World Journal of Research and Review (WJRR)
ISSN:2455-3956, Volume-2, Issue-4, April 2016 Pages 01-04

Exososome: Potential Biomarker for Cancer

Callie Mincy, Asha Eapen

***Abstract*— Transformation is a common phenomenon that occurs in a cancer environment. As a result of tumorogenesis, normal cells are transformed into cancer cells. Researchers have shown that multicellular vesicles known as exosomes secreted by malignant cells have the potential to induce this normal cell transformation. Accumulating evidence indicates that exosomes play important roles in cancer. Exosomes are known to play decisive roles in tumorogenesis, growth, progression, metastasis, and drug resistance by transferring oncogenic proteins and nucleic acids that modulates the activity of recipient cells. In this review, we will unveil the role of exosomes as communication molecules in cancer. Exosome shuttle proteins and nucleic acids and so have been suggested as novel diagnostic and prognostic indicators for a variety of cancers. Currently, tumor-derived exosomes are utilized as vaccines and as carriers for drugs and small molecules in pre-clinical studies and clinical trials.**

***Index Terms*— Exosomes, Paracrine, Synaptic, Biomarkers, Metastasis, Cancer, Clinical Trials**

I. INTRODUCTION

Intercellular communication is an essential for the development of multicellular organisms and is mediated through the secretion of molecules into the extracellular environment. A new mechanism for intercellular communication has emerged that involves extracellular vesicles (EVs). These vesicles are also referred to as microvesicles, ectosomes, shedding vesicles, or microparticles. Exosome are vesicles ranging from 40 to 1,000 nm that are released by a variety of cultured cells and are abundantly present in body fluids, carry RNA, and show a wide range of regulatory functions. Hence, exosomes are used as potential biomarkers for cancer diagnosis and therapy.

II. MECHANISM OF CELL COMMUNICATION

Re Extracellular signaling molecules plays a role in communicating between cells via binding to receptors or cell surface or intracellularly. This binding can trigger contact dependent signaling by membrane-membrane contact, paracrine signaling(where local mediators affect s only cells in the local environment), synaptic signaling (in the case of neurons) and endocrine signaling (depending on the endocrine cells which secrete the hormones). These extracellular signaling molecules include proteins, lipids, neurotransmitters messenger RNA and micro RNAs. However majority of these signaling molecules or ligands are found in cell-derived vesicles that are present in cells and all biological fluids, including blood, urine, and cell cultured medium.

III. EXTRACELLULAR VESICLES

Extracellular vesicles are released from the cells by three distinct mechanisms namely cell budding, multivesicular endosomal cell compartment and apoptotic bodies (Fig 1). Exosomes are released from the multivesicular endosomal cell compartment into the extracellular matrix/space for circulation via the body fluids. Exosomes are small membranous vesicles of 30–100 nm size, which are secreted from numerous types of cells and function in intercellular communication by transporting intracellular contents, such as protein and RNA [1]. Studies have shown that these vesicles could be a promising source of biomarkers for the diagnosis of a variety of diseases.

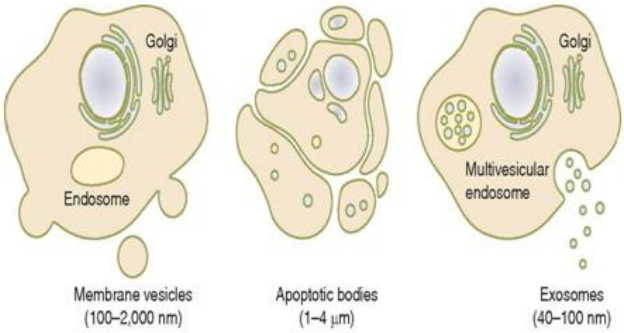


Figure 1:Various groups of extracellular vesicles form as a result of outward blebbing of the plasma membrane; apoptotic bodies form during the late stage of apoptosis; and exosomes form from the multivesicular endosomal cell compartment.(Adapted from Giuseppina Turturici et al. Am J Physiol Cell Physiol 2014;306:C621-C633)

IV. BIOGENESIS OF EXOSOMES: COMPOSITION AND FUNCTION

Exosomes are distinguished as extracellular vesicles (EVs) that are 30-100 nm in size, have a density of 1.13-1.19 g/cm^3, and are "cup-shaped" in their morphological appearance when viewed under an electron microscope [2]. These EV's have an endosomal origin, in that, they are formed by double inward budding of the endosomal membrane in a cells' during late endosome phase[3]. This causes the endosome to transform into a multivesicular body (MVB). MVBs create exosomes through the invagination of their membrane and therafterthey fuse with the membrane of the cell to release these exosomes. The development of exosomes through this

Callie Mincy, Biological Sciences, Southern Illinois University Edwardsville, IL, USA
Asha Eapen, School of Dental Medicine, Southern Illinois University Edwardsville, Alton, IL, USA

inward budding provide them with a double lipid bilayer that bears a large number of surface proteins and contain a variety of proteins, nucleic acids such as mRNA's and miRNA's, and lipids[4] (Fig 2). These contents are selectively loaded and are greatly characteristic of their parent cell type, however, the exosomes are not a direct copy of the parental cell. The resulting exosomes have a relative amount of RNA and protein that are different from the parent cell, although the sorting mechanisms responsible for this are only partly understood [5]. Once the MVB releases these exosomes to the extracellular space,an exosome can influence the function and phenotype of a recipient cell by emitting signal and regulatory molecules that other cells recognize and accept transfer from selectivelymolecules.This in turn could influence normal, healthy cells that come into contact with an infected exosome to become cancerous as well.

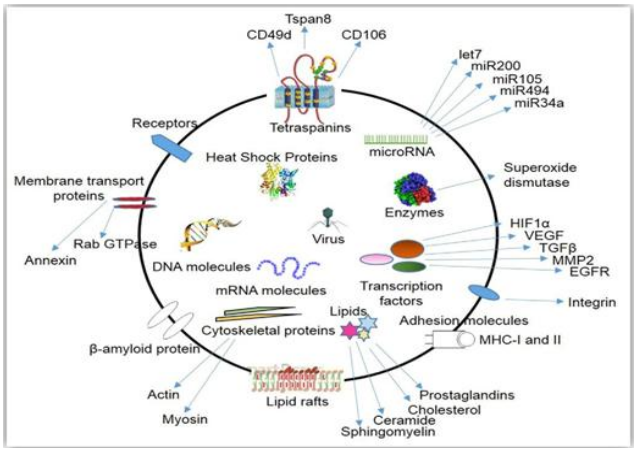
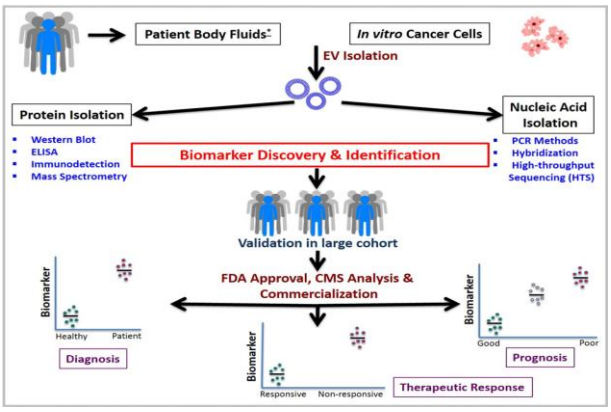


Fig 2: Exosomal content in cancer (Adapted from Lara Milane, Amit Singh, George Mattheolabakis, Megha Suresh, Mansoor M. Amiji, Journal of Controlled Release 219 (2015) 278–294)

Fig3: Biomarker validation schematic for Exosome screening. Extracellular vesicles are isolated biomarker identification and discovery then validated and submitted to the FDA for approval. (Adapted from Milane,L. et al. Exosome mediated communication within the tumor microenvironment 2015; 219:278-294)



V. ISOLATION OF EXOSOMES

Exosomes can be isolated from cell culture supernatants/medium or body fluids by different techniques. Isolation of exosomes are currently based on size, density and specific exosomal markers. Supernatant/medium containing the exosomes is subjected to a series of centrifugation steps followed by isolation with sucrose density gradients. However, higher yields of exosomes are obtained by incorporation of ultrafiltration membranes of varying pore sizes. Other techniques employed are PEG, magnetic beads coated with monoclonal antibodies, high-performance liquid chromatography (HPLC) etc.Recently, quantitative RT-PCR, nucleic acid sequencing, Western blot, or ELISA are used for exosome RNA and protein identification.

VI. THE ROLE(S) OF EVS AS BIOMARKERS

There is a need for biomarkers to make earlier detection of cancer and other diseases for easy and improved prognosis. Studies on using exosomes as effective biomarkers started when it was discovered that these extracellular vesicles can transport their contents through circulation to other distant cells. Exosomes are found in several different body fluids, such as blood and urine, and contain tumor-specific proteins that are important biomarkers[6].

Exosomes are very stable when frozen and thawed for research; their content is associated with the treatment and different stages of multiple tumors andis very abundant[7]. Exosomal protein levels in ovarian cancer patients have been observed before and after chemotherapy and showed an unchanged number in patients that were not responsive and a significant decrease in responsive patients which indicated that exosome protein content could be used for predicting treatment response [8]. Commercial companies have started developing an exosome based diagnosis strategy and studies have shown encouraging results in the success of serum differentiation from ovarian cancer, glioblastoma, melanoma, colorectal cancer, and pancreatic cancer against non-cancer controls [9]. This suggested that exosomes could be used as diagnostic biomarkers for multiple tumor types (Fig 3).

VII. EXTRACELLULAR VESICLES: NOVEL MEDIATORS IN METASTASIS

Exosomes have demonstrated a role in promotion of metastasis by physical invasion, transformation, and a pre-metastatic niche establishment [10]. Exosomes also show an association with invadopodia that degrade the extracellular matrix and encourage the invasion of cancer cells [11]. Experiments have shown that some exosomes even have the ability to induce formation of invadopodia in non-invading cells [11]. Pre-metastatic sites are developed by exosomes released from the primary tumor and are prime areas for metastasis showing that exosomes act as mediators of metastatic node formation [12]. Metastasis is a series of events that include epithelial-mesenchymal transition, the mobilization of cells, and mesenchymal-to-epithelial transition, establishment of a secondary tumor site. A study lead by Judy Lieberman showed that metastatic cancer cell exosomes could transfer the capability of metastasis, which is controlled by a known mediator of the mesenchymal-to-epithelial transition (MET) event, to non-metastatic cells [13].Cells used were highly capable of metastasis to test induction of metastasis in human and mouse

xenografts. These metastatic capable cells produced exosomes that transferred this known MET mediator, a family of micro RNA called miR-200, to the human breast cancer cells that were xenografted and displayed a promotion of metastasis in the xenografts. This demonstrated that exosomes can also influence and transform distant cells into metastatic cells (Fig 4).

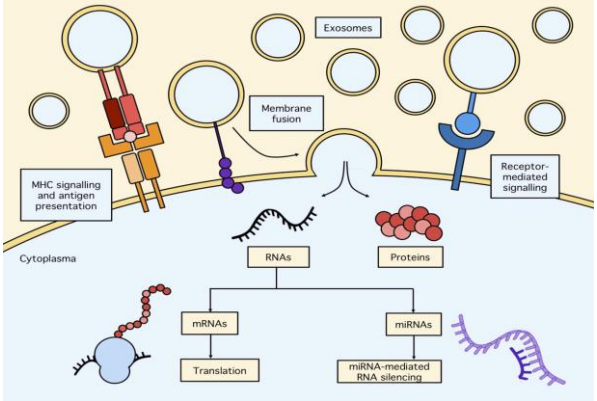


Fig 4: Receptor-ligand contact or the horizontal transfer of molecular information are ways an exosomes can communicate with target cells. Exosomes can also transport antigens to major histocompatibility complexes (MHC). The horizontal transfer of exosomal content begins with adhesion to the plasma membrane that leads to fusion and the internalization of the exosome membrane which release its contents into the target cells cytoplasm. (Adapted from Miller, I., Grunewald, T. Tumor-derived exosomes: Tiny envelopes for big stories 2015; 287-301)

VIII. EXOSOME UPTAKE IN RECIPIENT/TARGET CELLS

Exosomes take several different pathways to gain entrance into target cells all of which depends on transmembrane proteins and are non-random [4]. These pathways include phagocytosis, pinocytosis, endocytosis, and exosome fusion that occurs when an exosome fuses with a cellular membrane and releases its contents directly into the host cells cytoplasm (Fig 5). Since the membrane of exosomes have a high variety of proteins present, they tend to have a higher probability of gaining access through specific pathways into target cells. Once inside the target cell, a horizontal transfer of the contents from the exosome occurs[14]. A tetraspanin-integrin complex has been indicated to be a main contributor to the binding efficacy of exosomes to target cells and a pro-inflammatory environment has been shown to increase the expression of receptor molecules that further help exosomes to adhere to cells[15]. Research has not yet determined what homing and recognition mechanisms, that control the pathway of selective exchange,occur between an exosome and target cells.

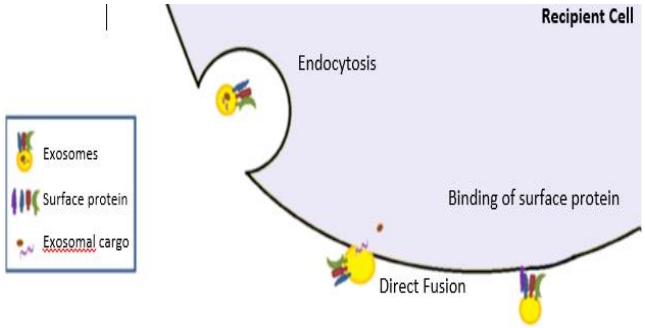


Fig 5: Exosomes can interact with the recipient cells via endocytosis, direct fusion, or binding of surface proteins. (Adapted from Tang, M., Wong, A. Exosomes: Emerging biomarkers and targets for ovarian cancer 2015; 367:26-33)

IX. EXOSOMES IN CLINICAL DIAGNOSIS AND CANCER THERAPY

Exosomes carry a wide variety of peptides, tumor specific antigens, and proteins that they are created with or they incorporate on their surface while also having the ability to target specific cells which makes them a strong candidate for cancer diagnosis and prognosis[16]. There is an increasein number of exosomes in cancer patients when compared to healthy individuals and there appears to be a correlation between malignant tumor growth and these increased numbers [9]. High levels of exosomes in colorectal patients showed a high association with an overall decreased survival rate from poorly differentiated tumors [17]. Studies done on the protein and transcriptional levels of these extracellular vesicles have shown noticeable differences in nucleic acid and protein content while reports suggested that they can also promote endothelial angiogenic responses that tumors depend on for growth [18]. MiRNA from exosomes and tumors showed a dysregulated expression of 43 out of 218 detectable miRNAs [19]. Using this expression dysregulation, miRNAs derived from exosomes could prove very useful in the classification of patients into risk categories providing a more modified and beneficial cancer therapy.

X. EXTRACELLULAR VESICLES: EMERGING TARGETS FOR CANCER THERAPY

Exosomes have come forward as a prime target of cancer treatment due to their role in intercellular communication and their distinctive properties in cancer tumor cells. Most of the studies on using these exosomes center on finding a way to use them as gene delivery systems to target tumor cells and effect tumor growth. Exosomes have also been seen to target immune system cells and cause them to promote growth of the tumor or stop the immune system from acting on the tumor [16]. Use of exosomes and their contained miRNA has been researched for an immunotherapy based exosome cancer treatment. A large variety of miRNAs can influence immune system cells to perform certain responses including the

elimination of tumor cells [20]. Another method of exosome treatment is the removal of afflicted exosomes from the blood through a type of dialysis called extracorporeal dialysis [4]. This method includes the use of an affinity plasmapheresis platform that can filter potentially harmful exosomes from the body by using an antibody-coated matrix. The impregnation of exosomes with antigens on melanoma, non-small-cell lung cancer, and ovarian cancer patients is another potential method looked at in France through phase 1 clinical trials showing results of prolonged disease stabilization. More natural approaches to exosome treatment methods, such as plant-based exosomes, are under study as well. Curcumin is a chemo preventative agent that, in conjunction with the chemotherapy drug triptolide, demonstrated the ability to induce apoptosis of ovarian cancer cells. However, it has low bioavailability and stability [16]. Research has shown that plant-based exosomes have the ability to increase the bioavailability and stability of these agents, and these exosomes are effectively taken up in immune and human intestinal cells [16]. This paved way to suggest that these foreign exosomes could be used as a treatment delivery vector in human diseases.

XI. CONCLUSION

Research on exosomes have been an exciting challenge and with the rapid expansion of published data, exosomes have establishes itself to be emerging players in intercellular communication. However, the roles of exosomes in cancer have been studied in detail. Published reports have shown that the level of circulating exosomes is increased in cancer patients and thus correlate with tumor progression. Thus exosomes are ideal biomarkers for cancer diagnosis and targeted therapy. With technology evolving, exosomes have been used as a transport vesicle to load small molecules or drugs for cancer therapy. Improvements in developing new strategies of exosomes will not only shed lights on their roles in the pathogenesis of cancer but will open new avenues for early cancer diagnosis and therapeutics

REFERENCES

[1] Ogorevc, E., Kralj-Iglic, V., Veranic, P. (2013) The role of extracellular vesicles in phenotypic cancer transformation. Radiolo Oncol. 197-203

[2] Thery, C. et al (2006) Isolation anf characterization of exosomes from cell culture supernatants and biological fluids. Curr. Protoc. Cell Biol. Chapter3 Unit 3 22

[3] Miller, V., Grunewald, T. (2015) Tumor-derived exosomes: Tiny envelopes for big stories. John Wiley & Sons Ltd. 287-301

[4] Vader, P., Breakefield, X., Wood, M. (2014) Extracellular Vesicles: Emerging Target for Cancer Therapy. Cell Press. Elsevier Ltd. 385-391

[5] Vander Pol, E. et al (2012) Classification, function, and clinical relevance of extracellular vesicles. Pharmacol. Rev. 64, 676-705

[6] Brinton, L., Kelly, K., Kester, M., Sloane, H. (2015) Formation and role of exosomes in cancer. Cellular and Molecular Life Sciences. Springer. 659-667

[7] Bryniarski, K., Nazimek, K., Park, W., Santocki, M. (2015) Exosomes as mediators of intercellular communication: clinical implications. Medycyna Prktyczna, Krakow. 370-378

[8] Szajnik, M. et al (2013) Exosomes in plasma of patients with ovarian carcinoma: potential biomarkers of tumor progression and response to therapy. Gynecol. Obstet. (Sunnyvale) 3 (Suppl. 4)

[9] Tang, M., Wong, A. (2015) Exosomes: Emerging biomarkers and targets for ovarian cancer. Elsevier Ireland Ltd. 26-33

[10] Milane, L. et al. (2015) Exosome mediated communication within the tumor microenvironment. Elsevier B.V. 219:278-294

[11] Hoshino, D. et al (2013) Exosome secretion is enhanced by invadopodia and drives invasive behavior. Cell Rep. 5, 1159-1168

[12] Peinado, H., Lavotshkin, S., Lynden, D. (2011) The secreted factors responsible for premetastatic niche formation: old sayings and new thoughts. Semin. Cancer Biol. 21, 139-146

[13] Le, M.T. et al (2014) Mir-200-containing extracellular vesicles promote breast cancer cell metastasis. J-Clin. Invest. 124, 5109-5128

[14] Valadi, H. et al (2007) Exosome-mediated transfer of mRNAs miRNAs is a novel mechanism of genetic exchange between cells. Nat. Cell Biol. 9, 654-659

[15] Kahlert, C., Kalluri, R. (2014) Exosomes in Tumor Microenvironment nfluemce Cancer Progression and Metastasis. J Mol Med. Berl. 1-6

[16] Tran, T.H. et al. (2015) Exosomes as nanocarriers for immunotherapy of cancer and inflammatory diseases. Elsevier Inc. 46-55

[17] Garcia, S. et al (2012) Analysis of exosome release and its prognostic value in human colorectal cancer. Genes, chromosomes and cancer. 51:409-418

[18] Al-Nedawi, K. et al (2009) Endothelial expression of autocrine VEGF upon the uptake of tumor-derived microvesicles containing oncogenic EGFR. Proc. Natl. Acad. Sci. U.S.A. 106, 3794-3799

[19] Taylor, D.D. (2008) Micro-RNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecologic oncology. 110:13-21

[20] Chaudhuri, A.A. et al (2011) MicroRNA 125-b potentiates macrophage activation. J. Immunol. 187, 5062-5068