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Review Article

Exosomes as Nanocarriers for Theragnostic miRNA Markers in Nonsmall Cell Lung Cancer Therapy

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Nonsmall cell lung carcinoma (NSCLC) is the leading cause of deaths related to carcinomas of lung by the involvement of several risk factors. Tumor cells, in general, exude larger quantities of biological macromolecules in comparison to their noncancerous opposites. Vesicular bodies or cavities created by the folding back of endosome membranes mingle with the plasma membrane and result in the release of exosomes into the extracellular space after which they enter proximal or distant cells of target. Exosomes are nanovesicles that can carry microRNAs (miRNAs) and other such macromolecules as cargos into the tumor environment by means of cell-to-cell communication. These materials transported by exosomes can act as indicators for oncogenesis and metastasis and result in resistance among therapy-sensitive cancer cells. The cargos inside the vesicles loaded with miRNAs vary according to their particular state and therefore can act as potential prognostic or diagnostic markers for a variety of diseases including lung cancer, especially NSCLC. Although the roles of exosomal miRNAs are unclear or contradictory, the possibility of using exosomes as efficient nanovesicles for the treatment of NSCLC using biological molecules such as miRNA remains critical. Hence, this review focuses on the roles of exosomal and cell-free miRNA in NSCLC therapy at preclinical and clinical levels.

1. Introduction

In the United States, 228,820 new cases and 135,720 deaths related to cancers of lung and bronchus were estimated to happen in 2020. Men are more prone compared to women according to the American Cancer Society Statistics of 2020 [1]. Among Afro-American population of the USA, 25,390 new cases and 16,550 deaths were estimated to occur in the year 2019 [2]. According to the 2021 estimate, 235,760 new cases and 131,880 deaths were projected for cancers of the lung and bronchus [3]. In 2022, the number of new cases was projected at 236,740 along with 130,180 deaths [4]. Lung cancer was the top-most cancer according to GLOBOCAN estimate of 2018 with 2.1 million new cases and was the leading cause of cancer-related deaths in 93 countries with 1.8 million deaths [5]. In the 2020 global estimate, the number of new cases did rise to 2.2 million although the estimated deaths did not vary significantly in comparison to the 2018 estimate [6].

NSCLC is a type of epithelial carcinoma that originates in the bronchial tubes. The symptoms such as persistent cough, dyspnea, and loss of appetite and weight are usually diagnosed at a very advanced stage [7, 8]. Adding to this, NSCLC is related to the majority of lung cancers (more than 80%) and is one of the deadly cancer types across the world which is generally identified by either a histological or cytological approach [9, 10]. Among the three types of NSCLC, squamous-cell carcinoma accounts for 25–30% cases, adenocarcinoma relates to highest percentage (40%) of cases, whereas, large-cell carcinoma comprises about 5-10% of all lung cancer cases [11]. Relating to the cancer status in China of 2018, 4.3 million (24%) new cases and 2.9 million (30%) deaths happened, with lung cancer being the foremost cause with 774,323 cases (18.1% of total) [12]. Since NSCLC

accounts for a higher incidence rate related to lung cancers, this review will focus on this type of cancer, excluding the information on small cell lung cancer (SCLC).

There are several factors and associated risks in oncogenesis which can be intrinsic or nonintrinsic. Exogenous nonintrinsic risk factors are modifiable and include carcinogens, viruses, and life-style linked factors. Endogenous nonintrinsic risk factors include aging, genetic vulnerability, inflammation, hormones, and several other reasons which could be modified to some extent based on the individual. Intrinsic risks arise due to errors in reproduction of human genome and are irreversible [13]. Age of onset of tobacco smoking, consumption of alcohol, dietary fat, and fermented milk products are risk factors for lung cancer based on life-style that occur in both sexes [14–22]. Heritable genetic susceptibility in the family, geographical location, air pollution, and infections are risk factors independent of life-style of an individual. Epstein-Barr virus, human papillomavirus, hepatitis B and C viruses, human T-cell lymphotropic virus-1, human herpesvirus-8, and Merkel cell polyomavirus are oncogenic viruses. *Opisthorchis viverrini, Clonorchis sinensis*, and *Schistosoma haematobium* are oncogenic flukes. *Helicobacter pylori, Chlamydia*, and *Mycoplasma* are prominent carcinogenic bacteria [23–36]. With this background, it is important to note that NSCLC is prominent in humans who carry gene variations. This set of population with gene variations is three times more vulnerable to the NSCLC when they do smoke in comparison to nonsmoking population [37].

Exosomes and their components can be used as a biomarkers, vaccines, and drug carriers in exosome theranostics [38–40]. They are recently identified as drug carriers of nanoscale with typical characteristics such as enhanced permeability and retention effect and passive targeting [41, 42]. There are several advantages of using exosomes as nanocarriers as they can surpass presystemic metabolism, cross blood-brain barrier effectively, and avoid undesired accumulation in nontargeted sites such as liver [43]. Correlating to this advantage, the interaction with the blood-brain barrier results in the transfer of exosomes across the barrier by mechanisms such as endocytosis, micropinocytosis, and phagocytosis [44].

Exosomal miRNAs are on an average 22 nucleotide in length and are significant, small, endogenous, noncoding RNA constituents of the exosomes. They can regulate the levels of several target mRNAs after the occurrence of transcription [45–47]. These RNA molecules are released from many cell types which can regulate the changes in neighbouring cells or cells that are faraway. Since 1993, the year of discovery of miRNAs, a minimum of 1% of human genome has been identified to have the ability to produce miRNAs and each miRNA is designated to function in the regulation of 200 mRNAs [48]. They may possess altered profiles compared to their parent exosomes and can play significant roles in cancer cells [49].

Research on exosomes and their role as nanocarriers is still at its early years. Hence, this review was aimed at analyzing the biogenesis, loading, and delivery of miRNA by exosomes and the roles of miRNA as biomarkers and suppressors of NSCLC.

2. Conventional Therapeutic Approaches for NSCLC

Surgery, radiation therapy, chemotherapy, targeted therapy for selective mutations and using inhibitors in addition to Immunotherapy are conventional means for therapy of NSCLC [50–52]. Lobectomy, wedge resection, segmentectomy, and pneumonectomy are surgical options for NSCLC [53–56]. Carboplatin or cisplatin, paclitaxel, docetaxel, gemcitabine, and vinorelbine are the chemotherapeutic drugs used [57–59]. Inhibition of epidermal growth factor receptor (EGFR) using erlotinib, gefitinib, entrectinib, afatinib, dacomitinib, and icotinib and anaplastic lymphoma kinase (ALK) using alectinib, brigatinib, ceritinib, crizotinib, and lorlatinib can stop or slow the growth of NSCLC [51, 60, 61]. Atezolizumab, avelumab, durvalumab, cemiplimab, nivolumab, and pembrolizumab are monoclonal antibodies used for immunotherapy of NSCLC [62–64]. Although these therapies are significant for the management of NSCLC, the side effects remain a concern for cancer care [11, 65]. The systemic toxicity remains critical, and therefore, alternative means of NSCLC treatment is the need of the hour.

3. Understanding the Biogenesis and Structure of Exosomes as Nanovesicles for miRNA Transfection

According to the International Society of Extracellular Vesicles, extracellular vesicles could be classified into exomers (< 50 nm), exosomes (30 to 150 nm), microvesicles (100 to 1000 nm), large oncosomes (1 μm to 10 μm), migrasomes (500 nm to 3 μm), and apoptotic bodies (100 nm to 5 μm) [66, 67]. Among these vesicles and exosomes, which are extracellular nanovesicles of size less than 150 nm, can help defend against diseases such as cancer by creating a difference in cellular homeostasis. This can help in trafficking of cargos including components at the genomic and proteomic levels between cells. Till date, more than 9000 proteins, 3000 mRNAs, 2500 miRNAs, and 1000 lipids have been identified to be a part of exosomes. Endocytosis results in production of early endosomes which is rich in intracellular vesicles. These early endosomes mature into late endosomes or intracellular multivesicular bodies which are later degraded by lysosomes and released into the extracellular space as exosomes. Rab27a and Rab27b are responsible for the secretion of exosomes that perform cell-to-cell communication predominantly in tumor microenvironment. These exosomes can be isolated by techniques that include ultracentrifugation, ultrafiltration, immunoaffinity and size-exclusion chromatography, immunoassays, precipitation (using polyethylene glycol for example), microfluidics, and magnetic-activated cell sorting. These techniques have unique principles and in their own way yield exosomes with low to high purity. Among these techniques, size-exclusion chromatography is the most suitable for the isolation of exosomes [49, 68–73].

After isolation, exosomes are transfected with mature miRNAs by means of methods using lentivirus, transfection

kits, or electroporation [74–78]. The transfected exosomes are usually distinguished by their dimensions, structure, density during floating, and the presence of proteins identified as markers including Alix, TSG101, flotillin 1, HSP70, and CD9. They are identified by adopting techniques such as transmission electron microscopy and nanoparticle tracking analysis [79–81]. After transfection, exosomes intended for drug delivery are taken up by recipient cells through endocytosis, macropinocytosis, and phagocytosis leading to delivery of the intended cargo such as proteins, mRNAs, and miRNAs [70, 82].

A clear understanding of the structure and composition of exosomes is necessary to utilize them better in nanomedicine. The lumen of exosomes is rich in RNA, rRNA, lncRNA, mRNA, miRNA, DNA, proteins, and lipids. Cell adhesion molecules such as integrins and tetraspanins are found on exosomal surface. Cytosolic and membrane-bound proteins such as tubulin, actin, ANNEXINS, RAB, GTPases, Rab GTPases, SNARE, integrins, tetraspanins (CD9, CD63, CD37, CD81, CD82, and CD53), endosomal sorting complex required for transport (ESCRT) proteins (Alix, TSG101), flotillin, dynamin, and syntaxin are identified to be involved in biogenesis, transport, and uptake of exosomes. Heat-shock proteins such as HSP70, Hsp90, and cytochrome C are used in improving the therapeutic ability of exosomes by involving in exosome release and signaling [83]. Online databases such as Vesiclepedia and Exocarta are used to determine the contents of these nanovesicles. Taken altogether, the research on exosomes suggests that these nanobodies are either flat or round lipoprotein bilipid layered with sizes of 30 to 150 nm and membrane potential ranging between -14 and -24 mV [84].

Activities of endonucleases such as Drosha and Dicer can lead to the formation of miRNAs via the canonical pathway. The noncanonical pathway is Drosha- and Dicer-independent [85]. In the canonical pathway, pri-mRNA synthesized by the transcription of specific genes are processed into pre-miRNA with the help of a complex that contains a RNA binding protein named DiGeorge syndrome critical region 8 (DGCR8) and Drosha, eventually leading to the formation of one end of the mature miRNA. The pre-miRNA is released into cytoplasm by Exportin-5 and is further processed by Dicer into mature miRNA [Figure 1]. The noncanonical pathway is either Drosha/DGCR8-independent or Dicer-independent [86, 87].

4. Exosome Mediated Delivery of miRNA

Signature miRNA patterns are useful in designing miRNA therapeutics directed towards several signaling pathways for intervening majority of pathological conditions that may either be an autoimmune condition, communicable, or noncommunicable [88, 89]. Studies show that exosomes are preferred choices for deriving miRNAs to be used in biomarker-associated research. Significant percentage of published research recommends the preferred use of exosomal miRNAs in comparison to nonexosomal miRNAs with regard to quality and stability [90, 91]. This statement seems appropriate since exosomes encompass a variety of proteins and genome constituents that can help in the detection of cancer. The volume of exosomes in blood and other body fluids is higher and therefore can aid in early detection of cancer. In addition, they can improve the stability of miRNAs with enhanced protection against degradation [92].

Exosomes are efficient drug delivery agents for miRNA and can modulate signal transduction between cells thereby leading to inhibition of tumor development [93]. Inhibition of tumors by extracellular vesicles like exosomes may involve several mechanisms such as apoptosis involving upregulation of enzymes such as Caspase 9 and downregulation of macromolecules such as Myc, TCF7, ki-67, and CD31 [94]. After being synthesized or identified, they can target tumor environment specifically in case they are derived from tumor cells. Specific antigens on vesicular surface can be primed for targeted cancer immunotherapy [95].

Cellular stress involving the endoplasmic reticulum can indeed increase the secretion and release of these nanovesicles into the extracellular space [96]. The released exosomes can interconnect or crosstalk via the involvement of several molecular mechanisms and result in the release of numerous components. These vesicles are usually emitted in plasma, urine, milk, bronchial lavage, bile, cerebrospinal fluid, amniotic fluid, and saliva and thereby act as biomarkers for cell-to-cell contact [97–99]. The comparison of the above-mentioned mechanisms with relation to the release of exosomes still remains uncertain. Other than considering lipid composition and endosome membrane properties, biogenesis of exosomes is closely related to the specificity of cargo molecules involved that are involved in multiple cellular processes of tumor [100].

After being added onto the target cell, exosomes are internalized by endocytosis or phagocytosis and their contents are delivered into cytosol. After such internalization occurs, the cancer cells and stromal cells communicate with each other and produce exosomal miRNA. This can lead to a communication that can affect both these cells. During this communication, the exosomal miRNA released can modify the invasive and metastatic behaviors of cancer cells thereby turning them into aggressive phenotypes of cancer. Hence, exosomes and exosomal miRNAs, the influence of both which remain the same in cancer, play significant roles in the tumor environment [101–105]. Yet, exosomes are synthesized more in cancer patients compared to normal subjects [106].

5. Advantages of Using Exosomes Loaded with miRNA for Cancer Therapy in Comparison to Conventional Means

Recent research at the nanolevel is growing rapidly which focuses on the use of exosomes and other sources for the treatment of cancer [107]. This can be achieved by communication between cells, identification of suitable miRNA and their potential as biomarkers and for other applications [72]. Targeting oncogenic miRNA by miRNA-based drugs (e.g., TargomiR) via injection into the tumor interstitial fluid or space can increase the specific targeting and decrease the

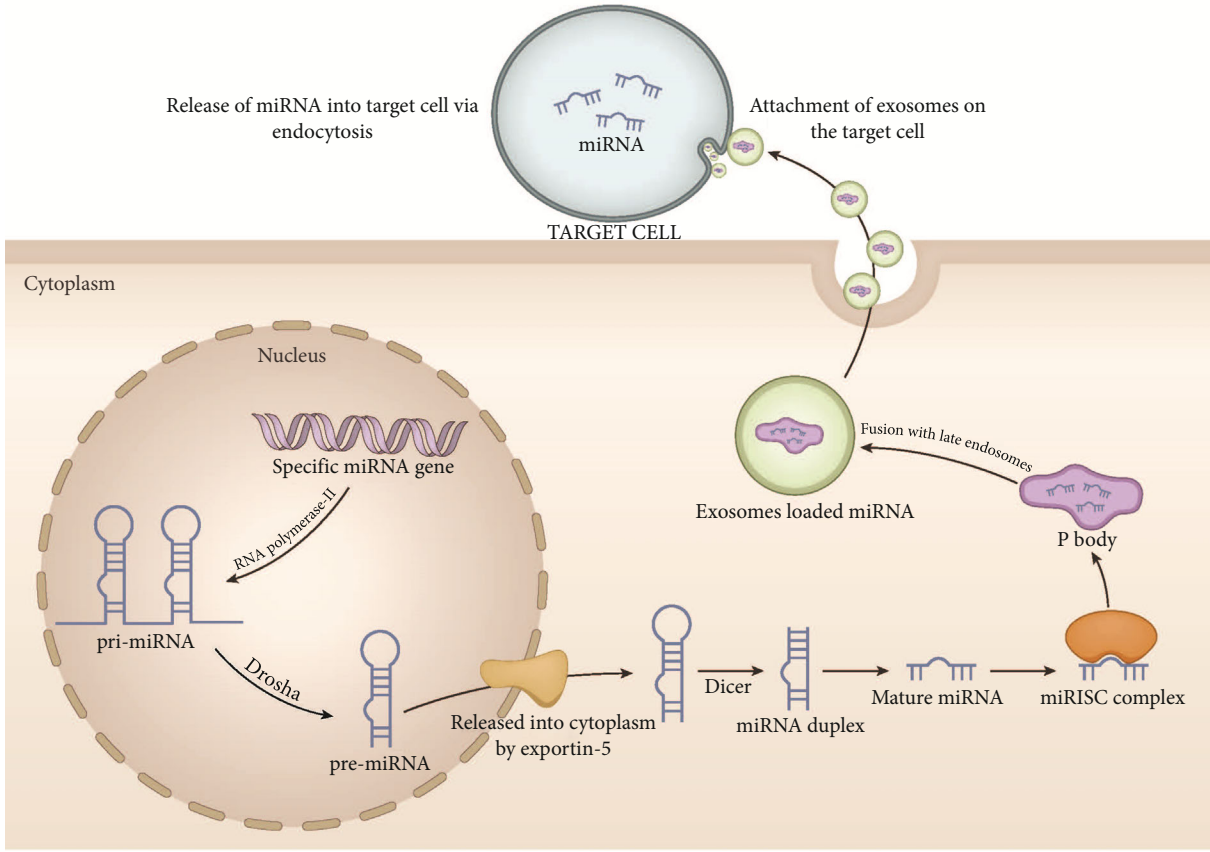

Figure 1: Biogenesis, isolation, miRNA transfection into exosomes, and release into the target cell.

levels of oncogenic miRNAs which is a huge prospect in cancer therapy [108].

Exosomes loaded with miRNA showed improved accumulation of more than 25000 times in comparison to the control exosomes observed in NSCLC cells. These miRNA were antiproliferative, anti-invasive, and antiangiogenic in such tumor models [109]. Owing to their nanosize and membrane integrity, exosomes can cross blood-brain barrier and escape immune surveillance. Therefore, they possess increased half-time in circulation, can improve the bioavailability of miRNA cargo for the intended use in nanomedicine, and suppress the resistance among cancer cells [110, 111]. Additional benefits of using exosomal miRNA include their disclosed origin, their therapeutic target, cellular responses which can help monitor tumor resistance, and their use as prognostic and diagnostic biomarkers [103]. Due to these benefits in comparison to conventional therapeutic approaches, exosomal miRNAs could be considered potential candidates for the personalized therapy of NSCLC.

Although the therapeutic and diagnostic tools are improved every day, the disease prognosis for NSCLC is poor due to understanding drug resistance and the mechanisms involved. With their auspicious role as biomarkers in diagnostics and prognostics, miRNAs can be a valuable addition to NSCLC therapy in humans [112]. Therefore, further studies at *in vitro* and *in vivo* levels are necessary and these studies remain critical to elucidate the actual role or involvement of miRNAs in therapy of NSCLC. Therefore, the following sections of this review will focus on a set of *in vitro* and *in vivo* studies on NSCLC therapy using miRNAs and the discussion of the associated mechanisms.

6. Mechanistic Studies for miRNA as Tumor Suppressors on NSCLC at the Preclinical Level

There are several studies supportive of miRNA interaction with its targets as a major direction for drug design and use in cancer therapy [113]. To begin, miR-6839-3p acted as a tumor suppressor by targeting and causing a decline in the expression of transcriptional enhancer associate domain transcription factor 4 (TEAD4) which is known to promote lung cancer. The cell lines used in the study were A549, SPC-A1, NCI-H1299, H1650, PC9, H1975, and NCI-H1703 [114]. miR-148b is known to regulate key mechanisms in tumor and normal cells and declined the cancer cell population at the G2/M phase in PC14/B and A549 cells. This tumor suppressor enhanced apoptosis and inhibited the MAPK/JNK signaling of these two NSCLC cells possibly by targeting MAP3K9 [115]. miR-7, an endogenous noncoding tumor-suppressor RNA, is downregulated in A549, H1299, and H1355 NSCLC cells. Yet, it induces apoptosis via the downregulation of Bcl-2 and suppresses the growth of A549 cells by inhibition of migration [116]. The tumor suppressor miR-608 promoted apoptosis induced

by doxorubicin in A549 and HCC4006 via the inhibition of expression of transcription factor activating enhancer-binding protein 4 (TFAP4) [117]. miR-377, miR382, and miR-498 were identified as possible tumor suppressors in A549, 95-D, HCC827, H1299, and SK-MES-1 cells. EZH2 is a target for these three miRNAs [118]. miR-448 is a tumor suppressor in A549, SK-MES-1, Calu-3, and H1299 cells by the suppression of proliferation, migration, and invasion of these cells [119].

Lentivirus-mediated delivery of miR-218 decreased the cellular proliferation and reduced the growth of human lung A549 cells injected into 6-week-old nude mice. The tumor tissues showed low levels of STAT3 and Ki-67 in comparison to tissues infected with the control virus without miRNA. The study indicated the involvement of IL-6/STAT3 pathway and identified its role in prognosis of lung cancer [74]. miR-200c as a tumor suppressor did improve the sensitivity of A549 cells towards methotrexate by targeting EZH2 and suppressing its invasive property. Apoptosis was induced via the P53/P21 pathway [120]. Overexpression of miR-144-3p inhibited the propagation and invasiveness of NCI-H1975, NCI-H441, NCI-H1792, and SPC-A1 lung adenocarcinoma cells. This miRNA downregulated the expressions of VEGFA, MMP2, and MMP9 and inhibited the growth of NCI-H1975 xenograft tumor in nude mice. The miRNA suppressed the expressions of EZH2, an oncogene associated with lung cancers to yield such effects [121]. miR-26a, with antioncogenic properties and known roles in several pathways, decreased the proliferation and induced apoptosis in docetaxel-resistant SPC-A1 and H1299 lung adenocarcinoma cells by the downregulation of EZH2 [122]. miR-101-3p with potent characteristics of an antitumor agent decreased the viability, migration, and invasive properties of H520, H1703, H2170, and SK-MES-1 lung squamous carcinoma cells and induced apoptosis by causing an inhibition of the target EZH2 [123].

miRNA-4465, known to possess roles of a prognostic indicator in cancer, suppressed the proliferation and metastasis of A549 and H2170 cells by regulating its target oncogene EZH2 [124]. Proapoptotic miRNA-557 suppressed the proliferative and invasive properties of A549 and NCI-H460 by causing a decrease in lymphocyte enhancement factor 1 (LEF1) [125]. Lung cancer suppressor miR-1244 inhibited the proliferation and induced apoptosis in cisplatin-resistant A549 and NCI-H522 cells by regulating the myocyte enhancer factor 2 named MEF2D [126]. Yet, another lung cancer suppressor miR-218 reduced the transcription factor MEF2D in A549, H450, and H1229 cells thereby causing a decline in proliferative, survival, and invasive properties of those cells. In H1975 and A549 cells, the miRNA affected the proliferative and invasive properties. The molecular targets were IL-6R and JAK3. The STAT3 signaling was inhibited after treatment [74, 127]. Transfection of miR-137 caused a decline in expression of an oncogenic histone demethylase named lysine-specific demethylase 1 (LSD1) in A549 and H460 cells by the downregulation of EZH2, HDAC1, and HDAC1 [128]. The influence of miR-26b on A549, 95D, and H520 cells was studied. The miRNA inhibited the migration and invasive properties of these cells using migration and invasion enhancer 1 (MIEN1) as a target by the involvement of NF-κB/MMP-9/VEGF pathways [129].

The overexpression of miR-582-5p decreased the proliferation and colony-forming ability of human NSCLC lines H460, H661, H647, H358, H1975, H661, H1299, and H226 and nullified filamentous actin (F-actin), despite increasing cellular apoptosis and YAP/TAZ phosphorylation. miR-582-5p targeted the actin regulators NCKAP1 and PIP5K1C resulting in suppression of YAP/TAZ-driven cell proliferation [130]. Similarly, the overexpression of miR-567 decreased the A549 cell proliferation, induced apoptosis, and cell cycle arrest at sub-G1 and S phases. The cyclin-dependent kinase 8 (CDK8) gene was the therapeutic target to obtain such effects as an outcome [131]. Adding to this, miRNA-377 prevented the proliferation and induced apoptosis in A549 and Calu-6 cells by targeting and downregulating the expressions of EGFR, MAPK1, and PAK2 of ErbB signaling pathway [132].

The oncogenic and therapeutic effects of nonprotein-coding transcripts in NSCLC are presented in Table 1. These studies indicate that several miRNAs have crucial roles, and EZH2 is a possible and valuable target for the treatment of NSCLC.

7. Exosomal miRNA as Biomarkers for the Detection of NSCLC

At the preclinical level, miR-19, miR-20, miR-21, miR-125, miR-205, miR-155, miR-let-7, miR-148a, miR-148b, and miR-320a are potential prognostic biomarkers for lung cancer. The miR-test for circulating miRNAs has a specificity of approximately 75%, and therefore, miRNAs could be considered as efficient prognostic markers for lung cancer [133–135]. Diagnostic biomarkers for lung cancer detection include let-7a, let-7b, let-7e, miR-15b, miR-17, miR-19a, miR-19b, miR-20a, miR-21, miR-21-5p, miR-22, miR-24, miR-25, miR-26b, miR-28-3p, miR-30b, miR-30c, miR-31, miR-92a miR-93, miR-106a, miR-125a, miR-126, miR-140-3p, miR-140-5p, miR-142-3p, miR-145, miR-146a, miR-148a, miR-150, miR-152, miR-155, miR-182, miR-190b, miR-193a-3p, hsa-miR-195-5p, miR-197, miR-203, miR-205, miR-210, miR-210-3p, miR-221, miR-222, miR-223, miR-320, miR-375, miR-425, miR-451, miR-486, miR-566, miR-660, miR-1260b, miR-1290, miR-3182, and miR-5100 [136–146].

NSCLC patients exhibit distinctive exosomal miRNA profile in comparison to healthy controls [147]. As an example, exosomal miR-620 were significantly lower among NSCLC patients in comparison to healthy controls [148]. Exosomal miR-126 could target and inhibit the gene ITGA6 and prevent NSCLC cells from progressing further [149]. NSCLC samples could be identified in comparison to control samples by the presence of miRNAs such as hsa-miR-451a, hsa-miR-486-5p, hsa-miR-363-3p, hsa-miR-660-5p, hsa-miR-15b-5p, hsa-miR-25-3p, and hsa-miR-16-2-3p [150]. Interestingly, in progressive NSCLC patients, miR-320d, hsa-miR-320c, and hsa-miR-320b were upregulated before treatment, whereas, hsa-miR-125b-5p was downregulated in plasma exosomes after being treated with checkpoint

Table 1: Therapeutic effect of nonprotein-coding transcripts in NSCLC.

miRNA	Cells treated	Target	Mechanism	Reference
miR-6839-3p	H1299	TEAD4	Decline in the expression of transcriptional enhancer associate domain	[114]
miR-148b	PC14/B and A549	MAP3K9	Enhanced apoptosis and inhibited the MAPK/JNK signaling	[115]
miR-7	A549, H1299 and H1355	Bcl-2	Apoptosis and inhibition of migration	[116]
miR-608	A549 and HCC4006	TFAP4	Apoptosis	[117]
miR-200c	A549	EZH2	Initiation of P53/P21 pathway	[120]
miR-144-3p	NCI-H1975, NCI-H441, NCI-H1792 and SPC-A1	EZH2	Downregulation of the expressions of VEGFA, MMP2 and MMP9	[121]
miR-26a	Docetaxel- resistant SPC-A1 and H1299	EZH2	Apoptosis	[122]
miR-101-3p	H520, H1703, H2170 and SK-MES-1	EZH2	Apoptosis and inhibition of the mitosis, invasion and migration	[123]
miRNA-4465	A549 and H2170	EZH2	Inhibition of proliferation and metastasis	[124]
miRNA-557	A549 and NCI-H460	LEF1	Decrease in migration and invasion	[125]
miR-1244	Cisplatin-resistant A549 and NCI-H522	MEF2D	Inhibition of proliferation and induction apoptosis	[126]
miR-218	A549, H450, and H1229	MEF2D	Decline in proliferation, survival and invasion	[127]
miR-218	H1975 and A549	IL-6R and JAK3	Inhibition of STAT3 signaling	[74]
miR-137	A549 and H460	LSD1	Downregulation of EZH2, HDAC1 and HDAC1	[128]
miR-26b	A549, 95D, and H520	MIEN1	Involvement of NF-κB/MMP-9/VEGF pathways	[129]
miR-582-5p	H460, H661, H647, H358, H1975, H661, H1299 and H226	NCKAP1 and PIP5K1C	Nullified filamentous actin (F-actin), increased cellular apoptosis and YAP/TAZ phosphorylation	[130]
miR-567	A549	CDK8	Decreased the cell proliferation, induced apoptosis and cell cycle arrest at sub-G1 and S phases	[131]
miRNA-377	A549 and Calu-6	EGFR, MAPK1, and PAK2	Prevented the proliferation and induced apoptosis	[132]

inhibitors of PD-1/PD-L1 pathway [151]. Exosomal miR-23b-3p, miR-10b-5p, and miR-21-5p are representative of poor overall survival [152]. In addition, miR-19-3p, miR-21-5p, miR-221-3p, and miR-17-5P are upregulated in NSCLC patients when compared to their healthy counterparts [153, 154]. Yet, miR-141 was expressed lesser in NSCLC patients than patients with no tumour [155], whereas, miR-181-5p, miR-30a-3p, miR-30e-3p, miR-361-5p, miR-10b-5p, miR-15b-5p, and miR-320b were specific to a group of cancers categorized into NSCLC [156].

Reports suggest sputum miRNAs such as miR-21, miR-143, miR-155, miR-210, and miR-372 to be clinical markers for early detection of NSCLC [157]. Polymorphisms in hsa-miR-196a2 have been identified to play insignificant roles in toxicity observed among individual cells or organs, whereas, the overall toxicity was significantly higher in NSCLC patients treated with platinum-based drugs [158]. High expressions of miR-16 in patients with NSCLC are assigned to be associated with poor disease-free and overall survival [159]. Contrasting to this report, high exosomal miRNA-32 levels have been correlated to improved progression-free and overall survival in patients treated with platinum-based drugs [160]. Similarly, miR-4782-3p could inhibit NSCLC proliferation by targeting the protease named ubiquitin specific peptidase 14 (USP14) [161]. These studies indicate that exosomal miRNA could be considered as potential diagnostic and prognostic noninvasive biomarkers for NSCLC, which can improve the chances of an intended targeted therapy.

8. Systemic Safety Profile of Exosomal miRNAs

Analysis in the major organs such as the lung, liver, spleen, and kidneys of tumor-bearing mice after intravenous injection of exosomal miRNA-142-3p indicates that exosomal miRNA are systemically nontoxic. This mRNA was

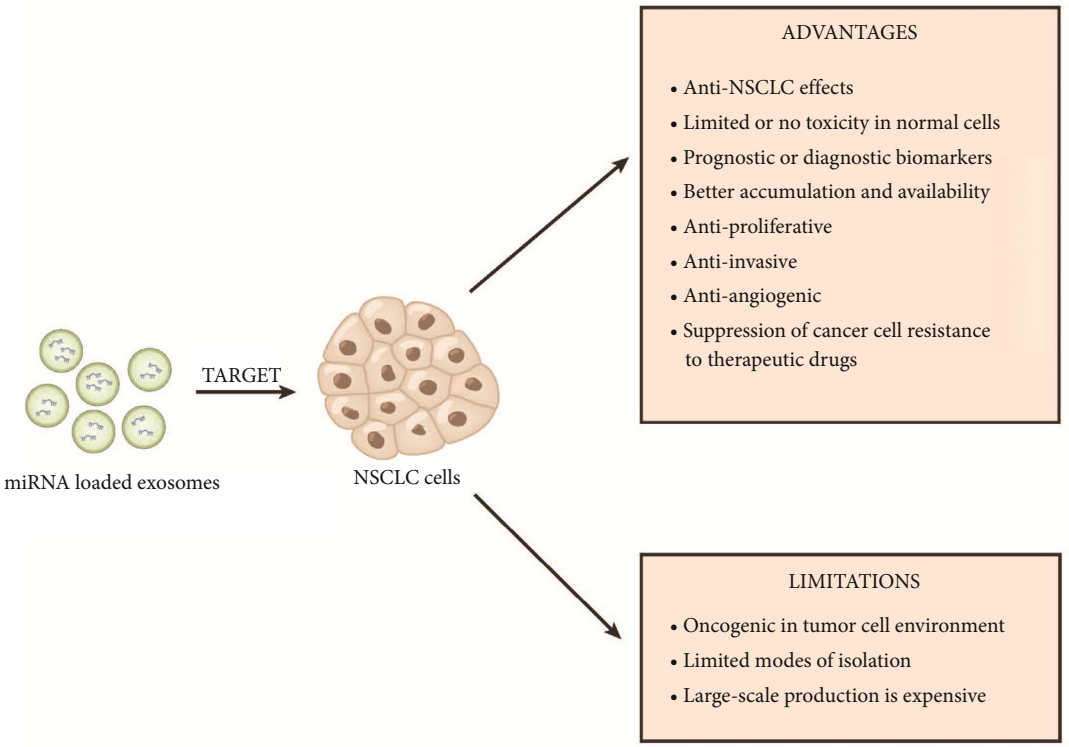

Figure 2: Multiple roles of exosomes loaded with miRNA in NSCLC.

identified to be tumor-specific as they were observed in the tumor environment even after 48 hours, whereas, absent in those major organs. This indicates that exosomes loaded with miRNA could be used effectively in cancer treatment. Although there are only limited reports on nanotoxicity of exosomes, current updates indicate them to be nanovectors with limited or no toxicity or immunogenicity against normal cells [162–167]. In short, exosomes possess significant biocompatibility, low immunogenic potency, and are relatively safe. They are taken up by cells adopting mechanisms such as phagocytosis, micropinocytosis, endocytosis, and fusion [168].

9. Future Perspective and Conclusions

With regard to future research, target specificity is an important criterion for miRNA delivery into the tumor environment. Biogenesis of each exosome should be identified and monitored for specific type of cancer rather than following an unsystematic approach. Exosomal membranes could therefore be modified for such purposes. This can improve targeting and improve yield of the desired effect. Furthermore, synergism with existing drugs (e.g., doxorubicin) can eliminate the side effects and improve the anticancer efficacy [169]. Isolation methods and purity check for exosomes have to be optimized better to control the limitations that may arise in the future [170]. Low-cost methods should be developed for large-scale production of exosomes. Improved insights and understanding of exosome usage such as route of administration and specific targeting are the issues to consider [165, 171]. The advantages and limitations of the use of exosomal miRNA are presented in Figure 2.

NSCLC, the leading cause of deaths related to lung cancers, are both metastasized and treated by exosomes loaded with microRNAs through the communication of nearby and far cells. Exosomes which are released as several vesicles into the extracellular space could be considered as biomarkers for lung cancers. Although, both miRNA and exosomes are involved in cancer therapy, exosomal miRNA are comparatively nontoxic and possess improved bioavailability in the tumor environment. Studies elucidate EZH2 as a possible and valuable target for the treatment of NSCLC at preclinical levels using miRNA. Although several preclinical and few clinical reports determine the roles of miRNA as biomarkers and tumor suppressors, further studies are warranted to determine the efficacy of exosomal miRNA in treatment and management of NSCLC.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Authors' Contributions

Ziyu Jiang and Yun He contributed equally to this work.

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