

Exosomes as Drug Delivery Systems to Treat Cancer

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Abstract: Exosomes are intended for targeted drug delivery systems which are extracellular vesicles and are surrounded by phospholipid bilayers. These are similar to Nanoparticulate drug delivery systems but have various advantages, these are released by erythrocytes of multivesicular bodies. Exosomes are secreted by immune cells and are enclosed by a lipid bilayers and few cytosols which are devoid of any kind of cellular organelle. The method of loading exosomes is electroporation, incubation, and sonication which results in effective drug loading into the exosomes. Tumour derived exosomes can selectively enter the cancer cells and therefore act as a carrier for enhanced drug delivery to tumour cells.

Keywords: Exosome, Tumour, Carriers, Targeted drug delivery systems.

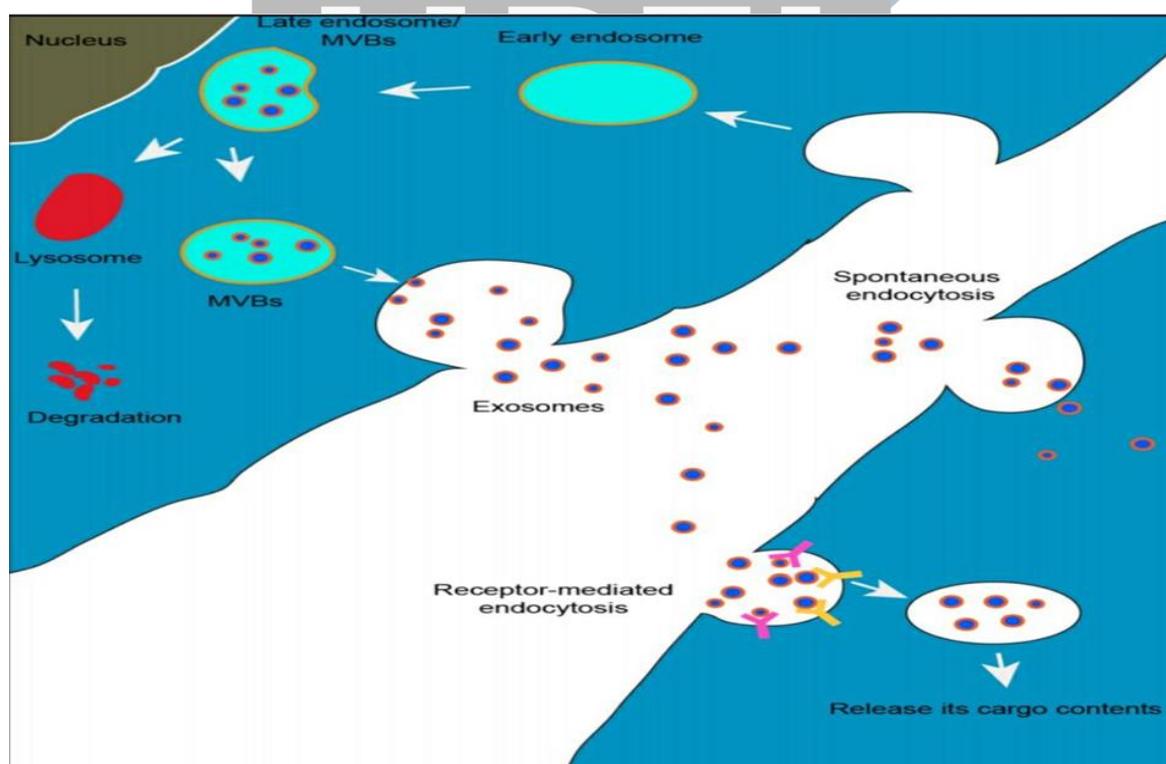
Introduction:

Extracellular vesicles which release from the multivesicular body may interact with the plasma-membrane called as exosomes (2). These are first discovered by Trams in 1981(1). It has a diameter of 30 - 150nm and roughly overlaps with viruses and mostly generated inside the multi vesicular bodies. When exosomes are released into the extracellular milieu and are taken up by the target cells.

Exosomes actually bind to the cell membrane with the help of receptor-ligand interaction and this will mediate the antigen presentation, cancer progression, etc. They also travel in the extracellular fluids and enter into the desired targeted site with high specificity and affinity, so these are said to be natural delivery systems (3). Exosomes will originate due to the inward budding of late endosomes into the MVBs, i.e. (multivesicular bodies) these are directed into the lysosomal compartment for the degradation and also recycling, Inward budding of endosomal membrane fills the lumen of early endosome called as intraluminal vesicles they fuse with the plasma membrane and releases into extracellular vesicles called as exosomes (4-5).

Biogenesis and release of exosomes:

Exosomes are enclosed by a lipid bilayer and a small fraction of cytosol which are devoid of any kind of cellular organelles. Moreover, exosomes have potential role in intercellular communication in cancer, While cellular communication can be successful through the soluble regulatory molecules such as cytokines and growth factors, exosomes function as a vehicular mediated exchange of intracellular contents, delivering proteins, lipids, nucleic-acid based molecules and metabolites to the adjacent and distant cells of tumour microenvironment(6).



Extracellular vesicles also play role in the cell communication, it includes exosomes, the process of formation involves firstly the cytoplasmic membrane form a hollow structure to form the intracellular vesicles, then these intracellular vesicles further get modified into multivesicular bodies and finally these get interact with the plasma membrane and release the exosomes (7-8). The recipient of exosomes with the recipient cell can be mediated by the ligand or the receptor interaction(8), various proteins are involved in the fusion of MVBs with the plasma membrane such as soluble N-ethylmaleimide (SNARE's) i.e. soluble N-ethylmaleimide-sensitive factor attachment protein receptors, Rabs and RasGtpases(9). Exosomes are released by cell organelles such as immune cells, these are also present in the physiological fluids such as a serum, urine, breast milk, cerebrospinal fluid, etc(10-11).

Exosomes are used to deliver interfering RNA (siRNA) or API substances; there are various methods of exosomes loading such as:

- Electroporation
- Incubation
- Sonication.

In the electroporation method, the electric field is applied to the exosomes suspension or to the cells and the therapeutic carriers of choice, now the pores are created into the lipid bilayer membrane results in the movement of carriers into the lumen of exosomes.

In the simple incubation method, the carrier is used as one of the loading methods of exosomes. An anti-tumour drug was efficiently loaded into the exosomes, shows the effect on the diseased site.

Another method of exosome loading is sonication, in this method drug exosomes mixture was sonicated results in effective drug loading into the exosomes (12).

Exosomes for drug delivery to breast cancer:

Breast cancer is major cancer in the females that develops in the breast tissue, symptoms such as lumps in the breast and patches around the skin, it can be diagnosed by biopsy, mammography, etc (13). Exosomes have various biological functions such as antigen presentation, immune regulation, evading apoptosis, drug resistance and escape from immune surveillance. These are also used as a biomarker for estimation in the metastatic spread, targeted drug delivery, anti tumour vaccination and also in gene therapy (15). Exosomes are released from the breast cancer cells and fibroblasts cells these are released into the extracellular milieu in the tumour microenvironment, exosomes have been found to play role in the cancer invasion and metastasis by the in vitro and involve of breast cancer model and epithelial cells, also in stem cell stimulation such as mesenchymal and adipose-derived stem cells, interaction with the cells of immune system and drug resistance (14).

Tian et al loaded exosomes with an anticancer drug such as doxorubicin which is widely used for the treatment of breast cancer and are used for the targeted delivery to triple-negative (MDA-MB-231) is a specific subtype of breast cancer, estrogen receptor-positive (MCF-2) human breast cancer cells. Ohno and colleagues modified the exosomes with the GE11 peptide (dodecapeptide that binds specifically to the epidermal growth factor receptor) (16) and were loaded with LET-7-a-miRNA is the lethal 7 gene (which are a group of small noncoding RNA, capable of regulating specific gene expression) (17) a regulator for the cell division reduction and alteration of the cell cycles, these were used to test the exosomes effectiveness. These drug loaded exosomes are delivered to the EGFR (epidermal growth factor receptor) expressing the xenograft breast cancer tissue in RAG2 (recombination activating gene) mice. So these studies have resulted a reduction of tumour growth (14-15).

Exosomes for drug delivery to lung cancer:

Lung cancer is the uncontrollable proliferation of cells in the tissues of the lung, the risk factors for lung cancer is due to long term smoking of tobacco, genetic factors and exposure to the radon gases (18). The symptoms are consistent cough, chest pain, shortness of breath and loss of appetite (19), it can be diagnosed by imaging tests such as x-ray, sputum cytology and tissue biopsy (20), where as exosomes are also useful in detection of lung cancer at early stages, exosomes which are originated from the cancer cells they carry genetic materials, proteins, and lipids which allow identifying the biomarker for cancer detection.

Kim et al loaded paclitaxel into the exosomes via sonication method, paclitaxel is shown to be promising strategy for the drug delivery to multi resistant pulmonary cancers, later the exosomes were modified with the aminoethylanisamide- polyethylene glycol it result in increased survival of the mouse models and reduction in toxicity (15). In the recent research studies Munagala, Aquil, Gupta, they used the passive loading techniques in the milk derived exosomes so as to deliver the paclitaxel, withaferin A and doxorubicin it reduces the IC50 values i.e. inhibitory concentrations values of the chemotherapeutic and also succeeded in vivo mouse model anti tumour activity for the lung cancer models. These exosomes model has emerged for the diagnosis and treatment of lung cancer (21).

Exosomes for drug delivery to pancreatic cancer:

Pancreatic cancer is responsible for the formation of cancerous cells in the pancreatic gland. The major function of the pancreas is the production of the bile juice and secretes insulin to maintain the blood glucose level (22-23). Major risk factors for pancreatic cancer are drinking alcohol, smoking, hereditary and diabetics. It can be diagnosed by CT scan, MRI, PET scan and Biopsy tests (23-24). Exosomes facilitate high drug delivery with a small dose of the drug and results in reduced tumour growth for pancreatic cancer. Recently Wang et al. Worked on Gemcitabine loaded exosomes. Gemcitabine is a chemotherapeutic drug, in this study;

they used various methods such as cell culture, various characterizations, cellular uptake, cell viability assay, in vivo bio distribution, animal studies, and analysis. And found that, ExoGemcitabine has improved cellular uptake and target drug delivery to cancer. Whereas injection of ExoGemcitabine resulted in reduction of the tumour growth and prolonged survival of mice in animal studies (25).

Overview of type of cancer, exosomal cargo and source:

Type of cancer	Exosomal cargo	Source of exosomes
Breast	Doxorubicin	MCF 7 cells and MDA-MB-231(14-15)
Lung	Paclitaxel	Macrophage cells (15)
	Paclitaxel Withaferin A Doxorubicin	Milk derived exosomes (21)
Pancreatic	Gemcitabine	Autologous Exosomes(25)

Tumour exosomes as potential biomarkers:

Biomarkers are the measurable indicators of biological conditions these are the tissues and bodily fluids which include DNA, RNA, proteins, and metabolites which indicate the state of health condition (26). During the carcinoma development, the cells secrete most of the exosomes which result in changes in the composition, many potential biomarkers are detected by the various biopsy detection such as liquid biopsy include physiological fluids and studies are found that biomarkers are present in exosomes, these could achieve a higher diagnostic and prognosis.

Exosomal micro RNAs are the potential targeted biomarkers for the detection of colorectal cancer, these micro RNAs are the small non-coding RNA with mRNA translation and induce mRNA degradation (27). For evaluating the cfDNAs (cell free DNAs) and CTC (Circulating tumour cells) in the liquid biopsy these can carry mutations to the primary cancers, while CTC is extremely and rarely observed in the blood so these are difficult to isolate the cells and detection is needed. Exosomes which are derived from serum samples with glioblastoma includes a specific EGFR VIII (Epidermal growth factor receptor) is a source of biomarkers reflecting the cancer cells, these studies imply the potential role in exosomes for the disease progression(32).

Tumour exosomes as therapeutic targets:

Tumour derived exosomes of carriers are attributed to cancer development, the therapeutic targets include exosome secretion, production and mediated cell-cell communication, these are said to be novel cancer interventions (32). The different composition and origin of the exosomes which are present in the physiological blood results in the enhancement of the biomarkers with the selective immune capture of exosomes to a particular environment.

In the recent research Rabinowits and colleagues resulted in the differences in exosomes and miRNA levels within the lung cancer patients and controls, the circular mRNAs are suggested and these are useful for the diagnosis of screening test for lung adenocarcinoma (30). For cell free vaccines in the tumour which is proposed by the Zitvogel et al showed DC-derived exosomes enhances the T-cell-dependent anti tumour immune responses in vivo (33).

Recently the cancer treatment for extracorporeal haemofiltration in exosomes with the help of circulation by the affinity plasmapheresis, which suggests the removal of exosomes in the circulatory system results in therapeutic agents to block oncogenes signal in cancers, so these studies suggest the efficient development of the anti cancer therapy (32).

Role of exosomes in drug delivery:

The drug delivery system should be capable of targeted delivery systems; they should avoid recognition, premature degradation by the immune defences. To enhance the exosomal activity Sato et al combined the exosomal membrane with the liposomes by the freeze thaw method, these results in optimization of the surface exosomes to decrease the immunogenicity and to improve half life of exosomes in blood, these are novel bio transporters of the hybrid exosomes (11).

In the targeting specificity alpha v integration specific RGD peptide is fused with the exosomes which are loaded with the anticancer drug, so as to improve the exosomal uptake by the alpha v integration positive cancer cells results in the inhibition of the cancer growth (32).

Current challenges:

Exosomes have increased research in recent years due to its unique activities, early diagnosis and treatment of the tumour targeted drug delivery. Isolating exosomes is the challenging approach due to its size and diameter which are similar to the extracellular vesicles (31).

In the proteomic cargos, exosomes carry genetic materials, miRNA, non coding RNAs mRNAs and mitochondrial RNAs, the loading of these cargos mechanism is not known though it is said to be RNA cargo assimilates with sphingomyelin and cholesterol enriched regions of a budding membrane in order to bud formation.

Cancer cells developed the exosomes mediated strategies so as to influence the numerous steps in metastasis. In triple negative breast cancer cells lives such as MFA-MD-231 which overly express the miR-10band these are derived exosomes transfers the miR-10b to non-malignant HMEC (Human mammary epithelial cell line) sequentially induces invasion ability.

In exosome gene carrier the naked therapeutic Gene's does not have the ability to cross the plasma membrane due to high negative charge and cellular uptake makes them limit cellular uptake. Exosomes are also involved in the cellular communication by the transport of bio macromolecules from host to recipient cell, it is unclear that which molecule is transported, or is also important to choose the appropriate donor cells to prevent the triggering of the immune response(15-32).

Conclusion:

Exosomes are useful for the early detection and treatment of cancer which involves cellular communication with the biomarkers for estimation of metastatic spread. In the cell free vaccines of tumour the DC derived exosomes enhances the T-cell dependent antitumour immune responses in vivo, there are many challenges in exosomes such as isolation and characterization of exosomes. Moreover, the lung cancer and breast cancer are the deadliest cancers and still research should give on for better treatment.

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