REVIEW

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Exosome Mediated Cancer Therapeutic Approach:Present Status and Future Prospectives

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Abstract

The unique extracellular vesicles (EVs) or exosomes formed by the sequential invagination of the plasma membrane are diverse and encompass important constituents with biological functions. Speculations on its cell independent biological functions are significant and pose them as vital biomarkers and as drug delivery vehicles especially in cancer. EVs possess theragnostic values and are known to elicit specific immune response. Exosomes can also serve as potential nanocarriers for delivering miRNA, siRNA, anti-cancer drugs and membrane-associated proteins. Exosomes play a crucial role in regulating tumour progression, metastasis, and angiogenesis. This review thus portrays the multiple facets of exosomes, in concert with the source for exosomes production and further on its regulation and intercellular communication. The review also explores the recent advances, present status and the future prospective in the application of exosomes in cancer therapeutics and cancer diagnostics.

Keywords: Exosomes- cancer-therapeutics- drug delivery vehicles- immuno-therapy

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Introduction

Exosomes are membrane-bound extracellular vesicles (EVs) released by all eukaryotic cells with a significant role in communication between the cells. EVs are considered as small organelle-free cytosol secreted from host cells with a lipoidal bilayer and act as an effective carrier for many biological and synthetic therapeutic molecules (Zhang et al., 2019). EVs carry a complex cargo of proteins, nucleic acids and lipids that were initially synthesized, and proposed ascellular waste that can actually damage the cells, or cellular homeostasis with no significant impact on neighbouring cells. They can also participate in signal transduction pathways, regulate the innate and adaptive immune responses and additionally in the intercellular exchange of RNA and proteins (Zhou et al., 2020). Unlike other biological delivery systems, it can cross the cytoplasmic membrane and blood-brain barrier, making them ideal targets as drug therapeutic and chemotherapeutic delivery molecules (Pardridge, 2012).

Exosomes are involved in re-modelling of the extracellular matrix (ECM) and mediates the intercellular transmission of signals and molecules (Xu et al., 2020). The major sources of exosomes include macrophages, B cells, dendritic cells, T cells, mesenchymal stem cells, epithelial, endothelial, cancer and immune cells (Oves et al., 2020). The exosomes hydration state exists intwo different morphological categories such as dehydrated sphered and hydrated-round structures (Liao et al., 2019). The ability of the exosomes to easily pass through the capillaries have transformed them as significant drug delivery molecules. Additionally they possess the properties of biocompatibility, high stability, low toxicity, low immunogenicity and permeability across the biological barriers (Li et al., 2021). EVs can successfully persist for longer periods, as they are anti-phagocytic evading from the phagocytosis process or degradation by macrophages. Moreover, they escape from the endosomal pathway or lysosomal degradation during delivery of the drugs in the cytoplasm (Ha et al., 2016).

A noteworthy fact about exosomes is that they are largely described as the promoters of tumor progression in various forms of cancer. Its presence in most of the body fluids serve as a diagnostic source in prostate, ovarian and breast cancers. In the context of theragnostic marker in cancers, EVs represent their complexity of their parent cells and have intrinsic ability to regulate complex biological functions (Nam et al., 2020). The DNA

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in the exosome provides information on cancer specific mutations as well and have high implications in medicine as nano-carriers (Batrakova and Kim, 2015). Its role in clinical application is also attributed to its biocompatible nature when it is present in the tissues and thus easily fits into the context of 'ease of engineering'(Liang et al., 2021). Proteomic profiling of these vesicles in signalling proteins substantiates its role as novel drug-delivery systems for targeted delivery to treat infectious diseases, cancers, as well as in diseases of cardiovascular system (Oves et al., 2020).

Exosome-based therapy is thus considered as a novel approach to combat the menace of cancer and can be applied in cancer immunotherapy to deliver anti-cancer drugs.A better understanding on the functional heterogeneity possessed by the exosomes must be enhanced in order to harness their theragnostic values. This review thus highlights on the biological functions of exosomes in concert with its immunobiology, regulation in the tumour microenvironment (TME), immunogenicity, immuno-toxicity, bio-distribution and mechanism of cellular uptake. Additionally, the review also portrays the application of exosomes as a potential drug carrier, siRNA, miRNA, proteins, cancer immuno-therapeutics along with its present status and future perspectives.

Exosomes as drug delivery vehicles in anti-cancer therapy

In recent decade, exosomes based cancer therapy is possible based on the following well-established mechanisms such as (1) suppressing cancer cells with naturally derived exosomes from immune cells; (2) suppressing cancer-derived exosomal release; (3) by use of exosomes as gene carriers; and as (4) anti-cancer agents. Due to their wide-spread bio-distribution and biocompatibility, exosomes act as an excellent anticancer drug delivery vehicle and are known to enhance the cancer treatment with reduced drug toxicity.To curb tumor progression,experimental evidences are documented towards administering genetically engineered exosomes by delivering suicide mRNA's and proteins. EVs have a high functional ability as a drug delivery system in targeting brain metastasis as evidenced from a research study on zebra fish (Yang et al., 2015).

Cell sources for exosome production:
Metastatic cancer cell-derived exosomes

Exosomes play a vital role in tumour progression by modifying the microenvironment of the primary tumor. They are capable of dissemination through blood leading to metastatic cancers.Exosomes exert tumor growth, and may aid in invasion due to cellular motility, angiogenesis encouraging cell formation, adhesion and cell polarity (Sung and Weaver, 2017). In view with this, studies document the purified and standardised exosomes from the metastatic cancer cells of the ovary leading to the peritoneal dissemination of the cancer cells (Yokoi et al., 2017). In the same line, exosomesof the melanoma cells results in pre-metastatic cancer of the lung (Peinado et al., 2012). In tumour progression, exosomes mediate intracellular communication aiding thrombin export through RhoA/Rock pathway among the recipient cell and control the host stromal's response to produce a pro-tumorigenic or anti-tumorigenic settings (Schillaci et al., 2017). From these reports, it is obvious that exosomes can be derived from the metastatic associated tumors.

Malignant mesothelioma (MM) cellsand bone derived exosomes

The malignant mesothelioma cells from humans have an immuno-regulatory function throughout the progression of cancer. Malignant mesothelioma (MM) involves serosal tissues and particularly pleura and is incurable usually.The immune producing capability of exosomes indicate that MM derived exosomes also can induce antigen-specific immune responses and acts as a marker for diagnosing mesothelioma and its progression (Greening et al., 2016). The present study could prove themigratory capacity of fibroblast/endothelial cells bytheoncogenic exosomes supporting the systematic model of MM progression andalso theregulatory capacity of exosomes in cell migration/tube formation assays.MM exosome components are able to regulate the tumour micro-environment as they are rich in immune-regulatory components, tumour-derived antigens, components of cancer signalling networks. In the same line, bone-derived exosomes have significant role in skeletal metabolism and exert varying effects on skeletal disorders, prostate cancer, multiple myeloma and breast cancer.Exosomes can be derived from both osteoclast precursors and mature osteoclasts. Mineralizing osteoblasts (MOBs) can also release exosomes (Behera and Tyagi, 2018; Lyu et al., 2020).

Pancreatic cancer cell (PCC) derived exosomes

Pancreatic cell–derived exosomal miR27a has therapeutic role in the angiogenesis of microvascular endothelial cells in pancreatic cancer (Shang et al., 2020). Studies also show that, exosomes from the serum of pancreatic cancer patients had resulted in cancer metastasis from pancreatic cancer patients (Tang et al., 2021). Similarly the circulating microvesicles, took part in efficient migration and further proliferation of the PANC-1 cells (An et al., 2018). In the pancreatic stellate cells, studies document the effects of pancreatic cancer cell-derived exosomes on cell functions, stimulating and activating the profibrogenic functions (Masamune et al., 2018). These suggest that pancreatic derived exosomes could be a good source for cancer therapy with immune modulations.

Mesenchymal stem cell (MSC) derived exosomes

MSCs are derived from adult mesodermal layers and in recent years, human MSCs are explored for their applications in clinical medicine. MSCs can be naturally obtained from bone marrow, umbilical cord blood, amniotic fluid, adipose tissue, dental pulp, placenta, brain, kidney, liver, lung, spleen, pancreas and thymus with the capacity to differentiate, self-renew and to form biological colonies (Lai et al., 2015). The exosomes derived from MSCs can serve as an alternative to whole cell therapy since they can reiterate the biological activity of MSCs with potentially reducing undesirable side effects such

as infusional toxicities and acting as paracrine mediators among the target cells (Mendt et al., 2018).

MSC-derived exosomescan exert their effects in different types and stages of the tumor through their inter-cellular communications in tumour development. MSC-derived exosomes show many unique advantages such as strong plasticity and availability for the recipient cells with their own limitations.Cancer-associated fibroblasts and tumor-associated mesenchymal stem cells promote development of tumour, however, inhibition of the growth of tumours is also reported from the exosomes derived from mesenchymal stem cells (Zhao et al., 2020).

Macrophage derived exosomes

During the course of different disease conditions, macrophage secretes exosomes and contributes to the progression of cancer, atherosclerosis, diabetes and heart failure. The macrophage-derived exosomes are used to deliver drugs, genes, and proteins in clinical applications and hence can play an important role in the diagnosis, prevention, and treatment of diseases. Exosomes were also derived from pro-inflammatory M1-macrophage cells and M2-macrophages (Wang et al ., 2019). Nicotine treated macrophages were studied for their role in atherosclerosis (Zhu et al., 2019). Adipose tissue macrophages derived exosomal miRNA- was reported for the modification of insulin sensitivity (Ying et al., 2017).

Regulation of exosome cancer cells and their microenvironment

Tumour micro-environment (TME) is an intertwined and inter-dependant phenomena of tumor occurrence, development, and metastasis. The imperative cellular components of the TME encompass vital biomolecules such as fibroblasts and immune cells.Additionally, exosomes are vital factors in TME, and in concert with hypoxia, inflammation and angiogenesis, they attribute for the growth of tumor with further invasion, and dissemination. (Semenza, 2016). TME interact with cancer cells through the matrix cells and non-cellular material via exosomes, during the progression of tumours. Exosomes are integral part of TME and recently it has been increasingly linked with the progression of cancer growth with nucleic acids, heat shock proteins, and several enzymes are few specific cargoes borne by exosomes (Figure 1).

Experimental evidences substantiate that, exosomes can facilitate cancer metastasis and affect other cells through various mechanisms such as (1) tumor-secreted exosomes causing epithelial-mesenchymal transformation and matrix degradation; (2) disruption of endothelial cells directly or indirectly by stimulating macrophages; (3) the released exosomes affectingthe immune cells by triggering the platelets (4) up-regulation of adhesive molecules on endothelial cells mediated by the attachment of the exosomes to the tumortissues; and (5) creation of micro-metastasis via the circulating exosomes. In the same line, exosomes can aid in the release of miRNA into the tumor microenvironment, and can induce the proliferation and cellular death of the tumor cells with further spread and cellular invasion. Also, the distribution of mRNA in patients with tumor exosomes makes it a precise target in

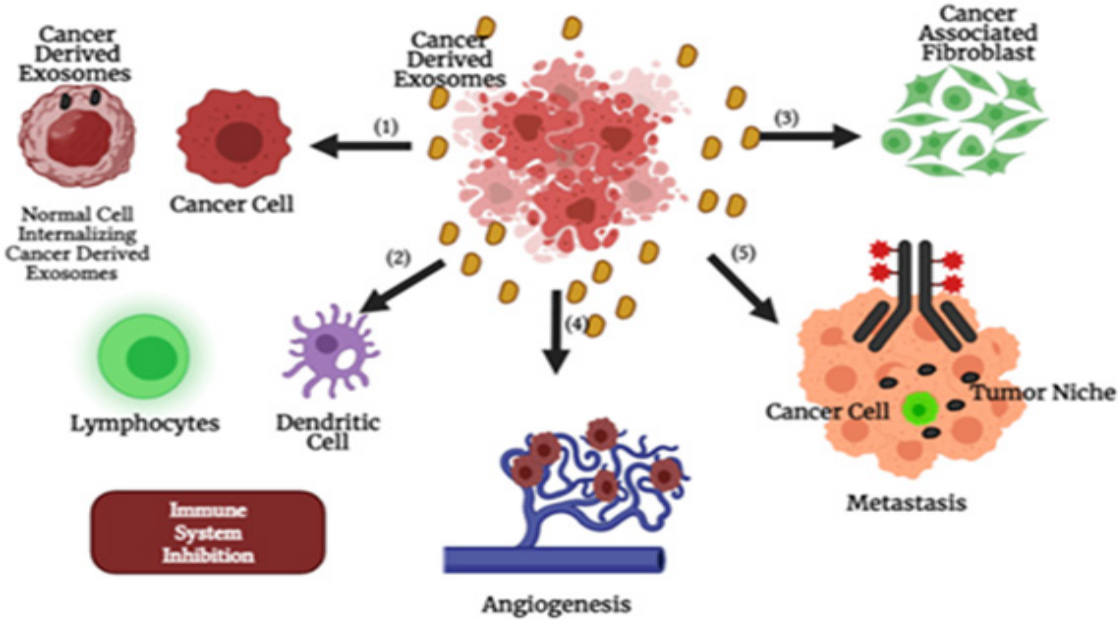

Figure 1. Role of Exosomes in the Development and Progression of Tumour Microenvironment (TME). 1. Exosome-mediated immune regulation modules the TME resulting in immune-evasion exhibiting immunosuppressive and tolerogenic characters. 2. The cellular elements in the TME, viz., immune cells (T lymphocytes, B lymphocytes, NK cells, natural killer T cells, and tumor-associated macrophages or TAMs), and endothelial cells (ECs), play a critical role in tumor-stromal interactions and can modulate the biological activities of cancer cells 3. Exosomesacts in the differentiation of fibroblasts (cancer-associated fibroblasts or CAFs) and mesenchymal cells into myofibroblasts. 4. Exosomes induce angiogenesis by changing the biological characteristics of endothelial cells and directly regulates the pro-angiogenic and pro-permeability factors. 5. Additionally, exosomes may lead to metastatic invasion, extracellular matrix degradation, and vascular leakage.

classifying tumor biomarkers, transforming it for cancer prediction and treatment (Ann et al., 2015).

Intercellular communication mediated by exosomes

Exosomes are known to interact effectively within the cell in four distinct ways: (1) exosomes stimulate target cells by functioning as signalling complexes; (2) receptor transmissions between cells (3) transporting functional proteins towards the receptor cells; and (4) delivering genetic information through mRNA and miRNAto the recipient cells (Camussi et al., 2010). Tumor cells produce more exosomes than normal cells, together with specific variations in its functional aspects in comparison to normal cell exosomes (Milane et al., 2015). The presence of miRNA in cancer cell exosomes is more than in normal cell exosomes and can be distinguished. Contact is mediated by the extracellular vesicles with the surrounding microenvironment, by a two-way communication with the stroma or tumour cells and thus may have a major impact on disease progression and treatment sensitivity (Han et al., 2017).

Few exosomescan deliver the epidermal growth factor receptor to regulate the signalling pathways of the endothelial cells. Suppression of the upstream regulation of miRNA (miR-26a/b), had resulted in the activation of hepatocyte growth factor (HGF) (Zhang et al., 2017). Furthermore, up-regulation of HGF facilitated the metastasis of the gastric cancer and liver metastasis, while down-regulation of the liver HGF had suppressed the metastasis. In this context, squamous cell carcinoma (SCC) based exosomes produce TGF type II receptor (TRII), which can induce TGF signalling in the tumor microenvironment. Similarly, tumor-associated macrophage (TAM's) based exosomes from the cancer cells of the ovary had inhibited the migration of the endothelial cells migration targeting the miR-146b-5p/ TRAF6/NF-B/MMP2 pathway (Jung et al., 2017).

Similarly, exosomes from glioma containing linc-POU3F3 affects the angiogenesis in the TME. Expression of vital biomolecules like VEGF, bFGF are up-regulated with high protein expression leading to pro-angiogenesis. Elevated levels of miR-210 are observed in the hypoxic environment of tumours and they do inhibit Ephrin-A3 and PTP1B, the known miR-210 target genes (Jung et al., 2017). In the same line, up-regulation of miR-23a is evidenced in the lung cancer cells in hypoxic conditions than in normal tissues of oxygen tension. Stimulation of vascular endothelial cells is observed in the lung cancer cells that are inducing prolyl hydroxylase 1/2 with a close junction protein ZO-1 together with hypoxic conditions (Wang et al., 2016). As evidenced by various experiments, the hepatocellular carcinoma derived exosomes shows resistance to sorafenib resistance, leading to cellular apoptosis. Increase in the levels of miR-21 is also observed in the M2 macrophage derived exosomes than inactivated macrophages (Huang et al., 2016). It is thus evident that these specific cellular interactions may highly influence the TME modulation through exosomes.

Immunogenicity and immunotoxicity of exosomes

Exosomes, as nano-vesicles with immunogenicity and molecular transfer roles, hold great promise in cancer immunotherapy. Exosomes have recently been recognized for their role in immune response regulation and advancements in immunotherapy. Exosomes from TME and immune cells possess specific biomolecules playing a direct role in anticancer immunotherapy. Exosomes deliver their vital molecules to targeted cells, influencing the phenotype and immune-regulatory functions of those cells. Exosomes play an important role in numerous cellular processes that contribute to the tumour immunology and further therapeutic effects, demonstrating their dual role intumour progression and tumour suppression (Syn et al., 2017). Exosomes do possess repercussions for their theragnostic values and thus become crucial to unravel its role in its application to curb cancer progression.

Exosomes had spurred renewed interest in recent decade for its immune-system boosting properties, and long-lasting effects. Exosomes possess promising anti-tumor activity in both lung and melanoma type of cancer, and kidney cancer, among other tumors. Exosomes can modify various types of stromal cells, with an invasive property towards cancer progression. Further, exosomes are effective in activation of the signalling ofautocrine VEGF in endothelial cells, promoting the tumour angiogenesis (Bebelman et al., 2018) .[41]Immunosuppression is expressed by the production of molecules like PD-L1 and TGF- β resulting. Cancer-derived exosomes can prevent CD8+ T cells from proliferating and activating while it promotes the expansion T regulatory cells (Sharma et al.,2020).

In relation to this, several studies have reported the expression of numerous MHC-1 molecules on the exosomal surface and tumour markers like heat shock proteins in mediating anti-tumor responses. Basically, a vast array of immune cells encompassing the phagocytes and NK cells are imperative in innate immunity playing a synergistic role in adaptive immunity.T and B lymphocytes and inflammatory cytokines are involved in the adaptive acquired immune response to create complex and diverse effects (Zech et al., 2012).In this view, it is intriguing to develop inhibitors of low-toxicity and high bio-safety vectors as delivery molecules in cancer immunotherapy.

Exosomes produced by tumors and their immune response have been studied extensively in various types and forms of cancers. Exosomes from a rat pancreatic adenocarcinoma acting in correlation with dendritic had triggered cytotoxic T cell (CTL) affecting the proliferation of the leukocytes (Zech et al., 2012). Exosome protein depletion in miRNA can serve as agonists and activate DC/cytokine-induced killer cells (DC/CIK), according to a pancreatic cancer report (Que et al., 2016). In vitro and in vivostudies have demonstrated that exosomes from Rab27a-overexpressing tumor cells promote DC maturation by up-regulating the MHC class II molecules, co-stimulatory CD80, and CD86 molecules, and substantially increase CD4+ T cell proliferation Li et al., 2013.

Exosomes derived from tumor cells with the glycolipid

based tumor-associated antigen MAGE-1 as well as the tumor rejection antigens G250 and GPI-IL-12 in renal cancer. The exosomes also promote the proliferation of the T cells, interferon (IFN)-γandcan efficiently activatecytotoxic T-lymphocyte throughFasL/Fas signalling pathway Zhang et al 2010. Cancer cells from the breast suppress both effector T cell proliferation through apoptosis and NK cell cytotoxicity controlling pathways, leading to the suppression of the anticancer immune response (Maybruck et al.,2017). In head and neck carcinoma, exosomes associated with the tumor tissues provoke a T-suppressor cells mediated via synergistic molecules and RNA(Maybruck et al.,2017). B16F0 melanoma derived exosomes modifies thetranscriptome of cytotoxic T-lymphocyte-associated proteins, making their mitochondrial respiration independent of substrates or hypoxia (Bland et al., 2018). Proliferation of T-cells and secretion of IFN-γ and IL-2 effectively stimulate the tumor-antigen-specific cytotoxic T cell response and NK cell cytotoxicity as well (Mirzaei et al., 2018). Exosomes secreted by brain tumour initiating cells to produce the extracellular matrixproteintenascin C, which is known to inhibit the proliferation of T-cells, by interacting with V-1 and V6 integrins and reducing mTOR signal transduction (Kibria et al., 2013; Du et al., 2018). Thus numerous studies substantiates the immunogenicity and immuno-toxicity effects of exosomes, urging further research for better understanding behind its immuno-therapeutic values.

Bio-distribution and stability of exosomes

Suitability of exosomes for its clinical application is often determined by their in-vivo bio-distribution and its ability to target specific tumour cells or tissues. The nanoparticles size and surface tension, in particular, decides not only their bio-distribution in the inter-tissues and stability but also their intra-tissue distributions as well Wiklande et al., 2015). Exosomes can be thus advantageous to be used in cancer therapy due to their size, lipid bilayer presence, surface property, and also its associated bio-molecules. Initially, the specificity of targeting the cells by the engineered exosomesare established in-vitro, with further assessment on their bio-distribution characteristics in-vivo. In this line, DiR-labeledexosomal bio-distribution and accumulation in vital organs and its further accumulation occurring within 24hr of time period (Wiklande et al., 2015). In athymic nude mice, Lai et al., 2014 found that the highest bioluminescent signals of Gaussia luciferase (Gluc)-labeledexosomes in the liver and spleen that can be further cleared after 6h. Smyth et al. recently found that exosomes, liposomes produced synthetically and from extracts may aid in the sufficient profiling to assess the bio-distribution profiles and clearance rates (Smyth et al., 2015). miRNA-loaded EVs administered intravenously had showed increased liver sickness compared to other vital organs. These studies show that systemically administered exosomes scan be efficiently cleared by the immune system, especially in the liver and spleen, exhibiting a relative short life in the systemic circulation (Kamerkar et al., 2017).

On the other hand, unmodified exosomes have bio-stability and clearance patterns similar to synthetic nanoparticles like non-PEGylated liposomes. Exosomes demonstrated improved circulation retention, presumably mediated by CD47 based defence and could promote tumortargeting in mice. The superiority of exosomes in the stability and targeting tumour cells over synthetic nanoparticles need to be optimized, by modifications by bioengineering. PEGylated nanoparticles with a size of >100 nm home to tumour tissues through a novel mechanism known to enhance the permeation and the retention (EPR) effect (Nakamura et al., 2015). Due to the micro size (range of 100nm) exosomes are expected to exhibit EPR mechanism whenever administered intravenously. Exosomes have additional intrinsic mechanisms to evade from the immune system, in addition to the surface specific markers or PEGylation. PEGylation of exosomes are known to improve their presence in the circulation for longer periods with more stability. In mice, non-PEGylated exosomes shows variations in the stability time, ranging from 10-60 mins. To improve targeted immuno-therapy, more research is needed to produce exosomes with longer half-lives and improved vascular permeability. Finally, origin of exosomes from various cells with their modifications of the surface molecules, and administration route also influences the bio-stability in cancer immunotherapy

Mechanism of Exosomes in cellular uptake

Exosomes are complex extracellular membrane vesicles which can interact and invade into the cells via different pathways, such as phagocytosis, receptor mediated endocytosis, micropinocytosis or direct fusion with plasma membrane (Mulcahy et al., 2014). Phagocytosis is a first line order of immune defence mechanism by which specialised cells engulf foreign particle by the way of receptor-ligand interaction. The receptors allow binding to specific ligands facilitating the phagocytes playing an important role in clearing apoptotic cells (Arandjelovic and Ravichandran, 2015). Exosomes can specifically bind to specific ligands on the phagocytes transforming them as ideal recipient cells. It is also evident that the fusion of exosomes and phagocytes is essential to regulate the response against immunity by antigen presentations on the surface of the phagocytes (Gordon, 2016). Similarly, endocytosis is a basic cellular process by which the cell absorbs all the substance externally, finally engulfing it within the cellular membrane by forming an intracellular vesicle. The endocytosis pathway involved with the PC12 cell-derived exosomes uptake, with clathrin-mediated endocytosis and micro-pinocytosis are also documented (Tian et al., 2014). In the same line, macro-pinocytosisis achieved by the formation of unique projections on the surface of the cells before fusion into the cell membrane making the uptake of exosomes in an effective manner (Conner and Schmid, 2003).

Exosomes can bind with recipient cells, inducing specific intracellular signals and can modify the molecular processes leading to variations in their pathophysiological states either via binding with surface receptors or internalization and release of their cargo content.Once attached onto the surface receptor (recipient cell), the

cell signal gets activated through specific interactionson the proteins on their surface and on the receptors of the cells. By intracellular signalling, the exosomes thenefficientlyelicit signal transduction (juxtacrine signalling) leading to fusion with the cellular membrane and further release of their bio-contents and transfer into the cytoplasm of the recipient via different mechanisms (McKelvey et al.,2015).

Present prospects of exosomes:
Exosomesas cancer diagnostic biomarkers

The bioactive moleculesin exosomes reflects the conditions of their originated cells, thus serves as biomarkers. Studies document that exosomes are present in in common body fluids and also in ascites (Yuana et al.,2013; Madison et al.,2014; Aqrawi et al., 2017; Halvaei et al., 2018). Exosomes are highly associated with miRNA profile and it is intriguing to note that most of the circulating microRNA's are concentrated in exosomes. Exosomal miRNAs are thus suggested as diagnostic and prognostic indicators for carcinoma, as exosomal miRNA's are directly connected with different grades and stages of cancer and metastasis. Long non-coding RNA's (LncRNAs) from exosomes are known for its clinical applications (Takahashi et al ., 2014). LncRNA was also reported as a completely unique biomarker(Figure 2) (Li et al., 2019).

Exosomes mediated cancer therapy

Cancer causes exorbitance in the mortality rate globally, and chemotherapy and/or radiotherapy are the routine therapeutic strategies available with adverse reactions and complications. Cancer immunotherapy is thus a sort of a treatment that controls the tumors by modulating the immune system for an effective anti-cancer immune response leading totumor evasion. The therapeutic approach mainly includes non-specific stimulation of the therapeutic blockades including adaptive and vaccination strategies (Xu et al., 2020). Exosomes are often absorbed by cells and can efficiently transfer the drugs, miRNAs and proteins. In comparison to liposomal, metal and polymer based nano-materials, exosomes can efficiently overcome the limitations of less stability, toxicity and immunogenicity. Moreover exosomes possess specific anchoring proteins on their transmembrane layersfor enhancing the endocytosis process and to promote the efficient delivery of the bio-molecules (Figure 2).

Exosomes as efficient delivery vehicles of miRNA and siRNA

Small interfering RNAs (siRNAs) and microRNAs (miRNAs) plays a vital role in the regulating the functional genes and are actually considered as noncoding RNAs. These are employed in gene-silencing to suppress the tumour growth together with its dissemination (Lam et al., 2015). siRNA can effectively destroy a corresponding sequence of mRNA and each specific loci are known to encode for a specific protein to induce a disease and have been extensively explored for cancer therapy. The only limitation of its clinical application is that they are easily degraded by nuclease but reports suggest unique mechanisms to overcome the same. Experimental evidences prevail for the delivery of siRNA with exosomes against RAD51, a vital therapeutic target of cancer cells effectively reduce RAD51 protein level with cellular apoptosis (Shtam et al., 2013). In the same context, exosomes from brain endothelial bEND.3 cells loaded with VEGF siRNA could effectively inhibit aggregation of xeno-transplanted cancer cells in zebra fish (Yang et al., 2017). Scientific evidences are also documented that exosomes can efficiently bind to EGFR-specific peptide (GE11) in order to deliver the tumour inhibitor, let-7a miRNA, in breast cancer cells (Ohno et al.,2013).[75] Effective suppression of tumour cell growth in hepatocellular carcinoma is also observed through the application of miRNA loaded exosomes (Fonsato et al., 2012). Exosomes containing miR-335-5p have also been shown to prevent cancer growth and metastasis especially in hepatocellular carcinoma (Wang et al., 2018). Exosome-mediated siRNA delivery has been studied using functional assays and imaging, and the findings were promising (El-Andaloussi et al., 2012). Exosomal miR-204-5p could induce apoptosis by enhancing the sensitivity of cancer cells and efficiently inhibiting the cancer cell proliferation and reversed chemo-resistance (Table 1) (Yao et al., 2020).

Table 1. Source of Exosomes Derived from Different Organs and Their Therapeutic Targets

S. No	Exosome	Therapeutic Targets	Reference
1.	PCC-derived exosomes	Pancreatic stellate cells	Masamune et al., 2018
2.	LINCO1133	Pancreatic ductal adenocarcinoma	Liu et al., 2021
3.	Pancreatic cell–derived exosomal miR27a	Pancreatic cancer	Shang et al., 2020
4.	Circulating microvesicles	PANC-1 cells	An et al., 2018
5.	Mesenchymal stem cell derived exosomes	Whole cell therapy	Bagno et al., 2018
6.	Tumor-associated mesenchymal stem cells	Inhibit the tumour growth	Zhao et al., 2020
7.	Macrophages derived exosomalmiRNA	Insulin sensitivity	Ying et al., 2017
8.	Bone-derived exosomes	Skeletal disorders, prostate cancer, multiple myeloma and breast cancer	Lyu et al., 2020
9.	Malignant mesothelioma exosomes	Diagnosing mesothelioma	Greening et al., 2016
10.	Metastatic cancer cell-derived exosomes	Primary tumor	Yokoi et al., 2017

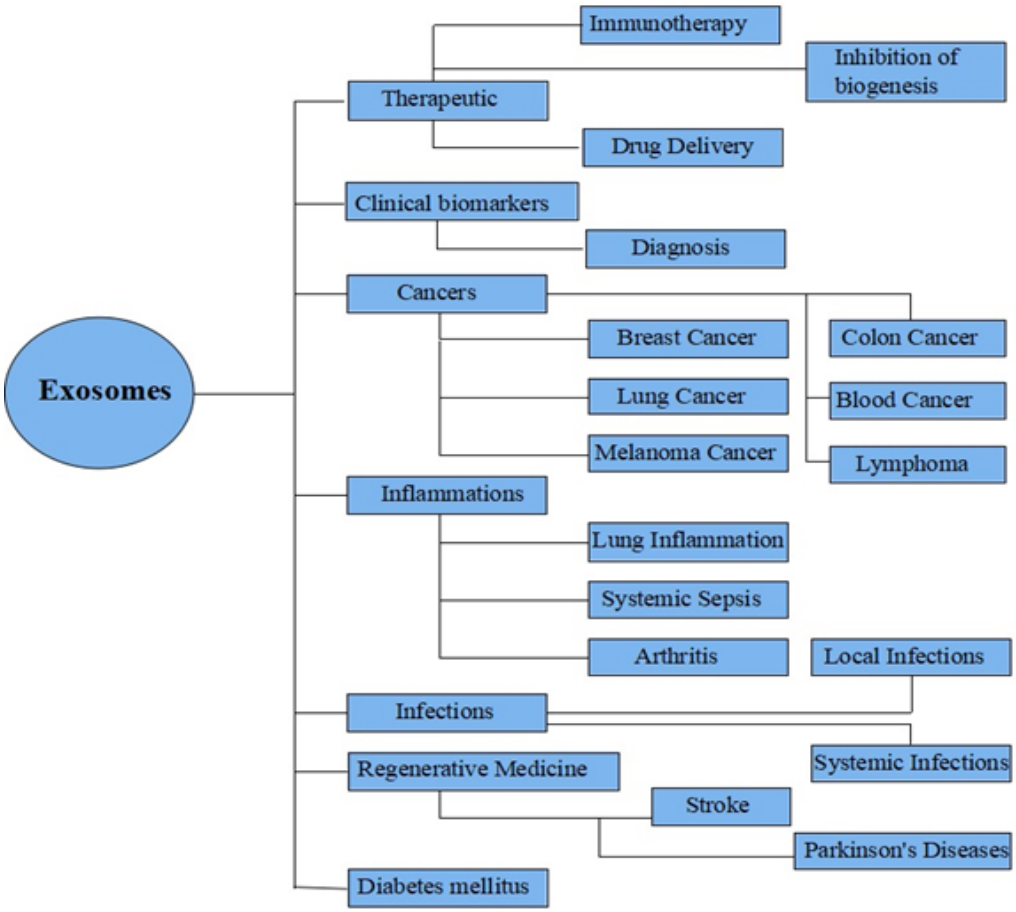

Figure 2. Promising Applications of Exosomes as Natural Cargoes to be Implemented as Drug Delivery Vehicles to Treat Different Types of Cancers, Systemic Diseases and as Theragnostic Marker.

Role of exosomesin the delivery of chemotherapeutics

Chemotherapeutics, the widely employed treatment strategy for cancer are specifically designed to disrupt cell function. Chemotherapy is applied for several type of cancers such as leukemia (Sakai et al., 2020), Burkitt's lymphoma (Patton et al., 1990), Hodgkin's lymphoma (Perre and Markman, 2011), small cell lung cancer (Nishino et al., 2011), Wilm's tumour (Xu et al., 2013) and testicular cancer (Kaufman, 2013). The chemotherapeutic drugs affects both normal cells as well as cancer cells and renders adverse effects on the normal cells. Thus as an alternate strategy, anti-cancer drug molecules can be incorporated into exosomes and can be used to deliverthe drugs to host cells and into the specific tissues to render targeted drug delivery. Such approach ofspecific targeted drug delivery can increase the local concentration of therapeutics and minimize the side effects (Liang et al., 2021). In-situ, the NPs are directly encapsulated inside the exosomes and are introduced into the exosomes by passive diffusion towards their membrane. Further, the Pd-nanosheets were successfully developed inside the exosomes by the conversion of P2+ to P0 using CO as a reducing agent. The end product of active Pd nanoparticles are loaded into exosomes and it is known to mediate Pd-triggered de-alkylation reactions inside the cells (Sancho-Albero et al., 2019).

An in-vivo study has revealed that miR159 and Dox delivery in a vesicular system could effectively silence the *TCF-7* gene without any adverse effect, together with miR159 and Dox delivery system exhibiting improved anticancer effects (Gong et al., 2019). Another interesting study states that exosomes derived from plants could effectively deliver phyto-compoundsas evidenced by the fruit-derived exosomes in delivering curcumin for the treatment of colon cancer (Luan et al., 2017). Electrophoration-mediated loading of doxorubicin into exosomes, internalized the RGD-modified recombinant methionines and significantly decreased the proliferation of cancer cells.

PTX incorporated into exosomes has greatly increased cytotoxicity >50 times in drug resistant MDCKMDR1 (Pgp+) cells. The exoPTX-AA co-localization of airway-delivered exosomes and intravenously delivered vectorizedexosomes with cancer cells, showed a potent anticancer effect of exoPTX in the mouse model (Kim, 2016). In-vivo study in mice model showed that chemotherapeutic drug loaded with nano-vesicles rendered no adverse effects but efficient tumour suppression. The bio-engineered nano-vesicles thus serve as novel exosome-mimeticsas drug delivery molecules to treat malignant tumors (Jang et al., 2013).

Exosome mediated delivery of membrane associated protein

Membrane proteins (MPs) are necessary to mediate communication between the cells and transduce

chemical signals in cells through interacting protein and downstream cellular processes (Almen et al., 2009). The membrane proteins, encoded by >30% of open reading frames, seem to be promising in delivering the drugs and vaccines (Miles and Wallace, 2016). Exosomes provides membrane scaffold proteins (MSP's) to display their action and further distribution. Exosomes specific proteins are displayed on exosome membranes and include tetraspanin proteins that are involved in cellular adhesion and membrane trafficking such as CD37, CD63, CD81 and CD82 (Théry et al., 2009). Tumor derived exosomes consist of membrane associated TGF-β and have potential to inhibit T cell effector and cytotoxic functions mediated through the FasL and TRAIL markers (Söderberg et al., 2007). Dendritic cell derived exosomes possess MHC-class I molecules that can specifically bind to the protein from the tumours and can activate the immune cells to render anti-tumour immunity.Similarly, they can also activate the T-cells and mediate CD4+ T-cells by an endocrine mechanism and this complex DEXs can improve cardiac function in post myocardial infarction (Liu et al., 2016).

Xie et al., (2010) have experimentally proved that, exosomes incorporated heat shock protein 70 (HSP70), may express and stimulate immune response against tumor, mediated by both PIA-specific CD8+ cytotoxic T lymphocyte (CTL) and non-PIA specific natural killer (NK) response. The data suggested that membrane-bound HSP70 can activate and induce dendritic cells (DC) maturation and simultaneously stimulate CD4+ Th1, CD8+ CTL and NK cell responses leading to increased and efficient antitumor immunity. Exosomes with signal regulatory protein alpha (SIRPα) modified exosomes could potentiate the antitumor responses by enhancing immune response against cancer (Koh et al., 2017).

Exosome-based immunotherapy

Exosomes serve as specific markers for predicting the immune activation and also serve as vehicles to stimulate an anti-cancer immune response.The unique features of exosomes make it as ideal candidate for cancer immunotherapy as they can be loaded with sensitive proteins and antigens for targeted delivery. Moreover, it depicts the features of the parent cell from which they are derived. Dendritic cell-derived exosomes possess high immunogenicity and have the ability to alter contents and function due to differentiate on maturation (Shenoda and Ajit, 2016). Exosomes derived from dendritic cells were used for preparation of vaccines which was superior to dendritic cell vaccines in terms of management and cost effectiveness. Moreover evidences suggest that strong anti-tumor immune responses can be obtained when exosomes derived from cancer cells are leveraged for therapeutic purpose (Santos and Almeida, 2021).

Immunotherapy is a widely used for treatment of cancers that trigger and activate the body immune system to fight against cancer cells. Cancer immunotherapy consists of antibodies that bind and inhibit the specific functional proteins expressed by cancer cells. The exosomes are mostly hydrophilic in nature which makes them suitable to carry water soluble drug molecules for the treatment of cancer (Yousefpour and Chilkoti, 2014). Recently tumor derived exosomes (TEX) are widely employed in cancer immunotherapy. TEX consists of tumor antigens and heat shock proteins (HSP70 and HSP90) which can efficiently elicit an immune response against cancer cells. It also contain inhibitory immuno suppressive molecules such as Fas ligand (Fas-L), prostaglandin E2 (PGE2), programmed death ligand (PD-L), tumor related apoptosis-inducing ligand (TRAIL), NKG2D ligands and transforming growth factor beta (TGF-β) leading to immune-suppression in the tumor environment (TME) (Taghikhani et al., 2020). Tex induce an immunosuppressive environment regulates the T-cells by inducing down regulation of MHC on a variety of immune cell types (Figure 2).

Exosomes derived from B lymphocytes and dendritic cells, loaded with peptide molecules, can induce systemic cellular immune response. Dendritic cell derived exosomes (Dex) are another source employed in cancer immunotherapy, where the Dex, the nanometer-sized membrane vesicles exhibit strong immune stimulatory properties (Pitt et al., 2016). Dai et al., (2008) have documented the use of ascites-derived exosomes (Aex plus) and granulocyte-macrophage colony-stimulating factor (GM-CSF) to treat colorectal cancer (HLA-A0201(+) CEA(+), patients. They observed that Aex plus GM-CSF could induce the cellular immune responseresponse with low adverse effects. DEXs derived from tumor peptidase could be applied in priming the tumor specific T-cells (CTL) response leading to destruction of the tumor cells in murine models (Zitvogel et al., 1998).

MSCs derived exosomes also trigger the secretion of various pro-inflammatory and inflammatory cytokines. Similarly, MSC derived exosomes fused with HOxB4, affects the maturation of the dendritic cells in promoting the T-cell proliferation via WNT signalling (Staal et al., 2008). Dutta, 2021 has suggested that DEXs, TEXs and MSC derived exosomes can be genetically engineered to fuse specific anticancer drugs, DNA, RNA and peptides. The fused complex particles can again be considered as a novel bio-carrier to specifically deliver the genes and drugs in the target site under cancer therapy.

Future Perspectives

Exosomal biology had thus opened new avenue of research especially in cancer immunotherapy. The advent of scientific technologies and experimental manipulations in utilizing the exosomes as drug delivery molecules must be well explored at the earliest, in order to harness the theragnostic application of exosomes to treat cancers. To reveal the functional heterogeneity of the exosomes in cancer biology, the isolation, purification and the standardisation of the analytical procedures must be made avid for the researchers and oncologists. Albeit, the role of exosomes have been vastly documented, much more genetic models to explore its actual role in the tumour micro-environment is yet necessary. Additionally, the associated host determined factors such as age and immune-senescence during the administration of exosomes need to be unveiled. Speculations on the physiological and biochemical alterations while

exosomes are in the delivery system must be revealed and substantially need to be improved.

In conclusion, this review pinpointed exosomes' potential and clinical applications as an alternative strategy to treat cancers. Exosomes can efficiently elicit an immune response in the TME, in concert with tumour immune-suppression in various forms of tumour tissues. Exosomes being the natural cargoes of potential biological agents, are known to be derived from various natural sources, and thus their applications must be substantially monitored in both cancer progression and tumour suppression. Promotion on its application and its prominent utility as a therapeutic agent must be regularized in complex intracellular pathways. Exosomes may be utilized by enhancing its bioavailability with minimal adverse effects to treat complicated grades and stages of cancers. Its use as a promising biomarker is also ascertained and may be thus developed for its therapeutic and diagnostic versatility to treat cancers.

Author Contribution Statement

AR and SG had equally contributed for the conceptualization, design, drafting, review and editing of the manuscript. PSG contributed for the drafting and figures of the manuscript. MS and BS contributed for review and editing.

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Ethical approval

Not applicable being a review article.

Availability of data

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Conflict of Interest

The authors declare that there is no conflict of interest.

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