Microparticles and Exosomes in Gynecologic Neoplasias

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ABSTRACT

This review presents an overview of the functions of microparticles and exosomes in gynecologic neoplasias. Growing evidence suggests that vesicles released from cancer cells in gynecologic malignancies contribute to the hypercoagulable state of these patients and contribute to tumor progression by suppressing the immune system, facilitating extracellular matrix degradation and removal of cytostatics from the tumor cell. Exosomes from ovarian carcinoma cells were shown to be present in peripheral blood and to augment tumor growth, suggesting that these vesicles directly support growth of tumor cells.

KEYWORDS: Microparticle, exosome, cancer, gynecologic malignancy

In 2007 we provided an overview of the functions of microparticles and exosomes in normal and complicated pregnancy.[1] In the present review, we focus on the presence and functions of cell-derived vesicles in gynecologic neoplasias. It must be stressed, however, that our knowledge with regard to cell-derived vesicles is still fragmentary. Two limitations must be mentioned about the present review. First, until now most of the evidence with regard to the occurrence and function of vesicles has been obtained in vitro or ex vivo. To what extent vesicles present in conditioned culture media, cell-free plasma or serum, or ascites reflect the in vivo situation is unclear because subpopulations of vesicles are prone to associate with cells and become lost when cells are removed before the vesicles are isolated. Second, we use the terms *microparticles* and *exosomes* throughout this review, but we have to be careful to assign functions specifically to those subtypes of microvesicles because their isolation is based mainly on protocols involving differential centrifugation without laboratory tools to monitor contamination by cells such as platelets or the cross-contamination of those microvesicles.

In this review, the reported functions of microparticles and exosomes are discussed (Fig. 1). In fact, we will see that although this field of research is relatively small, it has contributed considerably to the current interest and understanding of the biology and clinical relevance of cell-derived microvesicles.

FUNCTIONS OF MICROPARTICLES AND EXOSOMES IN GYNECOLOGIC MALIGNANCIES

Coagulation

In 1981 and 1983, Dvorak and coworkers reported that various cultured carcinoma cell lines, including TA3-St mouse breast carcinoma cells, "release procoagulant

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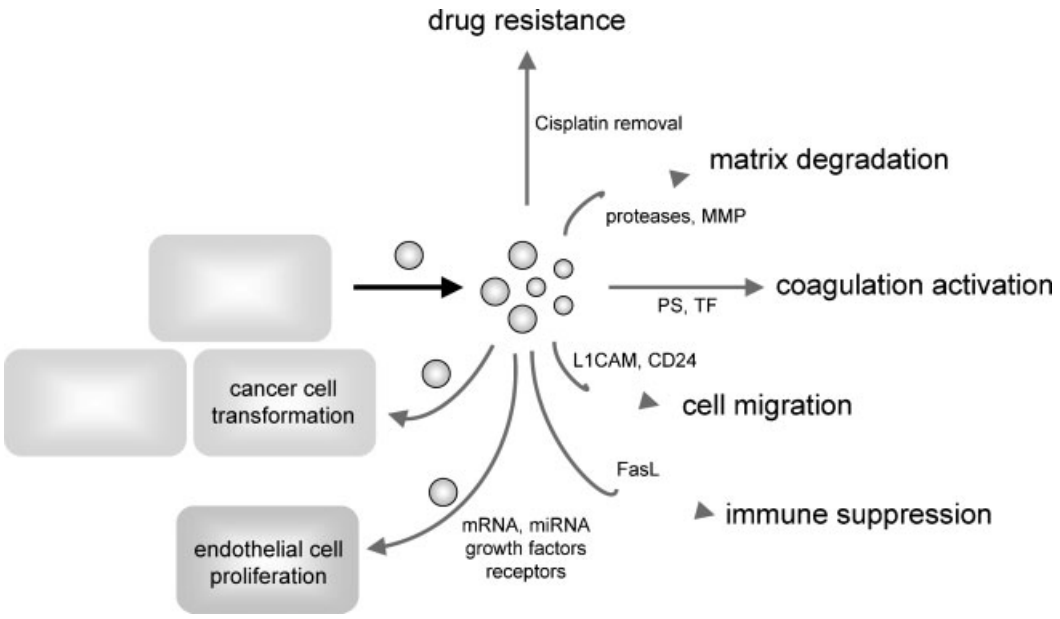

Figure 1 Overview of the functions of microparticles and exosomes in gynecologic neoplasias. Cancer cells release microparticles and exosomes, which in turn contribute to drug resistance, matrix degradation, coagulation activation, cell migration, suppression of the immune response, and promote tumor growth, endothelial cell proliferation, and tube formation. FasL, Fas ligand; L1CAM, L1 cell adhesion molecule; miRNA, microRNA; MMP, matrix metalloprotease; PS, phosphatidylserine; TF, tissue factor.

activity both in tissue culture and in tumour ascites in vivo, that this activity is associated with membrane-bound vesicles, and that it acts, at least in part, late in the common coagulation pathway, presumably at the level of prothrombinase generation."[2,3] They provided compelling evidence that these vesicles contribute to coagulation in two ways: "one procoagulant activity associated with shed human tumour vesicles behaved as tissue factor, requiring factor VII for activity and specific anti-bovine tissue factor antibody for inhibition," the other activity was acting "at a second step late in the clotting cascade at the level of prothrombinase generation, presumably by providing a phospholipid surface."[3]

Patients with gynecologic malignancies such as breast cancer or ovarian carcinoma are characterized by a hypercoagulable state, as reflected by elevated plasma levels of non-cell-bound tissue factor (TF) (antigen), factor VIIa (antigen), and prothrombin fragment F_{1+2},[4] and these patients have an increased risk of developing venous thromboembolism (VTE).[5,6] In a study by Uno et al, 10 of 32 patients with ovarian cancer (31%) developed VTE, and expression of TF by the cancer cells was strongly associated with the development of VTE.[7] Plasma samples from breast cancer patients presenting with VTE contained elevated levels of microparticle-associated TF activity compared with healthy controls, subjects with idiopathic acute VTE, and non-metastatic cancer patients, but whether or not this activity contributed to the development of the thrombosis is unclear.[8] The question remains whether coagulant TF in plasma of patients with (gynecologic) malignancies originates from the tumor. Ovarian cancer cells release TF-bearing microparticles,[9] and one may speculate that such vesicles enter the blood and increase the risk of VTE. Two independent studies demonstrated that exosomes released by ovarian cancer cells into (malignant) ascites entered the blood.[10] The finding of epithelial mucin exposing microparticles in the plasma of breast cancer patients strongly suggests that such tumors release microparticles that enter the blood.[8]

Taken together, although gynecologic cancer cells express TF and release TF-bearing vesicles in vitro, there is still insufficient evidence to support their direct contribution to the hypercoagulation state and the development of VTE in those patients.

Immune Suppression

Cancer cells may use vesicles to escape from immune surveillance and to suppress the immune system. Exosomes from murine breast cancer cells accelerated the growth of implanted tumor cells by inhibiting the cytotoxic activity of NK cells in vitro and ex vivo, and by reducing the fractions of NK cells in lungs and spleen in vivo by blocking the interleukin 2-mediated proliferation of NK cells.[11] With regard to suppression of the immune system, vesicles from various human breast cancer cell lines inhibited [^{3}H]-thymidine incorporation of stimulated and nonstimulated lymphocytes, suggesting that vesicles can indeed facilitate immune suppression.[12] Furthermore, several studies have provided evidence that the Fas (CD95)/Fas ligand (CD95L, FasL) system

may play a role not only in normal fetal and placental development but also in obtaining an immune privilege status of a tumor by triggering Fas-mediated apoptosis of tumor-specific lymphocytes.[13–15] For instance, Abrahams and coworkers showed that microvesicles from epithelial ovarian cancer cells isolated from ascites contained a 37-kDa FasL and a glycosylated form of 48 kDa-FasL, whereas normal ovarian epithelial cells did not release FasL. Both the 37-kDa and 48-kDa forms of FasL induced (Fas-mediated) apoptosis of Jurkat T cells.[13]

Drug Resistance

The causes of drug resistance in ovarian carcinoma patients are incompletely understood. Recurrent ovarian carcinoma occurs in patients with platinum-sensitive disease and in patients with platinum-resistant disease.[16] Several lines of evidence suggest that microparticles and exosomes contribute to cellular survival by protecting the cells against accumulation of waste or against environmental hazards, such as complement-mediated cell lysis.[17] Safaei and coworkers showed that cisplatin-resistant human ovarian carcinoma cells had a reduced lysosomal compartment compared with cisplatin-sensitive cells and that exosomes released from the resistant cells contained 2.6-fold more platinum than the exosomes released from the cisplatin-sensitive cells, suggesting that cancer cells can use vesicles to escape from such anticancer drugs.[18]

Extracellular Matrix Degradation and Protease Activity

Vesicles from human breast cancer cell lines contain several metalloproteinases, including the 97-kDa gelatinase precursor of matrix metalloproteinase (gelatinase B) and membrane vesicles isolated from conditioned culture medium of a human ovarian cancer cell line and from ascites and serum of patients with ovarian carcinoma that contained matrix metalloproteinase 2 (gelatinase A), urokinase-type plasminogen activator, and plasmin.[19–22] Some evidence indicates that the rate of membrane vesicle shedding by cancer cells may be related to their invasive capability. Compared with fluids of benign serous cysts, malignant tumor fluids contained large numbers of vesicles, and a positive association was present between tumor malignancy and both the number of vesicles and the amount of vesicle-associated matrix metalloproteinase 2.[23] In addition, ovarian carcinoma cells release exosomes containing functional ADAM10, a disintegrin also known as metalloprotease 10, and ADAM17 (TACE, tumor necrosis factor α converting enzyme). Depletion of ADAM10, but not ADAM17, blocked intravesicular cleavage of CD171 (L1; also known as cell adhesion molecule 1).[24] L1 is overexpressed in human ovarian and endometrial carcinomas, and it is associated with a bad prognosis. Ascites from ovarian carcinoma patients contains two different types of vesicles in which L1 cleavage occurs. One type of vesicles resembles exosomes; the other type resembles apoptotic bodies.[25] The soluble (nonmembrane) form of L1 from ascites is a potent inducer of cell migration and triggers extracellular signal-regulated kinase phosphorylation. Thus soluble L1 in tumor-derived vesicles can potentially regulate tumor cell function in an autocrine or paracrine manner. Similarly, CD24 is an established marker for poor prognosis in ovarian carcinoma patients. CD24 was identified in exosomes present in ascites of ovarian carcinoma patients, although no correlation was observed between CD24 in tumor tissue sections from 16 patients and the corresponding exosomes.[21]

NEW DEVELOPMENTS AND CLINICAL RELEVANCE

Several attempts have been made to use exosomes derived from tumor cellss as diagnostic biomarkers. Taylor et al compared the profiles of microRNA (miRNA) from tumor exosomes, present in peripheral blood of patients with ovarian cancer and isolated with beads coated with anti-EpCAM, to the miRNA profiles of the ovarian tumors of the same patients. EpCAM-exposing exosomes were detectable in blood from patients with ovarian cancer as well as in patients with benign ovarian disease. The miRNA profiles of exosomes and ovarian tumors were comparable, but the miRNA profile of exosomes differed from those present in benign disease. Exosomal miRNA could not be detected in blood from healthy subjects with this procedure.[26] Tumor-reactive immunoglobulin (Ig)G from the sera of ovarian cancer patients showed more immune reactivity to a protein array based on proteins isolated from exosomes compared with IgG from control sera. Reactivity to nucleophosmin, cathepsin D, p53, and SSX common antigen were higher in patients than in healthy subjects or those with benign ovarian disease. Reactivity toward placental type alkaline phosphatase, TAG72, survivin, NY-ESO-1, GRP78, and Mac16 (CA125) allowed further differentiation between advanced (stage III/IV) and early stage ovarian cancer.[27]

Recently, three studies have provided novel insights into the presence and biological relevance of exosomes in the growth of breast and ovarian carcinoma cells. First, Li et al showed that ovarian cancer cells release exosomes containing claudin 4. To evaluate the potential use of claudin 4-containing exosomes as a diagnostic biomarker of ovarian cancer, the presence of these exosomes was assessed in plasma samples from patients with ovarian cancer ($n = 63$) and healthy controls ($n = 50$). Of the patient plasma samples, 32 samples (50%) had claudin 4-containing exosomes, whereas only one control sample was positive (2%). These data suggest that exosomes released from ovarian cancer cells can be

present within the blood and as such may provide a diagnostic biomarker.[28] Second, Ochieng and coworkers isolated exosomes from fetal bovine serum because this is one of the critical ingredients in anchorage-independent growth assays of tumor cells. Viable colonies of breast cancer cells were formed in both soft agar and matrigel but only in the presence of the isolated exosomes from the fetal bovine serum. The exosomes were transiently taken up by cancer cells and then recycled back into the medium. Low concentrations of exosomes activated MAP kinases, and this mechanism may explain or contribute to the observed anchorage-independent cell growth in the presence of exosomes.[29] The second study is confirmed by the study of Keller and coworkers, who examined the presence of exosomes in ascites and blood from patients with ovarian carcinoma. They found that exosomes from malignant ascites contain several tumor progression-related proteins, including L1CAM, CD24, ADAM10, and EMMPRIN. The exosomes entered the blood and were taken up by NK cells. This uptake was partially phosphatidylserine dependent, and, perhaps most interesting, application of these exosomes to tumor-bearing mice augmented tumor growth. Thus exosomes may play a role in tumor progression.[10]

Although the underlying mechanisms explaining how vesicles augment tumor growth in vivo are unknown, microvesicles or "oncosomes" from glioblastoma tumor cells in vitro were shown to promote the intercellular transfer of an oncogenic form of the epithelial growth factor receptor (EGFR) vIII to nontransformed cells in vitro. They transferred mRNA transcripts and proteins to recipient cells including endothelial cells, which probably explains their ability to promote endothelial tubule formation.[30,31]

CONCLUSION

Several lines of evidence indicate that microparticles and exosomes facilitate tumor progression. The presence of tumor-derived vesicles within the blood is associated with collateral damage such as venous thrombosis but may also offer novel diagnostic and prognostic tools. Thus this field enhances our insights into the biological importance of microvesicles in general. This is a role we are unlikely to overestimate if we realize that the biological role of microvesicles seems to be an evolutionary conserved process because microvesicles facilitate intercellular communication not only between eukaryotic cells but also between bacteria.

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