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# MicroRNAs and its Dysregulation in Cancer Progression

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**Abstract:**
MicroRNAs (miRNAs) are small, conserved, non-coding RNA plays important role in in post-transcriptional gene regulation. Recent studies have elucidated the function of dysregulatedmiRNA in plethora of diseases commonly in human cancer. Various mechanisms, by which miRNA gets dysregulated include: abnormal transcription of miRNA, dysregulation in miRNA biogenesis, genomic aberrations and dysregulated epigenetic modification. Aberrantly expressed miRNA has been studied to affect the hallmark of cancer. Recent studies have highlighted on the potential of miRNA to act as therapeutic target and biomarker. In this review paper, we focused on the biogenesis, regulation of miRNA along with the dysregulation of it in human cancer and some other diseases, along with it highlighting miRNA roles in therapy.
Key words: mRNAs, therapeutic, biomarker, dysregulation

## 1. Introduction

MicroRNA (miRNA), refers to single stranded, small (approx 22-24bp in length), evolutionary conserved, non-protein coding regulatory gene found in eukaryotes (first described in *C. elegans*) (Bertoli et al. 2015), which plays a very vital role in gene silencing and its regulation, at the post-transcriptional level (Banerjee et al. 2020). Nearly 1-5% of the total human genome is composed of miRNA and regulates nearly 30% of protein coding genes (MacFarlane and Murphy 2010). miRNA genes are transcribed by RNA polymerase II, and are found in either antisense or sense orientation, mainly in the intergenic region (they are also found in exonic and intronic regions). It has been found to regulate various biological processes along with cellular functions such as cell proliferation, differentiation, metabolic pathways,apoptosis (Banerjee et al. 2020) and modulates gene expression of 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, by binding miRNA to the 3’ UTR of the target mRNA, leading to inhibition of translation via miRISC formation (Suzuki et al. 2013).

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 along withdownregulation of miR-16 and mir-15. These two miRNAs works on the antiapoptotic factor B cell lymphoma 2 (BCL2) gene of the host cell which leads to reduced apoptosis of

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the cancer cells, enhancing cancer progression (Banerjee et al. 2020). Apart from deletion, other genetic mutation in TARBP2 and XPO5 (the proteins involved in miRNA processing and maturation), and epigenetic alterations (such as aberrant DNA methylation and histone modification) lead to miRNAdysregulation 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) (Suzuki et al. 2013). Further, the role of dysregulated miR-33, miR-122, miR-208, miR-103 has been elucidated in the regulation of diseased condition, such as, HCV infection, Atherosclerosis, Cardiac diseases, Diabetes (Rupaimoole and Slack 2017).

Henceforth, the ability of miRNA to target altered mRNA, has been utilised to study therapeutic potential of miRNA in treating various diseases through drug sensitization (Banerjee et al. 2020). miRNA-based therapeutics in the form of miRNA mimics (as a therapeutic), and, antimiRs (as target of therapeutics) have been studied. RNA-delivery techniques in-vivo, has made this technique feasible in treating diseases (Rupaimoole and Slack 2017). miRNA has also been proposed as potential biomarker of various cancer due to its stable presence in body fluid as an circulating miRNA and easy detection in tumour biopsies as non-circulating miRNA(Shen et al. 2013). 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

### 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. The intergenicmiRNAs are in relation to their host gene expression while all intragenic miRNAs have independent transcription units (Acunzo et al. 2015) and regulation by their own promoters. miRNA is biosynthesised generally by two pathways; the canonical and the non-canonical pathway.

### 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, which leads to the generation of a long primary transcript known as the pri-miRNA which is several kilobases long (Acunzo et al. 2015). In the nucleus, the pri-miRNA is processed with the help of a microprocessor complex comprising of a ribonuclease III enzyme known as Drosha and DiGeorge Syndrome Critical Region 8 (DGCR8)an RNA binding protein. An N6-methyladenylated GGAC along with other motifs in the pri-miRNAarerecognised by DGCR8. At the base of the hairpin structure of the pri-miRNA,Drosha is involved in the cleavage of the pri-miRNA duplex (O'Brien 2018). This leads to the production of a transcript of a 70 kb miRNA precursor known as the pre-miRNA (Acunzo et al. 2015). This leads to the formation of a 2 nt 3' overhang on the pre-miRNA. As soon as the pre-miRNAs are generated, they

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 09, 2021

arefurther exported to the cytoplasm with the help 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 a double strand RNA binding protein TAR RNA binding protein (Saliminejad et al. 2018).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 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. The thermodynamicstabilityis dependent for the selection of the the 5p or 3p strandat the 5' ends of the miRNA duplex along with a 5' U at nucleotide position one. The strand with 5' uracil or a lower 5' stability is loaded to the AGO and is known as the guide strand while the unloaded strand is called as the passenger strand, which unwounds from the guide strand by various mechanisms based on its complementarity (O'Brien et al. 2018). The passenger strand of the miRNA duplex leaves o 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 at the 3' end of mRNA. There is recruitment of deadenylase complexes. Deadenylases causes the shortening of the mRNA poly (A) tail leading to the destabilisation of the mRNA by decapping and 5' to 3' exonuclease activity. TNRC6 results in low translation efficiency aided by CCR4-NOT along with DEAD-box helicase 6 (DDX6) further attaches to the decapping complex and inhibit translation. mRNAdestabilisation is the most common repression mediated by the mammalian mRNAs and it leads to translation repression though its effect is weaker.

**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 (Saliminejad et al. 2018). 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 ($m^7$G)-capped pre-miRNA. The exportin-1 facilitates in the export of the nascent RNAs without the Drosha cleavageto the cytoplasm. There is a strong 3p strand bias for the $m^7$G cap hence preventing the 5p strand from loading to the Argonaute. On the other side,by Drosha Dicer- independent miRNAs are further processed from endogenous short hairpin RNA (shRNA) transcripts. Within the cytoplasm AGO2 is required for the complete maturation of the pre-miRNAs, this promotes loading of pre-miRNA entirely into AGO2 and AGO2-dependent slicing of the 3p strand.

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 09, 2021

Further, this maturation is completed by 3'- 5' trimming of the 5p strand (O'Brien et al. 2018).

**miRNA regulation**

miRNAs have the ability to reduce gene expression by various modes and pathways. Various observations have proved that miRNAs in the form of effector complexesperform their functions known as miRgonaute,miRISC or miRNP, along with Argonaute, which is one of the most important constituent of all miRNPs, instead of working as naked RNAs (Steitz and Vasudevan 2009). The Watson-Crick pairing of 5'-proximal "seed" region in the miRNA into the seed match site in the target mRNA that is mostly positioned mostly in the 3' UTR which acts as a specific determinant for miRNA recognition of the target. A small subset of miRNAs are claimed to modulate the 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 target site and the miRNA seed region (Valinezhad Orang et al. 2014). 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-catalysed degradation of the target mRNA sequences by mRNA cleavage process (Wahid et al. 2010).

**Translational repression**

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 was proposed by Peterson et al, 2006. 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 the translation initiation failure (Mathonnet et al. 2007) (Thermann and Hentze. 2007). 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 the 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 therefore may be associated with miRISC translation inhibition (Wahid et al. 2010). The third model suggests that there may be inhibition of

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 09, 2021

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 (Chendrimada et al. 2007; Wang et al. 2008).

**mRNA degradation**

The target mRNA degradation processed 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 the role in mRNA degradation. Other mechanisms along with Ago- catalysed mRNA degradation such as deadenylation, decapping and exonucleolytic digestion of mRNA also play a role in mRNA degradation. Ago, 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 the miRNA/mRNA duplex play an important role in the selection of degradation or translational repression (Wahid et al. 2010).

mRNA degradation is initiated first by the 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, missing poly (A) tail can be exposed to the 3'- 5' degradation by cytoplasmic exonucleases. Parallely, sequence-specific endonucleolytic mRNA cleavage may occur by polysomalribonuclease 1 (PMR1) (Valinezhad Orang et al. 2014).

**3. miRNA and its dysregulation in cancer**

Both genetic and epigenetic mechanisms have been elucidated to induce miRNAdysregulation 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. 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. Glucocorticoid resistance in acute lymphoblastic leukaemiacell due to the blocking of pri-miR-128b processing has been found by Point mutation in miR-128b. Apart from genomic alterations, by the activity of aberrant transcription factoraltered miRNA expression in cancer is regulated. pri-miRNA transcription is controlled by the alteration in the oncogenic factors and tumor suppressors acting as transcriptional repressors and activators (Zhang et al. 2006). the pathways are discussed in the successive paragraphs.

**Tumor suppressive miRNAdysregulation**

During DNA- damage response p53 (a tumor suppressor) transcriptionally regulates the expression of miR-34 family 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 cancers.

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 09, 2021

Dysregulated let-7, has also been studied in breast cancer stem cell differentiation and self-renewal (Johnson et al. 2005). 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, it has been studied in human tumors that, ZEB2 along with ZEB1 interacts with the regulatory element in the promoter of miR-200 thereby repressing the transcription of miR- 200 leading to down regulation 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 (Burk et al, 2008). miR-520 has been studied to be downregulated in breast and ovarian cancer, thereby promoting tumor growth and metastasis (Keklikoglou et al. 2012). 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 (Yang et al. 2013). 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 (Pekarsky and Croce 2015).

**Oncogenic miRNAdysregulation**

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 tumour- suppressive miRNA in BCL (B-cell lymphoma progression (Bracken et al. 2008). 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 cell, resulting in increased HIF1α and cancer cell survival. It has also been studied to increase tumor angiogenesis by downregulatingephrin A3 (hypoxia-responsive angiogenesis inhibitor (Fasanaro et al. 2008). In various cancer studiesmiR-21 with antiapoptotic role has been seen to be up regulated. Withupregulation of AP-1 (a transcription factor) which binds to the promoter of miR-21, the chromosomal locus containing miR-21 is amplified during cancer. TGFß1 (Transforming Growth Factor Beta 1) and STAT3 (Signal transducer and activator of transcription 3) has been studied to play an important role in the upregulation. Moreover, TGFß1 stimulates its receptor TGFß1R 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) (Li et al. 2013). miR-21 targets PDCD4 (programmed cell death protein 4) leading to decreased expression of it, resulting in reduced apoptosis and increased metastasis (Frankel et al. 2008). The transcription factor, NF-κB (Nuclear factor- κB) further increases

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 09, 2021

its expression in cancer cells by binds to the promoter region of miR-155. This links cancer with inflammation (Tili et al. 2009).

**Dysregulation of the enzymes involved in miRNA biogenesis**

Drosha and Dicer, two of the important proteins involved in miRNA biogenesis has beenwell studied to be down regulatedspecifically 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) binds to 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 (Rupaimoole and Slack 2017). It is seen that reduced Droshaand Dicer expression have been associated with shorter metastasis and high grade Breast Cancer -free survival (Khoshnaw et al. 2013). This reduced Dicer phenomenon is also observed in other kinds of like Prostrate (Bian et al. 2014), gastric (Zhang et al. 2014), or squamous cell carcinoma (Gao et al. 2014). More to this, it is seen that in Breast Cancer nucleolin, a component of Drosha/DGCR8 microprocessor complex, which has been demonstrated to promote the maturation of a set of metastasis promoting miRNAs (miR-21,miR-103, miR-221/222 cluster and miR-15a/16) (Pickering et al. 2011; Davis et al. 2008). Dysregulation and alternation are also seen the Nuclear Exporting protein, XPO5, a key protein for pre-miRNA export to the cytosol has been also suggested as possible biomarker for Breast Cancer (Pichiorri et al. 2013).

## 4. miRNA and its involvement in CSC, EMT and Chemoresistance

Subset of cells having the ability of 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 has been studied to show CSC phenotype thereby regulating the self-renewal and differentiation. Role of miR- 34a has also been elucidated in the regulation of CSC by supressing 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 (Saito 2014). 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.

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 09, 2021

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 corelated 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, vital process in formation of blood vessels around the 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 formation of blood vessels. miR-126 and miR- 34a has also been associated with tumor angiogenesis, thus playing an important role in cancer cell growth, proliferation and invasion (Harquail et al. 2012).

**5. miRNAdysregulation 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 a 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 has 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 found to be oncogene, the cancer does not develop(oncosupressor-miRNAs) where as if the target gene is tumour suppressor, the cancer develops(oncomiRNAs) (Bertoli et al. 2015). There are several mechanism and dysregulations that can affect the degree of miRNAexpression. Often it is seen that Tumours 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: -

**Epigenetic Mechanism**

A large proportion of miRNAloci 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 very well-known method for gene silencing. The relation between gene methylation and miRNA expression can be explained by the miRNA-200 family which tells 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 (Castilla et al. 2012). It is also seen in case of another kind of miRNA called let-7e-3p which shows a level of down regulation during the case of Breast Cancer (Aure et al. 2013). Not only in Breast Cancer but also in Renal Cell Carcinoma (RCC), it is seen that there are 166 miRNA that undergoes significant dysregulation. It is seen that about

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 09, 2021

77 out of 166 miRNAs had decreased expression in Clear cell RCC which also led to the pathogenesis of the RCC.

**Genetic Alteration**

The Genetic alteration or a frameshift mutation results in a microsatellite instability. Hence, for this reason there is an alternation in the expression of several mRNA. For instance, mRNA of TARBP2 which stabilises 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 the colorectal and gastric cancer (Yamamoto et al. 2012) and as well as in case of Breast Cancer (Calin et al. 2004). It is also seen that some miRNA family like let-7 are more involved in tumour development (Reinhart et al. 2000). In the case of Breast Cancer, several let-7 family along with the cooperation from other miRNA families like miR-100, miR-34aand miR-125b which found to be located at fragile sites of human chromosomes which potentially contributing to miRNA expression.

**Transcriptional repression by other upstream protein**

A large group of transcription factors influence the degree of expression levels of single miRNA. It is also suggested that transcription factors and miRNAs work cooperatively. miRNAs are involved in the functional feedback loop in which transcription factors influence miRNA expression and vice versa (Tsang et al. 2007). This gives a notion that tumorigenic miRNA expression alteration could be due to the activity of tumour related transcription factors such as SMAD (Davis et al. 2010), p53 family proteins (p53, p63 and p73) (Suzuki et al. 2009). In Breast Cancer, the BC 1, early onset (BRCA 1) transcription factor (Kawai et al. 2012) and the epidermal growth factor receptor EGFR/HER1 which is a hypoxic transcription factor which is involved in regulation of RISC and are also able to inhibit miRNA maturation, thus enhancing cell invasiveness and survival.

**6. miRNA and other diseases**

Studies have found that certain miRNAs were associated with altered expression of the genes which were the causative factor 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 (Schonrock and Gotz 2012). Amyloid precursor protein was reported to be a target for dysregulation in miRNA in this disease (Kamal et al. 2015). A comparative sequence analysis of alpha-synuclein gene which is associated with Parkinson's disease has revealed that the 3'UTR of alpha-synuclein gene is conserved suggesting anmiRNA regulation. miR-153 and miR-7 have been shown to target alpha- synuclein so far, these two miRNAs bind to the 3'UTR of alpha-synuclein and also downregulate its mRNA and protein levels (Doxakis et al. 2010). Various miRNAs have 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

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 09, 2021

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-mediateddownregulation of these proteins lead to beta-cell apoptosis and thereby a decreased insulin production (Rupaimoole and Slack 2017). 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 (Maurer et al. 2010).

**Table1 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. | (Johnson et al. 2015) |
| miR-34a | CD44, NOTCH1, RAS | Cancer stem cell regulation | (Saito 2014) |
| let-7 | KRAS<br><br><br><br><br>3' UTR of DROSHA | Progression of Breast cancer stem cell, leading to self-renewal and differentiation properties.<br>Cancer progression. | (Johnson et al. 2015)<br><br><br><br><br>(Rupaimoole and Slack 2017) |
| miR-200 | ZEB1 and ZEB2<br>Interleukin-8 | Enhancement of EMT.<br>Angiogenesis in cancer. | (Burk et al. 2008) |
| miR-506 | RAD1, SNAI2 | Enhanced Metastasis. | (Yang et al. 2013) |
| miR-15/16 | Bcl-2, CDC2 | Cancer progression. | (Pekarsky and Croce 2015) |
| miR-17-92 | E2F1, THBS1 | Cell cycle progression and angiogenesis in cancer | (Bracken et al. 2008) |
| miR-210 | SDHD<br>Ephrin A3 | Cancer cell survival<br>Tumor angiogenesis | (Fasanaro et al. 2008) |

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 09, 2021

| | | | |
|---|---|---|---|
| miR-21 | PDCD4<br><br>JAG1, Bcl2, PTEN | Reduced apoptosis with increased metastasis.<br>Enhanced EMT | (Frankel et al. 2008) |
| miR-155 | NF-κB | Cancer progression with inflammation. | (Tili et al. 2009) |
| miR-221/222 cluster, miR-21, miR-103, miR-15a/16 | Component of DROSHA/DG CR8 microprocessor | Enhanced metastasis in cancer. | (Davis and Hilyard 2008) |
| miR-10b | HOXD10 and Syndecan-1 | Cancer cell migration and invasion | (Harquail et al. 2012) |
| miR-9 | JAK-STAT pathway | Tumor angiogenesis | (Harquail et al. 2012) |
| miR-519c | HIF1-A | Tumor angiogenesis | (Harquail et al. 2012) |
| • miRNAdysregulation in other Diseases | | | |
| miR-146, miR-106, miR-9, miR-29, miR-107, miR-81, miR-34 | Amyloid Protein | Alzheimer's disease | (Schonrock and Gotz 2012) |
| miR-7, miR-153 | 3' UTR of alpha-synuclein | Parkinson's disease | Doxakis et al. 2010 |
| miR-21 | SPRY1, an ERK-MAPK pathway molecule | Cardiovascular disease | (Rupaimoole and Slack 2017) |
| miR-200a | Gene encoding caspase inhibitor X-linked inhibitor of the apoptosis protein (XIAP)<br><br>Beta-cell chaperone p58 | Beta cell apoptosis leading to insulin development | (Rupaimoole and Slack 2017) |
| miR-29 | COL1A1 and COL2A1 | Systemic Sclerosis | (Maurer et al. 2010) |

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 09, 2021

**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 but their invasive and unpleasant nature of diagnosis have limited their application. To overcome this, miRNAs provide the accessibility 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 fluids (i.e. Blood, plasma, serum, saliva, urine etc). miRNAs are very much responsible of gene expression 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 case of Breast Cancer, they are found to be specifically and stably expressed in mammary tissues and in the body fluids of the area of the disease (Zhang et al. 2014). Hence, this detection of same miRNA molecules can be used as affordable, easy and clinically suitable molecular biomarkers in the retrospective analysis of large tissue collection and for the diagnosis, prognosis and prediction of the therapeutic outcomes in Breast Cancer. Few examples of miRNAs that dysregulate and contribute to the oncogenic development and metastasis is denoted in the table given in the next page. Several other miRNAs have also been validated to be overexpressed in Breast Cancer and these include miR-221/222 cluster (Christodonulators and Dalamaga 2014), miR-9, miR-10b,miR-520c miR-29a, miR-96,miR-375, miR-146a, miR-181, miR-589and miR-373, highlighting their potential use for Breast Cancer diagnosis, Prognosis and therapeutic studies (Tang et al. 2012), (Iorio et al. 2005). A very unique miRNA signature was associated with prognostic factors and also the disease progression in Chronic lymphatic Leukaemia (Calin et al. 2004) and Lung cancer, where let-7a down regulation and miR-155 overexpression were able to predict poor disease outcome which also supported the fact that miRNAs can be a be used as a prognostic biomarker. It is also seen in case of Breast Cancer that miR-10b binds HOXD10 geneunder the influence of the TWIST transcription factor, enhancing cell invasion and migration. HOXD10, in turn,favouring metastatic diffusion of tumourby inhibiting the Ras homolog gene family, member C (RHOC) protein. Among the downregulatedmiRNAs in Breast Cancer, miR-30a, miR-503, miR-31, miR-34, miR-93, miR-125, miR-126,miR-205, mR-146a, miR-195, miR-200, let-7 andmiR-206 have been shown to have role in the pathogenesis through the loss of tumour suppressor properties (Ma et al. 2007).

**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

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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 (Valinezhad Orang et al. 2014). 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 regulation and function of miRNA processes (Harquail et al. 2012). In the tumours, miRNAs are involved in various chemoresistance-related signalling pathways for regulating tumour resistance. There are various mechanisms concerning the dysregulation of miRNA which include the dysregulated epigenetic changes, defects in miRNA biosynthesis machinery and abnormal transcriptional control of miRNAs. The cancer cells with abnormal miRNA expressions have evolved to sustain proliferative signalling, evasion of growth suppressors, resist cell death, and activate invasion, metastasis and induction of angiogenesis. miRNAs may either act as tumour suppressors or oncogene 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 (Peng and Croce et al. 2016). Recent findings suggest miRNA'sdysregulation 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 miRNAs and epigenetic regulations might lead to the discovery of therapeutic targets and novel biomarkers (Suzuki et al. 2013). miRNA functions in controlling gene expressions in cancer as well as other disease making it ideal for therapeutic applications (Christopher et al. 2016). 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 (Balacescu et al. 2018).

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