Chapter 3

Gene Silencing Agents in Breast Cancer

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Abstract

MicroRNAs (miRs) are a class of non-coding RNAs, approximately 20–25 nucleotides long, discovered in the nematode, *Caenorhabditis elegans*, in 1993. There are two primary categories of non-coding RNA (ncRNA): (1) short interfering RNAs (siRNA) and (2) microRNAs (miRs). In general, miRs control protein production via partially complementary binding of the mRNA 3′UTRs. Both siRNAs and miRNAs are critical regulators of developmental and homeostatic processes as well as disease pathogenesis. While the treatment of advanced stage breast cancer presents several challenges, the development of therapeutic resistance contributes to a high mortality rate. Dysregulation of miR expression has been implicated in progression of breast cancer disease. Moreover, miRs have been found to play a role in the development of drug resistance. In this context, one of the therapeutic potentials of miRNAs is the correlation of circulating miR levels with breast cancer progression stages and disease phenotypes. Secondly, researchers are investigating novel delivery strategies for the substitution or silencing of ncRNAs involved in the disease. This chapter describes both the general miRNA mechanism of actions and the miRNAs related to breast cancer research. It is specifically designed for breast cancer researchers with expertise in gene delivery, clinicians, and clinical translational scientists.

Keywords: microRNA (miRs), short interfering RNAs (siRNA), gene regulation and gene silencing, target recognition, breast cancer, triple negative breast cancer, therapeutic agents, clinical trials, nanoparticle

1. Introduction

Gene silencing by RNA occurs when dsRNA induces cleavage of its complementary mRNA, which is known as RNA interference (RNAi). RNA interference (RNAi) is the mechanism that suppresses gene expression or translation through the activity of RNA interference molecules (RNAi), by the neutralization of mRNA molecules. RNAi is also part of the cell's endogenous

biological defense system which protects it against transposons and viruses. miRNAs and siRNAs are vital players in driving RNA interference mechanisms. These small RNAs bind mRNA molecules and either decrease or increase their activity and preventing their translation into protein.

In addition to RNAi, studies have identified several endogenous small RNA types including endogenous siRNA (endo-siRNA), small noncoding RNAs (miRNAs), and piwi-interacting RNA (piRNA) which has created a wider view of normal and pathological cellular mechanisms [1]. The above types are considered non-coding RNAs (ncRNAs) which describes any RNA that does not encode a protein [2, 3]. Since the early 1990s, ncRNAs including miRNAs and siRNAs have been the subject of intensive research. Although non-protein coding RNAs (ncRNAs) were first imagined as simply 'junk' RNA with no functional purpose, they have emerged as potent gene silencing factors [4]. Despite the importance of miRNA in medicine, mechanisms of genes targeting, their interaction with other cellular RNAs, as well as their mRNA editing capabilities are not yet fully understood [5]. It is clear that, the role of ncRNAs in the regulation of genes renders them putative targets for the development of novel drug therapies. As effectors, siRNAs and miRNAs can be used to silence or 'switch off' specific cancer genes. Thus, gene silencing via RNA interference (RNAi) is evolving as an aspect of cancer chemotherapy that could be personalized for individual patients. The therapeutic potential of miRNAs and siRNAs to play a key role in the treatment of cancer and other genetic diseases has been extensively investigated [2]. With the aim of mitigating side effects on healthy tissue, recent approaches have involved the selective targeting of mutant cancer cells.

At present, the main hurdle in implementing miRNA and siRNA therapy in the clinic is the absence of an efficient and targeted delivery system that would protect ncRNAs from degradation by endogenous RNAses and permit them to reach the precise tumor target [6]. Recently, nanoparticle technology and breast cancer targeting immunoconjugates for the delivery of siRNAs and miRNAs were demonstrated to control breast cancer tumor growth and metastasis [7].

MicroRNAs (miRs) are genomically encoded small, non-coding, single stranded RNA 18–25 nucleotides that regulate gene expression during embryonic development. In normal cells they act as a delicate switch in regulating cellular functions such as cell proliferation and cell differentiation [6]. Various preclinical research studies have sought to establish diagnostic, prognostic and therapeutic uses for miRNAs [3]. As a result, downregulated miRNAs in cancer cells are referred to as tumor suppressor miRNAs, while miRNAs that permit the expression of genes involved in cancerous processes are called oncomiRs [1]. siRNAs (with 21–25 nucleotides) are produced from exogenous double stranded (dsRNA); their structure is closely related to that of mature miRNAs. Nevertheless, similarities in the mechanisms and biogenesis of siRNAs and miRNAs have been observed. For example, both depend on Dicer enzymes and on Ago proteins. Most miRNAs, for example, are excised from the precursor (pre-miRNAs) by Dicer while the Ago will support the silencing [8].

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2. Molecular mechanisms for triggering silencing through microRNAs

In principle, miRNAs are generated in the nucleus via long primary miRNA transcript (pri-miRs), which are converted by the microprocessor complex into a 70-nucleotide stem-loop structure. This complex consists of Drosha (an RNase III enzyme) and a dsRNA binding protein critical region 8 (DGCR8) DNA sequence. After binding, the dsRNA component of pre-miRNA is cleaved. As partial cleavage occurs, the pre-miRNA is transferred from the nucleus to the cytoplasm through the exportin-5 pathway and further processed by Dicer; the dsRNAs now consists of an inactive passenger strand and an active mature strand. When the 'mature' miRNA is incorporated into the RISC complex it triggers a silencing effect on the target.

With regard to gene silencing and its possible applications in the clinic, miRNA can target multiple sites and thus modulate the expression of many genes. Recognition of mRNA occurs when it binds to a short sequence of nucleotides rather than to the total 21 nucleotides that form an siRNA.

To initiate RNAi, an miR can be partially complementary, binding to multiple mRNAs to block their expression. It is noteworthy that the mechanism of action of an miR is distinct from that of an RNAi in that miRs inhibit the translation of the mRNA [6]. In contrast, a small siRNA is perhaps the most frequently employed RNAi mechanism to silence protein coding genes in the short term. SiRNA is a synthetic RNA duplex structure designed to target specific mRNA in order to degrade it. In the laboratory, gene knockdown is commonly achieved in many cell types using siRNA. Normally, a perfect match is required between the siRNA oligonucleotide and the target mRNA sequence. Finally, siRNA can also be used to knockdown non-protein coding genes e.g. long non-coding RNAs (lnRNA) [9].

siRNAs and miRNAs are highly potent compared to small therapeutic molecules. In addition, both can act on proteins which lack enzymatic functions as well as those which cannot be reached by conventional drug molecules. Currently, two main therapeutic strategies are based on miRNAs, namely: (1) replacement of miRNA or (2) miRNA inhibition. Inhibition of miRNA can be conceptualized as antisense therapy, since synthetic, single stranded RNAs, act as miR antagonists which inhibit the activity of endogenous miRNAs. In contrast, synthetic miRNAs can replicate, substitute, or enhance the function of endogenous miRNAs. Thus, exogenous miR therapy results in mRNA degradation or inhibition leading to gene silencing.

siRNA, for its part involves the introduction of a man-made siRNA into the target cells to trigger RNA interference (RNAi), leading to inhibition of the mRNA expression and thus to gene silencing. Both miRNAs and siRNAs have similar physicochemical characteristics, but their functions are quite separate. Although their mechanisms of action are the same- both are short RNA duplexes that target mRNAs to silence gene, miRNA employs translational repression, the degradation of the mRNA and occasionally, endonucleolytic cleavage of mRNA, while siRNA works exclusively via the endonucleolytic cleavage of target mRNA [2].

3. Challenges in breast cancer diagnosis and treatment

In the Middle East, 25% of all reported cases of female cancer in 2012, were breast cancers. It has been projected that by 2020 about 2 million women will be diagnosed with breast cancer worldwide, a disturb increase of approximately 18.4% [3, 10]. Moreover, about 30% of Middle Eastern patients with newly diagnosed early stage breast cancer will go on to develop metastasis despite extensive therapy [6].

To prevent an advanced stage breast cancer diagnosis, women undergo periodic screening including self-breast examination and yearly mammograms beginning at the age of 40. More frequent monitoring is recommended for women at a high risk of contracting the disease, including those with a family history of it or genetic predisposition [11]. An epidemiological survey concluded in January 2017 reported that female breast cancer is the most frequently diagnosed cancer worldwide [12]. Though the gold standard for detecting breast cancer is mammography, it can cause significant discomfort, and it is not consistently reliable for the detection of smaller tumors at an early stage [3, 13].

3.1. Classification of breast cancer

Breast cancer has been traditionally classified in the clinic as either non-invasive or invasive, according to grade and stage. The classification system is based on the histological features of breast samples as well as the location of abnormal tissues. The two main forms of non-invasive breast cancer are ductal carcinoma *in situ* (DCIS) and lobular carcinoma *in situ* (LCIS). Non-invasive forms remain localized to the breast and do not migrate to nearby tissues and organs. However, most breast cancer are invasive and have been classified as infiltrating ductal carcinoma, tubular carcinoma, invasive lobular carcinoma, medullary carcinoma, inflammatory carcinoma and colloid carcinoma. Breast cancer is considered metastatic if it spreads to the other regions of the body through the blood or lymphatic systems.

Breast cancers are currently classified according to four molecular subtypes: luminal A, luminal B, HER-2 enriched and basal-like cancer. This classification is based on the expression level of the progesterone receptor (PR), estrogen receptor (ER) and the human epidermal growth factor receptor 2 (HER2). Seventy percent of breast cancers are responsive to hormone receptors and present an overexpression (abundance) of either PR or ER receptors or a combination of the two. Luminal A type cancers are known to overexpress the hormonal receptor (ER^{+ve} and/or PR^{+ve} and $HER2^{-ve}$), while luminal B type cancers overexpress all 3 receptors (ER^{+ve}/and/or PR^{+ve} and HER^{+ve}). Luminal A and B cancers are characterized by significant gene expression in the luminal epithelial layers of the mammary glands. However, breast cancers that have been shown to have an abundance exclusively of HER2 are referred to as HER2 enriched and account for about 20% of all cases. Basal-like breast cancers have distinct gene signatures. They are mainly triple negative breast cancer (TNBC), which means that the tumors do not express any hormone receptors. TNBCs are held to account for approximately 10% of all cancers of the breast that are known to have a high mortality rate. mRNA profiling

has revealed that to date there are ≥6 identified molecular subtypes of triple negative breast cancer. Variable MiR expression levels are observed in each type. These breast cancer classifications include the immunomodulatory (IM) subtype, luminal androgen receptor (LAR), basal-like (BL1 and BL2), mesenchymal stem cell-like (MSL) and mesenchymal (M) [14].

3.2. Diagnostic approach and treatment of breast cancer

The increasing use of modern adjuvant therapy (systemic) and diagnostic tools has resulted in enhanced treatment of early stage breast cancer patients, producing a substantial increase in the overall survival time from diagnosis. However, improvements in the treatment of relapsed metastatic cancer have been marginal at best. Therefore, there is an urgent demand to develop novel therapies to treat malignant and late stage breast cancer [9, 3]. At present the treatment options include surgery, chemotherapy, immunotherapy, hormonal supplements and radiotherapy [6]. Targeted hormonal therapy is available for patients with breast cancers that have been shown to aberrantly express an excess of receptors for hormones. Studies indicate that hormone receptor type breast cancers have a more favorable prognosis than other types. Hormonal therapies are often prescribed following surgery as an adjuvant treatment. For example, tamoxifen prevents the interaction between hormones and their target receptors, while aromatase inhibitors decrease the levels of circulating hormones. Physicians have administered tamoxifen for more than three decades to treat hormone receptor-positive breast cancer (PR^{+ve}, ER^{+ve} or both). Tamoxifen acts as an estrogen receptor antagonist; it blocks the binding of estrogen with its endogenous receptors. Similarly, herceptin, a human anti-HER2 monoclonal antibody, acts by binding to the HER2 receptor thus preventing the generation of growth factors signals in breast cancer cells. Herceptin and tamoxifen can be given alone or together with chemotherapy drugs such as doxorubicin or paclitaxel and radiotherapy if required. However, the heterogeneity of breast cancers remains a fundamental barrier against the accurate molecular classification of the cancers and the implementation of individualized therapy. It is noteworthy that not all patients with hormone receptor overexpression (ER^{+ve} and/or PR^{+ve}) respond favorably to tamoxifen therapy and not all patients that overexpress HER2 respond to Herceptin indicating the presence of other unknown factors that influence the response to breast cancer treatment.

At present, no effective targeted treatment options are available for triple negative breast cancer (TNBC) patients [6]. The degree of genetic aberrations and the absence of HER2, PR and ER receptors render TNBC patients unresponsive to traditional hormonal therapy. TNBC is all too often resistant to the cancer chemotherapy drugs available at present including paclitaxel and epirubicin, which are platinum-based drugs [2]. The definitions of luminal A and luminal B cancers in the clinic have been set using arbitrary criteria, rather than taking gene expression levels into account. This is probably due to the substantial heterogeneity in ER^{+ve} tumors, which can confound the selection of an appropriate treatment regimen. But the Ki-67 index can distinguish between luminal A and luminal B cancer types. Ki-67 is a protein that is used as a cellular marker for proliferation and may serve to identify a potential fifth molecular subtype, normal-like breast cancer, which is similar to luminal A but is less proliferative,

thereby showing decreased Ki-67: (ER^{+ve} /PR^{+ve} /$HER2^{-ve}$ /$Ki67^{-ve}$). Luminal B cancer can also be further subdivided into two subgroups: (ER^{+ve} /PR^{+ve} /$Her2^{-ve}$ /Ki-67^{+ve}) and (ER^{+ve} /PR^{+ve} / $Her2^{+ve}$ /Ki-67^{+ve}).

Nowadays, much research effort has focused on the further sub-classification of breast cancer types, based on gene expression and gene mutations including miRNA signatures. However, the prognostic value of this approach is still not clear [3, 6]. A better understanding of gene expression patterns and genetic mutations should help to determine predictive and prognostic markers and identify novel therapeutic targets that could induce the selective silencing of gene expression [6]. It is known that BRCA1 and BRCA2 genes are mutated in all types of breast cancer including TNBC. The expression of PLK1 has been reported as a possibly important marker for TNBC; in addition TP53, GADPH, HRAS, PCNA, CCND1, BIRC5, MYBL2 and IGFBP6 have been reported as being significantly down-regulated or up-regulated genes that are differentially expressed in TNBC cells compared to normal cells, while other research groups have reported the elevated expression of the AR and EGFR gene among TNBC patients [6].

During the last decade, the targeting of small molecular weight protein kinases and monoclonal antibodies against cell surface receptors has shown great promise in the ongoing struggle against cancer. Unfortunately, many of the key genes that cause cancer are still considered to be 'non-druggable', and as a consequence insufficient research has been applied to targeting them. Most tumors, due to their heterogeneity and their genetic instability are highly unlikely to present a single target that is found suitable for a long term treatment regimen [9]. RNAi has been shown to be able to rapidly and efficiently suppressing the expression of any gene in many cell types, thus highlighting the possibility of treating cancer by drug action on any gene that has been shown to induce cancer.

4. miRNA vs. siRNA for gene silencing in treatment of breast cancer

Current research on the drugs that silence genes has provided important insights into the possible value usefulness of these drugs for the treatment of various types of cancer. A number of mRNA targets, as well as interference in mRNA functions by miRs, will almost certainly make major contributions to our understanding and treatment of many diseases. Thus, the clinical application of miRs as diagnostic biomarkers is developing at a rapid pace, with the gene silencing effect of siRNAs on specific genes making them ideal tools for target identification and validation in drug discovery and development.

4.1. Mechanism of target recognition by miRNA and siRNA

The target recognition of miRNAs is a multifaceted process due to the presence of various binding sites and differing degrees of complementarity between the miRNA and the target mRNA; that is, to induce a functional effect, miRs need to be partially complementary to their target mRNAs. Complementary pairing between mature miRNA and mRNA takes place at

the 3′UTR group on the mRNA and the seed region of the miR. In contrast, other miR binding sites, such as the 3′ supplementary sites, centered sites and bulged sites are believed to be aberrant. Therefore, since perfect matching is not required, one miR strand can target an extensive number of mRNAs; thus one miR often has multiple targets. For example, due to partial complementary base pairing between the miR and the mRNA, AGO2, which plays a vital role in RNA silencing, miRISC is not stimulated. Alternatively, the mRNA targets themselves, silence miR via degradation by deadenylation or translation repression via exonucleases or decapping. Rarely, a high degree of complementarity between the miR and its mRNA target may produce endonucleolytic cleavage of the mRNA through the activity of the AGO protein. This activity is quite similar to the gene silencing mediated by siRNA [2]. In contrast, the siRNA must be matched fully with its target mRNA. The activation of AGO2 after complementary binding, leads to the cleavage of the phosphodiester bonds of mRNA bases 10 and 11 relative to the guide strand 5′ end. Any mRNA fragments produced as a result of this activity undergo rapid degradation through the actions of exonucleases [2].

4.2. Role of RNAi technology in breast cancer therapy

In the context of breast cancer therapy, target genes can be silenced by inhibiting miRNA with drugs or by substituting exogenous miRNA. This type of inhibition therapy is deemed useful when tumor cells are known to overexpress the target miRNA. The therapy involves the introduction of single-stranded, synthetic, RNAs that effectively act as miRNA antagonists. In this regard, the inhibition of miRNA is analogous to antisense inhibition. In contrast, cancer be treated by replacing miRNA when the target miRNA is deactivated or repressed, a common feature of cancer. This strategy involves introducing double stranded synthetic miRNAs into cells, which will mimic the actions of the target miRNAs. They bind to the target gene and initiate mRNA degradation which silences the target gene. miRNA mimics are more straightforward to use than siRNA, because the sequences are nearly identical to the endogenous miRNA [6].

In an alternative approach, siRNAs can be produced through gene expression or by chemical synthesis. The strategy governing chemical synthesis is to create synthetic RNAs that can be introduced into cells by a number of methods. The gene expression strategy can produce siRNA using expression constructs, including viral vectors and plasmids, that can be transcribed inside the cell and express short hairpin RNA (shRNAs) that are the precursors of siRNAs. When siRNAs are chemically synthesized this method provides fine control over the purity and quantity of the siRNAs produced. Furthermore, the chemical structure can be modified to improve the stability of the product, which is important when the delivery technique has to be considered. Synthetic siRNAs can also incorporate fluorescence labels using high-resolution fluorescence microscopy to analyze siRNA and its localization in cancer cells [6]. After the introduction of the siRNA into the cell, gene silencing is initiated, a process directed by the endogenous RNAi machinery. Within the cell, duplex siRNAs are incorporated into the RNAi pathway. The antisense strands are loaded onto the RNA induced silencing complex (RISC), which acts as a guide to permit the recognition of complementary mRNAs. Once the target sequence is identified, AGO2 protein, which is a constituent of the

RISC complex, cleaves the mRNA decreasing the protein levels produced by silenced gene. The main advantage of using siRNAs vs. conventional drug therapy is that they exhibit very low toxicity and a high degree of specificity. However, non-specific targets can be affected mainly because the miRNA-like activity of the siRNAs can act at the seed-like sequence of the 5′ end leading to a stimulation of the innate immune system induced by dsRNA [6].

It should be recalled that both siRNAs and mimetics engage with the same related RNAi pathways and interact with the RISC complex. Although siRNA and miRNA appear to be similar in all respects profound differences have been identified. For example, it is known that miRNAs are reproduced as primary miRNA transcripts (pri-miRs) through the activity of miR genes. The controlling nuclear complex partially cleaves the miRNA and resulting stem loop pre-miR is translocated from the nucleus to the cell cytoplasm where it is acted on by Dicer, thus transforming it into dsRNA. The dsRNA now consist of a mature or active strand or may incorporate an inactive "passenger strand". miRNA which is mature then interacts with the RISC complex to silence genes. Short dsRNAs, termed siRNAs, are assemblies of 21- to 23 base pairs, with typically 2 nucleotide overhangs at the 3′ ends, although numerous variations have been detected in their length and the degree of overhang. Structurally, they contain an active guide strand and an inactive passenger strand and can be considered 'exogenous RNAs' that can engage with endogenous RNAi pathways. It should be noted that miRNA often has defective complementarity, usually because of changes in the seed sequence at the 5′ end [6]. This finding stands in stark contrast to siRNA and its target mRNA, where there is a perfect fit (full complementarity) between the two composite molecules.

5. miRNA and its expression in breast cancer

It is well known that in most of the cancer cells studied so far the expression of miRNA has generally decreased. This is not surprising, for miRNAs generally act as tumor suppressors in most cells. Moreover, lower levels of miRNA are found in tumor cells that are poorly differentiated in contrast to those with a higher degree of differentiation. This fundamental finding suggests that global changes in the expression levels of miRNA are intimately associated with the degree of differentiation of aberrant cells. It has been demonstrated recently that a number of miRNAs are expressed at lower levels in tumor-derived cell lines than in the corresponding human tissue. Another study has shown that down-regulation of the expression of miRNA levels in cancer cells resulted in tumorigenesis and that the knockout of Dicer and Drosha, molecules necessary for miRNA biogenesis, led to the complete loss of expression of miRNA. Finally, accelerated growth with enhanced invasive properties occurred when tumor cells were injected into nude mice, strongly suggesting that the loss of miRNA expression leads to enhanced tumorigenesis [7].

Several studies have tackled miRNA profiling and their results indicate that many miRNAs are overexpressed in breast cancer. Although several functions of miRNAs have been investigated, it has become clear that many types of experiment will be required to establish whether miRNAs can be used as novel therapeutic agents or as diagnostic markers. One fact is clear:

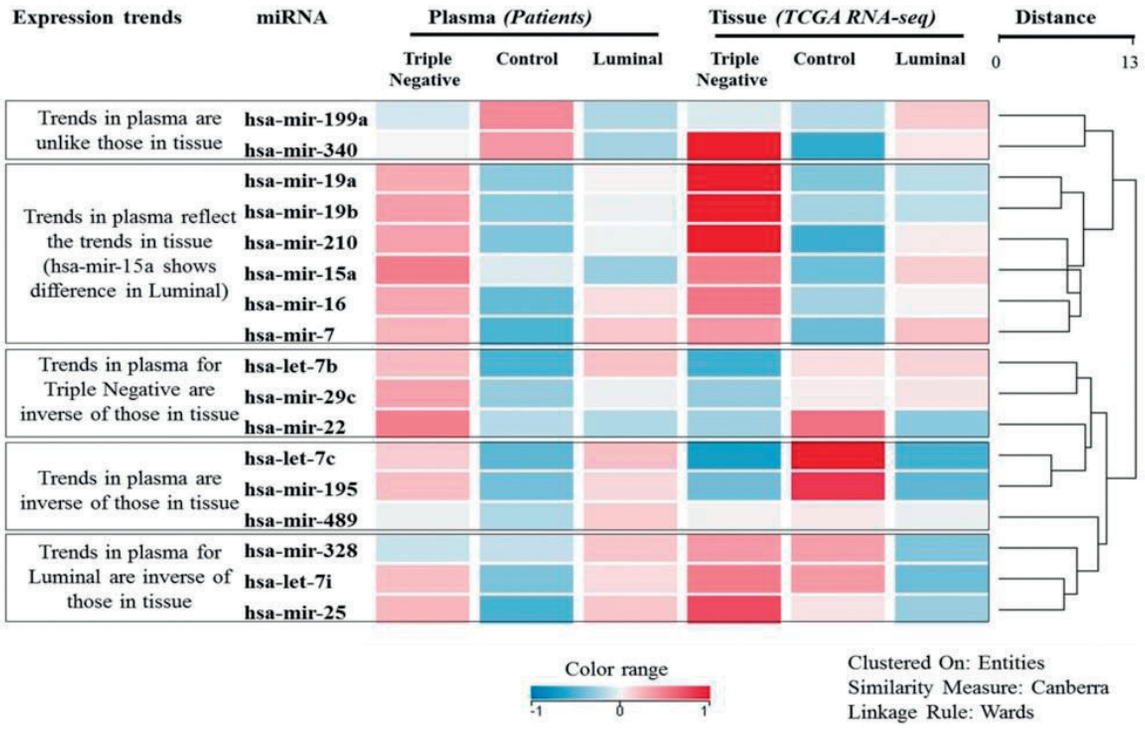

Figure 1. Hierarchical clustering view of the normalized expression levels of miRNA measured in plasma and corresponding miRNA precursor levels in tissue obtained from TCGA RNA-seq data.

tumor metastasis may be promoted through the enhanced expression of pro-oncogenic/pro-metastatic miRNAs or via the down-regulation of anti-oncogenic/anti-metastatic miRNAs. A number of miRNAs have been shown to be deregulated in breast cancer, indicating that particular miRNAs may be involved in the modulation of oncogenesis [3, 15]. miR-10b, miR-125b and miR-145 have been shown to be down-regulated, while miR-21 and miR-155 were up-regulated, strongly suggesting that these miRNAs play important roles as tumor suppressor genes or contribute to creating an oncogene supportive environment [16]. Recently it has been demonstrated that miR-19a, miR-19b, miR-210, miR-15a, miR-16 and miR-7 are overexpressed in plasma as well as in TNBC tissue (**Figure 1**) [3]. At present, a number of research studies have reported on the oncogenic role of miR-19a/b in TNBC tumor development, which occurs due to the repression of PTEN and activation of NF-kB [3, 17]. In addition, the circulating levels of miR-19 have been associated with the efficacy of the epirubicin + paclitaxel chemotherapy regimen in Stage II and III patients with luminal A breast tumors [3, 18]. In a Japanese TNBC patient cohort study, it was reported that a high hsa-miR-210 expression level was an independent risk factor for poor prognosis [3, 19].

6. RNAi as therapeutic in clinical application

In terms of possible clinical applications, a key difference between siRNA and miRNA is that an siRNA specifically targets a single site on a unique mRNA, and initiates inhibition

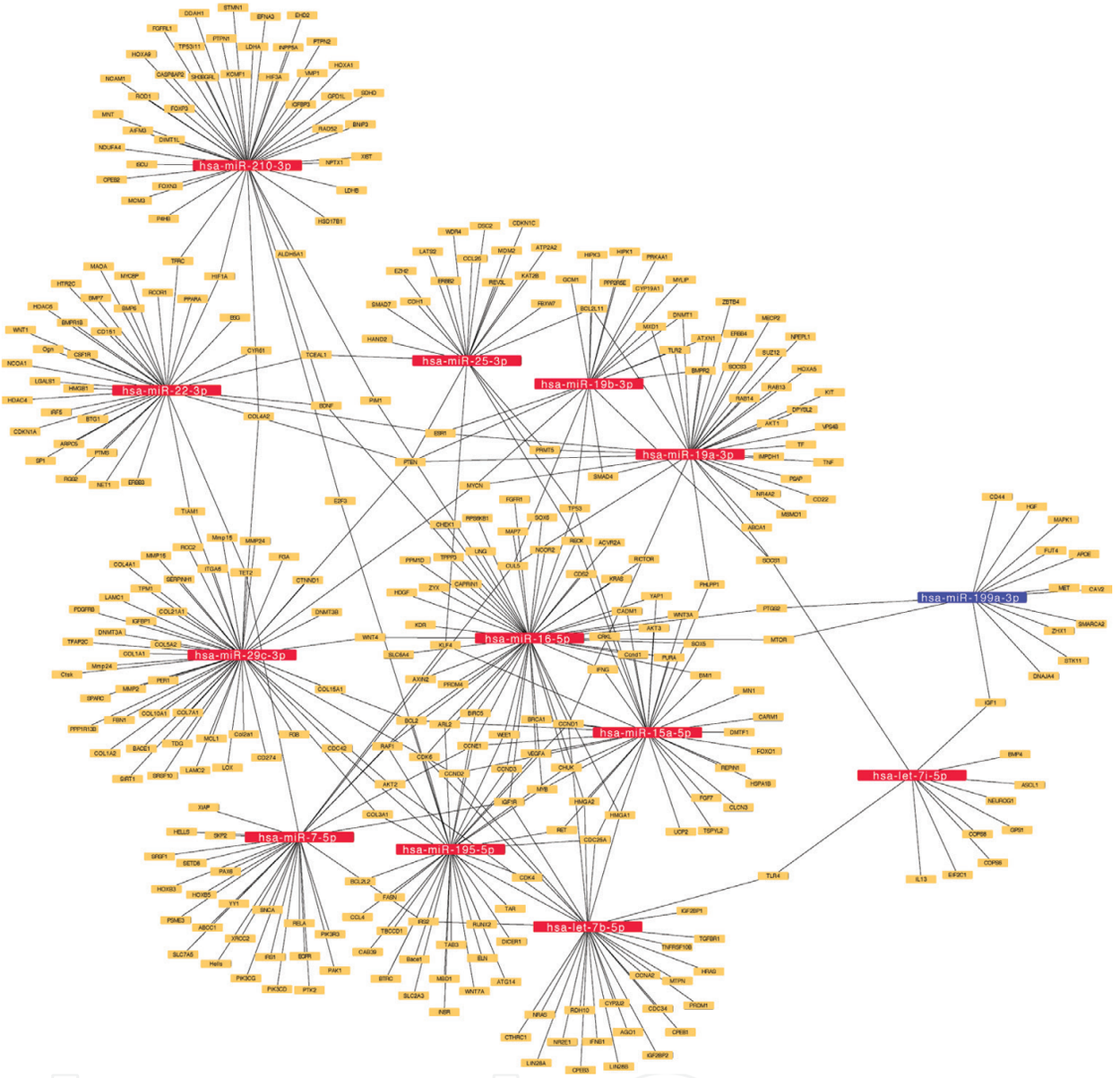

Figure 2. microRNA-mRNA interaction network.

of expression of a single target gene. In contrast, an miRNA molecule usually has multiple mRNA targets and regulates the activity of a number of genes (**Figure 2**). For an mRNA to be recognized by an miR, just a short binding sequence is necessary rather than the entire nucleotide sequence of siRNA. In contrast, the siRNA must be entirely complementary with its target mRNA to enable it to initiate RNAi. However, miRNAs partially complement and bind to multiple mRNAs to inhibit their expression. In addition, their mechanism(s) of action are quite different: siRNAs are known to cleave mRNAs, but miRNAs in contrast act to inhibit the translation of mRNAs. This leads to the inevitable conclusion that scientists will have to develop techniques for targeting specific cells, thus avoiding the down-regulation of the genes expressed by normal cells. The objective will be the delivery of siRNAs and miRNAs to specific targets exclusively in tumor cells. Taken together, three key procedures must be

undertaken before RNAi treatment can be given to cancer patients in the clinic, namely: (1) the target gene(s) driving the development of a specific cancer need to be unequivocally identified; (2) an siRNA that specifically targets a particular gene must be fabricated and (3) the man-made siRNA must be capable of being delivered into the target cell cytoplasm [6].

About 20 clinical trials have been initiated to study the great potential of siRNA- and miRNA-based therapy. Only 1 miRNA therapeutic drug, SPC3649, an inhibitor of miR-122, known on the market as miravirsen, has entered clinical trial [20]. A number of other miRNA-based therapeutic agents are currently in the preclinical stage of testing with the aim of introducing the most promising candidates into clinical trials in the near future. At present, many siRNA-based drugs have begun clinical trials for many researchers believe that miRNAs will become a unique category for RNAi-based therapy [20, 21]. The actions of miRNAs are similar to those of siRNAs regarding the post-transcriptional silencing of genes. This chapter has reviewed the biological features of miRNAs and described them as endogenous short RNAs that interact with Argonaute proteins and regulate the expression of many genes. While both siRNA- and miRNA are important for gene regulation at the translational level, endogenous siRNAs help to maintain the stability of the genome. Both are single-stranded and have been shown to be associated with RISC complex components. However, there are fundamental differences between the siRNA and miRNA mechanisms of action. miRNAs most often attach to 8 nucleotides that comprise the 5′ end to enable them to bind to the target mRNA sequences and thus use their inhibitory activity to limit the translation processes. On the other hand, siRNAs use nearly all of their full sequences to identify a particular target and thus mediate cleavage of the targeted mRNA [20]. Taken together, both siRNA and miRNA based therapies are currently under pre-clinical development as breast cancer treatment options.

7. Conclusion

The silencing of gene through RNAi is a natural process that can be found in cells which are mainly involved in the degradation of mRNA and occur in the post-transcriptional phase. Their current status in treatment and diagnosis faces severe challenges because the traditional drugs are not very efficient and also because resistance to the treatment has developed. Silencing the gene that causes this resistance or the genes which cause tumors to form can be regulated by inhibiting them. RNAi i.e., miRNA and siRNA hold great promise in this situation in that these RNAs are involved in silencing the gene. Since their discovery, just over 20 years ago, these genes have been applied as therapeutics and for this they are synthesized synthetically. Not only in cancer, but also in the treatment of other disease as well, they have had a great contributory effect. The genes are very attractive in therapeutic approaches: they have the potential to target any gene in a virtual aspect since they can be synthesized as complementary to their target gene. If compared with conventional drugs, however, their mechanism of action depend on binding with the target site of the target molecule. Though the use of siRNA and miRNA as therapeutics has been found effective, it faces technical barriers such as its delivery to the target tissue, specificity and chemical modification. Delivery to the target tissue requires proper administration to obtain the desired effect. The gene should

design carefully, lest it bring about the silencing of other related genes and unwanted effects. A proper safety profile should be maintained in designing the delivery of the RNAi which is highly important for its efficacy. Along with these factors, information regarding silencing effect of the gene, the dosage required for the desired effect, the stability of the RNA molecules, the release of the RNA from the delivery system, and its half-life and turnover of the target proteins are equally important.

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Conflict of interest

The authors declare that they have no competing interest.

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References

[1] Irimie AI, Zimta AA, Ciocan C, Mehterov N, Dudea D, Braicu C, Berindan-Neagoe I. The unforeseen non-coding RNAs in head and neck cancer. Genes. 2018;**9**(3):1-20. DOI: 10.3390/genes9030134

[2] Lam JK, Chow MY, Zhang Y, Leung SW. siRNA versus miRNA as therapeutics for gene silencing. Molecular therapy. Nucleic acids. 2015;**4**:e252. DOI: 10.1038/mtna.2015.23

[3] Qattan A, Intabli H, Alkhayal W, Eltabache C, Tweigieri T, Amer SB. Robust expression of tumor suppressor miRNA's let-7 and miR-195 detected in plasma of Saudi female breast cancer patients. BMC Cancer. 2017;**17**(1):799. DOI: 10.1186/s12885-017-3776-5

[4] Wright MW, Bruford EA. Naming 'junk': Human non-protein coding RNA (ncRNA) gene nomenclature. Human Genomics. 2011;**5**(2):90-98

[5] Li Z, Rana TM. Molecular mechanisms of RNA-triggered gene silencing machineries. Accounts of Chemical Research. 2012;**45**(7):1122-1131. DOI: 10.1021/ar200253u

[6] Ahmadzada T, Reid G, McKenzie DR. Fundamentals of siRNA and miRNA therapeutics and a review of targeted nanoparticle delivery systems in breast cancer. Biophysical Reviews. 2018;**10**(1):69-86. DOI: 10.1007/s12551-017-0392-1

[7] Venugopal ANaSK. Translational research on breast cancer: miRNA, siRNA and immunoconjugates in conjugation with nanotechnology for clinical studies. In: Done S, editor. Breast Cancer – Recent Advances in Biology, Imaging and Therapeutics. InTech Europe: InTech; 2011. pp. 361-380

[8] Carthew RW, Sontheimer EJ. Origins and mechanisms of miRNAs and siRNAs. Cell. 2009;**136**(4):642-655. DOI: 10.1016/j.cell.2009.01.035

[9] Shen H, Mittal V, Ferrari M, Chang J. Delivery of gene silencing agents for breast cancer therapy. Breast Cancer Research. 2013;**15**(3):205. DOI: 10.1186/bcr3413

[10] Albeshan SM, Mackey MG, Hossain SZ, Alfuraih AA, Brennan PC. Breast cancer epidemiology in gulf cooperation council countries: A regional and international comparison. Clinical Breast Cancer. Jun 2018;**18**(3):e381-e392. DOI: 10.1016/j.clbc.2017.07.006

[11] Coughlin SS, Ekwueme DU. Breast cancer as a global health concern. Cancer Epidemiology. 2009;**33**(5):315-318. DOI: 10.1016/j.canep.2009.10.003

[12] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA: A Cancer Journal for Clinicians. 2017;**67**(1):7-30. DOI: 10.3322/caac.21387

[13] Weinberger M, Saunders AF, Samsa GP, Bearon LB, Gold DT, Brown JT, Booher P, Loehrer PJ. Breast cancer screening in older women: Practices and barriers reported by primary care physicians. Journal of the American Geriatrics Society. 1991;**39**(1):22-29

[14] Qattan A. Metabolic reprogramming of triple-negative breast cancer: The role of miRNAs. MicroRNA Diagnostics and Therapeutics. 2017;**3**:1-8. DOI: 10.1515/micrnat-2017-0001

[15] Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR. MicroRNA expression profiles classify human cancers. Nature. 2005;**435**(7043):834-838. DOI: 10.1038/nature03702

[16] Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, Magri E, Pedriali M, Fabbri M, Campiglio M, Menard S, Palazzo JP, Rosenberg A, Musiani P, Volinia S, Nenci I, Calin GA, Querzoli P, Negrini M, Croce CM. MicroRNA gene expression deregulation in human breast cancer. Cancer Research. 2005;**65**(16):7065-7070. DOI: 10.1158/0008-5472.CAN-05-1783

[17] Mathe A, Scott RJ, Avery-Kiejda KA. miRNAs and other epigenetic changes as biomarkers in triple negative breast cancer. International Journal of Molecular Sciences. 2015;**16**(12):28347-28376. DOI: 10.3390/ijms161226090

[18] Li Q, Liu M, Ma F, Luo Y, Cai R, Wang L, Xu N, Xu B. Circulating miR-19a and miR-205 in serum may predict the sensitivity of luminal A subtype of breast cancer patients to neoadjuvant chemotherapy with epirubicin plus paclitaxel. PLoS One. 2014;**9**(8):e104870. DOI: 10.1371/journal.pone.0104870

[19] Toyama T, Kondo N, Endo Y, Sugiura H, Yoshimoto N, Iwasa M, Takahashi S, Fujii Y, Yamashita H. High expression of microRNA-210 is an independent factor indicating a poor prognosis in Japanese triple-negative breast cancer patients. Japanese Journal of Clinical Oncology. 2012;**42**(4):256-263. DOI: 10.1093/jjco/hys001

[20] Chakraborty C, Sharma AR, Sharma G, Doss CGP, Lee SS. Therapeutic miRNA and siRNA: Moving from bench to clinic as next generation medicine. Molecular Therapy. Nucleic Acids. 2017;**8**:132-143. DOI: 10.1016/j.omtn.2017.06.005

[21] Hydbring P, Badalian-Very G. Clinical applications of microRNAs. F1000Research. 2013;**2**:136. DOI: 10.12688/f1000research.2-136.v3