasis formation. Today, the mechanisms responsible for TEX-mediated re-programming of recipient cells are under intense investigation, and as our knowledge of TEX expands so does the spectrum of cellular activities that TEX can apparently regulate and alter in a variety of recipient cells.

In a recent paper published in *Oncotarget* (5), Rahman and colleagues report that exosomes derived from supernatants of highly metastatic lung cancer cells or from sera of patients with lung cancer drive the epithelial to mesenchymal transition (EMT). The EMT is a cell process that drives differentiation of epithelial cells into mesenchymal cells. Epithelial cells undergoing EMT dramatically alter their shape, phenotype (lose E-cadherin, down-regulate EPCAM; acquire vimentin, Zeb1, Twist, Snail) and behavior (e.g., increase motility). Importantly, carcinoma cells that have undergone an EMT not only acquire a distinct molecular signature but become resistant to chemotherapy and immunotherapy (6). TEX have been previously reported to carry a pro-EMT program that includes EMT inducers such as TGF-β, HIF1α, β-catenin, caveolin-1 or vimentin, which increase invasive capabilities of

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recipient cells and promote the pre-metastatic niche formation [reviewed in (7)]. While the morphological, phenotypic and functional changes accompanying the EMT are well characterized, molecular and genetic mechanisms responsible for driving the process remain unclear. More recent data suggest that TEX carry factors necessary for activation, initiation and support of the EMT (7).

The *Oncotarget* report (5) provides *in vitro* evidence that lung-cancer-derived exosomes activate the metastatic process in human bronchial epithelial cells (HBECs) by increasing their metastatic properties such as migration, invasion and vimentin expression. In this report, TEX were isolated from supernatants of non-metastatic and metastatic lung cancer cell lines by ultracentrifugation and shown to carry the epithelial (E-cadherin, ZO-1) and mesenchymal (N-cadherin, vimentin) markers, respectively. HBECs were co-incubated with TEX and tested for migration in wound healing “scratch” assays; for invasion in matrigel assays; and for expression of mRNA for vimentin as well other EMT markers by RT-PCR. Only TEX produced by the metastatic lung cancer cell line induced activation of the EMT program in recipient HBECs. Importantly, exosomes isolated from sera of patients with the late-stage lung cancer (but not those isolated from sera of normal donors) similarly increased vimentin expression as well as migration and invasion capabilities of recipient HBECs. Finally, exosomes isolated from lung cancer patients sera and labeled with the PKH67 dye (but not exosomes from normal donors’ sera) were shown to be taken up by HBECs and to up-regulate vimentin expression. Further, a successful knockdown of vimentin in serum-derived exosomes reduced migration of the recipient HBECs. These data suggested that vimentin carried by TEX and delivered to recipient HBECs may be one of the key proteins necessary for induction of the EMT. However, the precise mechanism of how vimentin transferred by TEX contributes to the initiation of the metastatic program in recipient HBECs remains unsolved.

The EMT is a complex multistage process that involves progressive changes of the molecular pathways in the tumor and neighboring cells. It has been suggested that TEX play a critical role in all stages of the EMT—from initial activation of the invasive phenotype to metastasis (7). To initiate and sustain the process, TEX have to deliver autocrine or paracrine signals to neoplastic epithelial cells and other cells in the TME. The targeting of TEX to specific recipient cells is probably dependent on the content of TEX cargo, e.g., membrane-associated integrins. It is known that TEX can interact with recipient cells via the receptor/ligand type signaling or integrin-mediated adhesion or they can be internalized by endocytosis or phagocytosis (8). The type of recipient cell probably determines which of these mechanisms are engaged in TEX cross-talk with a recipient cell. Disrobing of the internalized TEX in recipient cells and delivery of nucleic acids, including miRNAs, leads to genetic re-programming and to changes in the proteome and/or transcriptome of the cell. Evidence that TEX may serve as a conduit for EMT-initiating signals is based on observations that: (I) TEX carry and deliver known EMT inducers such as TGF-β, IL-6, β-catenin and others to recipient cells (9); and (II) epithelial neoplastic cells exhibit morphologic, phenotypic and functional alterations that are consistent with the EMT after co-incubation with TEX (10). The pre-metastatic niche formation is then followed by progressive shift toward metastasis, which is also facilitated by TEX delivering signals and cues that culminate in the formation of metastasis at secondary sites (7).

To fully understand how TEX drive the EMT, it will be necessary to better characterize their unique molecular and genetic cargos and to *in vivo* model cellular changes mediated by TEX delivery in a suitable animal model of the EMT. To do so, TEX isolation from body fluids rather than supernatants of tumor cell lines will have to be accomplished. As body fluids of patients with cancer, while variably enriched in TEX, contain a mix of exosomes derived from different normal cells, capture of TEX for molecular, genetic and *in vivo* modeling in animal models of the EMT will be essential for comprehending of TEX mechanisms of action and of their relative contributions to the initiation and progression of metastasis. It is expected that rapidly emerging technological advances enabling TEX capture from body fluids of cancer patients and TEX characterization will soon be at hand to help in a mechanistic definition of the role TEX play in the EMT.

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