MicroRNA heterogeneity: As a Drug Target and Treatment Option in Cancer

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Abstract

Standard cancer therapies for solid malignancies, such as chemo and radiotherapy do not achieve a target-specific strategy against the cancer cells, and do not have full efficiency in numerous cases. Application of chemo or radiotherapy may cause side effects, showing the necessity for urgent need to develop additional strategies for cancer treatment.

MicroRNAs (miRNAs) are small non-coding RNAs, with heterogeneous functions described in almost every known cancer model. Besides their basic function in cancer, as tumor suppressive or oncogenic, they have the ability to modulate chemo or radiotherapy, and to be manipulated with different chemical compounds that make them chemically suitable to be delivered to cancer cells, with no noteworthy side effects, developing another aspect of microRNA benefits-usage of miRNAs as targets for cancer treatment, or therapeutics themselves.

In order to make the efficiency of treatment stronger, the investigation of the expression level of particular miRNAs has been suggested to anticipate the stage of chemo/radio sensitivity. Application of miRNAs solely, or together with standard therapeutic strategies may significantly improve cancer treatment success.

Key Points:

One miRNA can be used as target for cancer therapy or therapeutic itself.

Heterogeneity of miRNAs reflects in the fact that different miRNAs or miRNA combination can be used in various ways of treatment options.

MicroRNAs as therapeutics or drug targets may significantly increase therapy success, applied along with standard cancer therapies.

Short title

MicroRNA as targets and treatment options in cancer

1 Introduction

Malignant transformation is a result of combinatorial effect of genetic and epigenetic changes that lead to cancer formation of various types of cells. Among genetic and epigenetic changes such as promoter hypermethylation, cancer pathogenesis is frequently associated with small non-protein coding genome elements, microRNAs molecules (miRNAs). miRNAs are approximately 19-24 nucleotide long single-stranded molecules that tune gene expression on posttranscriptional level, by binding to the 3' untranslated region (3'UTR) of its partially complementary mRNA targets. The effect of their activity is translational repression, and reduced protein synthesis [1]. Messenger RNA (mRNA) levels and levels of translation of mRNA into the protein depends on miRNA levels and on nucleotide complementarities between 3'untranslated reagion (3'UTR), a sequence that miRNA with its 'seed' sequence [2, 3]. Considering the fact that they repress translation of tumor suppressor and oncogenes, they can be oncogenic, and tumor suppressive miRNAs, respectively [4], and they have the ability to cause side effects that form different cancer phenotypes, and progression.

Diverse miRNA signatures (changes in different combinations of various miRNA expression levels) were linked with hematological and solid malignancies [5]. miRNA expression level profiling may be also used in near future as an additional factor and criterion for classifications of human cancers, rather than expression profiling of mRNA, according to Lu et al. [6], which proposes them as potential therapeutic targets in cancer researches.

Besides miRNA features used in tumor classification, and disease progression prediction, miRNA molecules are discussed, examined, and proposed to be powerful tool for the development of additional drug and therapeutic options in cancer treatment and therapy. The heterogeneity in their function and the heterogeneity in their behavior in different cancer types and subtypes make them usable for various therapeutic approaches and strategies. There is additional level of their heterogeneity; the ability to be manipulated chemically in different ways and used as parts of replacement therapy as miRNA mimics, or to be inhibited by different chemical compounds and genetic systems.

2 Limitations of current cancer therapies

Classical chemotherapy, disrupting the activity of cell vital elements like the cytoskeleton, ribosome, etc., blocks the key enzyme action to inhibit replication, transcription, or translation, or simply harms DNA to arrest the cancer cells proliferation and induces production of free radicals cells. Yet, the classical cancer therapy does not have a target-

specific strategy against the cancer cells. Besides, it causes the toxicity in fast-dividing normal tissue cells in gastrointestinal tract and in bone marrow, giving the rise to the side effects. Accordingly, the target-specific approaches were advanced to particularly inhibit molecular targets managing cancer formation and progression, such as tyrosine kinase inhibitors [7]. The inhibitors generally targets small molecules overexpressed in the carcinomas and having intracellular location. For instance, the tyrosine kinase inhibitor, targeting the growth factor receptors or the effectors in the downstream of the pathway, latterly appeared as the systemic cancer treatment. However, the inhibitors occasionally bind to a wide range of receptors or the downstream elements, causing to decreased specificity and raised toxicity.

On the other hand, more than 50% of patients with solid malignancies undergo radiotherapy (RT) [8]. Radiotherapy results in prevention of highly proliferative cancer cells to divide. Irradiation also induces cell and DNA damages and activates repair mechanisms. If activation of repairing systems of cells and microenvironment is too strong, tumor may develop resistance to radiation treatment. Resistance of tumor to radiation results in earlier tumor recurrence and poor prognosis [8]. Problems such as radioresistance and radiotoxicity could in near future be overcome by introducing therapeutics such as miRNA mimics or antimiR molecules, or can be used to modulate radiotherapy [9]. Considering the heterogeneity of microRNAs involved in biology of malignant tumors, described in our previous article published in this journal [10], it is also important not to neglect their heterogeneity from the aspect of drug resistance. Their heterogeneity reflects in the fact that miRNAs may be used as drugs, or drug targets in cancer treatment as major therapeutic, and as adjuvant therapeutics to sensitize tumors to treatment, to prevent or reduce resistance to therapeutics/therapy, and to reduce toxic effects of treatment. Seeking agents or molecules to enhance cancer cell sensitivity to therapy is the long-term goal to improve the therapeutic efficacy [11].

3 Advantages of miRNA-based cancer therapies

miRNAs can control levels of wide range of genes used in cancer therapy at the same time. It has been presented that targeting a group of associated oncogenic pathways or genes concurrently was associated with good outcome in cancer patients. Besides targeting only cancer cells, miRNAs can further target the tumor-triggering stromal cells like tumor-related fibroblasts and endothelial cells to block tumor fibrosis and angiogenesis needed in the course of tumor formation, progression, and metastasis [12]. Furthermore, miRNAs, which are natural antisense nucleotides, displayed lower toxicity and more decreased immune response

than protein-based drug complexes and plasmid DNA-based gene therapy. Therefore, miRNAs can take a remarkable function in cancer treatment [13].

Majority of drugs bind to, and inhibit protein interactions, and their enzyme or receptor activities. In cancer biology and therapy, in some cases, it is not sufficient to block enzyme and receptor activities, and there is an emerging need for developing novel strategies, among them, miRNAs emerged [14]. There are several strategies, developed to target oncogenic miRNAs in cancer therapy, such as miRNA inhibitors, that bind to mature overexpressed miRNA disabling its processing with RNA induced silencing complex (RISC), maturation, and translational repression of tumor-suppressor mRNA, targets of suppressed miRNA [15].

The basic strategy lies in the oncogenic feature of miRNAs overexpressed in a large number of various cancers. Their oncogeinc ability can be repressed with various molecules named miRNA inhibitors. Inhibitors of miRNA molecules represent synthetic single stranded oligonucleotides, which complementary bind to the endogenous miRNA. We will describe and give the examples of several synthetic anti-miRNA strategies that have the same targets-miRNA molecules, such as locked nucleic acid (LNA) anti-miRNAs, anti-miR oligonucleotides (AMOs), small molecule chemical compounds (SMIRs), antagomiRs, miRNA-zippers [16], and miRNA sponges [15, 17].

Modulation of and targeting specific miRNAs in cancer by using different anti-miRNA techniques can block miRNAs with altered and non-desirable activities and loosen up previously silenced gene transcripts to translate into proteins, usually tumor suppressors and activate their downstream regulatory networks including proapoptotic or DNA-repairing signaling pathways, or can increase drug/therapy sensitivity, thus improving treatment outcome and patients survival.

4 miRNA in drug, chemo, and radiotherapy resistance and sensitivity

Because of the fact that caner is a very heterogeneous disease, and that malignant tumors are frequently comprised of genetically heterogeneous cells, the tumor might develop resistance to chemotherapy [18]. Recently, microRNAs have been reported to play an important role in inducing resistance to anti-cancer drugs. Specific microRNA alterations occur selectively in cancer cells, rendering these cells resistant to various chemotherapeutic agents. For example, resistance to 5-fluorouracil is mediated by alterations in miR-21, miR-27a/b, and miR-155; the sensitivity to docetaxel is influenced by miR-98, miR-192, miR-194, miR-200b, miR-212, and miR-424, and miR-214 [18].

For example, miR-451 was overexpressed in non-small cell lung carcinoma (NSCLC) tissues when compared to normal lung tissue, and overexpression of miR-451 increases cisplatin (DDP) based chemosensitivity in A549 cells by suppressing cell development and triggering apoptosis formation. Bian et al. [19] displayed in their research that miR-451 upregulation increased caspase-3-dependent apoptosis via Akt signaling cascade inactivation, which in turn reduced B-cell lymphoma 2 (Bcl-2), while elevating B-cell lymphoma 2-associated X (Bax) protein levels. Moreover, miR-31 is overexpressed in NSCLC cell lines and was shown to trigger resistance to DDP. To prove this, Glavinas et al. [20] transfected DDP-sensitive SPC-A-1 cells with miR-31 mimics which caused remarkable elevation in the resistance of human lung cancer SPC-A-1 cell line, while transfection with miR-31 inhibitors made firstly resistant NSCLC line (NCI-H1299) sensitive to DDP therapy.

Adjuvant treatment is applied after, or along with the first treatment to boost its efficiency and enhance disease management. It would be advantageous if adjuvant therapy could suppress general resistance mechanisms coming out via genetic and epigenetic alterations in the cancer cell or in their microenvironment by making probably resistant cancer cells sensitive to related agent. Already, numerous preclinical studies have demonstrated miRNA-based therapy agents as hopeful targets for this type of adjuvant treatment. For instance, colorectal carcinoma (CRC) cells were made sensitive to methotrexate by miR-192 [21], to 5-fluorouracil by miR-143 [22], while miR-222 has been thought to take role in drug resistance via controlling a desintegrin and metalloprotease 17 (*ADAM17*) [32]. In lung cancer, miR-379 increases chemosensitivity to cisplatin via eukaryotic translation initiation factor 4 gamma 2 (*EIF4G2*) [24].

Folinic acid, fluorouracil and oxaliplatin (FOLFOX) is the most commonly used treatment option as the primary chemotherapy in advanced colorectal cancer, however just a half of the patients benefits from this therapy and there is no perfect indication in anticipating resistance in such primary treatment administration [25]. The results proved upregulation of serum miR-19a in FOLFOX chemotherapy resistant patients, proposing serum miR-19a level may be a probable molecular biomarker for anticipating and screening resistance to primary FOLFOX chemotherapy application in advanced colorectal cancer patients [25].

Targeting miRNA in radiotherapy may be used to reduce radioresistance, to induce radiosensisitvity, or to predict or reduce acute or late radiotoxicity [9]. miR-21 is one of the important regulators of radioresistance in aplethora of solid tumors [26]. The same authors showed the importance of miR-21 avoiding radiation-induced cell death in leading to

radioresistance in malignant glioma model. Conversely, inhibition of miR-21 activity with anti-miR-21, sensitized glioma cells to radiation, describing novel therapeutic clue for future treatment of glioma malignancies [26]. In NSCLC model, miR-7 and miR-885 were described to be potential prognostic biomarkers of better overall survival after chemo and radiation therapy treatments [27]. Another study showed that RT-resistant cases had significantly lower levels of miR-126 and let-7a in the NSCLC tumor model, comparing with patient group sensitive to radiotherapy. Lower levels of miR-126 and let-7a were associated with poor overall survival, as well. miR-126 overexpression in a lung cancer cell line model induced sensitivity of cells to radiation, by inducing apoptosis [28]. miRNAs may be used as indicators of radiotoxicity in surrounding non-transformed tissue miRNAs may be used in personalized approach for radiotherapy application (dosage) and prediction of response to therapy.

5 Selected examples of miRNAs as drug/therpeutic targets of miRNA inhibitors

The characterization of specific microRNAs involved in oncogenesis has made it possible to formulate new miR-based anticancer therapies finalized to rehabilitate normal physiological functions of deregulated microRNAs. Therapeutic approaches are focused on inhibiting oncogenic miRNA activity (miRNA inhibitors) or by restoring the function of tumor suppressor miRs (miRNA mimics). Selected examples of miRNAs as targets of drugs and therapeutics of miRNA inhibitors are summarized in Table 1.

AntagomiRs are artificial molecules completely complementary to mature miRNA sequence of interest. Song and Rosi [29] identified antagomiR-21 targeting miR-21 on two levels: transcriptional and post-transcriptional in human colon carcinoma HCT 116 cell line model. AntagomiR-21 also significantly increased rates of intracellular mature miR-21 degradation, by competing for and compensating RISK components, necessary for complete miRNA maturation. Interestingly, antagomiR-21 also decreased rates of pro-angiogenic miR-30, ‘hitting’ two oncomiRNAs by one type of molecule. These results indicate the benefits of potential usage of miR-21 as a drug target in colon cancer, and multifunctional role of antagomiR-21 [29].

miR-17/92 members of oncogenic miR-17-92 cluster, were described as potentially good targets for anti-miR therapy by Dereani et al. [30]. Authors introduced specific oligonucleotide having the ability to bind to miR-17. The levels of miR-17 were significantly lowered as well, as the proliferation of chronic lymphocytic leukemia (CLL)-like MEC-1 cells, whose proliferation upregulated onco miR-17 increase. In in-vivo experiment after the

induction of tumor formation by MEC-1 cells injection in severe combined immunodeficient (SCID) mice, showed tha anti-miR oligonucleotide significantly reduced disease progression and significantly improved mouse survival [31].

miR-21 was proposed as potentially good anti-miR therapeutic target for breast cancer models, especially in combination with 4-Hydroxytamoxifen (4-OHT), delivered by poly (d,l-lactide-co-glycolide)-block-poly (ethylene glycol) PLGA-b-PEG polymer coated nanoparticles (NPs) [32]. Devulapally et al. [32] used antimiR-21 and 4-OHT drug combination, and administrated it in ER+ MCF7, BT-474, and ZR-75-1 human BC cell line models and 4T1 mouse BC cells in vitro. Anti-miR-21 administrated by PLGA-b-PEG polymer NP did not show significant antiproliferative feature, but significantly influenced on proliferation rates delivered with 4-OHT. Furthermore, they found significant reduction of proliferative rates when 4-OHT was combined with antimiR-21 in NPs, suggesting PLGA-b-PEG polymer NPs may be good choice for co-delivery of these two molecules and that the combination of these two drugs may be effective for the treatment of estrogen receptor positive (ER+) BC.

Application of antisense-miR-21 in BC MCF7cells has been shown to suppress tumor cell growth in cell culture model, as well, as to suppress tumor growth in xenograft mouse tumor model [33]. The authors have also shown that inhibition of cell proliferation depends on a dose of anti-miR agent, and that reduced levels of miR-21 make tumor cells more sensitive to anticancer factors, and increased apoptosis rates by neutralizing translational silencing of *Bcl-2* [33]. This example is another evidence for potential significance of miR-21 targeting in cancer therapy. There is another experimental confirmation on how miR-21 molecule might be targeted by antago-miR molecule in cancer. miR-21 was silenced by Griveau et al. [34] in an in vitro experiment with locked nucleic acid (LNA)-modified anti-sense oligonucleotide complexed with lipid nanocapsule (LNC), resulted in significantly lower rates of miR-21 molecules and higher rates of caspases, which reduced U87MG glioblastoma (GB) cell viability, by sensitization of GB cells to radiation, and resulted in radiated cell deaths.

Brognara et al. [35] targetetd miR-221 in MDA-MB-231 breast cancer cell line with peptide nucleic acid (PNA), which resulted in significant reduction of onco miR-221 binding to is target mRNA in this case mRNA that translates into cyclin-dependent kinase inhibitor 1B ($p27^{Kip1}$) protein, the product of tumor suppressor gene that was upregulated, which allows to control cell cycle progression. Targeting miR-221/222 with antisense oligonucleotides AS-miR-221 and AS-miR-222 can restore the sensitivity to tamoxifen treatment in [36], and can

regenerate tumor suppressor activity of miR-221/222 targets such as *TIMP3* tumor suppressor, associated with ER positive invasive BCs progression [37, 38].

As a target for antisense therapy (39) used negatively charged liposomal delivery system to apply antisense miR-221 oligonucleotide (anti-miR-221) in hepatocellular carcinoma (HCC) derived HepG2 cells overexpressing transferrin (Tf) receptor. In in vivo experimental model, chemically modified antisense miR-221 molecules coated with Tf-targeted negatively charged liposome, were injected into xenograft mouse model with tumor. It resulted in increased expression levels of phosphatase and tensin homolog (*PTEN*), cyclin dependent kinase inhibitor 1B (*CDKN1B*), and metalloproteinase inhibitor 3 (*TIMP3*), suggested such a delivery system for targeted anti-miR therapy for HCC treatment [39].

miR-10b is overexpressed in numerous tumor types. Ma et al. [40] showed that miR-10b suppression, with the activity of a particular antagomiR, have not decreased primary tumor development, but blocked lung metastasis formation and elevated the expression of the target Hoxd10 in a 4T1 mammary tumor cells transplanted in a Balb/c mouse model [40]. The molecule was extremely specific and well tolerated by healthy animals, administered with the antimiR-10b therapy under the similar settings.

Another interesting feature of miRNAs that contributes to miRNA heterogeneity as drug target is noteworthy to mentione; besides the fact that miRNAs persist in every cell, every tumor cell, and biofluids, miRNAs frequently trafficking in and out of tumor cells can reach all parts of the tumor, or metastatic tumors coated with plasma membrane in the form of exosomes. In this case, it was suggested that let-7, miR-21, and miR-29a may be potenitaly good exosomal target miRNAs, and can be formed by cells of various types of cancers (15). Some cancer cell lines have higher ability of cancer cells of other types of cancer to form exosomes with particular miRNA, introducing additional level of heterogeneity in targeting miRNAs based future cancer treatments [41]. For example, macrophages that express Toll-like receptores (TLRs) frequently absorb exosomes realeased form tumorous cells containing miR-21/29a or let-7 oncomiRNAs described as potential drug targets. The effect would be inhibited oncomiRNAs in cancer cells, exosome levels containing described miRNAs will be decreased, and trafficing by those oncomiRNAs will be reduced [41]. Considering the findings of Lemmann et al. [42], some miRNAs can activate TLRs, so inhibitory molecules of such miRNAs can also prevent TLR signaling cascade activation.

Recently, another model of miRNA targeting approach was tested, miRNA-zippers, miRNA inhibitors, that result in a miRNA loss-of-function phenotypes. miRNA-zipper contains a

nucleotide gap between two miRNAs generating the space to assure the formation of a stable form and to assure specificity in binding for miRNA. In fact, miRNA zipper molecule form a structure that at the 5' end/part of a zipper 5' part/end of miRNA binds, connecting with its 3'end with 5' another miRNA molecule, forming specific structure where miRNA molecules are connected to each other [16]. In the study of Meng et al . [16] miR-17 and miR-221 and, are tested in human breast cancer cell lines, reducing almost 90% of miRNA levels. Moreover, the authors concluded that this strategy, miR-221 zipper reverses doxorubicin resistance with greater efficiency than anti-miR-221 in BC cell lines.

miRNAs can also be the targets of miRNA sponges in cancer. Long non-coding RNAs (LncRNAs) represent a class of epigenetic endogenous elements that saturate miRNA molecules as 'sponges', and by binding to them, they disable miRNAs to bind to its target genes, preventing translational repression or transcript degradation [43]. LncRNAs usually contain several binding sites on its sequence endogenous microRNA (miRNA) sponges to bind to miRNAs and regulate their function, described as competitive inhibitors of microRNA function. Liu et al. [44] showed that (LncRNA) LncSPRY4-IT1 positively regulated the expression of histone-lysine N-methyltransferase-enhancer of zeste homolog 2 (EZH2) via sponging miR-101-3p, an oncogene molecule in bladder cancer, and suggested this lncRNA as therapeutic that effects miR-101-3p activity on its downstream targets. As the second example of miRNA targeted by sponge-acting molecules is [45] in BGC823 and SGC7901 gastric cancer (GC) cell lines, who proposed long non-coding RNA PVT1 as sponge molecule for suppressor miRNA miR-152. miR-152 overexpression suppresses translation of cluster of differentiation 151 (*CD151*) and fibroblast growth factor 2 gene *(FGF2*). In this case, miR-152 is not desirable target, but long non-coding RNA PVT1 is proposed to be targeted by a drug, to prevent sponging and miR-152 activity [45]. These two different examples show how miRNA biology and actions adds a level of heterogeneity in drug selection and drug targeting in a world of small RNAs.

Small molecule chemical compounds (SMIRs) are presented as potential drugs targeting oncomiRNA molecules. Firstly, Melo et al. [46] described them as small compounds targeting specific miRNAs, with the role to inhibit predominantly oncomiRNA activities. Small molecules can inhibit miRNA oncogenic activities on each of the three levels: pre-transcriptional, transcriptional, and post-transcriptional, by preventing their maturation. These small molecules would target either primary (pri-miRNA) or precursor (pre-miRNA) or mature miRNA sequence, on one hand, and on the other molecules, factors involved in

miRNA processing and biogenesis, on the other hand. In addition, these small molecules may also target promoter region of miRNA genes, thus changing/decreasing the transcription rates of a specific miRNA [47]. Gumireddy et al. [48] described infibitors of components of miRNA biogenesis pathway and described the action of small-molecule inhibitor of miRNA function. For example, small molecules regulate transcriptional regulation of miR-21 rather than inhibition of target recognition by miR-21. Small molecule enoxacin, is antibacterial fluoroquinolone, that binds to the miRNA TAR RNA-binding protein 2 (*TRBP*), a miRNA biosynthesis protein. Conversely, [49] described and investigated two compounds; trypaflavine (TPF) and polylysine (PLL), which suppress RISC function. Small molecules that modulate miRNA activity may be used to restore activity and amounts of deregulated miRNAs in cancer pathology.

6 Systemic delivery options of miRNAs as therapeutic molecules

MicroRNAs encoded by expression vectors or miRNA mimics can be utilized to restore the physiological function of miRNAs [50]. Delivery mechanisms upon viral or non-viral (polymers, liposomes) approaches to target cells in vivo are nowadays investigated to increase the efficiency of the molecules remarked above. Nanotechnology-based strategies have being improved and analyzed for their probable clinical usage in solid tumors. Transportation by nanoparticles presents numerous benefits in vitro and particularly in vivo, because of their immunogenicity, low toxicity, target specificity, and uniform nano-size. Many researches on pre-clinical in vivo models have defined the possible efficiency of miR-based treatment choices in various types of tumor [51].

Virus-based transporters like, adenoviruses, adeno-associated viruses, or lentiviruses can more effectively carry miRNAs to interested cells and to save them from the activity of nucleases, extending half-live of miRNAs in the blood. Unfortunately, viral transporters have drawbacks like limited DNA packaging capacity, restricted vector generation, and most scary they can lead life-threatening consequences like immunogenicity reaction or systemic toxicity [52].

Nanoparticle-based non-viral transport mechanisms were improved for optimizing specific transport with fewer problems. Nanoparticles protect miRNAs from lysosomal and/or endosomal degradation, and possess the capability to transport miRNAs to the nucleus or the cytoplasm of the target cell without producing a high toxicity or powerful immune response. The cationic polymers are the most widely preferred nanoparticles; these are positively charged molecules, which can readily be, combine with nucleic acids and display low

immunogenic reaction and toxicity. The artificially derived polymer polyethylenimine (PEI) and its conjugates have been commonly used for gene transport objectives thanks to their low molecular weight, which supports their efficiency and fast uptake and release of the nucleic acid inside the cell [53]. Yet, the main restriction using PEI is the weak biodegradability inside the cell, which causes to its aggregation and cytotoxicity.

Another set of widely used transporters for miRNA transport are the biodegradable and biocompatible liposomes. They imitate the phospholipide cell membrane, providing them to readily infiltrate the cell membrane to transport their content into the cell. However, liposomes possesse low specificity, low sensitivity, and are possibly toxicant [54]. Actually, modified liposomes were benefited as transporters for the first miRNA replacement treatment in a clinical trial, MRX34 (https://clinicaltrials.gov/show/NCT01829971).

7 Selected examples of miRNA molecules as treatment options

miRNA mimics or microRNAs coded by expression vectors can be used as best choices to restore physiological function of miRNAs. Thanks to their biological features and functions, miRNAs can be used as agents suitable for the therapeutic purposes, and biomarkers of chemotherapy, radiotherapy and targeted therapy success. Suppression or restoration of miRNA actions displays high potential in controlling cancer. Numerous researches on pre-clinical patterns have presented the efficiency and applicability of miRNA-based treatments. Nevertheless, in spite of the exciting great potential, some restrictions have to be regarded to achieve passage to clinical administration, due to specific ways of bio-distribution or to probable hazardous effects [55]. Selected examples of miR mimics combined various delivery systems are summarized in Table 2.

Lung cancer is one of the most deadly cancer types, globally. Therefore, there is an urgent requirement to figure out the pathological molecular mechanisms and create innovative treatment choices. For example, let-7 has the ability to directly suppressed lung carcinoma progression both in vitro and in vivo in xenograft immunodeficient mice [56]. Moreover, they found that the intranasal application of an adenovirus expressing let-7 inhibited growth and lung carcinoma production in LSL-K-ras G12D mice, having G12D K-Ras mutation. Furthermore, Trang et al. [57], verified these outcomes, promoting the therapeutic potency of let-7 in NSCLC. Systemic transport of let-7 and miR-34a mimics to lung carcinomas in mice was realized by intravenous administration of miR mimics coated with NLE neutral lipid-based carrier. These complexes do not include cationic lipids and can make easier the

transport of microRNAs within the tumor mass. Mice exposed to miR-34a and let-7 presented remarkable decrease in tumor sizes, supporting the efficiency of the therapy [57].

Hepatocellular carcinoma (HCC) is the third major reason of cancer-associated deaths. miR-124 was defined as a downstream stimulator of the hepatocyte nuclear factor 4α (HNF-4α). miR-124 is important molecule that activates hepatocyte, and liver growth. It presents a central component of an inflammatory feedback mechanism consisting of miR-629, miR-24, signal transducer and activator of transcription 3 (*STAT3*), and interleukin 6 receptor (*IL6R*), responsible for starting tumorigenesis by repressing the same HNF-4α when activated [58]. Administration of miR-124 with liposome complexes restored its activity in HCC mouse model and blocked tumor growth via tumor-specific apoptosis and tumor progression [58]. In addition, miR-122 is a liver-specific tumor suppressor generally downregulated in HCC [59]. Cationic lipid LNP-DP1 nanoparticles enclosing miR-122 were used in an in vivo miR-122-knockout mouse model exposed to the carcinogenic DEN and a Sk-Hep-1 xenograft nude mice model. miR-122 mimics, coated by LNP-DP1, was convenient without any toxicity and, when administered intra-tumor, provided 50% decrease in xenograft tumor development, showing a probable use of this nano-complex in HCC therapy [40].

Also, miR-145 has been stated to be downregulated in breast carcinoma and control the regulation of insulin-like growth factor 1 receptor (*IGF-1R*), fascin-1, *SMAD2/3* and myelocytomatosis oncogene (*c-myc*). Ad-miR-145 adenoviral assembly was applied to MDA-MB-231 breast cancer mice. Up-regulation of miR-145 remarkably reduced breast cancer development. Combinatorial therapy of 5-FU and Ad-miR-145 construct fortified the curative efficiency [60]. To evaluate miR-34a curative potency, Li et al. [61] constructed a T-VISA-miR-34a plasmid, which was particularly triggered in breast carcinoma cases. With the help of a liposomal transport mechanism, T-VISAmiR-34a was intravenously administered to MDA-MB-231 human breast cancer mouse model. T-VISA-miR-34a administration provided miR-34a activity within cancer cells and reduction in tumor mass without noteworthy side effects [61].

Colorectal cancer (CRC) is the third most frequent cancer type all over the world [62]. It has been defined that p53 activation triggers the cyclin-dependent kinase inhibitor p21, which can further move for blocking p53-mediated apoptosis [63]. A recombinant adenoviral vector, which facilitated co-cistronic expression of p53 and synthetic miRNAs targeting p21 (Ad-p53/miR-p21), was constructed to assess its therapeutic efficiency in an in vivo model of nude mice administered with DLD1 or SW480 CRC cells. When the tumor size ranged a stable

volume, adenoviral constructs were applied directly inside the tumor mass. A remarkable elevation in apoptosis, chemosensitivity and tumor downsizing were seen in Ad-p53/miR-p21 applied animals when compared to those exposed to Ad-p53 alone [64]. Moreover, a HCT116 or LS1741T colon carcinoma cells xenograft nude mouse model was intraperitoneally administered with PEI (polyethylenimine)-complexed miR-145, a miR downregulated in colon cancer which targets *c-myc* and extracellular receptor kinase (*ERK5*). A 50% decrease in tumor progression was detected. The same animal model was further exposed to PEI-miR33a, leading to Pim-1 oncogenic kinase suppression and blocking of in tumor development [65]. Dai et al. [66] engineered a vector-based plasmid to evaluate the anti-cancer efficiency of tumor suppressor miR-15a/16-1, whose activity was inversely related to cyclin B1 (*CCNB1*) in CRC [66]. Systemic transport of structures enclosed in cationic liposomes led remarkable supression of angiogenesis and tumor development. Zhai et al. analyzed the function of miR-502, a miRNA downregulated in CRC, which inhibits autophagy via targeting Rab1B, on CRC tumor development in a HCT116 xenograft mouse model. They displayed that miR-502 intra-tumor administration provided reduction in tumor development [67].

8 Application of miRNAs in therapy success prediction

In a study investigating the probable usage of circulating miR-451 in serum to predict neoadjuvant chemotherapy (NACT) resistance in breast cancer, the relative expression of miR-451 was remarkably reduced in both the NACT-sensitive group and the NACT-resistant group when compared with the control group of patients [68]. Furthermore, they detected that the relative miR-451 expression was decreased in the NACT-resistant group when compared to the NACT-sensitive group. Therefore, these findings suggest that the circulating miR-451 level might possess a functional importance to predict the NACT resistance in BC patients [68]. Anti-EGFR monoclonal antibodies (anti-EGFRmAb) are used in metastatic colorectal cancer (mCRC) treatment, but patients having a Kirsten rat sarcoma viral oncogene homolog/ v-raf murine sarcoma viral oncogene homolog B1 (*KRAS/BRAF*) mutation, and nearly one-half of those not having mutation do not benefit from the treatment [69]. miRNA investigation was realized to detect miRNAs anticipating anti-epdermal growth factor receptor (anti-EGFR) mAb efficiency. Significant miR-592 underexpression and miR-31 overexpression were found in progressive disease comared with control group. let-7 miRNA family members presented notable upregulation in the patient group with poor overall survival. Moreover, miR-1224-5p underexpression and miR-140-5p overexpression were

associated with poor overall survival. In mCRC patients having wild-type *KRAS/BRAF*, miRNA profile may effectively predict the success of anti-EGFR mAb therapy [69].

Treatment success ratio of preoperative chemoradiotherapy (CRT) ranges from complete regression to resistance in locally advanced rectal carcinoma. Local resection (LR) applications are recently investigated to lessen surgical morbidity and to enhance functional consequences for those patients responding well to CRT. Suitable grading processes are necessary to keep up the oncologic efficacy. Yet, latest clinical evaluation and imaging methods require further enhancement. Five miRNAs related with rectal carcinoma (miR-31, miR-18b, miR-17, miR-193-3p and miR-20a) were investigated in the plasma of rectal cancer patients. Expression levels were evaluated before during and after the CRT, and were tested with respect to lymph node status of the patients. Four of miRNAs showed up trustworthy outcomes in the plasma. miR-17, 18b, 20a, and 193-3p levels changed at previously described time points. The expression of miR-20a and miR-18b during CRT were associated with negative lymph node status. The coincidence of decreased miR-20a and miR-18b expressions with lymph node negativity after preoperative CRT may assist to stratify the surgical procedure in the matter of complete meso-rectal excision [70].

9 Conclusion

The worth of miRNAs as therapy agents is widely accepted. Recently, the eternal precise molecular specification of cancers made it feasible to anticipate the responses to treatments, and generate targeted drugs and personalized schemes to manage cancer. Expression statuses of miRNAs are associated with tumor growth and aggressiveness, and the estimation of chemo/radio/targeted treatment success. In vivo and in vitro experimental studies have displayed the applicability of rehabilitating the normal or, contrarily, repressing the abnormal activity of deregulated miRNAs in cancer: the miRNA formation presents low antigenicity, and mimics have been readily transported via effective and well-tolerated vectors, like nanoparticles. miRNA activity has been repressed independently by short synthetic structures, particularly designed to enhance specificity, stability and binding efficiency to target. According to literature listed in this review, targeting miRNAs might be much more effective in cancer treatment along with standard therapy approaches, suggesting that miRNA targeting may also improve existing therapeutic models. Some miRNA molecules can be excellent targets for various types of drugs that silence their oncogene activity and reverting resistance to other drugs and radiotherapy or both, adding a new level of heterogeneity in cancer

treatment strategies. MicroRNAs can be very valuable targets for various therapeutic mechanisms, increasing the success of cancer treatment.

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