Exosomal Communication Goes Viral

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Exosomes are small vesicles secreted from cells that participate in intercellular communication events. Accumulating evidence demonstrates that host exosome pathways are hijacked by viruses and that virally modified exosomes contribute to virus spread and immune evasion. In the case of tumor viruses, recent findings suggest that alterations in normal exosome biology may promote the development and progression of cancer. These studies will be discussed in the context of our current knowledge of Epstein-Barr virus (EBV)-modified exosomes.

Cell-to-cell communication is absolutely essential to a multitude of cellular processes, including cell growth, migration, differentiation, neuronal signaling, and immune cell modulation. It has only recently been established that extracellular vesicles called exosomes are key facilitators of intercellular communication with broad biological and medical implications. In addition to having a role in normal physiologic functions, exosomes have been described as important players in human diseases. For example, in cancer, exosomes have been linked to tumor growth, progression, and metastasis (1). These nanoscale membrane-enclosed vesicles are found at high levels in circulating biological fluids and contain specific lipids, proteins, and RNAs from their cells of origin (1). The availability of abundant molecular information within circulating exosomes and their function as delivery vehicles have generated tremendous interest in their use in diagnostics and therapeutics.

Exosomes are 30- to 150-nm vesicles generated by inward budding events on the cytoplasmic, endosome-derived membranes of multivesicular bodies (MVBs). MVBs can either be targeted for lysosomal degradation or be transported to the plasma membrane, where they fuse and release intraluminal vesicles (exosomes) into the extracellular space. It is not surprising that the mechanisms of the exosome biogenesis pathway have considerable overlap with the assembly and egress of numerous enveloped viruses (1). For example, various cellular factors implicated in the construction of MVBs interact with herpesvirus proteins and have been documented to be important for virus budding and egress (1).

Following virus exposure, infected cells release highly specific exosome populations with distinct molecular repertoires (2). Studies of virally modified exosomes have proven critical to laying the framework for determining the roles of exosomes in intracellular communication, an area of research currently receiving much attention (1). Exosomes released from virus-infected cells have been most extensively studied with regard to human immunodeficiency virus (HIV) and members of the herpesvirus family. More recently, the contents of exosomes have begun to be investigated for hepatitis C virus (HCV), HBV, human T-lymphotropic virus (HTLV), and human papillomavirus (HPV). Early studies on herpes simplex virus (HSV) exosomes, also known as L-particles, demonstrated that these microvesicles contain functional viral tegument proteins and can enhance the infectivity of viral DNA (3). Exosomes from HCV-infected cells contain viral RNA that can facilitate infection of new cells, suggesting potential functions of exosomes in the spread of HCV (4). There is even evidence that the exosomal pathway is utilized by nonenveloped viruses for cell-to-cell spread and immune system avoidance (5). Based on the contents and functions of exosomes and viruses, the lines separating them continue to blur. Their similarities point toward host pathways evolutionarily exploited by viruses for replication, spread, and immune evasion. Despite tremendous growth in this area of research, our understanding of the functions of exosomes during virus infection remains in its infancy. Further investigation into these exciting virus-host interactions will better clarify the mechanisms of exosome formation and protein sorting and their functions during viral infections. Here, I will focus on our existing understanding of exosomes in the context of Epstein-Barr virus (EBV) infection, with emphasis on current discoveries regarding their contributions to tumor microenvironment remodeling.

EBV is an important human pathogen that persistently infects more than 90% of the world’s population. The ability of EBV to establish lifelong infections and, in most cases, produce no noticeable disease is due to the exploitation of host regulatory networks by the virus. Following primary infection of naive B cells, EBV initiates a proliferative and potentially oncogenic program to drive B cell differentiation and maturation into memory cells, where the virus establishes latency. This complex and multifaceted process requires interactions between the infected B cells and the cellular microenvironment, consisting of T cells and other surrounding stromal cells. If not properly controlled, the growth program induced by EBV can result in malignant transformation. Under normal circumstances, the host T cell response restrains malignant outgrowth; however, in immunocompromised individuals, EBV-positive lymphomas can arise, resulting in death of the host.

EBV is able to persist for the life of the infected individual because of the capacity of the virus to evade the immune response and establish latency. EBV-modified exosomes are emerging as important cellular communicators in protecting infected tumor cells from destruction. Latent membrane protein 1 (LMP1) is the

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major viral oncogene expressed in most EBV-associated cancers and is essential for the proliferation program and B cell immortalization. More than a decade ago, LMP1 was discovered to be secreted from EBV-infected cancer cells in exosomes, raising the important question about the function of exosomal LMP1 (6). Early work pointed to a potential role of exosomal LMP1 in altering the immune response. Specifically, a conserved region within the first transmembrane domain of LMP1 was found to exhibit immunosuppressive effects by inducing T cell anergy (7). Both EBV-infected lymphoblastoid and nasopharyngeal carcinoma cells were found to secrete LMP1-containing exosomes that inhibit T cell activation and proliferation, likely through the conserved transmembrane domain of LMP1 (7, 8). The exact mechanisms controlling exosome-mediated immunosuppression are still not clear. Other viral and cellular factors present within exosomes secreted from EBV-infected cells may also contribute to the observed effects. For example, exosomes from EBV-associated nasopharyngeal carcinoma cells contain galectin-9, which induces apoptosis through interactions with TIM3 surface receptors present on recipient CD4+ T cells (8, 9). EBV has recently been found to also utilize the host exosome pathway to avoid the innate immune response by shutting important immune effectors, like IFI16, cleaved caspase-1, interleukin 1β (IL-1β), IL-18, and IL-33, out of infected cells (10). The viral factors that control the specific trafficking of immunoregulator proteins from EBV-infected cells into exosomes remain to be determined.

One potential candidate is LMP1, a viral protein that has emerged as a key mediator of the components of exosomes and their functions in intracellular communications (2, 11). FGF-2, a potent angiogenic factor, was the first protein whose exosomal secretion was demonstrated to be influenced by LMP1 expression (12). Increased exosomal packaging of epidermal growth factor receptor (EGFR) and phosphoinositol 3-kinase (PI3K), two proteins frequently activated in cancers and important for LMP1-mediated transformation, were later observed in nasopharyngeal carcinoma cells latently infected with EBV (11). The enhanced exosomal trafficking of PI3K, EGFR, and FGF-2 may simply be a consequence of their increased expression in LMP1-positive cells, or it may reflect changes in cellular pathways that favor their increased transport to exosomes. The mechanisms governing protein targeting to exosomes is an important, yet understudied, area of research.

Exosomes expelled from EBV-infected cells possessing LMP1, EGFR, and PI3K are efficiently taken up by uninfected cells through paracrine mechanisms, leading to the activation of AKT and extracellular signal-regulated kinase (ERK) signaling pathways in the recipient cells (11). These data revealed new ways that EBV can manipulate the cellular microenvironment through the exosomal transfer of viral and cellular factors, and they suggest important signaling functions of LMP1-modified exosomes in recipient cells. In EBV-associated cancer, these virally modified exosomes may influence the tumorigenic growth of neighboring cells, inhibit immune cell function, or stimulate angiogenesis. Thus, the mechanisms regulating protein sorting to exosomes offer clear therapeutic targets for EBV-associated malignancies.

Attempting to understand how EBV modulates the components of exosomes, we performed quantitative proteomic analysis of exosomes purified from 10 different cell lines that were uninfected or latently infected with EBV, Kaposi’s sarcoma-associated herpesvirus (KSHV), or both viruses (2). Gamma-herpesvirus infection resulted in substantial modifications in the proteomes of exosomes with changes common and unique to each virus. Interestingly, many of the alterations within exosomes correlated with levels of LMP1 in the exosome-producing cells, providing further evidence to support the hypothesis that LMP1 is a critical effector of the cargo and functions of exosomes. Molecules enriched in LMP1-positive exosomes included major histocompatibility complex (MHC) class I and II molecules, tumor necrosis factor (TNF) receptor-associated factor 2 and NF-κB-induced kinase (TN1K), the Fgr kinase, the p85 regulatory unit of PI3K, intercellular adhesion molecule 1 (ICAM1), ezrin, annexins, Rab GTPases, ARF6, integrins, filotinin 1 and 2, growth factor receptor-bound protein 2 (GRB2), neuroblastoma Ras viral oncogene homolog (NRAS), Lyn, mitogen-activated protein kinase 1 (MAPK1), RAC2, and PIP4K2A (2) (Fig. 1). Computational analysis of the differentially expressed proteins predicted that LMP1-modified exosomes would affect molecular and cellular functions of cell growth and proliferation, cellular movement, cell death and survival, development, and cell-to-cell signaling (2). These predictions were corroborated by three independent studies demonstrating that LMP1-modified exosomes do indeed enhance proliferation, migration, invasion, and B cell differentiation toward a plasmablast-like phenotype when incubated with noninfected cells (13–15). Together, these data suggest that EBV-infected cells can manipulate the tumor microenvironment and contribute to the pathogenesis of EBV-associated cancers through the transfer of LMP1-modified exosomes. The significance of exosomes in oncogenicity was recently demonstrated in breast cancer, where nontransformed cells were found to form tumors in mice when incubated with exosomes from malignant cells or the blood of patients alone (16). It remains to be determined whether EBV exosomes facilitate tumorigenesis in vivo; however, it is likely that they play some role, based on the findings of Melo et al. and the potent growth signaling properties of LMP1 and other cellular proteins enriched in EBV-modified exosomes (Fig. 1).

While it is clear that LMP1 is an important mediator of exosome content and function, EBV exosomes also contain other notable viral factors, like viral mRNAs (vRNAs) (17), viral microRNAs (vmiRNAs) (11, 18), EBV-encoded small RNAs (EBERs) (19), and the LMP2a protein (2, 20), that warrant further investigation into their specific roles in EBV pathogenesis and persistence (Fig. 1). Moreover, it will be important to determine whether there are different exosome populations secreted from infected cells with unique components that are destined for specific target cell types. It is interesting that most of the studies on virally modified exosomes have been limited to pathogens that induce persistent infections like those described for EBV. Is this because virus-host interactions that take advantage of the exosome pathway for intercellular communications are specific to persistent infections? The ability of viruses to alter the cellular microenvironment in the absence of virus replication through the transfer of virally modified exosomes is an attractive model for viral persistence. However, based on the functions of exosomes in immune cell modulation and indications that extracellular vesicles from HSV- and HIV-infected cells can enhance infectivity, it is likely that exosomes and other microvesicles also perform important functions during acute and lytic infections. In the case of EBV, it would be interesting to know if the content of host exo-
FIG 1 Effects of EBV infection on the components and functions of exosomes. Many proteins have been found to be incorporated at high levels in exosomes released from EBV-infected cells or as a result of LMP1 expression in exosome-producing cells (black text). Other viral and cellular factors that are uniquely packaged into EBV-modified exosomes compared to their packaging in exosomes from control uninfected cells are highlighted in red text. These changes in exosome components due to EBV and the proposed physiological changes in recipient cells following exposure to EBV-modified exosomes are summarized here.

IVL, intraluminal vesicles.

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