Analogies Between Cancer-Derived Extracellular Vesicles and Enveloped Viruses with an Emphasis on Human Breast Cancer

Toni M. Green 1 · Mark F. Santos 1 · Sanford H. Barsky 1 · Germana Rappa 1 · Aurelio Lorico 1

Published online: 27 August 2016
© Springer Science+Business Media New York 2016

Abstract

Purpose of Review Cancer cells utilize extracellular vesicles (EVs) as a means of transferring oncogenic proteins and nucleic acids to other cells to enhance the growth and spread of the tumor. There is an unexpected amount of similarities between these small, membrane-bound particles and enveloped virions, including protein content, physical characteristics (i.e., size and morphology), and mechanisms of entry and exit into target cells.

Recent Findings This review describes the attributes shared by both cancer-derived EVs, with an emphasis on breast cancer-derived EVs, and enveloped viral particles and discusses the methods by which virions can utilize the EV pathway as a means of transferring viral material and oncogenes to host cells. Additionally, the possible links between human papilloma virus and its influence on the miRNA content of breast cancer-derived EVs are examined.

Summary The rapidly growing field of EVs is allowing investigators from different disciplines to enter uncharted territory. The study of the emerging similarities between cancer-derived EVs and enveloped virions may lead to novel important scientific discoveries.

Keywords Extracellular vesicles · Cancer · Breast cancer · Enveloped viruses · Viral vesicles

Introduction

Breast cancer, the leading cause of cancer-related deaths worldwide, is a very heterogeneous disease. While the classical distinction between the two main forms of breast cancer, ductal and lobular, is still valid, DNA microarray analyses in the past decade have allowed the subclassification of breast cancer based on gene expression patterns in normal-like, basal-like, human epidermal growth factor receptor 2/neu-overexpressing, luminal A, and luminal B cells [1]. These subtypes have both prognostic and therapeutic relevance, although heterogeneity persists within each subtype. Independent from its subclassification, metastatic spreading is mainly responsible for mortality of breast cancer patients, with overt metastases appearing in several cases 5–10 years after removal of the primary tumor. Extracellular vesicles (EVs), including ectosomes, also known as microvesicles (MVs), shed from the plasma membrane, and exosomes, derived from multivesicular bodies, are released from nearly all cells, but in increased quantities from cancer cells [2], and have come to the limelight as biological entities involved in development and progression of breast cancer as well as most types of malignancies. Moreover, their possible exploitation as breast cancer biomarkers and potential components or targets of novel therapeutic strategies is increasingly evident.

Enveloped viruses spread infection through the replication of viral nucleic acids, assembly of viral elements, budding of mature viral particles, and subsequent fusion to surrounding cells [3]. Both viruses and cancer-derived EVs
contain proteins and nucleic acids that are transmitted to
target cells in order to promote disease progression.
Common morphological characteristics, protein content,
and entry and release pathways between viruses and EVs
produced from cancer cells, with a special emphasis on
breast cancer, as well as the ability of viruses to utilize EV
trafficking to spread pathogenesis are discussed in this
review.

Physical Properties and Morphology

A variety of techniques have been used to determine the
morphology and size of EVs, including electron micro-
scopy, nanoparticle tracking analysis (NTA), atomic force
microscopy, and flow cytometry. Both exosomes and MVs
are spherical in shape [4–6]. Although exosomes have
traditionally been described as having a cup shape when
viewed using transmission electron microscopy (TEM),
this observation is an artifact of sample preparation and
does not accurately reflect exosome morphology [7].
Cancer-derived exosomes are typically smaller than MVs,
with the former often ranging from 30 to 100 nm in size
[5, 8–10], and the latter having a larger size distribution,
frequently being described between 100 nm to over 1 µm
in diameter [6, 10, 11]. Recently, an even smaller subset of
EVs, 8–12 nm in diameter, called homogeneous
nanovesicles from various cancerous cell lines and bio-
logical samples (human MDA-MB-231 breast carcinoma
cells, 4T1 mouse mammary carcinoma cells, plasma from
colon cancer patients and from mice implanted with 4T1
mammary tumor cells) has been reported [12]. The size of
EVs also can also vary depending upon the originating cell
line as well as type of cancer. For example, exosomes from
oral squamous cell carcinoma were 50–200 nm as mea-
sured by scanning electron microscopy [13], while using
the same NTA technique those derived from SKBR3 breast
cancer were 183 ± 34 nm and those from murine B16F0
metastatic melanoma were 162 ± 23 nm [7].

Due to the size similarities between them, there are
problems obtaining purified viral particles because they are
often contaminated with EVs and vice versa, so specific
methodologies have to be employed when isolating virions
or EVs to ensure a pure preparation devoid of unwanted
materials [14, 15]. Like EVs, enveloped viruses can have a
variety of sizes, ranging from 40 to 300 nm for those that
are spherical in shape [16–18], although larger sizes have
been reported as well [19]. Similarly to EVs, enveloped
viral particles can have different sizes depending upon the
host cells from which they are released. For example,
electron cryotomography showed the measles virus pro-
duced from Vero-SLAM cells to be 50–510 nm in size
[20], although measles are pleomorphic in shape, while
measles virions produced from HeLa cells were determined
to by 180–600 nm by TEM [21].

Protein Content

It is well known that viruses incorporate host proteins
within the viral core as well as in the envelope [22].
However, there are also many proteins identified in EVs,
including those derived from breast cancer, that are
observed in enveloped virions as well [23, 24, 25, 26].
For example, proteins involved in endosomal trafficking
pathways, such as syntaxin-12 and VAMP3, have also been
identified within pseudorabies virus [27], as well as in EVs
derived from ovarian, colon, and non-small cell lung can-
cer [28–30]. The Epstein–Barr virus (EBV) and human
cytomegalovirus (HCMV) have been found in breast can-
cer patients, although to date, no viruses have been con-
clusively proven to induce breast cancer [31]. One
interesting study examined the proteomics of SKBR3
breast cancer-derived EVs, using a 10,000×g centrifuga-
tion step to isolate larger plasma membrane-derived vesi-
cles, 100,000×g centrifugation to isolate exosomes,
followed by another 100,000×g centrifugation using a
density gradient, or using size exclusion chromatography
instead of centrifugations, prior to LC–MS/MS [25].
Table 1 lists proteins that are common to both breast
cancer-derived EVs and enveloped EBV and HCMV viral
particles. These virions and EVs contained a variety of
shared protein classes, including cytoskeletal (i.e., moesin,
β-actin, and filamin A), those involved in endocytosis (i.e.,
clathrin, PIK3C2A), autophagy (i.e., heat shock proteins,
14-3-3 proteins), and endocytic transport (i.e., annexins,
RAB1A, CD81). There are also proteins in common
between SKBR3 EVs and EBV/HCMV that are involved
with protein folding (i.e., t-complex 1 (TCP1), chaperonin-
containing TCP1 (CCT) proteins), cell adhesion/migration
(i.e., galectin-1, 2-3 enolase), and cell division (i.e., cell
division cycle 42 (CDC42), RAN binding protein 2
(RANBP2)), indicating EVs and viral particles share more
than transport and degradation routes, including proteins
involved in significant cellular functions. Additionally,
EBV, which has been shown to support the growth of
nasopharyngeal cancer (NPC) [32], expectedly shares
many proteins with EVs derived from C666-1 (EBV-in-
fected) NPC EVs (Table 1) [33–35]. Interestingly, there
seem to be more cellular proteins associated with EBV
particles that have also been identified in breast cancer-
derived EVs compared to those that have been identified in
NPC EVs (Table 1), further supporting a link between
EBV and breast cancer. These studies suggest EBV and
Table 1  Cellular proteins common to viruses and cancer-derived EVs

| Protein class | Protein name | Virus          | Cancer          | Protein class | Protein name | Virus          | Cancer          |
|---------------|--------------|----------------|-----------------|---------------|--------------|----------------|-----------------|
|               |              | EBV [33]       | HCMV [26]       | skBR3 [25**]  | C666-1 [34, 35] (breast ADCA) |               |                 |
| Cytoskeletal  | β-actin      | +              | +               | +             | CD81          | +              | +               |
|               | γ-2-actin    | +              | +               | +             | Annexin A2    | +              | +               |
|               | Actin-related protein 3 | + | + | + | Annexin A6 | + | + + |
|               | Alpha actinin 1 | + | + | + | GAPDH | + | + + |
|               | Alpha actinin 4 | + | + | + | RAB1A | + | + + |
|               | Plastin-2     | +              | +               | +             | EHD1          | +              | +               |
|               | Filamin A     | +              | +               | +             | PRKAR2A       | +              | +               |
|               | Moesin        | +              | +               | +             | Thioredoxin   | +              | +               |
|               | Tubulin beta chain | + | + | + | VCP | + | + + |
|               | Tubulin alpha 1A chain | + | + | + | Mitosis | + | + + |
|               | Tubulin alpha 1C chain | + | + | + | CDC42 | + | + + |
|               | Coflin-1      | +              | +               | +             | eEF2          | +              | +               |
|               | Myosin-9      | +              | +               | +             | Signal transduction | + | + + |
|               | Profilin-1    | +              | +               | +             | SLC3A2        | +              | + +             |
|               | S100A6        | +              | +               | +             | RISC complex  | +              | +               |
|               | Keratin Type I cytoskeletal 10 | + | + | + | Keratin Type II cytoskeletal 2 epidermal | + | + + |
|               | Keratin Type II cytoskeletal 2 epidermal | + | + | + | Keratin Type I cytoskeletal 17 | + | + + |
|               | Keratin Type II cytoskeletal 1 | + | + | + | Keratin Type II cytoskeletal 19 | + | + + |
|               | Keratin Type I cuticular Ha6 | + | + | + | Na+/K+ ATPase | + | + + |
|               | Keratin Type I cuticular Ha6 | + | + | + | Autophagy | + | + + |
|               | Keratin Type II cytoskeletal 5 | + | + | + | Keratin Type II cytoskeletal 2 oral | + | + + |
|               | Keratin Type II cytoskeletal 2 oral | + | + | + | Keratin Type I cuticular Ha6 | + | + + |
HCMV viral particles contain common cytoskeletal proteins, chaperones, and proteins involved in migration and cell division, as well as utilize similar intracellular trafficking routes as EVs, with particular similarities to breast cancer-derived EVs.

**Cellular Entry Pathways**

In addition to morphology and protein content, enveloped viruses and EVs also share cellular entry and release pathways. It is important to mention that EVs and enveloped viruses often utilize multiple pathways for cellular entry [36–40], perhaps to increase the efficiency of uptake. Figure 1 outlines cellular entry pathways shared by cancer-derived EVs and enveloped viruses.

**pH**

Tumors are known to be acidic in nature, which results in a low pH surrounding the cancer cells and enhances cancer progression [41]. Low extracellular pH also influences EV uptake, as shown by the significant increase in internalization of EVs from melanoma cells grown at low pH (6.0) as opposed to the physiological pH of 7.4 [42]. However, an inhibition in internalization of EVs from metastatic breast cancer and prostate cancer cells was observed when grown at low pH (6.3) as opposed to the physiological pH of 7.4. It should be noted, however, that internalization of EVs was not affected by the pH of the medium where target cells were growing, but rather the surrounding pH of the cells which released the EVs [43]. These conflicting results could indicate that extracellular pH variably affects EV entrance depending on cell type. Endosomal pH is also a determining factor for entry of enveloped viruses into host cells. When ammonium chloride, concanamycin, bafilomycin A1, or chloroquine, which make endosomes less acidic, were added to host cells, the infectivity of HIV-1 virions pseudotyped with hepatitis C virus (HCV) glycoproteins, HCV virions, or oropouche virions was reduced [44–46]. Additionally, decreases in extracellular pH can enhance vaccinia virus entry by promoting cellular membrane fusion and overcome the inhibition of viral entry caused by endosomal acidification inhibitors [47]. Another study demonstrated that treatment of Huh 7.5 hepatocarcinoma cells with bafilomycin A1 or lansoprazole (another compound which raises endosomal pH) decreased the entry of, and subsequent infection from, EVs from HCV infected cells, as well as HCV virus [48]. These studies suggest endocytosis of viral particles and EVs is affected by both extracellular and endosomal pH levels.
Clathrin- and Caveolin-Mediated Endocytosis

EVs isolated from the SKOV3 ovarian cancer cell line were shown to be taken up by target SKOV3 cells through clathrin-mediated (receptor-mediated) endocytosis [37]. The inhibition of PC12 pheochromocytoma EV uptake was observed upon addition of inhibitors of clathrin-mediated endocytosis, such as potassium depletion buffer and chlorpromazine, as well as the knockdown of the CHC clathrin subunit or the subunit of the clathrin adaptor complex AP2 within target cells [36]. Multiple enveloped viruses also enter hosts through clathrin-mediated endocytosis, such as rubella virus [39] and HCV [45]. Caveolin-1, an integral membrane protein often associated with lipid rafts, is also known to regulate endocytosis through lipid rafts [49]. Both EVs, including pancreatic cancer-derived EVs [38, 50], and viruses such as coronavirus [51], and Newcastle disease virus [52] utilize caveolin-mediated endocytosis for cellular entry.

Lipids/Lipid Rafts

Lipid rafts, which play an important role in cell signaling, are another mechanism for EVs and viruses to enter target cells. Glioblastoma EVs undergo lipid raft-dependent endocytosis, whose uptake is inhibited by caveolin-1, to enter HUVECs and glioblastoma cells [53], and ovarian cancer EVs also utilize lipid rafts, independent of caveolin involvement, for their cellular uptake [37]. Therefore, caveolin-1-dependent and lipid raft-dependent mechanisms are not necessarily synonymous. Additionally, breast cancer BT549-derived EVs enter cells through lipid rafts, which was indicated through the inhibition of EV internalization by treatment with methyl beta-cyclodextrin (MβCD), a disruptor of lipid rafts, as well as EV colocalization with cholera toxin B, which enters cells through lipid rafts [54]. Herpes simplex virus (HSV) also employs lipid rafts for entry into a variety of host cells, including I143 epithelial cells, as well as SW480 and HT29 colon cancer cells, as shown by a decrease in viral infectivity upon addition of the cholesterol depletion compounds filipin and nystatin [55]. Lipid raft involvement has also been observed in regard to the cellular entry of other viruses such as Japanese encephalitis virus and dengue virus (DENV) [40, 56]. Lipid content plays an important role in EV uptake, and the type of phospholipids present within EVs determine the extent of cellular entry [43]. Sphingomyelin, phosphatidylserine (PS), and phosphatidylinositol are upregulated, while phosphatidylcholine is downregulated in EVs compared to their originating cell lines [43]. Lipids such as PS on the EV/viral surface have also been reported to be involved in EV entry into squamous carcinoma cells [57] and ovarian carcinoma cells [58] as well as DENV [59], and ebola virus [60] entry into host cells.

![Common routes of entry between EVs and enveloped viruses.](image)
Actin-Dependent Mechanisms

Cytoskeletal components such as actin have a functional role in cellular uptake mechanisms, including phagocytosis, a process in which a cell engulfs foreign material into phagosomes, macropinocytosis, where large materials are taken up into vesicles called macropinosomes, in addition to endocytosis [61]. Several studies have shown the actin polymerization inhibitor cytochalasin D was shown to decrease the uptake of EVs, including those derived from CEM T-cell lymphoblastic-like cells [62], Jurkat T lymphocyte [63], and K562 erythroleukemia cells [64]. Similarly, treatment of host cells with cytochalasin B or D decreased the infectivity of rubella virus [39] and amphotropic murine leukemia virus (A-MLV) [65], respectively. More specifically, one group showed EV uptake occurred through phagocytosis by identifying K562 or MT4 (HTLV-transformed T-cell leukemia)-derived EVs colocalized with phagosomes in macrophages and showed their entry was dependent upon actin and dynamin-2, the latter protein being important for phagocytosis [64]. HSV was determined to enter host cells through phagocytosis by a significant decrease in viral entry in host cells expressing a mutated form of dynamin-2 [66]. Macropinocytosis as a mechanism for EV entry was determined through the decrease in uptake of PANC-1 pancreatic cancer-derived EVs upon addition of amiloride (a macropinocytosis inhibitor) [38]. The inhibition of macropinocytosis and resulting decrease in PC12 pheochromocytoma-derived EV uptake was also achieved using a Na"−H"+ exchange inhibitor (EIPA) and a phosphoinositide 3-kinase inhibitor (LY294002) [36]. A separate study demonstrated that significantly more EVs produced from HeLa cervical adenocarcinoma cells entered A431 epidermoid carcinoma cells upon stimulation with epidermal growth factor, a protein known to induce macropinocytosis [67]. Another study suggested that mRNA levels within EVs derived from 4T1 cells were quickly degraded within endocytic compartments after internalization and further observed EVs derived from HEK293FT cells colocalized with endocytic vesicles through the use of the macropinocytosis marker FITC-dextran [68]. EIPA and/or amiloride were also used to determine that macropinocytosis was involved in the entry of A-MLV into host NIH 3T3 fibroblasts and HeLa cells [65] and rubella virus into host Vero-E6 kidney cells [39].

Filopodia Surfing

Recently, an interesting mechanism of EV entry has been shown to occur through "surfing" along cell extensions in order reach the cell membrane prior to endocytosis and colocalization with the endoplasmic reticulum [69•]. EV uptake was reduced after addition of the actin polymerization inhibitor SMIF2, which decreased the numbers of cellular filopodia, and indicated these protrusions were an essential component for EV uptake [69•]. This type of transport into host cells can also be achieved by a variety of viruses [70, 71]. In particular, the murine leukemia virus (MLV) uses actin and myosin within the length of cellular filopodia to transport viral particles to the cell for subsequent infection [72].

Cellular Release Pathways

Endocytic Vesicles

Interestingly, one common thread shared by EVs and viruses is the recruitment of the endosomal sorting complexes required for transport (ESCRTs) machinery to aid in cellular release [73]. However, within the two major types of EVs—ectosomes and exosomes—the role of the ESCRT complexes differs. Exosome biogenesis utilizes all four types of ESCRTs, ESCRT-0, -I, -II, and -III. ESCRT-0 recognizes ubiquitinated proteins to be packaged into intraluminal vesicles (ILVs) within multivesicular bodies (MVBs) and recruits ESCRT-I [73]. ESCRT-I and ESCRT-II also bind cargo and cluster at the endosomal membrane, prior to inward vesicular budding by ESCRT-III [73]. One model suggests ESCRT-III, along with VPS4 ATPase, constricts the membrane to then facilitate fission [74]. Upon maturation of the exosomal cargoes, MVBs proceed toward the outside of the cell and release their contents through fusion with the plasma membrane. This process is facilitated by a host of proteins belonging to the Rab GTPases family, notably RAB11, RAB35, and RAB27A/B [75]. Bobrie et al. [76] illustrated the importance of RAB27A in the secretion of exosomes from TS/A (non-metastatic) and 4T1 (metastatic) murine mammary carcinoma cells, as knockdown of this protein resulted in significantly decreased exosome numbers.

In contrast, the biogenesis of ectosomes is not well established. Generally, ectosomes are formed through the outward budding and fission of the plasma membrane. One particular study showed that the ESCRT-I subunit TSG101 may play a role in the direct release of ectosomes through plasma membrane budding [77]. Upon recruitment to the plasma membrane, TSG101 bound to the arrestin 1 domain-containing protein 1 (ARRDC1) via a tetrapeptide motif, PSAP, and, along with VPS4 ATPase activity, resulted in ectosome release [77].

The discovery of ARRDC1’s function in vesicle formation and release gives light to the similarity of viral recruitment of the ESCRT machinery to mediate their release. Arrestin-related trafficking proteins such as...
ARRDC1 and thioredoxin interacting protein that bind to ESCRT machinery are also found to also be recruited by viruses [78]. The Gag structures in viruses employ specific tetrapeptide motifs, including P(T/S)AP, YPXL, and PPXY, to recruit ESCRT and ESCRT-associated proteins. Similar to exosome formation, Gag PTAP binds to TSG101, which along with VPS4, are required for viral release [79]. Additionally, YPXL and PPXY bind to ALIX and NEDD4 proteins, respectively, which are important in viral release [80, 81]. The ability of viruses to mimic these interactions provides evidence of their efficiency in utilizing their host cells’ endogenous vesicle-releasing machinery.

**Actin-Dependent Mechanisms**

There are conflicting reports about the involvement of actin in EV release. One study reported that EV release from cervical cancer cells was dependent upon actin, as treatment of cells with cytochalasin D markedly reduced the amount of secreted EVs [82]. However, another group demonstrated a significant increase in EV release upon treatment of HT29 colorectal cancer cells with cytochalasin D [83]. Another study supported this increase in EV release from ovarian cancer cells after addition of cytochalasin D [84], suggesting non-actin-dependent mechanisms of EV release. This discrepancy could have to do with the type of cell from which the EVs are released, with different cancer cell types utilizing separate release mechanisms. However, in MDA-MB-231 cells, as well as other cancer cell lines, the inhibition of ARF6, a regulator of ERK/MLCK actomyosin-based contraction, decreased EV release, further supporting an actin-based mechanism [85] and confounding the overall necessity for actin in the release of cancer EVs. The role of actin in regard to virus release is more apparent, with actin depolymerization producing a decrease in enveloped vaccinia virus, measles virus, and respiratory syncytial virus (RSV) particle release [86–88].

**Lipids/Lipid Rafts**

Ceramide is a sphingolipid that is located within lipid rafts and has a functional role in their organization and protein recruitment [89]. Knockdown of neutral sphingomyelinase 2 (nSMAse2), which produces ceramide, resulted in a decreased amount of EVs released from 4T1 mammary carcinoma cells [90]. However, EV release from prostate PC-3 cells was not affected by inhibition of nSMAse2 nor inhibition of ceramide synthase, although the knockdown of lipid raft proteins flotillin-1 and flotillin-2 altered the amounts of caveolin-1 and annexin A2 in exosomes [91]. Therefore, the influence of lipid raft components on EV release varies from cell to cell. RSV [92] and MLV release [93] have also been reduced upon addition of MβCD, indicating viral release can also be dependent upon lipid rafts.

**Viral Exploitation of EVs**

Due to the many similarities shared between EVs and enveloped viruses, it is not surprising that viruses have been postulated to exploit the exosome transport pathway as a means to incorporate host proteins and avoid immune detection, which has been coined the “Trojan exosome hypothesis” [94]. Viruses also have the unique ability to transfer nucleic acids and proteins into host exosomes to promote infectivity. The utilization of the exosomal sorting and release pathway by viruses is especially evident in cancer cells, which secrete large amounts of EVs. One such example involves the HIV-1 Gag protein, which was localized to endosome-like regions and released into exosomes from infected Jurkat and K562 chronic myelogenous leukemia cells [95]. Viruses can take advantage of these EVs and use them as a means to promote infectivity, as shown by viral transfer of sever fever with thrombocytopenia syndrome virus through EVs to infect host HeLa cells [96]. In addition to proteins, viral nucleic acids can be transferred to host cells via EVs, which was demonstrated through the transfer of functional EBV miRNA to host monocyte-derived cells and resulting downregulation of miRNA targets [97].

**Viral-Regulated EV Content**

Cancer-associated viruses not only use the exosome transport pathway to promote viral infection, but to also spread oncogenes and proteins that will promote cancer progression. NPC-derived EVs have the ability to transfer the EBV latent membrane protein 1 (LMP1) as well as viral miRNAs to HUVEC cells and activate the AKT and ERK signaling pathways, which are known to promote cellular transformation and enhance tumor growth [35]. A separate study also demonstrated that LMP1 upregulated hypoxia-inducible factor-1a (HIF-1α) in EVs derived from NPC cells [98]. Additionally, they observed EV-associated HIF-1α was involved in the increase in levels of N-cadherin in HEK293T cells, which is a marker of the epithelial to mesenchymal transition [98]. Therefore, enveloped viral particles associated with traditional pathogenesis as well as cancer transmission both utilize EVs within host cells to increase infectivity and enhance tumor growth, respectively. The miRNA content, including those that target oncogenes as well as tumor suppressors, of EVs has been shown to be altered by viral infection. For example,
numerous EV miRNAs with multiple targets were identified to have altered expression profiles in patients with chronic hepatitis B, including miR-520b, miR-149, and miR-150 [99]. While there is no direct correlation between EBV infection and EV miRNA content, there are a number of miRNAs that have been identified in viral-associated cancer (i.e., breast and prostate) EVs that have also shown to be modulated by EBV infection. A variety of miRNAs have been identified in metastatic and/or non-metastatic breast cancer EVs, including oncogenic miR-21, -23b, -27b, -181a, -378, as well as miR-26a, -151-3p, 151-5p, and let-7i [100]. These miRNAs have been found to have differential expression upon EBV infection of both B cells and diffuse large B cell lymphoma, with upregulation of the former miRNA group, and downregulation of the latter miRNA group [101, 102]. Additionally, EBV-infected host cells showed increased miR-146a and miR-34a levels, with the latter occurring through LMP1 expression and NF-κB activation [101]. miR-34a and miR-146a were also identified as two prostate cancer EV biomarkers, with significantly decreased expression of miR-34a observed in prostate cancer tissue compared to healthy controls [103]. Taken together, these studies suggest that EBV, a virus not only causally associated with NPC but also found in breast and prostate cancers, can regulate oncogenic or tumor-suppressive miRNA levels secreted into EVs, perhaps to enhance tumor growth in cancer patients.

Breast Cancer and Viral-Regulated EV Content

Over the years numerous viruses including the mouse mammary tumor virus, HPV, the bovine papilloma virus, and EBV [31] have been associated with human breast cancer, although the association has not risen to causal significance. Perhaps the reason for this is that some viruses may promote rather than initiate human breast cancer. The analogies mentioned in this review between mammalian EVs and enveloped viruses may explain the promotional role of both in the pathogenesis of human breast cancer. For example, HPV DNA sequences from the E6 gene region have been detected in human biopsy samples of invasive ductal carcinoma [104]. The transduction of E6/E7 into MCF-7 and BT-20 breast cancer cells increased cell invasion and metastasis [105]. A link between HPV and the content of cervical cancer EVs has been observed by Honegger et al. [106]. This group showed EV-associated miRNAs are regulated by the HPV oncogene E6/E7 within cervical cancer cells. For example, let-7d-5p, miR-378a-3p, and miR-92a-3p are significantly downregulated, and miR-21-5p, miR-100-5p, and miR-30c-5p are significantly upregulated in EVs by inhibition of E6/E7 in HeLa and/or SiHa cells, which led to the conclusion that E6/E7 expression influences EV miRNA content to enhance the levels of those with pro-tumorigenic properties [106]. These miRNAs that are influenced by E6/E7 are also expressed in EVs from breast cancer cells, as mentioned above for miR-21 and miR-378 [100]. EVs from MCF-7 cells have been found to contain and transfer miR-100 to target cells [107], and miR-92a, let-7d, and miR-30c have also been located in MCF-7 EVs [108]. The combination of these studies raises the possibility that HPV within infected breast cancer cells can affect the miRNA content of breast cancer-derived EVs. Future studies could determine if HPV-regulated EV miRNAs or proteins have a direct promotional role in the progression of breast cancer.

Conclusions

Cancer-derived EVs, including both exosomes and MVs, are membrane-bound particles that share multiple characteristics with enveloped viruses, including morphology, protein content, and entry and release pathways. The entry of EVs and a variety of enveloped viruses into target cells is pH sensitive, dependent upon lipid rafts, and utilizes actin-dependent processes such as phagocytosis and macropinocytosis, among others. The release of both particle types includes similar mechanisms as those used for entry, including those involving actin as well as lipid rafts, but both viruses and EVs utilize ESCRT machinery for cellular release as well. In order to disseminate infection, enveloped viruses exploit the endosomal trafficking pathway, incorporating host proteins during intracellular transport. Viruses, including those associated with cancer, take advantage of the large quantities of EVs released from normal and transformed cells, using them to incorporate viral oncogenes and nucleic acids into target cells, which can enhance the spread of infection and cancer development. In the case of human breast cancer, although a causal viral association has not been demonstrated, the presence of EBV and HPV DNA in human breast cancer biopsies suggests a promotional role. Many cancer-associated miRNAs that are regulated by HPV are also present in breast cancer-derived EVs, suggesting the possibility of viral-linked EV content and breast cancer promotion. Furthermore, breast cancer-derived EVs have a particularly large number of proteins, including those found in the endocytic pathway, which have also been identified in viral particles, supporting mutual cellular transport routes. The multitude of these similarities suggests EVs, including those derived from breast cancer, and viruses may have evolved from a common origin, with both serving as tools for the pathogenesis of disease.
Compliance with Ethical Guidelines

Conflicts of Interest  Toni M. Green, Mark F. Santos, Sanford H. Barsky, Germana Rappa, and Aurelio Lorico declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
• Of importance
** Of major importance

1. Koboldt DC, Fulton RS, McLellan MD, for the Cancer Genome Atlas Network et al (2012) Comprehensive molecular portraits of human breast tumours. Nature 490:61–70
2. Verma M, Lam TK, Hebert E et al (2015) Extracellular vesicles: potential applications in cancer diagnosis, prognosis, and epidemiology. BMC Clin Pathol 15:6
3. Welsch S, Muller B, Krausslich HG (2007) More than one door—budding of enveloped viruses through cellular membranes. FEBS Lett 581:2089–2097
4. Stec M, Szatanak R, Baj-Krzyworzeka M et al (2015) Interactions of tumour-derived micro (nano) vesicles with human gastric cancer cells. J Transl Med 13:376
5. Sharma S, Das K, Woo J et al (2014) Nanofilaments on glioblastoma exosomes revealed by peak force microscopy. J R Soc Interface 11:20131150
6. Stratton D, Moore C, Antwi-Baffour S et al (2015) Microvesicles released constitutively from prostate cancer cells differ biochemically and functionally to stimulated microvesicles released through sublytic C5b-9. Biochem Biophys Res Commun 460:589–595
7. Wu Y, Deng W, Klinke DJ 2nd (2015) Exosomes: improved methods to characterize their morphology, RNA content, and surface protein biomarkers. Analyst 140:6631–6642
8. Paolini L, Zendrini A, Noto GD et al (2016) Residual matrix of human cytomegalovirus pp65 of human cytomegalovirus acts as an optional scaffold protein that optimizes protein uploading into viral particles. J Virol 88:9633–9646
9. Kim H, Shao H, Park YI et al (2014) Label-free detection and molecular profiling of exosomes with a nano-plasmonic sensor. Nat Biotechnol 32:490–495
10. Xu R, Greening DW, Rai A et al (2015) Highly-purified exosomes and shed microvesicles isolated from the human colon cancer cell line LIM1863 by sequential centrifugal ultrafiltration are biochemically and functionally distinct. Methods 87:11–25
11. Saari H, Lazaro-Ibanez E, Viitala T et al (2015) Microvesicle- and exosome-mediated drug delivery enhances the cytotoxicity of Paclitaxel in autologous prostate cancer cells. J Control Release 220:727–737
12. Zhang HG, Cao P, Teng Y et al (2016) Isolation, identification, and characterization of novel nanovesicles. Oncotarget 7:41346–41362
13. Li L, Li C, Wang S et al (2016) Exosomes derived from hypoxic oral squamous cell carcinoma cells deliver miR-21 to normoxic cells to elicit a prometastatic phenotype. Cancer Res 76:1770–1780
14. Segura MM, Garnier A, Kamen A (2006) Purification and characterization of retrovirus vector particles by rate zonal ultracentrifugation. J Virol Methods 133:82–91
15. Keryer-Bibens C, Poche-Durieu C, Vilmant C et al (2006) Exosomes released by EBV-infected nasopharyngeal carcinoma cells convey the viral latent membrane protein 1 and the immunomodulatory protein galec tin 9. BMC Cancer 6:283
16. Gelderblom HR (1996) Structure and classification of viruses. In: Baron S (ed) Medical microbiology, chap 41, 4th edn. University of Texas Medical Branch, Galveston, TX
17. Sherman MB, Weaver SC (2010) Structure of the recombinant alphavirus Western equine encephalitis virus revealed by cryo-electron microscopy. J Virol 84:9775–9782
18. Setitz S, Urban S, Antoni C et al (2007) Cryo-electron microscopy of hepatitis B viruses reveals variability in envelope capsid interactions. EMBO J 26:4160–4167
19. Malkin AJ, McPherson A, Gershon PD (2003) Structure of intracellular mature vaccinia virus visualized by in situ atomic force microscopy. J Virol 77:6332–6340
20. Liljeroos L, Huiskonen JT, Ora A et al (2011) Electron cryo- tomography of measles virus reveals how matrix protein coats the ribonucleocapsid within intact virions. Proc Natl Acad Sci USA 108:18085–18090
21. Nakai M, Imagawa DT (1969) Electron microscopy of measles virus replication. J Virol 3:187–197
22. Cantin R, Methot S, Tremblay MJ (2005) Plunder and stowaways: incorporation of cellular proteins by enveloped viruses. J Virol 79:6577–6587
23. Doellinger J, Schaade L, Nitsche A (2015) Comparison of the cowpox virus and vaccinia virus mature virion proteome: analysis of the species- and strain-specific proteins. PLoS One 10:e0141527
24. Hosseini-Behesti E, Pham S, Adomat H et al (2012) Exosomes as biomarker enriched microvesicles: characterization of exosomal proteins derived from a panel of prostate cell lines with distinct AR phenotypes. Mol Cell Proteomics 11:863–885
25. ** Clark DJ, Fondrie WE, Liao Z et al (2015) Redefining the breast cancer exosome proteome by tandem mass tag quantitative proteomics and multivariate cluster analysis. Anal Chem 87:10462–10469. This study provided an extensive proteomic analysis of breast cancer exosomes using multiple purification methods, including differential centrifugation and SEC
26. Reyd S, Tenzer S, Navarro P et al (2014) The tegument protein p65 of human cytomegalovirus acts as an optional scaffold protein that optimizes protein uploading into viral particles. J Virol 88:9633–9646
27. Kramer T, Greco TM, Enquist LW et al (2011) Proteomic characterization of pseudorabies virus extracellular virions. J Virol 85:6427–6441
28. Liang B, Peng P, Chen S et al (2013) Characterization and proteomic analysis of ovarian cancer-derived exosomes. J Proteomics 80:171–182
29. Tauro BJ, Greening DW, Mathias RA et al (2012) Comparison of ultracentrifugation, density gradient separation, and immunofinity capture methods for isolating human colon cancer cell line LIM1863-derived exosomes. Methods 56:293–304
30. Park JO, Choi DY, Choi DS et al (2013) Identification and characterization of proteins isolated from microvesicles derived from human lung cancer pleural effusions. Proteomics 13:2125–2134
31. Lawson JS, Gunzburg WH, Whitaker NJ (2006) Viruses and human breast cancer. Future Microbiol 1:33–51
32. Raab-Traub N (2015) Nasopharyngeal carcinoma: an evolving role for the Epstein–Barr virus. Curr Top Microbiol Immunol 390:339–363
33. Johanssen E, Luftig M, Chase MR et al (2004) Proteins of purified Epstein–Barr virus. Proc Natl Acad Sci USA 101:16286–16291
34. Chan YK, Zhang H, Liu P et al (2015) Proteomic analysis of exosomes from nasopharyngeal carcinoma cell identifies intercellular transfer of angiogenic proteins. Int J Cancer 137:1830–1841
35. Meckes DG Jr, Shair KH, Marquitz AR (2010) Human tumor virus utilizes exosomes for intercellular communication. Proc Natl Acad Sci USA 107:20370–20375
36. Tian T, Zhu YL, Zhou YY et al (2014) Exosome uptake through clathrin-mediated endocytosis and macropinocytosis and mediating miR-21 delivery. J Biol Chem 289:22258–22267
37. Escrevente C, Keller S, Allevogt P et al (2011) Interaction and uptake of exosomes by ovarian cancer cells. BMC Cancer 11:108
38. Javeed N, Sagar G, Dutta SK et al (2014) Pancreatic cancer-derived exosomes cause paraneoplastic beta-cell dysfunction. Clin Cancer Res 21:1722–1733
39. Kee SH, Cho EJ, Song JW et al (2004) Effects of endocytosis inhibitory drugs on rubella virus entry into VeroE6 cells. Microbiol Immunol 48:823–829
40. Das S, Chakraborty S, Basu A (2010) Critical role of lipid rafts in virus entry and activation of phosphoinositide 3’ kinase/Akt signaling during early stages of Japanese encephalitis virus infection in neural stem/progenitor cells. J Neurochem 115:537–549
41. Kato Y, Ozawa S, Miyamoto C et al (2013) Acidic extracellular microenvironment and cancer. Cancer Cell Int 13:89
42. Parolini I, Federici C, Raggi C et al (2009) Microenvironmental pH is a key factor for exosome traffic in tumor cells. J Biol Chem 284:34211–34222
43. Smyth TJ, Redic JS, Graner MW et al (2014) Examination of the specificity of tumor cell derived exosomes with tumor cells in vitro. Biochim Biophys Acta 1838:2954–2965
44. Hsu M, Zhang J, Flint M et al (2003) Hepatitis C virus glycoproteins mediate pH-dependent cell entry of pseudotyped retroviral particles. Proc Natl Acad Sci USA 100:7271–7276
45. Blanchard E, Belouzard S, Goueslain L et al (2006) Hepatitis C virus entry depends on clathrin-mediated endocytosis. J Virol 80:6964–6972
46. Santos RI, Rodrigues AH, Silva ML et al (2008) Oropouche virus entry into HeLa cells involves clathrin and requires endosomal acidification. Virus Res 138:139–143
47. Townsley AC, Weisberg AS, Wagenaar TR et al (2006) Vacinia virus entry into cells via a low-pH-dependent endosomal pathway. J Virol 80:8899–8908
48. Bukong TN, Momen-Heravi F, Kodyk S et al (2014) Exosomes from hepatitis C infected patients transmit HCV infection and contain replication competent viral RNA in complex with Ago2-miR122-HSPI90. PLoS Pathog 10:e1004424
49. Lajoie P, Nabi IR (2007) Regulation of raft-dependent endocytosis. J Cell Mol Med 11:644–653
50. Sagar G, Sah RP, Javeed N et al (2015) Pathogenesis of pancreatic cancer exosome-induced lipolysis in adipose tissue. Gut 65:1165–1174
51. Nomura R, Kiyota A, Suzuki E et al (2004) Human coronavirus 229E binds to CD13 in rafts and enters the cell through caveolae. J Virol 78:8701–8708
52. Cantin C, Holguera J, Ferreira L et al (2007) Newcastle disease virus may enter cells by caveolae-mediated endocytosis. J Gen Virol 88:559–569
53. Svensson KJ, Christianson HC, Wittrup A et al (2013) Exosome uptake depends on ERK1/2 heat shock protein 27 signaling and lipid Raft-mediated endocytosis negatively regulated by caveolin-1. J Biol Chem 288:17713–17724
54. Koumangojy RB, Sakwe AM, Goodwin JS et al (2011) Detachment of breast tumor cells induces rapid secretion of exosomes which subsequently mediate cellular adhesion and spreading. PLoS One 6:e24234
55. Gianni T, Gatta V, Campadelli-Fiume G (2010) αvβ3-integrin receptors hedge simplex virus to an entry pathway dependent on cholesterol-rich lipid rafts and dynamin2. Proc Natl Acad Sci USA 107:22260–22265
56. Diwaker D, Mishra KP, Ganju L et al (2015) Protein disulfide isomerase mediates dengue virus entry in association with lipid rafts. Viral Immunol 28:153–160
57. Al-Nedawi K, Meehan B, Kerbel RS et al (2009) Endothelial expression of autocrine VEGF upon the uptake of tumor-derived microvesicles containing oncogenic EGFR. Proc Natl Acad Sci USA 106:3794–3799
58. Keller S, Konig AK, Marme F et al (2009) Systemic presence and tumor-growth promoting effect of ovarian carcinoma released exosomes. Cancer Lett 278:73–81
59. Meertens L, Carnecc L, Lecoin MP et al (2012) The TIM and TAM families of phosphatidylinerse receptors mediate dengue virus entry. Cell Host Microbe 12:544–557
60. Moller-Tank S, Kondratowicz AS, Davey RA et al (2013) Role of the phosphatidylinerse receptor TIM-1 in enveloped-virus entry. J Virol 87:8327–8341
61. Stern ST, Adiseshiah PP, Crist RM (2012) Autophagy and lysosomal dysfunction as emerging mechanisms of nanomaterial toxicity. Part Fibre Toxicol 9:20
62. Yang C, Xiong W, Qiu Q et al (2012) Role of receptor-mediated endocytosis in the antiangiogenic effects of human T lymphoblastic cell-derived microparticles. Am J Physiol Regul Integr Comp Physiol 302:R941–R949
63. Bastos-Amador P, Perez-Cabezás B, Izquierdo-Useros N et al (2012) Capture of cell-derived microvesicles (exosomes and apoptotic bodies) by human plasmacytoid dendritic cells. J Leukoc Biol 91:751–758
64. Deng F, Zhao WL, Ye YY et al (2010) Cellular internalization of exosomes occurs through phagocytosis. Traffic 11:675–687
65. Rasmussen I, Vilhardt F (2014) Macropinocytosis is the entry mechanism of amphotropic murine leukemia virus. J Virol 89:1851–1866
66. Clement C, Tiwari V, Scanlan PM et al (2006) A novel role for phagocytosis-like uptake in herpes simplex virus entry. J Cell Biol 174:1009–1021
67. Nakase I, Kobayashi NB, Takatani-Nakase T et al (2015) Active macropinocytosis induction by stimulation of epidermal growth factor receptor and oncogenic Ras expression potentiates cellular uptake efficacy of exosomes. Sci Rep 5:10300
68. Kanada M, Bachmann MH, Hardy JW et al (2015) Differential fates of biomolecules delivered to target cells via extracellular vesicles. Proc Natl Acad Sci USA 112:E1433–E1442
69. * Heusermann W, Hean J, Trojer D et al (2016) Exosomes surf on filopodia to enter cells at endocytic hot spots, traffic within endosomes, and are targeted to the ER. J Cell Biol 213:173–184. This paper describes a novel mechanism for exosomal entry by traveling along cellular filopodia, an occurrence previously observed with viruses
70. Zamudio-Meza H, Castillo-Alvarez A, Gonzalez-Bonilla C et al (2009) Cross-talk between Rac1 and Cdc42 GT-Pases regulates formation of filopodia required for dengue virus type-2 entry into HMEC-1 cells. J Gen Virol 90:2902–2911
71. Dixit R, Tiwari V, Shukla D (2008) Herpes simplex virus type 1 induces filopodia in differentiated P19 neural cells to facilitate viral spread. Neurosci Lett 440:113–118
72. Lehmann MJ, Sherer NM, Marks CB et al (2005) Actin- and myosin-driven movement of viruses along filopodia precedes their entry into cells. J Cell Biol 170:317–325
