

miRNA and its Dysregulation in Cancer Progression

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ABSTRACT

MicroRNAs [miRNAs] are conserved group of small, non-coding RNA involved in post-transcriptional gene regulation. Several studies have found that downregulation of miRNA after post transcriptional changes and negative effects on miRNAs acting as the activators of gene expression. Aberrant expression in body fluids of miRNA is a helpful diagnostic approach to early detection of cancer. Various mechanisms by which miRNA gets dysregulated include: dysregulation in miRNA biogenesis, abnormal transcription of miRNA, dysregulated epigenetic modification and genomic aberrations. Aberrantly expressed miRNA has been studied to affect the hallmarks of cancer. Modified miRNA causes some diseases which could be avoided or overcome by controlling miRNA gene expression. A novel biomarker by miRNA could be utilized for therapy purposes because miRNA controls certain gene expression in cases of cancer as well as some other ailments. In this review, we focused on the biogenesis and regulation of miRNA along with the dysregulation of it in human cancer and other diseases, along with highlighting some of the role of miRNA in therapy. By comparing previously studies, it is anticipated that miRNA based treatment would be one of the reliable and highly recommended to diagnosis or suppress cancer cells.

Keywords: miRNA biogenesis, miRNA regulation, miRNA degradation, Oncogenic miRNA, Diseases

1. Introduction

MicroRNA [miRNA], refers to single stranded, small [nearly 22-24bp in length], evolutionary conserved, non-protein coding regulatory gene found in eukaryotes [originally described in *C. Elegans*] [1], which plays a very vital role in gene silencing and its regulation, at the post-transcriptional level [2]. Nearly 1-5% of the total human genome is composed of miRNA and regulates nearly 30% of protein coding genes [3]. There are around 2500 miRNAs recognized until now and miRNA genes are transcribed by RNA polymerase II, which are found in either sense or antisense orientation, mainly in the intergenic region [they are also found in intronic and exonic regions]. It has been found to regulate various biological processes and cellular functions such as cell proliferation, differentiation, apoptosis and metabolic pathways [2] and modulates gene expression in a cell by targeting the mRNAs in one of the two ways. Either the miRNA upon being nearly complementary to the mRNA, leads to the induction of RNAi [RNA mediated-interference] pathway, in which the miRISC [miRNA-associated RNA-induced silencing complex] formed cleaves the mRNA transcript leading to negative gene regulation, or, by binding of the miRNA to the 3' UTR [3' Untranslated region] of the target mRNA, leading to inhibition of translation via miRISC formation [4].

Apart from regulating the normal biological processes, aberrant expression of miRNA leads to plethora of diseases, along with driving the oncogenic pathway. Chronic Lymphocytic Leukemia [CLL], was the first studied cancer condition due to deletion and downregulation of miR-15 and miR-16. These two miRNAs target the antiapoptotic factor B cell lymphoma 2 [BCL2] gene of the host cell which leads to reduced apoptosis of the cancer cells, enhancing cancer progression [2]. Apart from deletion, other genetic mutation in TARBP2 and XPO5 [the proteins involved in miRNA

processing and maturation], and epigenetic alterations [such as histone modification and aberrant DNA methylation] lead to miRNA dysregulation in cancer. Studies have also reported the potential role of dysregulated miR-372, miR-373, miR-216a/217 in cancer cell proliferation and induction of Epithelial Mesenchymal Transition [EMT] [4]. Further, the role of dysregulated miR-122, miR-33, miR-208, miR-103 etc. has been elucidated in the regulation of diseased condition other than cancer, such as, HCV infection and related diseases, Atherosclerosis, Cardiac diseases, Diabetes etc. [5].

Henceforth, the ability of miRNA to target altered mRNA, has been utilized to study therapeutic potential of miRNA in treating various diseases through drug sensitization [2]. miRNA-based therapeutics in the form of miRNA mimics [as a therapeutic], and, anti-miRNAs [as a target of therapeutics] have been studied. RNA-delivery techniques in-vivo, has made this technique feasible in treating diseases [5]. miRNA has also been proposed as potential biomarker of various cancer due to its stable presence in body fluid [as circulating miRNA] and easy detection in tumor biopsies [as non-circulating miRNA] [6]. In this review, we focus on the biogenesis and regulation of miRNA, and its dysregulation in cancer, along with highlighting on the role of miRNA as therapeutic target and biomarker.

2. miRNA Biogenesis and Regulation

2.1 miRNA biogenesis

Genes for miRNA are located either within introns or exons of the coding genes [about 70%] or the intergenic areas [30%] and are evolutionary conserved. Intergenic miRNAs are in relation to their host gene expression while all intragenic miRNAs have independent transcription units [7] and regulation by their own promoters. miRNA is

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biosynthesized generally by two pathways; the canonical and the non-canonical pathway.

2.1.1 Canonical Pathway

This is the main pathway for the processing of miRNAs. In this pathway, the microRNAs are transcribed and processed by RNA polymerase II, this leads to the generation of a long primary transcript known as the pri-miRNA which is several kilo bases long [7]. In the nucleus, the pri-miRNA is processed with the aid of a microprocessor complex comprising of an RNA binding protein DiGeorge Syndrome Critical Region 8 [DGCR8] and a ribonuclease III enzyme known as Drosha. A N6-methyladenylated GGAC and other motifs in the pri-miRNA are recognized by DGCR8. Drosha is involved in the cleavage of the pri-miRNA duplex at the base of the hairpin structure of the pri-miRNA [8]. This leads to the production of a transcript of a 70 kb miRNA precursor known as the pre-miRNA [7]. This leads to the formation of a 2 nt 3' overhang on the pre-miRNA. As soon as the pre-miRNAs are generated, they are exported to the cytoplasm with the aid of a complex known as exportin 5 [XPO5]/RanGTP complex and following this is processed by RNase III endonuclease enzyme Dicer which is associated with TAR RNA binding protein [TRBP], a double strand RNA binding protein [9]. This processing is concerned with the removal of the terminal loop, therefore resulting in a mature miRNA duplex. The name of the mature miRNA form is determined by the directionality of the miRNA strand. There is the emergence of the 5p strand from the 5' end of the pre-miRNA hairpin while the 3p strand is originated from the 3' end. Both the strands that are obtained from the mature miRNA duplex can be loaded in an ATP-dependent manner to the Argonaute [AGO] family of proteins. The thermodynamic stability at the 5' ends of the miRNA duplex or a 5' U at nucleotide position 1 is dependent for the selection of the 3p or the 5p strand. The strand with a lower 5' stability or 5' uracil is loaded to the AGO and is known as the guide strand while the unloaded strand is called as the passenger strand, which unwinds from the guide strand by various mechanisms based on its complementarity [8]. The passenger strand of the miRNA duplex leaves to produce the single stranded mature miRNA thereby returning the AGO to its original conformation. AGO promotes assembling of a ribonucleoprotein complex known as RISC after loading, this mediates the recognition of the target mRNA. Mature miRNA are guided to their target specific mRNA with the help of base pairing. An adaptor protein known as the Trinucleotide repeat containing 6 [TNRC6] is recruited by the AGO which further interacts with the PABPC protein [poly [A] binding] at the 3' end of mRNA. There is recruitment of deadenylase complexes [most significantly the carbon catabolite repressor 4-negative on TATA [CCR4-NOT] complex]. Deadenylase causes the shortening of the mRNA poly [A] tail leading to the destabilization of the mRNA by decapping and 5' to 3' exonuclease activity. TNRC6 results in low translation efficiency aided by CCR4-NOT and its recruitment of the DEAD-box helicase 6 [DDX6] which attaches to the decapping

complex and is reported to inhibit translation. mRNA destabilization is the most common repression mediated by the mammalian mRNAs and it leads to translation repression though its effect is weaker.

2.1.2 Non-canonical Pathway

Mainly Drosha and DGCR8 are essential for processing the canonical miRNAs while in their absence non-canonical miRNAs are produced. In non-canonical pathway of miRNA biogenesis, there are several pathways under it which include the Drosha-independent and the Dicer-independent pathways [9]. The pre-miRNAs generated by Drosha/DGCR8 independent pathway have resemblance with the Dicer substrates. Mitrons is an example of such a pre-miRNA that are generated from mRNA introns during splicing process, another such example is of the 7-methylguanosine [m^7G]-capped pre-miRNA. The exportin-1 facilitates the export of the nascent RNAs to the cytoplasm without the Drosha cleavage. There is a strong 3p strand bias for the m^7G cap hence preventing the 5p strand from loading to the Argonaute. On the other side, the Dicer-independent miRNAs are processed from endogenous short hairpin RNA [shRNA] transcripts by Drosha. AGO2 is required for the complete maturation of the pre-miRNAs within the cytoplasm as they are insufficient in length to be Dicer substrates, this promotes loading of pre-miRNA entirely into AGO2 and AGO2-dependent slicing of the 3p strand. Further, the maturation is completed by 3'-5' trimming of the 5p strand [8].

2.2 miRNA regulation

miRNAs have the ability of reduce gene expression by various modes and pathways. Various observations have proved that miRNAs perform their functions in the form of effector complexes known as miRNP, miRgonaute, or miRISC, along with Argonaute, which is the most important constituent of all miRNPs, instead of working as naked RNAs [10]. The Watson-Crick pairing of 5'-proximal "seed" region [nucleotides 2 to 8] in the miRNA into the seed match site in the target mRNA that is mostly positioned mostly in the 3' UTR acts as a specific determinant for miRNA recognition of the target. A small subset of miRNAs are claimed to modulate expression by specific targeting of the 5' UTR and/or the coding region of some mRNAs. The most important factor is the exact base pairing between the miRNA seed region and the target site [11]. The degree of miRNA-mRNA complementarity of the regulatory mechanism acts as a significant determinant.

The miRNAs guide miRISC for specific recognition of the messenger RNA or mRNA and further downregulation of gene expression by either of the post-transcriptional mechanisms [PTM]: [i] translational repression and [ii] mRNA cleavage. As mentioned earlier, the animal miRNA binding sites are mostly located in the 3' UTR as multiple copies and the degree of complementarity enables the Ago-catalyzed degradation of the target mRNA sequences by mRNA cleavage process [12].

2.3 Translational repression

The exact mechanism for target mRNA translation repression by miRISC is still not clear and also whether it occurs at the translational initiation or post-translational level is unknown. A mechanism through which miRISC exerts its action by repression of the elongation process. Based on many studies, it was suggested that miRISC promotes the early dissociation of ribosome from mRNAs. Recent studies have suggested three models to explain the mechanism of miRISC mediated repression of the initiation mechanism. In the first model, the miRISCs were seen to compete with eIF4E for binding to the mRNA 5' cap structure that leads to translation initiation failure [13, 14]. Few studies contradict the model and suggest that GW182 or a downstream factor could be acting as the eIF4E competitor. The second model suggests that miRISC prevents mRNA from circularising thereby resulting in translation inhibition. The C-C chemokine receptor 4-negative on TATA [CCR-NOT] complex consists of multiple proteins, named chemokine [C-C motif] receptor 4 [CCR4], chromatin assembly factor 1 subunit [CAF1], and NOT1-NOT5. These are involved in the regulation of gene expression and therefore may be associated with miRISC translation inhibition [12]. The third model suggests that there may be inhibition of the assembly of the 60S ribosomal subunit with the 40S preinitiation complex by miRISC. Therefore, in the process the 40S ribosomes are attached to the targeted mRNA while the 60S ribosome subunit fails to bind the 40S subunit, thereby resulting in translation repression [15, 16].

2.4 miRNA degradation

The target mRNA degradation processes are aided through Ago protein slicer activity when miRNAs have a high degree of complementarity. A fall in the mRNAs along with the abundance of miRNA suggests that miRNAs play a role in mRNA degradation. Other mechanisms along with Ago-catalyzed mRNA degradation such as deadenylation, decapping and exonucleolytic digestion of mRNA also play a role in mRNA degradation. Also, GW182 and the cellular decapping and deadenylation machinery are essential for the mRNA degradation. It has been seen that the type, number, and the mismatch positions in miRNA/mRNA duplex play an important role in the selection of degradation or translational repression [12].

mRNA degradation is initiated first by deadenylation from the 3' end and/or decapping from 5' end by enzymes like DCP1/2. The missing poly [A] tail and cap structure exposes the remaining RNA for exonucleolytic action by the enzyme known as Xrn1p. The truncated mRNA, the missing poly [A] tail can be exposed to the 3'-5' degradation by cytoplasmic exonucleases. Parallel, sequence-specific endonucleolytic mRNA cleavage may occur by polysomal ribonuclease 1 [PMR1] [11].

3. miRNA and Its Dysregulation in Cancer

Both genetic and epigenetic mechanisms have been elucidated to induce miRNA dysregulation thereby driving

the cell fate towards an oncogenic pathway. A large amount of human miRNA genes being located at fragile genomic sites, are prone to alteration or mutation such as deletion, translocation and amplification in cancer. The Biogenesis pathway of miRNA is altered during cancer, where transcription of pri-miRNA, the initial stage in the miRNA biogenesis is mutated, leading to cancer initiation and progression. Point mutation in miR-128b leads to glucocorticoid resistance in acute lymphoblastic leukemia [ALL] cell due to the blocking of pri-miR-128b processing. Apart from genomic alterations, altered miRNA expression in cancer is regulated by the activity of aberrant transcription factors. pri-miRNA transcription is controlled by the alteration in the oncogenic factors and tumor suppressors acting as transcriptional repressors and activators [17]. The pathways are discussed in three successive paragraphs.

3.1 Tumor suppressive miRNA dysregulation

p53 [a tumor suppressor] transcriptionally regulates the expression of miR-34 family during DNA-damage response which represses growth-promoting genes and inhibits cell proliferation and induces apoptosis. But in cancer cells, the activity of p53 and DNA damage response is altered. A concomitant decrease in let-7, a family of miRNA which targets the mRNA encoding oncogenes such as KRAS, has been observed in various cancer. Dysregulated let-7, has also been studied in breast cancer stem cell self-renewal and differentiation [18]. miR-200 family targets the mRNA encoding ZEB1 and ZEB2 [Zinc-finger E-box-binding homeobox], the transcription factor involved in promoting Epithelial Mesenchymal Transition [EMT] associated with cancer metastasis, thereby downregulating it. But, in human tumors, it has been studied that, ZEB1 and ZEB2 interact with the regulatory element in the promoter of miR-200 thereby repressing the transcription of miR-200 leading to downregulation of it, with subsequent enhancement of EMT, a crucial step in cancer. Other than promoting EMT, downregulation of miR-200 leads to increased expression of Interleukin-8, involved in promoting angiogenesis in cancer [19]. miR-520 has been studied to be downregulated in ovarian and breast cancer, thereby promoting tumor growth and metastasis [20] miR-506 has been studied to target mRNA encoding proteins involved in DNA-damage response [RAD51], metastasis [SNAI2]. And its downregulation has been observed in ovarian cancer leading to enhanced metastasis [21]. During Chronic lymphocytic leukemia [CLL], the chromosomal section 13q14.3 where miR-15/16 lies, has been studied to be deleted, leading to the downregulation of it. Downregulation of miR-15/16, which otherwise targets BCL-2, CDC2, leads to the cancer progression [22].

3.2 Oncogenic miRNA dysregulation

MYC [a proto-oncogene] activates the expression of miR-17-92 cluster [a set of oncogenic miRNA] which targets E2F1, THBS1 [Thrombospondin] and other mRNA expression, thereby regulating cell cycle progression and angiogenesis in cancer. MYC has also been studied to

repress tumor-suppressive miRNA in BCL [B-cell lymphoma progression [48]. miR-210, which targets the mRNA coding for Succinate dehydrogenase complex unit [SDHD] in hypoxic condition, has been seen to be upregulated in various cancer types, thereby decreasing the expression of SDHD in cells, resulting in increased HIF1 α and cancer cell survival. It has also been studied to increase tumor angiogenesis by downregulating ephrin A3 [hypoxia-responsive angiogenesis inhibitor [23]. miR-21 with antiapoptotic role has been seen to be upregulated in various cancer studies. During cancer, the chromosomal locus containing miR-21 is amplified along with upregulation of AP-1 [a transcription factor] which binds to the promoter of miR-21. TGF β 1 [Transforming Growth Factor Beta 1] and STAT3 [Signal transducer and activator of transcription 3] have been studied to play an important role in the upregulation. Moreover, TGF β 1 stimulates its receptor TGF β 1R thereby leading to the formation of cancer associated fibroblast by activating SMAD2 and SMAD3 [the transcription factors] and targeting SMAD7 (An inhibitor of the signaling cascade leading to cancer-associated fibroblast formation) [24]. miR-21 targets PDCD4 [programmed cell death protein 4] leading to decreased expression of it, resulting in reduced apoptosis and increased metastasis [25]. The transcription factor, NF- κ B [Nuclear factor- κ B] binds to the promoter region of miR-155, thereby increasing its expression in cancer cells. This links cancer with inflammation [26].

3.3 *Dysregulation of the enzymes involved in miRNA biogenesis*

Drosha and Dicer, two of the important proteins involved in miRNA biogenesis has been studied to be downregulated in cancer. The transcription factor MYC regulates the expression of DROSHA, leading to reduced expression of pri-miRNA. Apart from this, during hypoxic condition, ETS1 and ELK1 [the hypoxia-responsive transcription factors] bind to the DROSHA promoter, leading to its downregulation in cancer. The downregulation of transcription factor TAp63 leads to the downregulation of DICER. Apart from this, miR-103/107, let-7 targets the 3'UTR of DICER and downregulates it. The epidermal growth factor receptor[EGFR]-dependent phosphorylation inhibits AGO2 [the biogenesis protein]. As a result of this inhibition, the AGO2 does not properly bind to Dicer, resulting in cell survival and increased invasiveness [5]. It is seen that reduced Dicer and Drosha expression has been associated with high grade Breast Cancer and shorter metastasis -free survival [27]. This reduced Dicer phenomenon is also observed in other kinds of cancers like Prostrate [28], gastric [29], or squamous cell carcinoma [30]. More to this, it is seen that in Breast Cancer nucleolin, a component of Drosha/DGCR8 microprocessor complex, has been demonstrated to promote the maturation of a set of metastasis promoting miRNAs [miR-221/222 cluster, miR-21, miR-103, and miR-15a/16] [31, 32]. Dysregulation and alternation are also seen in the Nuclear Exporting protein, XPO5, a key protein for pre-miRNA export to the cytosol has also been suggested as a possible biomarker for Breast Cancer [33].

4. **miRNA and Its Involvement in CSC, EMT and Chemoresistance**

A Subset of cells (having the ability for self-renewal, differentiation, resistance to chemotherapy and responsible for tumor initiation and growth) are referred to as Cancer Stem Cells [CSCs]. Existence of CSCs leads to therapeutic resistance, disease relapse and progression. miRNAs such as let-7 have been studied to show CSC phenotype thereby regulating self-renewal and differentiation. Role of miR-34a has also been elucidated in the regulation of CSC by suppressing the expression of CD44, NOTCH1, RAS and other target genes. miR-17-92 cluster has also shown its potential role in regulation of Glioma Stem Cells [GSCs]. Therefore, miRNA can serve as novel therapeutic strategy to target CSC by regulating the gene expression of it, mediated by miRNA [34]. Recent studies have discovered the role of miRNA in cancer progression and invasion apart from showing stem cell characteristics. miR-21, is the first studied miRNA having multiple targets such as JAG1, Bcl2 and PTEN leading to upregulation of EMT [Epithelial Mesenchymal Transition], one of the major steps prior to invasion of cancer cells, where the cells undergo phenotypic conversion to an invasive one, leading to metastasis and secondary tumor growth. Downregulation or inhibition of miR-200 has been studied to downregulate E-Cadherin expression thereby upregulating EMT. Migration and Invasion of cancer cells leading to colonization and dissemination has also been linked to miRNA. miR-10b has been correlated with metastasis in breast cancer. It has further been associated with migration and invasion by targeting the HOXD10 [repressor of genes involved in cell migration] and Syndecan-1. Other than these, miR-193b, miR-632, miR-125b etc. has been associated with increased migration and invasion of cancer cells. Angiogenesis, a vital process in the formation of blood vessels around solid tumors has also been associated with miRNAs. miR-9 has been studied to activate JAK-STAT pathway leading to angiogenesis. miR-519c, regulates HIF1-A, which changes according to oxygen content in the microenvironment, resulting in the formation of blood vessels. miR-126 and miR-34a have also been associated with tumor angiogenesis, thus playing an important role in cancer cell growth, proliferation and invasion [35].

5. **miRNA Dysregulation and other Aberration**

As the biogenesis of miRNA is explored in the previous topics, it is ensured that the biogenesis of miRNA is nothing but a series of steps that pri-miRNA has to follow after getting transcribed directly from the miRNA gene of interest. It is seen that if there is a slight change in the same steps that have been explored before in this article, the miRNA loses its function due to its dysregulation in various stages of biogenesis. Therefore, by this way miRNA loses its ability to silence a target gene. For instance, miRNA plays a pivotal role in the fate of cancer as seen in few cases of cancer genes, if the target gene is oncogene, the cancer does not develop (oncosuppressor-miRNAs) where as if the target gene is tumour suppressor, the cancer develops (oncomiRNAs) [1]. There are

several mechanisms and dysregulations that can affect the degree of miRNA expression. Often it is seen that tumors often present alternate versions of expressed mature miRNA as a result of which there are consequences in the Epigenetic mechanism, genetic alteration, further defects in the miRNA biogenesis pathway and also other Transcriptional repression, all of which is explained in the respective order.

5.1 Epigenetic Mechanism

In the recent studies, it is seen that a large proportion of miRNA loci on the genome are associated with CpG islands, giving strong bases for methylation and it is also been studied that in case of Breast Cancer aberrant DNA methylation is a well-known method for gene silencing. The relation between miRNA expression and genemethylation can be explained by the miRNA-200 family which tell us that in case of that same particular miRNA-200, it is seen that during BC, the primers of the miRNA of the same family gets silenced which results of the miRNA not getting expressed properly [36]. It is also seen in the case of another kind of miRNA called let-7e-3p which shows a level of down regulation during the case of Breast Cancer [37]. Not only in Breast Cancer but also in Renal Cell Carcinoma [RCC], it is seen that there are 166 miRNA that undergo significant dysregulation. It is seen that about 77 out of 166 miRNAs had decreased expression in Clear cell RCC which also led to the pathogenesis of the RCC.

5.2 Genetic Alteration

The Genetic alteration or a frame shift mutation results in microsatellite instability. Hence, for this reason there is an alternation in the expression of several mRNA. For instance, mRNA of TARBP2 which stabilizes the Dicer protein can be found to be altered since the Genome of the same protein gets altered. As explained earlier, the Dicer protein plays a vital role in the biosynthesis of miRNA and any alternation in that protein molecule might dysregulate the biosynthesis of the same. This is found in colorectal and gastric cancer [38] and as well as in cases of Breast Cancer [39]. It is also seen that some miRNA families like let-7 are more involved in tumour development [40]. In the case of Breast Cancer, several let-7 family along with the cooperation from other miRNA families like miR-125b, miR-100 and miR-34a have been found to be located at fragile sites of human chromosomes [11q23-q24D], potentially contributing to miRNA expression.

5.3 Transcriptional repression by other upstream protein

A large group of transcription factors can influence the degree of expression levels of single miRNA. Various evidence suggest that miRNAs and transcription factors work cooperatively. miRNAs are involved in the functional feedback loop in which transcription factors influence miRNA expression levels and vice versa [41]. This gives a notion that

Table 1: Highlighting the role of various mirnas in cancer and its progression along with its target genes.

miRNAs	Target	Role	References
miRNA in Cancer			
miR-34	p53	Altered DNA damage leading to cancer.	[18]
miR-34a	CD44, NOTCH1, RAS	Cancer stem cell Regulation	[34]
let-7	KRAS	Progression of Breast cancer stem cell, leading to self-renewal and differentiation properties. Cancer progression.	[18]
	3' UTR of DROSHA		[27]
miR-200	ZEB1 and ZEB2 Interleukin-8	Enhancement of EMT. Angiogenesis in cancer.	[19]
miR-506	RAD1, SNAI2	Enhanced Metastasis.	[21]
miR-15/16	Bcl-2, CDC2	Cancer progression.	[22]
miR-17-92	E2F1, THBS1	Cell cycle progression and angiogenesis in cancer	[48]
miR-210	SDHD Ephrin A3	Cancer cell survival Tumor angiogenesis	[23]
miR-21	PDCD4 JAG1, Bcl2, PTEN	Reduced apoptosis with increased metastasis. Enhanced EMT	[25]
miR-155	NF-κB	Cancer progression with inflammation.	[26]
miR-221/222 cluster, miR-21, miR-103, miR-15a/16	Component of DROSHA/DGCR8 Microprocessor	Enhanced metastasis in cancer.	[32]
miR-10b	HOXD10 and Syndecan-1	Cancer cell migration and invasion	[35]
miR-9	JAK-STAT pathway	Tumor angiogenesis	[35]
miR-519c	HIF1-A	Tumor angiogenesis	[35]
miRNA dysregulation in other Diseases			
miR-146, miR-106, miR-9, miR-29, miR-107, miR-81, miR-34	Amyloid Protein	Alzheimer's disease	[49]
miR-7, miR-153	3' UTR of alpha-synuclein	Parkinson's disease	[46]
miR-21	SPRY1, an ERK-MAPK pathway molecule	Cardiovascular disease	[5]
miR-200a	Gene encoding caspase inhibitor X-linked inhibitor of the apoptosis protein [XIAP] Beta-cell chaperone p58	Beta cell apoptosis leading to insulin development	[5]
miR-29	COL1A1 and COL2A1	Systemic Sclerosis	[47]

tumorigenic miRNA expression alteration could be due to the activity of tumour related transcription factors such as SMAD [42], p53 family proteins [p53, p63 and p73] [43]. In Breast Cancer, the BC 1, early onset [BRCA 1] transcription factor [44] and the epidermal growth factor receptor [EGFR/HER1], a hypoxic transcription factor which is involved in regulation of RISC, are able to inhibit miRNA maturation, thus enhancing cell survival and invasiveness.

6 miRNA and Other Diseases

Studies have found that certain miRNAs were associated with altered expression of the genes which were the causative factors of Alzheimer's disease. miRNAs which were identified to be dysregulated in this disease include miR-146, miR-106, miR-9, miR-29, miR-107, miR-81, miR-34. Amyloid precursor protein was reported to be a target for dysregulation in miRNA in this disease [45]. A comparative sequence analysis of the alpha-synuclein gene which is associated with Parkinson's disease has revealed that the 3'UTR of alpha-synuclein gene is conserved suggesting a miRNA regulation. miR-7 and miR-153 have been shown to target alpha-synuclein so far, these two miRNAs bind to the 3'UTR of alpha-synuclein and downregulate its mRNA and protein levels [46]. Various miRNAs play a key role in cardiovascular disease progression such as cardiac hypertrophy, fibrosis and myocardial infarction. miR-21 is upregulated during the fibrosis of myocytes and leads to cardiac hypertrophy which is a condition resulting from the gradual loss of myocytes and systemic hypertension. SPRY1, an ERK-MAPK pathway molecule acts as a direct target for miR-21. Several miRNAs are also involved in diabetes development by targeting genes related to inflammation, cholesterol and glucose metabolism. miR-200a targets genes which encode the caspase inhibitor X-linked inhibitor of the apoptosis protein [XIAP] and beta-cell chaperone p58. miR200a-mediated downregulation of these proteins led to beta-cell apoptosis and thereby a decreased insulin production [5]. Patients suffering from systemic sclerosis, miR-29 is significantly decreased resulting in fibrosis due to an elevated expression of the collagens COL1A1 and COL2A1, which are in normal conditions downregulated by miR-29 [47].

7 miRNA as Potential Biomarker and Therapeutics

If it's possible to discriminate and differentiate the tumour origin, subtypes, oncogenic mutations and cancer predisposition, and regulating the most important cellular processes, it is quite possible to counter the oncogenic development before it is too late. It is hypothesized that miRNA can be used to predict cancer prognosis and also response to specific therapies hence acting as a potential biomarker. Although in case of Breast Cancer diagnosis, tissue gene biomarkers have been greatly improved; their invasive and unpleasant nature of diagnosis has limited their application. To overcome this, miRNAs provide the opportunity to bypass the problems associated with tissue biopsy, which is required in the currently available genetic tests. miRNAs are small molecules and they are found in almost every body fluid [i.e. Blood, plasma, serum, saliva,

urine etc.]. miRNAs are very much responsible for gene expression and being dysregulated in several types of cancer diseases as described in the previous topic and hence any dysregulation in miRNA can be pertained as form of biomarker for any Cancer cases. In the case of Breast Cancer, they were found to be stably and specifically expressed in mammary tissues and in the body fluids of the area of the disease [29]. Hence, this detection of same miRNA molecules can be used as easy, affordable and clinically accessible molecular biomarkers in the retrospective analysis of large tissue collection and for the diagnosis, prognosis and prediction of the therapeutic outcomes in Breast Cancer. A few examples of miRNAs that dysregulate and contribute to the oncogenic development and metastasis are denoted in the table given in the previous page.

Several other miRNAs have also been validated to be overexpressed in Breast Cancer and these include miR-221/222 cluster [50], miR-9, miR-10b, miR-29a, miR-96, miR-146a, miR-181, miR-373, miR-375, miR-520c and miR-589 highlighting their potential use for Breast Cancer diagnosis, Prognosis and therapeutic studies [51, 52]. hsa-miR-21-3p, hsa-miR-1290, hsa-miR-338-5p, hsa-miR-491-5p and hsa-miR-485-5p are glioblastoma-related miRNA signatures that play a role in brain tumorigenesis and invasion [53]. MiRNA [miR-200c, miR-200a and miR-200b] has been an essential factor in the non-invasive diagnosis of ovarian carcinoma [54].

A very unique miRNA signature was associated with prognostic factors and also the disease progression in Chronic lymphatic Leukaemia [39] and Lung cancer, where miR-155 overexpression and let-7a downregulation were able to predict poor disease outcome which also supported the fact that miRNAs can be used as a prognostic biomarker. It is also seen in cases of Breast Cancer that miR-10b, under the control of the TWIST transcription factor, binds HOXD10 gene, enhancing cell migration and invasion. HOXD10, in turn, inhibits the Ras homolog gene family, member C [RHOC] protein, favoring metastatic diffusion of tumour. Among the downregulated miRNAs in Breast Cancer, miR-30a, miR-31, miR-34, miR-93, miR-125, miR-126, miR-146a, miR-195, miR-200, miR-205, miR-206, miR-503, and let-7 have been shown to have role in the pathogenesis through the loss of tumour suppressor properties [55].

8 Conclusion

A large number of reports suggest that the expression of important non coding RNAs like the miRNA are associated with numerous pathological outcomes and human diseases. Cell protection is an important role of miRNA due to its several characteristics. Among all studies related to miRNAs, the most elaborated is the downregulating role of miRNA-related post transcriptional modification, while recent studies have revealed an adverse role of miRNAs acting as the activators of gene expression [11]. miRNAs are used as biomarkers in a non-invasive diagnostic approach in patients suffering from cancer which is emerging as an interesting prospect of miRNA profiling in medical applications. There

have been recent discoveries concerning the aberrant expression of miRNAs in body fluids, including serum and plasma. The use of aberrantly expressed miRNAs as an effective screening method complementing other established cancer screening methods thereby aiding in a more complete method for early detection of cancer.

It is noted that miRNAs have a great impact in the malignancy of cancer and progression. The mechanism of miRNA action to regulate gene expression can be modulated by several factors, thereby adding complexity to the regulation and function of miRNA processes [35]. In the tumours, miRNAs are involved in various chemoresistance-related signaling pathways for regulating tumour resistance. There are various mechanisms concerning the dysregulation of miRNA which include the abnormal transcriptional control of miRNAs, dysregulated epigenetic changes and defects in miRNA biosynthesis machinery. Cancer cells with abnormal miRNA expressions have evolved to sustain proliferative signaling, evasion of growth suppressors, resist cell death, and activate invasion, metastasis and induction of angiogenesis. miRNAs may either act as tumour suppressors or oncogenes under particular situations. Hence, the challenges are in the identification of the specific targets of miRNAs involved in cancer progression and establishment into a malignant form [56]. Recent findings suggest miRNA's dysregulation and its relation to aberrant DNA methylation and histone modifications which leads to a wide genome range of epigenetic alterations. The extensive study of the relation between epigenetic regulations and miRNAs might lead to the discovery of novel biomarkers and therapeutic targets [4]. miRNA functions in controlling gene expression in cancer as well as other diseases making it ideal for therapeutic applications [57]. Studies suggest that miRNA modulation in tumour cells leads to phenotypic changes thereby leading to an increase in apoptosis and cell death, tumour development suppression, invasion, and metastasis by inhibition of oncogenic miRNAs and/or substitution of the deficient tumour suppressive miRNAs. In future, miRNA-based therapy for cancer and important diseases could be a reliable weapon [58].

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