IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
Review Article
October 2020 Vol.:16, Issue:4

The Biological and Clinical Role of Exosomes in Lung Adenocarcinoma

IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

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Submission: 23 September 2020
Accepted: 30 September 2020
Published: 30 October 2020

HUMAN JOURNALS
www.ijsrm.humanjournals.com

Keywords: Nonsmall cell lung cancer", "exosomes in lung cancer", "nanotherapy in lung cancer", "tumor derived exosomes", "targeted therapy for lung cancer", "exosomes in cancer"

ABSTRACT

Nonsmall cell lung cancer accounts for 85% cases of lung cancer and has been the most common cause of cancer related deaths worldwide due to diagnosis in advanced stages, rapid metastasis and recurrence. The current targeted therapy for NSCLC has made great progress and made tremendous improvement in survival of patients but can easily lead to drug resistance. New methods are needed to not only develop different and better ways for targeted therapy but also to diagnose, response to therapy and monitor cancer with minimally invasive techniques. Exosomes are nanosized extracellular vesicles containing proteins, lipids and genetic material which are secreted by different cells and can be detected in different body fluids like blood, pleural fluid, urine, bronchiolar fluid etc. They control cancer growth via multiple mechanisms. Exosomes could be used as nanoparticle to deliver therapy to NSCLC and they could be target for therapy as well. In this review, we will describe how exosomes are involved in NSCLC maintenance, growth, invasion and metastasis and how could we use them to target NSCLC for therapy.

1. INTRODUCTION

Lung cancer is the leading cause of death due to cancer related deaths in United States of America (USA) and worldwide(1, 2). Most common type of non-small cell lung cancer (NSCLC) is lung adenocarcinoma(3). It is estimated that total lung cancer related mortality worldwide would increase up to 10 million per year(4). Smoking habit is tightly adhered with the cause and lung cancer related deaths. With the increase in smoking habits, the incidence and death due to lung cancer increases before falling following the start of comprehensive tobacco control programs(5-7). Lung cancer incidence and mortality rate in USA and United Kingdom (UK) is decreasing since 1990s. Emerging nations like India, South Africa, China, Russia have higher rates of smoking and continue to rise. Their incidence rate of cancer is low, but high mortality burden as compared to developed countries like UK, USA etc. The cause could be unequal access to healthcare systems which ultimately results in late diagnosis (advanced stages) and treatment, sociocultural barriers and environmental contaminants exposure(8).

Genetically, lung cancer is very complicated and heterogeneous disease which is due to mutations in the oncogenes as a result of which the normal cells progresses to neoplastic and malignant cells under the influence of genetic and epigenetic changes(9). Lung cancer is broadly divided into 2 groups: Small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC is the predominant type (approximately 85% of cases among lung cancers)which originates from the epithelial cells of lungs. NSCLC is further classified in to adenocarcinoma, squamous cell carcinoma and large cell carcinoma. SCLC (approximately 15% of cases among lung cancers) has very particular distinction of neuroendocrine differentiation and small cells with scant cytoplasm(10).

Exosomes are nano sized vesicles found outside the cells. They contain different proteins, enzyme, nucleic acids and lipids(11). They have a role in the cancer spread and maintenance by controlling the microenvironment surrounding the tumor. Exosomes play this role by enhancing angiogenesis, tumor growth by evading from the immune system attacks and growth into the surrounding tissue and metastasis(12). Exosomes have also a role in guiding the targeted therapy to tumor cells and exosomes can be the target of tumor treatment. In this review we will discuss the biological role of exosome in cancer maintenance and growth and how can we use the exosomes to our advantage for the treatment of lung cancer.

2. MATERIALS AND METHODS

How exosomes cause spread of lung cancer and how can we use it to our advantage? With this question in mind we searched the data and collected the material. Pubmed and google scholar were used to collect the materials. We included only those relevant studies which is describing the role of exosomes in lung cancer spread and growth and use of these exosomes in the treatment of lung cancer.

2.1.Inclusion Criteria

1) We used studies referring the role of exosomes in lung cancer spread and treatment.
2) All studies published after year 2004 (including year 2004) were included in our paper.
3) All studies have open excess.
4) All papers were in English language.

2.2.Exclusion Criteria

1) All studies which published before year 2004 were not included in our study.
2) Those papers which were not in open excess, we didn't use those articles for our paper.
3) Papers published in any language other than English were excluded from our study.

Exosomes

Exosomes are small particles of endosomes which are produced by different cells like reticulocytes(13), dendritic cells (14), lymphocytes (15, 16) and neoplastic cells (17). The exosomes cannot be visualized by conventional optical microscope. The transmission electron microscope (TEM) tool is the best way to visualize the exosomes. The exosomes are isolated and mounted on metallic formvar grid. They are then observed with negative staining with uranyl acetate. Exosomes ranges from 30 nm to 150 nm in diameter. The morphology is described as double membraned, cup shaped structure (18, 19). Furthermore, exosomes have been effectively removed from different body fluids such as seminal plasma(20), blood(21), urine(22, 23), pleural effusion(24), ascites(25), amniotic fluid(22) and bronchoalveolar fluids(26, 27). Exosome has a very vital role of transferring information and material in the form of DNA, RNA and protein

from one cell to another cell via fusion with plasma membrane of receiving cells, endocytosis by phagocytosis or receptor ligand interaction with cells (28)and has very diverse nature of their functions as they are secreted by different kinds of cells with different performance (29). Thus, due to diverse nature of cells secreting them, exosome exhibit both physiological and pathological performance.

Exosomes has a role in immunological performance, supporting tumor microenvironment for cancer growth and maintenance. Exosomes affect the immunological regulations by activation of immune system, cell to cell communication, immunosuppression and regulation of antigen expression (30). Exosomes which are derived from the tumor cells can interfere with immune regulation and ultimately developing suppression to immune therapies (31). One study shows that there are around 2000 trillion exosomes found in the blood of a disease-free person whereas there are around 4000 trillion exosomes in the blood of a cancer patients(32), which demonstrate that tumor cells produce a lot of exosomes than their normal counterpart cells. Thus, they can be used as tumor markers as well(33). Hypoxia in the tumor cells results in the secretion of exosomes which promote angiogenesis and create a favorable microenvironment for cancer to receive more blood supply and chances of metastasis increases (34). Exosomes derived from tumor cells are very important for them as they are involved in the maintenance, growth, metastasis and drug resistance of tumor itself through which we can obtain information regarding the diagnosis, prognosis, prediction of treatment response, response to therapy (17, 35, 36).

2.3.Role of exosomes in angiogenesis

Angiogenesis is the formation of new capillaries through destabilizing of old blood vessels, migration and proliferation of endothelial cells, canalization and ultimately leading to stabilization of blood vessel wall which then leads to formation of new stable blood vessel(37). Angiogenesis play important role in tumor growth by providing nutrients to the tumor cells. Vascular endothelial growth factor (VEGF) is the main component involved in tumor angiogenesis by signaling through the VEGF receptor-2. VEGF binds to its receptor on the endothelial cells (EC), which then causes dimerization of the receptor and autophosphorylation of tyrosine residues, leading to activation of intracellular signaling cascade which lead to angiogenesis through increased cell survival, permeability, migration, and proliferation (38). Once the EC are activated by VEGF, they produce matrix metalloproteinase (MMPs). These then

breakdown the surrounding extracellular matrix (ECM) components such as proteins and polysaccharides, to aid the migration of EC(39). This allows the formation of capillary sprouts, which eventually form the mature network of blood vessels. In addition to VEGF, fibroblast growth factor (FGF) also plays a critical role in angiogenesis by secreting MMPs, plasminogen activator and collagenase, all of which help in ECM degradation (40). Expression of both VEGF and FGF from tumor cells is promoted by immune mediators such as IL-6 through STAT3 (41). Epithelial growth factor (EGF) plays its role in tumorigenesis by acting through three main signaling pathways viz. Ras/MAPK, PI3K/Akt, and JAK/STAT pathway, leading to uncontrolled angiogenesis(42). Exosomes play its role in angiogenesis by transferring protein, lipids and genetic material to the cells of tumor microenvironment. The materials transferred by exosomes for angiogenesis are; sphingomyelin(43), matrix metalloproteinases(44), vascular endothelial growth factor (VEGF), von Willebrand factor (vWF), fibroblast growth factor (FGF), epidermal growth factor receptor (EGF-R), interleukin-6 (IL-6), interleukin-8 (IL-8), and angiogenin (37, 45).

Nazarenko et al. observed in a rat adenocarcinoma model that when endothelial cells internalized the exosomes containing tetraspanin Tspan8-CD49d complex, the VEGF-independent regulation of several angiogenesis genes (von Willebrand factor, Tspan8, chemokine CXCL5, MIF, chemokine receptor CCR1 and VEGF receptor 2) will be induced. The EC also start proliferation, migration, sprouting and maturation of their progenitor cells (46). Another study by Cui et al. observed that tissue inhibitor of metalloproteinases-1 (TIMP-1) could lead to increase in miR-210 of lung adenocarcinoma. miR-210 then excreted in the form of exosomes from tumor cells which then cause an increase in activity of angiogenesis (47). Zhuang's team showed that microvesicles containing miR-9 reduced the suppressor of cytokine signaling 5 (SOCS5) which then activates JAK-STAT pathway and hence angiogenesis (48). Thus, exosomes secreted by cancerous cells contain materials which has role in stimulating angiogenesis and promote tumor growth.

2.4.Role of exosomes in tumor growth and progression

Exosomes secreted by lung adenocarcinoma affects the normal functioning of not only the surrounding cells but also the distant cells as well and changes the microenvironment in favor of cancerous cells growth(49). Lung tumor cells A549 derived exosomes could induce new kind of

pro-inflammatory mesenchymal cells which can secrete IL-6, IL-8 and MCP-1 which has role in tumor growth (50). Acting through STAT3, IL-6 plays several roles. It causes transcription of target genes including the cell cycle regulator cyclin D1 and proto-oncogene c-myc. IL-6 also induces the expression of anti-apoptotic proteins such as bcl2 and bcl-XL (41). Both IL-6 and IL-8 can attract circulating tumor cells (CTC), cells that have shed into the circulation from a primary tumor. These circulating cells have the ability to re-infiltrate the primary tumor in a process called 'Tumor self-seeding', and thus grow in their tumor of origin, further accelerating tumor growth (51). The cytokine IL-10 increases tumor cell proliferation and survival by upregulating anti-apoptotic genes(52).

There exosomes also play a very important role be escaping the tumor cells from being attacked by immune system. They do this by transferring proteins and genetic material into the receptor cells and enhance tumor growth by reprogramming the immune cells functions specially in its microenvironment (31, 53). Tumor cells can escape the anti-tumor attack from CD positive T cells by release of exosomes which induce, promote expansion and up regulate Treg cells (54). Treg cell immune suppressive viz.

1. Inhibition of costimulatory signals by CD80 and CD86 expressed by dendritic cells through cytotoxic T-lymphocyte antigen-4.

2. Expression of IL-2 receptor α-chain (IL-2Rα; CD25), consume IL-2 through high-affinity IL-2 receptors but are not able to produce IL-2. This limit the amount of IL-2 available for T-cell proliferation and activation.

3. Secretion of inhibitory cytokines, such as TGF-β, IL-10, and IL-35 all of which inhibit activation of effector T-cell.

4. Direct killing of effector T cells, by producing cytotoxic substances such as perforins and granzymes (55).

Chalmin et al. observed that exosomes secreted from tumor cells containing heat shock protein 72 (hsp72) suppress the myeloid progenitor cells in the bone marrow and hence inhibiting the activation of mature myeloid cells (56). The exosomes not only change the immune system response but also affect other cells and cancerous cells which enhance its invasion and growth. Exosomes derived from lung cancer cells (NCI-H1688 and NCI-H2228) has a role in increasing

IL-10 and tumor growth factor β (TGF-β) which then promote tumor growth and metastasis (57). Wu et al. observed not only exosome microRNA-96 (miR-96) is positively correlated with lung cancer but also as the lung cancer grade is increased so did the level of miR-96 (58). In conclusion, these studies suggest that exosomes derived from tumor cells could enhance tumor growth and progression via different mechanisms.

2.5.Role of exosomes in metastasis

Tumor metastasis is one of the major reasons of mortality in lung cancer which involves multiple mechanisms. Metastasis allows the tumor cells to migrate to different organ and start a new growth over there at distant site (59). Tumor derived exosomes can enhance the microenvironment in support of cancer maintenance, growth, invasion and metastasis by interacting with surrounding stromal cells(60, 61). Hypoxia in the tumor tissue cause excessive release of exosomes which then promote and enhance the microenvironment for its survival (62). Loss of epithelial like cells characteristics and gain of mesenchymal like cells features is known as epithelial mesenchymal transition (EMT), exosomes has important in completing this process (63) which is then involved in cancer spread (64, 65). The exosomes from the tumor cells transfer its miRNA to the non-tumor cells and changing the cellular structural of recipient cells for promoting metastasis. Rana and his team observed that exosomes (miR-494 and miR-542-3p) derived from the lung tumor cells downregulate the cadherin-17 expression and upregulate the matrix metalloproteinases level and modulate the surrounding tissue (lymph nodes and lung tissue) them to support the metastasis of cancerous cells (66). In some cases, the exosomes from the normal cells release exosomes containing miRNAs which promote tumor growth and metastasis. One study observed that exosomes containing miR-193a-3p, miR-210-3p and miR-5100 is secreted from bone marrow derived mesenchymal stem cells under hypoxic conditions which then promote lung cancer metastasis via STAT3 signaling (67). These studies give an impression that the exosomes from cancerous cells have important role in the tumor spread by changing the cancerous cells physiology, releasing enzymes cause cells to metastasize easily and weakening the cells to cells adhesion molecules causing break loose of cancer cells which then spread and seed other organs.

2.6.Exosomes as biomarkers

The proteins present in exosomes could be used as a biomarker to diagnose NSCLC (68). CD151 (Area under curve = 0.68, p = 0.0002), CD171 (Area under curve = 0.60, p = 0.0002) and tetraspanin 8 (Area under curve = 0.60, p = 0.0002) were present in the plasma of patients with NSCLC (69). Epidermal growth factor receptor (EGFR) expressed on exosomes was observed to be present in significantly higher amount in lung cancers patients than controls (70). Exosomal long noncoding RNA metastasis-associated lung adenocarcinoma transcript 1 (RNA MALAT1) found in the could be used as a diagnostic marker for lung adenocarcinoma (71). Exosomes with lipopolysaccharide-binding proteins (LBP) can also be used to distinguish between metastatic and non-metastatic lung adenocarcinoma (Area under the curve was 0.803 with a sensitivity of 83.1% and a specificity of 67%, $P < .0001$) (72). Vykoukal's team observed 108 expression of proteins in the vesicle preparation from plasma of lung adenocarcinoma cases, of which 43 were identified in the extrasomal vesicles of lung adenocarcinoma cell lines (73).

Exosomal microRNA can also be used to as a biomarker to diagnose lung cancer. The miRNA present in exosomes extracted from plasma is very similar to the exosomal miRNA extracted from the tumor tissue. Hence, we could use circulating exosomal miRNA for lung cancer diagnosis (74). One study reported that exosomal miRNA (miR-139-5p, miR-378a, miR-200b-5p and miR-379) could be used to differentiate between nodule (lung adenocarcinoma and carcinomas) and non-nodule (healthy former smoker). Exosomal microRNA that could be used to distinguish between adenocarcinoma and granulomas were miR-151a-5p, miR-30a-3p, miR-200b-5p, miR-629, miR-100, and miR-154-3p (75). Zhuo et al. studied the microsomal RNA in the plasma of patients suffering from lung adenocarcinoma. They observed many miRNA that were found only in plasma of lung adenocarcinoma patients. They also studied the exosomal miRNA and observed miR-19b-3p, miR-21-5p and miR-221-3p were significantly elevated in NSCLC patients (76). miR-96 level was observed to be in increased in the tissue of lung cancer as compared to the normal lung tissue. The higher the grade of tumor, the more the increase in level of miR-96. Based on this finding the team decided to check the exosomal miR-96 in the serum and observed the same results that exosomal miR-96 is raised in those suffering from lung cancer. Positive correlation was observed between cancer grade and serum exosomal miR-96 level (77). Exosomal miR-126, miR-181-5p, miR-30a-3p, miR-30e-3p, and miR-361-5p were

associated with lung adenocarcinoma (78, 79). Exosomal miR-205-5p, miR-483-5p, miR-375, miR-200c-3p, miR-429, miR-200b-3p, miR-200a-3p, miR-203a-3p, and miR-141-3p were collected from pleural fluid of patients suffering from lung adenocarcinoma (80). The exosomes reflect the cell physiology and pathology. These exosomes which are associated with lung adenocarcinoma, there raise level in plasma could be beneficial as they could be used to see the tumor presence and response to therapy because the cells will start to die due to therapy, thus reducing the exosomal content in blood.

2.7.Exosomes in Therapy

Surgery, chemotherapy and radiotherapy has been the main standard of therapy for lung adenocarcinoma or NSCLC. After surgery, chemotherapy is important regime to eliminate the remnant cancerous cells and improve the 5year survival rate for NSCLC. However, the chemotherapeutic resistance is increasing which results in cancer recurrence and metastasis(81). With the introduction and tremendous improvement in nanotechnology, we have developed different techniques and methods of delivering chemotherapeutic drugs to cancerous cells, especially in lung cancer. These nanocarriers were meant to deliver the drugs to targeted cells and minimizing unnecessary exposure to normal cells. Many techniques have developed for the delivery of nanoparticles but many of them didn't have many successful results due to poor bioavailability, nontargeted cytotoxicity and immunogenicity (82, 83). Due to stability, biocompatibility, permeability, low toxicity and low immunogenicity, exosomes are being used as a delivery mode for cancer therapy (84). Berries and there bioactives such as anthocyanins and there aglycones anthocyanidins (Anthos) which have anti-oxidant, anti-proliferative, apoptotic and anti-inflammatory properties could be used in cancer treatment (85-91). Because of their poor bioavailability and retention, these compounds do not have very successful use in cancer treatment. However, Munagala et al. when encapsulated these compounds in exosomes and delivered them to the mice xenografted with human lung cancer (A549 and H1299), breast cancer (MDA-MB-231 and MCF7), pancreatic (PANC1 and Mia PaCa2), prostate (PC3 and DU145), colon (HCT116) and ovarian (OVCA432) cell lines, they observed significant changes in anti-proliferation, cell survival and anti-inflammation (92). Kim et al. studied the effect of exosome encapsulated paclitaxal on lung cancer and observed that drug cytotoxicity is significantly increased in cancerous cells as compared when paclitaxal was delivered without

formulation with exosomes(93). One study observed that when lung cancer cells (H460) when restored with liver kinase B1 (LKB1), these cells exhibited higher ability in cell migration by increasing the cells motility and decreasing the release of exosomes which have migration-suppressing miRNAs (miR-125a, miR-126 and let7b) (94) and we could target these exosomes in lung cancer treatment (32). In another study when lung cancer cells (A549) when exposed to cisplatin they observed that exosomes release were increased and exomes containing miR-21 and miR-133b were detected in those exosomes. The increased level of exosomes cause the surrounding cells to increase resistance against cisplatin. Thus, exposure of cisplatin to these tumor cells result in the development of drug resistance against cisplatin of not only targeted cells of cisplatin but also of the surrounding cells due to excess release of exosomes containing miR-21 and miR-133b(95). A study observed the miRNA and exosomes expression from lung adenocarcinoma cells (A549) which are sensitive to cisplatin (A549) and resistance to cisplatin (A549/DDP). Decrease expression of miRNA-100-5p was involved in the resistance against cisplatin and the exomes alter the cisplatin sensitivity of surrounding cancerous cells. Qin et al. observed that A549/DDP cells have significantly low expression of miRNA-100-5p and exosomes containing miRNA-100-5p as compared A549(96). Exosomes containing miR-21 and miR29a can bind as a ligand to toll-like receptor (TLR) family, murine TLR7 and human TLR8 on immune system cells and inducing a TLR mediated inflammatory response that is supportive of tumor growth and metastasis (97). Thus, we could use exosomes as a carrier for different therapeutic agent to target those cancerous cells. This technique could be used for unresectable cancerous cells. We could also target those exosomes from releasing from cancerous cells for treating cancers.

3. CONCLUSION

Exosomes are nanovesicles secreted by different cells containing lipids, protein, DNA, RNA, miRNA. They play important role in transferring material from one cell to the other surrounding cells, thus participating in intercellular communications. By transferring material from one cell to another, it has an important role in regulating the microenvironment of the cancerous cells specially the NSCLC. They participate in angiogenesis, tumor growth, invasion and metastasis. We can also use these exosomes for the diagnosis and treatment of NSCLC. Since exosomes

closely mimic the condition of cells from which they are being released and relatively stable in the circulation, they are important markers for cancer diagnosis and treatment.

However, the use of exosomes for the diagnosis and treatment of lung cancer is still in its initial stages. The precise role of exosomes in regulating the microenvironment of tumor and its molecular biogenesis is still not cleared. In future, further work on exosomes regarding its implication in biogenesis, interaction with surrounding cells and target cells need to be done. Limitation in the role of exosomes for lung cancer that is observed so far are:

1. Sensitivity and specificity of exosomes for the diagnosis and treatment of NSCLC.
2. The precise mechanism and role of exosomes in supporting NSCLC spread and growth.
3. Preservation, identification and quantification of exosomes has technical limitations, high cost and not been standardized yet.
4. Limitations in the use of exosomes as a carrier for NSCLC therapy.

All these factors restrict the use of exosomes for the diagnosis and treatment of NSCLC. More work needed to be done to develop techniques, novel devices and strategies which must be cost effective and efficient in isolations of exosomes which then could benefit a lot to patients suffering from NSCLC.

4. DISCLOSURE OF STATEMENT

There is no conflict of interest in term of financial support or relationship.

5. CONTRIBUTION OF AUTHORS

All authors have contributed equally as a group to each of the key contributions i.e. conception and design of the study; acquisition and analysis of data; drafting the manuscript, or others.

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