

New understanding of exosomes as a miRNA delivery mechanism has led to advances in RNA cancer treatments.

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Abstract

With the introduction of mRNA-based vaccinations and RNA interference techniques like siRNA, a new age of RNA therapeutics has begun. Unfortunately, there are currently no RNA-based therapies for cancer that have been authorized by the FDA. MiRNAs are an exciting new frontier in the fight against cancer since they are RNA molecules. Despite miRNA-based medicines' potential to target numerous pathways, getting to the phase 3 clinical trial is difficult. The difficulty of delivering miRNAs just to the tumor cells that need them has contributed to the delay. This emphasizes the need of a reliable distribution network. In this regard, exosomes are seen as the safest and most efficient cargo delivery vehicles for miRNA-based therapeutics. As an added bonus, a new generation therapy for cancer treatment has been uncovered: harnessing exosomes to release superfluous miRNAs to counteract the tumor's potential to replicate. The latest developments in the use of microRNAs in cancer therapy are discussed, including the transport of miRNAs via exosomes and strategies to overcome the limitations of miRNA-loaded exosomes in clinical settings.

MiRNA, microRNA, microRNA delivery system, RNA-based medicines, cancer therapy, exosomes.

1. Introduction

Therapeutic methods based on RNA, such as messenger RNA (mRNA), RNA interference (RNAi) using small interfering RNA (siRNA), and microRNA (miRNA) treatments, show promise for the treatment of a variety of disorders (Damase et al., 2021). Evidence suggests that the therapeutic efficacy of these therapies is higher than that of DNA-based medications and lower than that of protein-based therapeutics (Li et al., 2021). There are now four small interfering RNA (siRNA) medications and two mRNA-based therapies available for clinical use. However, none of these has been utilized for cancer therapy. The thick tumor stroma, unstructured blood arteries, immunosuppression, multidrug resistance, and hypoxia all contribute to the challenging pathophysiological milieu in which cancer develops (Heinrich et al., 2021). To achieve therapeutic impact, it is necessary to treat many concerns. In the circulation, 'naked' RNA molecules must resist enzymatic breakdown, renal clearance, and phagocytic trapping due of their tiny size, poor stability, and immunogenicity (Kulkarni et al., 2019). RNA molecules have a large molecular weight, negative charge, and high hydrophilicity, making it difficult for them to pass negatively charged biological membranes and infiltrate cancer cells once within the tumor. Since foreign agents are internalized via endocytic and endo-lysosomal routes, RNA destruction is expected to occur (Singh et al., 2020), and even after internalization, few RNA molecules are able to escape endosomal entrapment. There have been several suggestions for chemical changes that might increase the stability of RNA therapeutics while also decreasing their immunogenicity. Conjugation of siRNA to N-acetylgalactosamine molecules is used to create the clinically-approved formulations Givosiran (GIVLAARI® Inc.),

Lumasiran (OXLUMO® Inc.), and Inclisiran (LEQVIO® Inc.). A problem still exists, however, with endosomal escape (Dammes and Peer, 2020).

The mRNA-based coronavirus illness 2019 vaccines emphasized the importance played by drug delivery systems in RNA-based therapeutics. Lipid nanoparticles (LNPs) for the delivery of nucleic acid- and mRNA-based therapies, are well known. After mRNA/LNP injection, it was observed that the liver was the primary site of mRNA translation. Therefore, the key difficulty is to efficiently transport RNA molecules to the tumor location without causing them to build up in the liver. Non-specific delivery and the inability of LNP to circumvent immune surveillance may lead to off-target effects, which might generate significant adverse effects and diminish the therapeutic effectiveness. In contrast to mRNA-based therapies, which work by increasing expression of targeted proteins, RNAi therapies like miRNA and siRNA downregulate expression of checkpoint proteins for disease treatment by complexing with the RNA-induced silencing complex (RISC) in the cytoplasm to induce cleavage of the mRNA sequence. This means that an ideal RNA delivery system for cancer therapy would prevent RNA degradation and have the capacity to release RNAs from endosomes into the cytoplasm. Additionally, essential qualities to think about while creating an effective nanocarrier are tumor targeting ability and immune system surveillance escape.

Here, we describe recent breakthroughs in the utilization of exosomes as miRNA transfer vehicles and present an overview of current RNA uses in cancer treatment, with a particular emphasis on miRNA-based medicines now in clinical trials. In this talk, we'll go over some of the latest strategies for using exosomes loaded with miRNA in clinical settings.

2. Application of RNA in Medicine

Due to its central involvement in biological processes, RNA-based therapeutics hold great promise for the prevention and treatment of a wide range of human disorders. approximately 1.5% of the human genome is responsible for encoding proteins, and approximately 10% to 15% of those proteins can be drugged with typical proteins or small compounds (Hopkins and Groom, 2002). To bind "undruggable" or altered targets, RNA therapies may be tailored to disrupt any gene or genomic area, including non-coding transcriptomes (Yu et al., 2019).

Although RNA treatment provides several benefits over conventional medications, only a small number of RNA-based drugs have been authorized for clinical use. Unfavorable physico-chemical features (negative charge, high molecular mass and size), instability and short circulation half-life, and severe immunogenicity have all been obstacles to the development of therapeutic RNAs (Damase et al., 2021). To achieve this goal, it is necessary to introduce a number of chemical changes to the saccharide and/or phosphodiester backbone. Phosphorothioate (PS) is used to substitute phosphodiester links in the vast majority of therapeutic oligonucleotides to prevent nuclease degradation, increase stability, and improve cellular absorption in vivo (Eckstein, 2014). Sugar-modified oligonucleotides have been demonstrated to be the most effective among the several chemical modifications. Both binding affinity and resistance to nuclease attack may be improved by modifying the ribose at its 2' position (Prakash, 2011). To prevent degradation, expedite distribution to the correct cells and tissues, and guarantee absorption by those cells, therapeutic RNAs need to be packaged in suitable delivery vehicles. These objectives may be met by the creation of various selected delivery agents (Bajan and Hutvagner, 2020).

There are three main types of RNA therapeutics: aptamers, which target specific proteins, RNA molecules that bind to specific nucleic acids, and mRNAs, which are translated into proteins. Antisense RNAs and small interfering RNAs (siRNAs) are within the first group of RNAs, which also includes double-stranded RNAs.

2.1. Inverse RNAs

Antisense oligonucleotides (ASOs) are short (15-20 bp) synthetic, single-stranded RNA/DNA molecules, perfectly complementary to the target RNA sequence. RNA cleavage (RNase H-dependent ASOs) and RNA blocking (RNase H-independent ASOs) are two methods by which ASOs that bind to a particular RNA via Watson-Crick base pairing might change gene expression. The mechanism of action of an ASO is determined by both the target sequence and the chemistry of the ASO. When it comes to causing protein knockdown, the former is much more popular and effective. RNase H recognizes the RNA strand of the RNA-ASO duplex and cleaves it (Wu et al., 2004; Wada et al., 2021). However, mRNA instability (inhibition of 5' cap formation) and splice-switching (alteration of splicing) are two non-RNase-mediated processes that may reduce target protein production.

Stability, transport, targeting, and pharmacological characteristics of ASOs are improved by chemical modification (Kurreck, 2003; Bennett et al., 2017). These alterations may happen anywhere along the ASO's backbone or on its side chains, and many ASOs are intentionally created to be chimeras, meaning they comprise a variety of nucleotides with widely varying chemical properties (Scoles et al., 2019). Here, PS was used to replace the phosphodiester linkages in the first generation of ASOs, while in the second generation, the -OH group at the 2'-position was substituted with 2'-O-methyl (2'-O-Me) or 2'-fluoro (2'-F) (Eckstein, 2014; Khvorova and Watts, 2017, 2017stergaard et al., 2017). Locked nucleic acids (LNAs)-modified anti-miRs are characterized by increased binding specificity and resistance to nucleases (Rupaimoole and Slack, 2017), and they are produced by using the C3'-endo conformation, which involves connecting the 2'-oxygen and 4'-carbon of the ribose with a bond bridge. In addition, liposomes, polymeric nanocarriers, and metallic particles, or ASOs linked with different peptides, are required to enable effective transport of antisense oligonucleotides to the target cells (Yang et al., 2020).

Cancer, diabetes, AIDS, and cholesterol-linked disorders are all characterized by dysregulated protein expression, and ASOs provide a viable therapeutic method for treating these illnesses (Crooke et al., 2018). For instance, Fomivirsen (Vitravene) was the first medicine to use antisense technology to treat cytomegalovirus (CMV) retinitis, and it was authorized by the FDA. By inhibiting the translation of early CMV proteins, the medication stops viral replication (Jabs and Griffiths, 2002). As well as eteplirsen (Sarepta Therapeutics® Inc.) for the treatment of Duchenne muscular dystrophy (Mendell et al., 2013) and nusinersen (Spinraza® Inc.) for the treatment of spinal muscular atrophy (SMA) (Khorkova and Wahlestedt, 2017), the FDA has approved several other ASOs for the treatment of various diseases. Pre-clinical and clinical trial findings are promising for ASOs in cancer treatment, although none have yet been licensed by the FDA (Xiong et al., 2021). The antisense method may be able to treat "undruggable" proteins since it is based on the mRNA of the target protein. As a result, ASOs are more adaptable, flexible, and targeted than traditional small medicines. Antisense medicines may be manufactured rapidly and delivered specifically to diseased tissues where they will have the most effect. However, drawbacks include hazardous side-effects, focused tissue-delivery (i.e., ASOs often do not cross the blood-brain barrier), and difficulty of finding the ideal dose, plus the high cost (Xiong et al., 2021; Crooke et al., 2021).

2.2. To RNA aptamers

RNA aptamers are small single-stranded RNAs that attach to a target with great specificity and affinity, and inhibit or modify its activity. RNA aptamers are molecular mimics of antibodies because of their features (Germer et al., 2013). Systematic Evolution of Ligands by Exponential enrichment (SELEX) (Zhuo et al., 2017) identifies aptamers with affinity for a chosen target from random pools of RNA. Many different types of targets, from tiny molecules and peptides to proteins and carbohydrates and even living cells, have been used in the development of RNA aptamers. Aptamer binding is based on the tertiary structure rather than the main sequence. In reality, RNA molecules fold into different and complicated tertiary structures that interact with the target ligand by means of hydrophobic and electrostatic interactions, van der Waals forces, hydrogen bonding, and base stacking (Zhang et al., 2021a).

RNA aptamers may be utilized for target validation and drug discovery in addition to their more traditional analytical, diagnostic, and therapeutic applications, making them a competitive alternative to antibodies. Compared to the usage of antibodies, therapeutic use of RNA aptamers offers various benefits, including being less immunogenic, more stable, more structurally flexible, easier to modify, and requiring less time and money to produce. RNA aptamers have the advantage over antibodies in that they can infiltrate biological compartments (such as tissues and cells) and bind non-immunogenic molecules (such as tiny targets or poisons) with greater ease. At the same time, the tiny size renders RNA aptamers sensitive to renal filtration and to nuclease-degradation. Chemical modifications are made to therapeutic aptamers to increase their pharmacokinetic profile and circulation half-life in vivo (Kovacevic et al., 2018). Their renal excretion may be decreased by coupling with polyethylene glycol (PEG), and their susceptibility to exonucleases can be increased by modifying their 3' and 5' ends (Adachi and Nakamura, 2019).

Tumors, retinal degeneration, viral infection, inflammation, diabetes, and cardiovascular and coagulation issues are only some of the many human disorders that have been targeted by therapeutic RNA aptamers (Xiao et al., 2021). Although numerous RNA aptamers have been discovered, only nine have been tested in humans. The only aptamer-based medicine currently available for the treatment of AMD is pegaptanib (Macugen: Eyetech Pharmaceuticals® Inc.), which is an RNA aptamer against vascular endothelial growth factor (VEGF) (Ng and Adamis, 2006). In addition to NOX-E36 targeting C-C motif-ligand 2 (CCL2) for the treatment of type 2 diabetes mellitus, NOX-A12 binding CXC chemokine ligand 12 (CXCL12) to inhibit lymphocytic leukemia cell motility, REG1 targeting factor IX, a protein linked to coronary artery disease, and ARC1905 acting against complement component 5 for the treatment of AMD are all undergoing clinical trials. Despite encouraging findings, further research is required to address concerns about their safety and their incapacity to passively diffuse over the plasma membrane.

2.3. Therapeutic use of messenger RNAs (mRNAs)

Therapeutic mRNA is an RNA analog that is synthesized or translated in vitro and used to stimulate the production of a target protein. It functions similarly to natural mRNA by transporting genetic instructions to the translational machinery. mRNA has a cap at its 5' end and a poly(A) tail at its 3' end, as well as a single-stranded open reading frame in the middle and untranslated regions (UTRs) on both sides (Sahin et al., 2014). Some common methods of treating with mRNA include cell therapy, in which target cells are manipulated ex vivo to introduce therapeutic mRNA, and replacement therapy, in which mRNA is delivered into the patient's cells to compensate for a defective or missing protein; vaccination, in which mRNA encodes for antigen(s) that activate the immune system to produce antibodies against a specific pathogen; and cancer treatment.

When compared to traditional small molecules or recombinant proteins, therapeutic mRNA has several benefits, including the potential for individualized treatment, rapid protein creation, and reduced manufacturing costs and time. The lack of genomic integration, the absence of persistence in vivo, the absence of external components in the RNA sequence, and the ease of downstream purification make mRNA-based therapies safer than those based on DNA (Orlandini von Niessen et al., 2019). However, there are a number of issues with using mRNA for therapeutic purposes, such as its short half-life, susceptibility to enzymatic destruction, poor transport to target cells, and undesirable immunological responses. To achieve the desired immunogenicity, therapeutic mRNAs are engineered and chemically modified to increase their intracellular stability, shield them from nuclease destruction, increase their translation efficiency, and narrow their specificity. Intriguingly, UTRs may be altered in order to encode regulatory elements, allowing for cell-specific regulation of RNA production (Wroblewska et al., 2015). N1-methyl-pseudouridine is an example of an RNA base alteration that boosts mRNA translation and decreases its immune-stimulatory function (Andries et al., 2015). More favorable half-life, better-controlled release, and cell type- and organ site-specificity may be achieved by encapsulating therapeutic mRNAs in nanocarriers (such as lipidic, polymeric, and polypeptidic systems, gold and silica nanoparticles, and dendrimers) (Lee et al., 2022).

Cancer, infections, rare diseases, metabolic, cardiovascular, and immunology disorders, and any other disorder associated with functional loss of proteins all seem treatable with therapeutic mRNA (Weissman and Karikó, 2015; Xiong et al., 2018). Numerous pre-clinical investigations and a small number of clinical trials have made use of the mRNA technology. In fact, despite the fact that mRNA's medicinal potential was found quite some time ago, progress in this area has been sluggish. There are just two mRNA-based medicines now licensed by the FDA (vaccines against SARS-CoV-2) and most are still in the early stages of clinical testing (Gómez-Aguado et al., 2020; Pawlowski et al., 2021). The immense promise of mRNA technology is shown, however, by the creation and global use of these mRNA vaccines. Finally, therapeutic mRNA encoding tumor antigen, antibodies, or immunomodulators (cytokines and co-stimulatory) have been shown to have promising results in stimulating and activating anti-tumor immune responses (Beck et al., 2021). Preclinical and clinical research employing mRNAs as therapies have shown encouraging results in the battle against SARS-CoV-2; nevertheless, significant challenges, including stability and delivery, remain.

2.4. Short hairpin RNAs (siRNAs)

siRNAs are double-stranded non-coding RNAs, approximately 20-25 base pairs in length, that block gene expression by binding complementary mRNA targets (Dana et al., 2017). Although siRNAs have many similarities with miRNAs, including comparable physical characteristics and the capacity to gene silencing via a process termed RNAi, they are two independent kinds of RNA with unique roles and modes of action (Davidson and McCray, 2011). Unlike microRNAs, which target many mRNAs, siRNAs target just a single mRNA. MiRNAs often only partly bind with their target mRNA, repressing translation while siRNAs cause nuclease cleavage of fully complementary mRNAs (Lam et al., 2015). Because of this distinction, therapeutic uses of siRNAs and miRNAs diverge; siRNAs are utilized to generate a gene-silencing effect by suppressing the production of a particular mRNA. The two main types of miRNA-based therapies are miRNA inhibition and miRNA substitution (Hu et al., 2020).

For siRNA-based therapies to be effective, siRNA molecules need to be designed to be highly active and selective with respect to the target. The stability, effectiveness, potency, resistance to nucleases, and circulation half-life of siRNAs, as well as the reduction of immunogenic and toxic effects, are all areas of research (Watts et al., 2008). Increased dsRNA-length increases its effectiveness, and it has been discovered that the presence of two-nucleotide overhangs at the 3'-end (often TT or UU) boosts recognition by the RNAi machinery (Walton et al., 2010). Substituting a 2'-O-Me, 2'-F, or 2'-methoxyethyl (2'-O-MOE) group for the 2'-OH group of ribose improves stability and decreases immune system activation, as was previously reported for various forms of therapeutic RNA (Cekaite et al., 2007). Creating reliable and efficient 'carriers' that transport siRNA to its target is essential for its usage in therapeutics. Non-viral vectors (including lipid-based carriers, peptides, polymers, and dendrimers) exist alongside viral vectors (Wang et al., 2010). A possible method is bio-conjugation of squalene (SQ) to siRNA to create siRNA effective nanoparticles (Massaad-Massade et al., 2018). Compared to traditional medications, there are benefits of siRNAs as treatments, including the unlimited choice of targets, safety, and high specificity and effectiveness to decrease gene expression (Xu et al., 2019). Diabetes, hypercholesterolemia, macular degeneration, respiratory illnesses, metabolic disorders, uncommon diseases, hepatitis, viral infections, and cancer are only some of the conditions for which the efficiency of siRNA-based treatment has been assessed. Clinical trials using siRNA therapies have been conducted, most often for loco-regional applications such intravitreal and intranasal delivery.

Currently, there are three FDA-approved siRNA medicines (patisiran, givosiran, and lumasiran) and seven siRNAs in late stages of Phase 3 clinical studies (Zhang et al., 2021b). In addition, clinical studies utilizing siRNAs have shown promising effects across a variety of cancer types. DCR-MYC, a synthetic double-stranded RNA encapsulated within lipid nanoparticles directed against MYC, has been used to treat solid tumors, multiple myeloma, non-Hodgkins lymphoma, and pancreatic neuraxial carcinoma. Phase I2 results for Atu027, a siRNA-based lipid nanoparticle that inhibits the expression of protein kinase N3 (PKN3). Although siRNAs show great promise as a therapeutic tool, their

clinical use is limited by a number of problems, including their instability, low cellular uptake, off-target effects, and immunological responses.

2.5. Ribozymes

Ribozymes are RNA molecules that can catalyze a cleavage reaction at a specified location. Many disorders, including cancer, have benefited from the use of ribozymes, which limit gene expression *in vitro* and *in vivo*. For instance, RPL4610 is an anti-VEGFR-1 ribozyme used in combination with carboplatin and paclitaxel to treat advanced solid tumors (Morrow et al., 2012), and Ad5CRT targets RNAs encoding human telomerase reverse transcriptase (hTERT) in patients with gastrointestinal cancer (Lee et al., 2019). However promising the results of the pilot study, more work has to be done to optimize stability, effectiveness, safety, delivery, and long-term expression (Khan, 2006).

2.6. miRNA

MiRNAs are small, single-strand RNA (ssRNA) molecules that have been around since 1993, when they were found in *C. elegans* (Lee et al., 1993) and are known to degrade or hinder translation of their intended mRNA target, hence reducing protein production (Bartel, 2018). MiRNAs bind to the 3'-UTR of their mRNA targets, which inhibits protein production at the molecular level. Indeed, mRNA degradation results from a perfect complementarity between miRNAs and their targets, whereas protein translation inhibition results from an imperfect match. Consequently, a particular miRNA sequence may target numerous mRNAs, thereby impacting related biological processes. MiRNAs are recognized non-coding transcripts, yet they control various biological processes critical to cellular homeostasis and organismal development (Bartel, 2018). Indeed, poor miRNAs biogenesis leads in dysregulation of their level, which has been established by scientific evidence to be connected with serious illnesses such as cancer (Peng and Croce, 2016).

MiRNA dysregulation may result from a number of causes, including as chromosomal defects, transcriptional misregulation, and epigenetic modifications, all of which must be understood. Cancer pathologies are characterized by these unfavorable occurrences, with the tumor communicating with the stroma via miRNAs dispersed across the TME to control the phases of cancer growth (Raue et al., 2021). Furthermore, miRNAs circulate in blood and other biofluids, where they might perform paracrine or endocrine signaling effects. The early identification of many cancers has prompted much research into the detection and assessment of miRNA levels in the extracellular environment (Wang et al., 2018a). While miRNAs are promising biomarkers because they can detect tumors at an early stage, circulating miRNAs have been shown to have low specificity and sensitivity issues, such as variations in miRNA levels between patients and between malignant and benign tumors (Wang et al., 2018a).

Despite extensive research on miRNAs as potential cancer biomarkers, no miRNA therapeutics have yet been licensed by the Food and Drug Administration. Since miRNAs may target either numerous or single components in cellular pathways, it may be possible to use miRNA mimics and inhibitors (antimiRs) together to restore depleted or overexpressed miRNAs in the TME, as was observed above. The first kind consists of synthetic double-stranded oligonucleotides designed to restore depleted miRNA levels in TME. The second kind is antisense ssRNA molecules, which are modeled after ASOs and were created to inhibit the pairing of miRNAs with their respective target mRNAs (Rupaimoole and Slack, 2017; Fig. 1). Below, we highlight the continuing state of miRNA in clinical use as prospective cancer therapies.

3. clinical studies using miRNA-based treatments

Interest in miRNAs as biomarkers has grown since their discovery, and their use in the clinic has been recognized by the scientific community. The modification of miRNA molecules and the loading of miRNA onto delivery devices have made considerable strides, however miRNA-based medicines have not yet advanced to phase 3 clinical testing. In fact, there are now diagnostic tools on the market that are specifically designed to identify miRNAs (Ciarletto et al., 2021).

Oncological miRNA medicines, in contrast, face significant barriers on the road from lab to bedside. Nonetheless, expenditures towards miRNA-based therapeutics by pharmaceutical firms are growing year by year (Chakraborty et al., 2020), showing that this innovation is not far from accessing the therapeutic market. Table 1 lists miRNA mimics and antimiRs that are presently being tested in clinical studies for the treatment of cancer.

In Mojdeh Mahmoudian and colleagues' study, it was discovered that certain microRNAs showed increased expression in BC tumor compared to the adjacent tissues. Specifically, hsa-miR-25-3p, -29a-5p, -105-3p, and -181b1-5p were upregulated, while hsa-miR-335-5p and -339-5p were downregulated. The upregulation or downregulation of these candidate microRNAs was found to be associated with TNM stages, except for hsa-miR-339-5p. Additionally, with the exception of hsa-miR-105-3p, each candidate microRNA correlated with HER-2 status. Furthermore, the analysis of ROC curves revealed that the combination of these six microRNAs could potentially serve as a biomarker to differentiate between tumor and non-tumor breast tissue samples.

3.1. Mirroring RNA Isoforms, or MiRNAs,

With MRX34, miRNA mimics took a significant stride toward entering clinical trials. The ionizable liposome NOV340 (SMARTICLES®, Marina Biotech, Bothell, WA; Mirna Therapeutics Inc., 2011) used to create MRX34 (Mirna Therapeutic Inc.) is laden with a miR-34 mimic, known as a tumor suppressor miRNA. MiR-34 inhibits tumor development by preventing the expression of several oncogenes. Since the tumor microenvironment (TME) is acidic, NOV340 becomes positively charged when it is in close proximity to a tumor, causing it to bind to cancer cells (Bader, 2012). The MRX34 complex was infused intravenously into patients with primary liver cancer, small cell lung cancer, lymphoma, melanoma, multiple myeloma, and renal cell carcinoma in a phase 1 clinical trial after showing promise in tumor suppression in a mouse model of lung cancer (Wiggins et al., 2010; Trang et al., 2011). Of the 66 patients, 3 had a partial response and 16 had stable illness for >4 cycles and >19 weeks, whereas 31 had progressive disease. Immune-related AEs resulted in the deaths of 4 patients (NCT01829971; NCT02862145) (Beg et al., 2017; Hong et al., 2020), leading to the suspension of the study despite the fact that the majority of reported AEs were of grades 1 and 2. Still unclear is whether miR-34's GC-rich seed sequence in the liposome carrier (Gao et al., 2018) or its effect on immune cells' signaling (Hart et al., 2020) provoked the severe immunological response.

Little progress has been achieved by a collaboration between EnGeneIC and the Asbestos Diseases Research Institute (Sydney, NSW, Australia), in which a miRNA mimic based on miR-16, a downregulated miRNA in malignant pleural mesothelioma (MPM), has been loaded in a minicell conjugated with anti-EGFR antibody named targomiR. MesomiR-1 is the name given to a clinical study of a miR-16-containing targeted minicell that has progressed to phase 1 (NCT02369198). Twenty-two patients out of a total of twenty-seven enrolled exhibited objective response, while fifteen patients showed SD. The authors proposed a phase 2 clinical study combining chemotherapeutics and immune checkpoint inhibitors with MesomiR-1 based on pre-clinical findings. Lymphopenia, inflammatory symptoms, and cardiac-circulatory issues were all reported as AEs in the research (van Zandwijk et al., 2017).

3.2. AntimiRs

Cobomarsen (MRG-106) created by MiRagen Therapeutics Inc. is the novel antimiR utilized for cancer therapy and now in clinical trial phase. Cobomarsen is an LNA and suppresses miR-155. Both cutaneous T-cell lymphoma (CTCL) and mycosis fungoides (MF) are characterized by an unchecked proliferation and survival of immune cells, which has been linked to an up-regulation of miR-155. Cobomarsen's altered chemical structure makes it highly absorbable by CD4+ T cells and mycosis fungoides tumor cells. In vitro research on human lymphotropic virus type 1 (HTLV-1+) CTCL and MF cell lines reveals that Cobomarsen suppresses cellular growth and caused apoptosis (Seto et al., 2018). There were no significant adverse events (AEs) recorded by any of the 38 patients in the phase 1 clinical study (NCT02580552) of Cobomarsen, and 29 patients reported an improvement in their mSWAT score. Duration of the research was 22 months and it evaluated CTCL population, in a phase 2 clinical trial that was halted owing to economic

concerns (NCT03713320). Importantly, a clinical study has demonstrated promising results with RGLS5579, an anti-miR-10b developed by Regulus Therapeutics Inc. (Carlsbad, CA, USA) for the treatment of glioblastoma multiforme (GBM). In a xenograft mouse model of GBM, this miR-10b inhibitor dramatically improved median survival when used in conjunction with temozolomide, from 27% to 159% (Wang et al., 2018b). This potential AntimiR, however, is still at the pre-clinical stage at the present time.

4. Strategies for treating cancer using microRNA-based therapies

Naked nucleic acid administration is a technique used in the use of miRNA treatments. However, various hurdles in terms of pharmacokinetics need to be addressed, particularly their destruction by cellular and extracellular RNases, endosomal escape, cellular uptake, immunogenicity, and specificity in targeted tissues (Winkle et al., 2021). In response to these challenges, scientists have developed two primary solutions: chemically modifying miRNA medicines to make them more stable (Rupaimoole and Slack, 2017) and loading them onto carriers (Roberts et al., 2020). Chemical modifications (ASO described previously) made miRNA mimics and antimiRs more stable, resistant to degradation and safer, however, the negative charges and hydrophilic nature of nucleic acids contributes to problems with cellular uptake resulting in higher dose administration that did not translate to high efficiency, mostly in the treatment of leukemia and metastatic cancers (Raue et al., 2021). Because of this, there is a pressing need for a reliable method of transporting therapeutics based on miRNAs. The scientific community has made significant strides toward this goal via the development of several oligonucleotide carriers, including viral or bacterial vectors, lipid- or polymer-based envelopes, and oligonucleotide bio-conjugation (Roberts et al., 2020).

In terms of targeting and cellular uptake, viral and bacterial vectors seem to be promising nanocarriers (van Zandwijk et al., 2017; Monahan et al., 2021), but the danger of AE induction remains significant. Lipid-based nanocarriers are the most standardized entities in terms of manufacture and clinical application among non-viral vectors (Hou et al., 2021). LNPs-PEG component has been reported to cause the so-called complement activation related pseudo-allergy (CARPA) and IgM and IgG production, potentially provoking at first, an anaphylactic shock (Zhou et al., 2021). Therefore, immune reaction in patient administered with LNPs therapeutics and already exposed to PEG-containing products should be monitored and studied. Considerable progress has been made in the area of polymer-based nanocarriers in terms of effective absorption and safety; for example, using biodegradable chitosan cationic polymers as carriers showed less toxicity than previously utilized polymers in vitro and in vivo (Raue et al., 2021). While bio-conjugated oligonucleotides have been shown to be safe in the short term, their long-term safety is still unknown (Ha et al., 2016). A more promising method is provided by bio-conjugation, which consists of covalent conjugation of oligonucleotides with biomolecules like as lipids, peptides and sugars, in order to leverage their receptor-mediated endocytosis. Nonetheless, research into the implications of receptor saturation is warranted (Raue et al., 2021). While there are many benefits to the miRNA delivery methods discussed above, there are also many problems that have yet to be resolved (Table 1). However, there are still many questions about the long-term safety of lipid- and polymer-based vehicles, as is the case with bio-conjugated oligonucleotides (Raue et al., 2021; Ha et al., 2016), and viral- and bacterial-derived vectors (immunogenicity and accumulation in certain organs). Exosomes have been found as a possible answer to the problems associated with nanocarriers. An introduction to this potential miRNA transfer vehicle is provided below.

5. MiRNA delivery via exosomes

It has been postulated that exosomes serve as a delivery method since they are natural carriers of miRNAs. It was established in 1981 that exosomes are present in most bodily fluids and are spontaneously generated by all cell types, including procaryotes (Trams et al., 1981). Extracellular vehicles (EVs) like exosomes, which range in size from 30-160 nm in diameter, are a type of nano- and micro-particles distinguished by their double phospholipid bilayer structure (Trams et al., 1981). Most EVs, such as microvesicles (MVs), micro-particles and big vesicles, emerge from cell

membrane by a budding process, and they are discharged in the extracellular environment. Multivesicular bodies (MVBs) are specialized endosomes that contain intraluminal vesicles (ILVs) and then fuse with the cell membrane to release the ILVs into the extracellular environment (Kalluri and LeBleu, 2020). Exosomes' fascinating properties and crucial biological significance have been elucidated by studies conducted since their discovery (Fig. 2-upper panel). Exosomes have been linked to immunological responses (Raposo et al., 1996) and intercellular communication (Simons and Raposo, 2009) and have also been identified as natural miRNA carriers (Valadi et al., 2007). Multiple illness models are being studied to see if exosomes may be used as therapeutic agents or prognostic indicators (Pegtel and Gould, 2019). Furthermore, recent research on exosomes and miRNAs has demonstrated that several elements of exosome biology might indicate natural features as MBTs carriers, including biogenesis, uptake, miRNA loading, safety profile, and effective delivery ability (Fig. 2).

According to reports, exosomes are released following fusion of endosomal MVBs with the plasma membrane and are generated as ILVs via an endosomal system process. Thus, exosomes retain the same membrane architecture as donor cells, although losing transmembrane lipid asymmetry and having phosphatidylserine (PHS) residues localized in the vesicle's outer leaflet. Currently, processes implicated in exosome formation include the endosomal sorting complex needed for transport (ESCRT)-dependent and -independent mechanisms. The ESCRT machine has five different complexes, or subunits. Vps4, ESCRT-0, ESCRT-1, ESCRT-2, and ESCRT-3. These multi-subunit complexes coordinate ILV formation and cargo sorting (Gurung et al., 2021). It has been proposed that biogenesis of exosomes takes place via formation of SL-enriched ceramide microdomains and their fusion into larger domains, by promoting inward vesicle budding (Trajkovic et al., 2008) (Fig. 3). This is in contrast to the ESCRT-dependent pathway, which relies on ceramide domains lacking sphingolipids (SLs) in the membrane of MVBs.

The principal SLs that operate as signaling molecules are ceramide and sphingosine-1-phosphate; they regulate a wide variety of cellular activities including growth, adhesion, migration, senescence, and cell death (Hannun and Obeid, 2008). GW4869, a selective inhibitor of neutral sphingomyelinase (nSMase) 1/2, and two structurally unrelated nSMase blockers, spiroepoxide and glutathione, were used to examine the function of ceramide in the synthesis and release of exosomes (Dinkins et al., 2014). Treatment of the cells with any of the nSMase inhibitors resulted in a significant decrease in exosome release, as validated by knockdown of nSMase-2 using siRNA (Trajkovic et al., 2008). The amount of exosomes produced from multiple myeloma cells was shown to increase dose-dependently with the addition of exogenous cell-permeable C6 ceramide (Cheng et al., 2018). Blocking nSMases does not prevent the release of all exosomes or decrease exosome synthesis in all cells (Colombo et al., 2013). Exosomes are a diverse population of vesicles. Of note, tetraspanin membrane proteins have been also identified to impact exosome synthesis (Trajkovic et al., 2008). Canonical regulators of exosome secretion include the nSMase and Ras-associated binding (Rab) proteins (Fig. 4). Inhibition of exosome release has been linked to autophagy activation, and studies have shown that Rab11 is involved in the docking of MVBs to the plasma membrane (Savina et al., 2002). Exosome secretion was inhibited by silencing Rab27A and Rab27B (Ostrowski et al., 2010).

5.2. The Exosome's Cancer-Fighting Role

Exosome levels have been shown to be higher in the bloodstreams of patients with tumors than in those of healthy participants (Kharaziha et al., 2012). Involvement in the metastatic process is facilitated by tumor-derived exosomes (TDEs), which are actively generated and released by tumor cells and transmit information from tumor cells to healthy cells or aberrant cells (Bai et al., 2022a). 'Transmission' of oncogenic activity was shown when EGFR was overexpressed in glioma cells, leading to enhanced release of exosomes that could be taken up by other glioma cells lacking EGFR (Al-Nedawi et al., 2008). Furthermore, exosomes containing HRAS DNA, RNA, and proteins were secreted at a higher rate when oncogenic RAS was expressed in non-tumorigenic epithelial cells (Lee et al., 2014). Exosome secretion in prostate cancer was shown to be reduced when RAS signaling was inhibited with either a farnesyl transferase inhibitor

(tipifarnib) or manumycin A (Datta et al., 2017, 2018). Conversely, restored expression of liver kinase B1 (LKB1/STK11), a tumor suppressor often altered or deleted in lung cancer, boosted exosome secretion (Zhang et al., 2018).

5.3. Exosome uptake

In general, the exchange of exosomes may be affected by a wide variety of factors controlled by cancer cells and the tumor environment. It is now known that exosome internalization is an active process that may occur via a number of different mechanisms, including as clathrin- and caveolae-mediated endocytosis, phagocytosis, macro-pinocytosis, and plasma or endosomal membrane fusion (Mulcahy et al., 2014). Donor cells change the composition of released exosomes, and lipid rafts and proteins present on the surface of exosomes have been found to impact the exosome absorption rate into recipient cells (Escrevente et al., 2011). PHS is essential for exosome uptake. Anx-V was discovered to significantly inhibit the integration of hypoxia-induced mesenchymal stem cell-derived microvesicles (MSC-MVs) into human umbilical vein endothelial cells (HUVECs). Although other surface molecules may potentially play a role in internalization, the data show that PHS on hypoxia-induced MSC-MVs is a major molecule responsible for internalization (Wei et al., 2016).

Tissue-selective exosomal absorption may, therefore, be influenced by variations in exosomal membrane phospholipids and protein expression. However, it has been shown that exosomes from A549 (lung cancer) cells, HCT116 (heart cancer), and COLO205 (colon cancer) cells are absorbed into both donor and recipient cells, with the exosome absorption level being higher in HCT116 cells regardless of the source cells. This shows that exosomal absorption capacity is not reliant on the production of exosome marker proteins but on the receiving cells (Horibe et al., 2018a). In fact, mesothelial cells, fibroblasts, and malignant mesothelioma cells, all of which are common in the tumor microenvironment, rapidly ingested exosomes from HUVECs (Monaco et al., 2019). Uptake may also occur via receptor-mediated endocytosis (RME). Several alternative types of endocytosis have been connected to the receptor/ligand interaction that facilitates uptake, despite the fact that RME has often been associated with clathrin-mediated endocytosis. Low-density lipoprotein (LDL) and its receptor (LDLR) or transferrin (Tf) and its receptor (TfR) are two examples of well-described receptor-ligand complexes. In order to degrade LDL into free cholesterol for cellular function, the LDL/LDLR complex is endocytosed and transported to the lysosome. However, TfR is able to release its iron payload inside the endosome before being recycled back to the cell surface. The receptor and endocytosis process determine the final destinations of the receptor and the ligand. Whether or whether exosomal uptake is a cell type-specific mechanism hath not been determined. Vesicular uptake is a very specialized process that requires the proper mix of ligand and receptor on both the cell and the exosome for uptake to occur (Zech et al., 2012), yet some studies have shown that exosomes can be picked up by practically every cell type examined (Svensson et al., 2013).

5.4. RNA loading into exosomes

It is possible to selectively load just certain miRNAs into exosomes. Indeed, the exosome composition is influenced by a number of sorting processes that vary with the kind of cell and the physiological state of that cell. Selective sorting during exosome formation is facilitated by RNA-binding proteins (hnRNPA2B1, Ago2, YBX-1, MEX3C, MVP, La protein) and membrane-bound proteins (caveolin-1 and nSMase2). These pathways determine whether miRNAs are able to perform their regulatory activities or become dysregulated, which plays a role in pathogenesis (Groot and Lee, 2020). For example, a group examining myocardial fibrosis revealed YBX-1-mediated sorting of miR-133 into endothelial progenitor cell-derived exosome that raised miR-133 levels in cardiac fibroblasts by boosting mesenchymal-to-endothelial transition (Lin et al., 2019). Exosomes originated from breast cancer cells include the RISC-complex components, but exosomes derived from normal breast cells do not (Melo et al., 2014). This intriguing paper details the tumorigenic impact and maturation of pre-miRNAs inside breast cancer cell-derived exosomes. Recently, it was shown that the KRAS-MEK signaling pathway regulates the preferential packing of Ago2 into colon cancer cell-derived exosomes (McKenzie et al., 2016). This data demonstrates that it is possible to alter the biological activity of miRNAs

in target cells by enriching exosomes with particular miRNAs by utilizing donor cell selective sorting machinery. However, the mechanisms that control miRNA sorting are poorly understood.

Despite their usefulness, exosomes have limitations when used in living organisms because of the difficulty of loading RNAs into them. Several methods exist for using exosomes as a therapeutic delivery vehicle for RNA (Amiri et al., 2022). Transfecting donor cells beforehand to load them up with RNA is one method. In this procedure, siRNA or miRNA is transfected into parental cells, and then exosomes are harvested. MiR-126-enriched exosomes from adipose-derived stem cells (ADSCs) were discovered to have a protective effect against acute myocardial infarction (Luo et al., 2017). Treatment with miR-126-enriched exosomes suppressed angiogenesis, caused cell death, and halted tumor development in vivo in MPM after transfection of HUVECs with miR-126 mimics (a 300-fold increase) (Monaco et al., 2019; 2022). Another research found that cisplatin-resistant gastric cancer might be treated by administering exosomes from HEK293T cells transfected with anti-miR-214 (Wang et al., 2018c). Furthermore, miR-34a-loaded exosomes effectively reduced breast cancer cell growth after transfection of MSCs (Vakhshiteh et al., 2021). Cytotoxicity, low specificity, and ineffective packing are some of the issues that prevent this technology from being as effective as it may otherwise be (Liu and Su, 2019). Donor cells that generate exosomes and naturally encapsulate the miRNA of interest may be able to overcome miRNA-induced cytotoxicity and inefficient miRNA packing when used to address these issues. HUVECs that generate and package miR-126 into exosomes readily and effectively load miR-126, as described by Monaco et al. (2019, 2022).

The cellular-nanoporation technique is yet another way to lessen the pre-loading restrictions. This technique was developed for large-scale synthesis of exosomes containing therapeutic mRNA molecules (Zarovni et al., 2015). Exosomes (55-fold more vesicles) containing transcribed mRNA (1000-fold increase) were secreted from plasmid-transfected cells in response to focal/transient electrical stimulations. Exosomes are electroporated, transfected with targeted reagents, and exosome-liposome hybrids are created as part of the post-RNA-loading method. While microRNAs (miRNAs), small hairpin RNAs (shRNAs), and messenger RNA (mRNA) insertion may all be accomplished using electroporation, this method is insufficient for loading siRNA into exosomes. Transfection of exosomes using particular reagents, such as lipofectamine or Exosome-Fect, allows for the insertion of small molecules, DNAs, or RNAs into isolated exosomes, providing an alternative post-loading RNA strategy. One major drawback, however, is that exosomes cannot be isolated from the transfection reagent using this approach (Li et al., 2018). Combining exosomes and liposomes led to the greatest results. To effectively package the enormous plasmid that is the CRISPR-Cas9 expression vector, a hybrid of exosomes and liposomes has been developed (Lin et al., 2018).

6. Exosomes: A Clinical Perspective

The characteristics of exosomes that we discussed in the preceding paragraph demonstrate their potential as natural MBT transfer carriers. Here, we investigate if and how exosomal characteristics are amenable to therapeutic use. High-yield production, safety, target specificity, cargo internalization, and release are only few of the criteria that exosomes, as a possible MBTs carrier for cancer therapy, must satisfy. Here, we'll explain how exosomes tick all those boxes, and we'll provide some advice for when things go wrong. In addition, we illustrate a novel and intriguing approach to treating cancer that makes use of control of exosome production inside the cell: the use of miRNA-loaded exosomes.

6.1. Synthesis of Exosomes

Exosomes may be obtained by donor cell culture, harvesting from conditioned media, and subsequent isolation or purification. The cells typically employed in the synthesis of exosomes include MSCs, ADSCs, dendritic cells, and HEK293 cells. The exosomes released by MSCs have been investigated extensively for their potential therapeutic applications. For exosome-based treatments to be useful in the clinic, exosome separation is essential, along with the careful selection of donor cells for exosome synthesis. Ultracentrifugation and density-gradient centrifugation are two techniques for isolating exosomes. The most extensively used technique for exosome extraction is ultracentrifugation,

which comprises of repeated centrifugation processes with increasing centrifugal power to successively pellet cells (300 g), cell detritus (10,000 g) and exosomes (100,000 g). In recent years, simple precipitation solutions like ExoQuick and Total exosome Isolation have been developed, eliminating the need for specialized equipment or labor-intensive procedures. When compared to the ultracentrifugation approach, the purification of exosomes from lesser amounts of cell culture medium and blood is an additional benefit of employing these kits. Although their method of action has not been published, these kits are frequently utilized. Precipitation-isolated exosomes, on the other hand, lacked or expressed weakly on exosome hallmark proteins such CD63, CD9, CD81, and HSP70 (Horibe et al., 2018b).

However, the yield of exosome manufacturing requires appropriate inputs and might be enhanced. In this context, the overexpression of exosome biogenesis proteins such STEAP3, SDC4, and NadB is of particular interest (Kojima et al., 2018). Increasing exosome synthesis by modulating the exosome release route is another option. The treatment of donor cells with monensin raised intracellular calcium concentration, which in turn boosted exosome secretion (Savina et al., 2003). Since exosome synthesis and release may be modulated, exosomes can be mass-produced for clinical use (Qu et al., 2023).

6.2. Assurance of Exosome Security

Each therapeutic use of exosomes must be risk assessed to ensure that the validated safety profile from in vivo investigations is not compromised. Indeed, 22 days of IV or IP administration of 1010 modified and wild type HEK293-derived exosomes had no cytotoxic impact or immunological response in immunocompetent mice (Zhu et al., 2017). Patients were routinely given MSC-derived exosomes (MSC-EXOS) with no reported adverse effects (Kordelas et al., 2014). Additionally, clinical trials have been conducted to test the efficacy of tumor and dendritic cells-derived exosomes in activating anti-cancer immune responses, and no toxicity higher than grade 2 has been reported (Dai et al., 2008; Escudier et al., 2005; Morse et al., 2005). Patients with malignant middle cerebral artery infarct (mMCAI) were included in a pilot trial to assess the safety of placental MSC-EXOS. Five patients with ischemic stroke who had intraparenchymal implantation of MSC-EXOS experienced no adverse events (AEs) after the operation. Patients with acute ischemic stroke and post-ischemic impairment may benefit in the future from the safe and effective use of exosomes injected locally for the treatment of mMCAI (Dehghani et al., 2022). All these studies show that exosomes are generally harmless, but it's important to be cautious with exosomes isolated from tumor cells since they could carry oncogenic cargo (Bai et al., 2022b). Tumor cells actively create, release, and use exosomes to promote tumor development (Whiteside, 2016).

6.3. Precision aiming, cargo internalization, and discharge

The distribution of MBTs-loaded exosomes in vivo across tissues is affected by the method of delivery. Malignancies are good candidates for intravenous injection of exosomes. The pharmacokinetic profile of exosomes demonstrated a short half-life in systemic circulation following intravenous administration, with just a trace remaining 4 hours later. The short half-life index in circulation of exosomes after intravenous delivery is one of the key drawbacks of this administration technique. Another option for cancer types is offered by intratumoral injection of exosomes laden with a therapeutic substance. The therapies may be delivered specifically to tumors thanks to the exosomes injected directly into the tumors (Takahashi et al., 2013). One hour after intraperitoneal injection, exosomes were located in the liver, lungs, kidneys, and spleen; following intranasal treatment, exosomes were found throughout the brain and intestines. However, the liver quickly eliminated them from the body's bloodstream (Sun et al., 2010).

It has been reported that in case more nanocarriers are present in circulation, there is a larger possibility that they reach a certain target. The question of whether exosomes have a longer half-life or quicker clearance because of their inherent capacity to overcome physiological barriers (Alvarez-Erviti et al., 2011) is still up for dispute. Since their concentration, circulation time, and half-life in the body are all low, exosome bioengineering has received a lot of attention. For optimal efficacy, exosomes should be designed to attack certain cell types that play a role in the development of a

disease. For instance, rabies virus glycoprotein may be utilized to design exosomes for CNS diseases. It has been shown that miRNA can be effectively delivered to EGFR-expressing cancer tissues via exosomes carrying the GE11 peptide on their surface (Ohno et al., 2013). However, CD47-coated exosomes impede phagocytosis by monocytes and macrophages, leading to a slow clearance rate and a long half-life (Kamerkar et al., 2017). More importantly, the idea of exosome heterogeneity must be addressed if we are to get insight into the targeting capacity of exosomes. As indicated, exosomes' targeting ability toward the receiving cell, as well as their internalization processes and therapeutic effects, are affected by the surface proteins they produce (Kalluri and LeBleu, 2020). Exosomes from B lymphocytes, dendritic cells, mast cells, and intestinal epithelial cells are enriched in major histocompatibility complex class II molecules (Simpson et al., 2008), whereas exosomes released from cancer cells contain higher levels of growth factors and their receptors.

The presence of major histocompatibility complex proteins poses a risk for immunological responses in allogeneic exosomal treatment. To achieve the desired therapeutic effect based on the route of entry, it is possible to choose cell-producing exosomes to target the desired tissue type (Ohno et al., 2013). The ability of a nanocarrier to evade endosomal degradation is crucial to the success of any delivery mechanism that relies on it. It has been discovered that the exosome membrane destabilizes and fuses with the endo-lysosomal membrane during a pH drop, releasing its content in the cytoplasm via a protein-mediated mechanism (Bonsergent and Lavieu, 2019; Joshi et al., 2020). However, the exact mechanisms by which exosomes deliver their cargo remain poorly understood. Prada and Meldolesi (2016) propose using fusion proteins including SYCY1, SYCY2, and EFF-1 to fuse exosome membranes as a technique to circumvent the endosomal process and boost cytosolic miRNA medication delivery.

6.4. A novel approach using exosome-released inhibitors and miRNA-exosomes

Exosome-delivered miRNA-cargo was shown to be quickly released by cancer cells into the tumor micro-environment via exosomes (Monaco et al., 2019), regardless of the mode of administration. Cells have been shown to employ exosomes as a waste disposal system for miRNAs they no longer need (Yu et al., 2016). Cell death was induced by miRNA accumulation inside the cell after exosome release was blocked by an inhibitor of nSMase2 (GW4869) (Monaco et al., 2022). This therapeutic strategy has the potential to circumvent clearance issues, opening the door to single-dose administration of very small amounts of exosomes. Malignancies have been shown to secrete tumor-suppressive miRNAs (Kanlikilicer et al., 2016; Munson et al., 2019), which play a role in controlling cancer cell survival. MiR-29b overexpression leads to caspase-3 activation and death in multiple myeloma (MM) cells (Zhang et al., 2011), while miR-15a/16 may increase MM apoptosis by reducing Bcl-2 expression (Li et al., 2016). Exosome release was inhibited by GW4869, resulting in a considerable decrease in exosomal miR-16-5p and an increase in cytoplasmic miR-16-5p (Munson et al., 2019).

Using an MPM-derived spheroid model, Monaco et al. used a novel strategy by combining the effects of miR-126-loaded exosomes with GW4869 and found encouraging results. In contrast to GW4869, which suppresses autophagy, miR-126 activates the AMPK-ULK1ser-555 pathway, which in turn stimulates autophagy and autophagosome formation. Necroptosis, a kind of cell death linked with the activation of PARP1, results from this short circuit of autophagy induction and inhibition. As stated before, GW4869 inhibits exosome release, leading to increased miR-126 activity and target-cell accumulation (Fig. 4) (Monaco et al., 2022). According to recent research (Mathieu et al., 2019), ceramide production is where exosome biogenesis and autophagy collide. When the MVB and autophagosome vesicles are not digested into the lysosome, they fuse together and are secreted into the cytoplasm via the originating amphisome (Salimi et al., 2020). Given that GW4869 blocks nSMase2, preventing the manufacture of ceramide, ILV biogenesis and MVB formation, and exosome release via the amphisome-forming pathway are all suppressed. In conclusion, both the MVB and autophagy pathways for exosome release are blocked. To maximize the therapeutic efficacy of miR-126, it is necessary to inhibit the crosstalk between exosome creation and autophagy activation after absorption of the miR-126-loaded exosome by cell targets.

6.5. Molecules that block metalloproteinase 2 (nSMase2)

Several nSMase2 inhibitors have attracted growing interest as a technique to manage ceramide levels in different disease states, including cancer (Hwang et al., 2015), due to nSMase2's specific involvement in the regulation of ceramide-dependent exosome release (Verderio et al., 2018). Because of its extremely hydrophobic nature (practically insoluble in water and low solubility in organic solvents like DMSO), the most extensively used research medication, GW4869, has poor solubility, which restricts its therapeutic usefulness. Cambinol was considered a more promising candidate than GW4869 because of its better solubility; nonetheless, its *in vivo* pharmacokinetic profile was found to be subpar. The nSMase2 inhibitors scyphostatin and manumycin A are well-known. However, clinical use of these inhibitors is hindered by a paucity of data on their selectivity, potency, and physicochemical features (Kumar and Kumar, 2021). These hydrophobic medications may be placed in exosomes to improve their solubility, stability, and bioavailability; the drug and exosomes can be incubated together, allowing the drug to diffuse into exosomes along a concentration gradient. Because hydrophobic medicines may interact with lipid bilayer membranes, their hydrophobicity affects how well they are loaded using this approach (Butreddy et al., 2021).

Datta et al. (2018) found that the farnesyl transferase inhibitor tipifarnib had an inhibitory impact on exosomes. Janssen has started phase 2 studies for RAS-dependent solid tumors, phase 3 trials for the possible treatment of pancreatic cancer and leukemia, and phase 2 trials for patients with advanced non-small cell lung cancer. According to Credit Lyonnais Securities, new drug applications (NDAs) for pancreatic cancer and other malignancies were expected to be filed in 2002 and 2003, respectively (Norman, 2002).

Recently, DPTIP (2,6-dimethoxy-4-(5-phenyl-4-thiophen-2-yl-1H-imidazol-2-yl)-phenol) was found to be the most promising compound for inhibiting human nSMase2 after screening 365,000 compounds from the Molecular Libraries Small Molecule Repository (MLSMR) and 2816 compounds from the NCGC pharmaceutical collection (NPC) library (Rojas et al., 2018). The anti-fungal drug ketoconazole (KTZ) has been demonstrated to inhibit exosome synthesis and secretion (Greenberg et al., 2021).

7. In conclusion, and looking forward

MiRNA-based therapy is one kind of RNA-based cancer treatment that has shown promise in clinical trials. A safe, effective, and targeted therapeutic carrier to shield miRNAs from degradation and promote their targeted distribution *in vivo* is missing, despite the fact that their onco-suppressor activity is well recognized. Natural compartmentalization features of exosomes have recently 'beaten' the conventional carriers to give substantial support for miRNA treatment. A novel approach would be to suppress nSMase, an enzyme involved in exosome release, and then deliver the miRNA into cancer cells via exosomes. This therapeutic strategy promotes miRNA accumulation in cancer cells at therapeutically relevant concentrations and times. nSMase is involved in both autophagy and the suppression of exosome release. Cell mortality was increased in cancer cells that rely on autophagy for survival, such as cancer stem cells, when autophagy and exosome release were both inhibited simultaneously.

It is expected that the combination of miRNA-based therapy with an FDA-approved inhibitor of exosome release would improve the onco-suppressive performance of miRNA-based therapy in cancer treatment.

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