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microRNAs IN CANCER METASTASIS

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ABSTRACT

MicroRNAs (miRs) are highly conserved, endogenous non-coding RNAs among different species. miRs involve in the regulation of gene expression, hence it controls various cellular processes such as cell proliferation, apoptosis, and metastasis etc. Additionally in-spite of their physiological roles, miRs are deregulated in various pathological diseases including cancer. Metastasis is the primary reason of mortality in cancer patients. So the management of tumor cell invasion and metastasis play crucial role in cancer therapies. Many cellular mechanisms and molecular pathways have been identified in metastasis, which provide the basis of anti-metastatic drugs. miRs regulate timorous genes related to metastasis also. Recently, many studies have evidenced about the role of miRs in cancer metastasis and also revealed the concepts, that they target many genes, play a pivotal role in epithelial mesenchymal transition (EMT), which promotes invasive and motility properties of tumor cells. Herein, in this review, we have focused on recent findings of miRs in the regulation of EMT, migration, invasion and metastasis of cancer cells.

Keywords: Metastasis; microRNA; Invasion; EMT

INTRODUCTION

Approximately twenty years ago, some investigators dogged the constituents of genome that were nonfunctional, named as miRs. MiRs are nonfunctional short oligonucleotide sequences, regulate the gene expression at post-transcriptional level Lee *et al*. (1993) discovered a short RNA LIN-4 in C.*elegans* which was involved in the developmental timing of the nematode by targeting the lin-14 protein (1). These investigators noticed that lin-4 gene does not code for proteins but it is small RNA which has antisense complementarily in the 3'UTR region of lin-14. Thus LIN-4 Mrna binds with lin-14 and represses it [2, 3]. These microRNAs are found in many organisms like plants, worms and humans [4].

The role of miRs has been identified in genetic studies of C.*elegans* and D. *malanogaster*, they play main functional role in cell proliferation, differentiation, stress resistant, metabolism of fat [5-8]. MiRs genes are found in introns, exons and in addition to intergenic sequences. The genomic sequences of micro RNAs are evolutionary conserved. MicroRNAs genes are transcribed likewise messenger RNA. MiRs are transcribed by RNA polymerase II as well as RNA polymerase III. Primary microRNAs are more than 100 nucleotides in length and their post transcriptional modification like 5' capping and 3' Poly A tail are similar to messenger RNA, RNAase polymerase III enzyme like DROSHA and DICER involves in the processing of pri-micro RNA in nucleus and cytosol and forms the 70 nucleotide pre-micro RNA and ultimately produces 19-24 nucleotides of mature double stranded micro RNA. Double stranded micro RNA include one guide strand and other passenger strand , guide strand incorporated into RNA inducing silencing complex (RISC) and pairs within the 3'UTR region of their target mRNA. On the basis of partial paired mRNA will either degrade or repressed by the micro RNA [9-13].

MiRs play a vital role in different biological pathways of multicellular organisms [14]. MiRs involve in the regulation of main key biological processes like cell proliferation, differentiation and apoptosis in their normal physiological condition [15,16].

Over the past years in many studies, it was noticed that altered expression of single or small subset of small miRs was found to have intensive effect on many mRNAs [17,18].Recent studies, show altered expression of different micro RNAs was reported to have a profound effect types of

cancer, stage of tumor and their therapeutic implications. Tumor biopsy procedures was very painful and extensive invasive procedure for cancer patients, it attracts the interest of researcher towards the less or non-invasive biomarkers such as circulating microRNA as biomarkers in biofluids of multicellular organisms. After the discovery of circulating microRNAs, they are considered as novel regulator of gene expression. Much microrna are found intracellularly but some are also found in extracellular fluids. In 2008, circulating micro RNA have been discovered in mammalian body fluids such as saliva, serum, plasma, sputum, These miRs are stable biomarkers and shows variability in expression patterns. Body fluids contain ribonuclease which can degrade microRNA. Extracellular microRNA evolves the new way to protect from the RNase digestion by packaging into vesicles and different proteins.

In this review we analyze in detail about the role of biofluids circulating microRNAs (serum, plasma, urine, CSF) in metastasis [19-21].

miRNA discovery

This breakthrough research of miR have increased the interest of researchers towards it after the discovery of the first miRs Lin-4 in C.elegans in 1993 [24, 25]. Lin-4 was first miR that was discovered in the laboratory of Ambros's and Gary Ruvkun's.In the C.*elegans* hetrochronic genes are involved in the patterning of the developmental stages of the larva. These hetrochronic genes include lin-4, mutation in these genes negatively affect the regulation of the development of C.*elegans* [26, 27]. Mutation in the lin-4 genes result in the absence of several adult structures.Lin-4 involves in the transition of larval developmental stages from L1 to L2 stages [27,28].Ambros's and Gary Ruvkun's also concluded that, these non-protein coding genes target lin-14 in the 3'UTR region and either repress it or degrade it [29-31].In 2000, the second miRNA that was discovered in C.*elegans* named as let-7.In the laboratory of Ruvkun's, Reinhart, Slack *et al*. have noticed that let-7 was 21 nucleotide long non coding RNA which helps in the regulation of the developmental stages from L4 to adult stage [32].

Biogenesis of microRNA

RNA polymerase II involves in the transcription of miRs genes, belong to class II genes and generate stem loop primary miR of different size from few hundred nucleotides to many kilobases (kb). Whereas sometimes, RNA polymerase III also involve in the transcription of miRs found in

repetitive sequences [33, 34]. Evidences have shown that 40% miRs loci are found in the intronic region, 10% in exonic regions of and 40% in protein coding genes of introns and remaining part of genes are found in other regions [35]. Similar to mRNA, miRs also show the characteristics of post transcriptional modification, such as 5'capping and 3' polyadenylation [36]. Splicing of mammalian miRs found in intronic regions, take place before miRNA processing. In the nucleus many proteins named as microprocessors such as RNase III enzyme, DROSHA, double stranded RNA binding protein and cofactor core component-DGCR8/Pasha involves in the processing of primary-miRNAs (pri-miRNAs) [37, 38]. These microprocessors cleave stem loop pri-miRNA and produce 70 nucleotide long precursor miRNA (pe-miRNA). The characteristics of RNase III mediated cleavage is 2-nt 3'overhang at the cleavage siteis recognized via nuclear export factor (NEF) Exportin-5, which transports pre-miR into the cytoplasm in a Ran–GTP dependent manner [39,40]. Another RNase III enzyme Dicer with dsRNA binding protein activator of the interferon induced protein kinase (PACT) and trans-activating response RNA binding protein (TRBP) further processed and cleaved pre-miRNA to produce mature 22 nucleotide short miRNA duplex within the cytoplasm [41,42].TRBP binds with Argonoute (Ago) protein and finally binds to Dicer to form a ternary complex in human cells, it progresses into the assembly of the RNA inducing silencing complex (RISC), this complex leads to degradation of miR [43,44]. One strand of miR is incorporated into RISC complex and the other strand is usually degraded [45]. When the strand is incorporated into the RISC complex the miRNA guides the RISC complex to bind their target RNA molecule by base –pairing interactions [38]. RISC comprises of Ago2 protein having endonucleolytic cleavage activity in mammals, whereas it is a major sole enzyme in flies possessing this activity [46]. In animals, miRs bind to their target mRNA via perfect or nearly perfect base pairing, as a result mRNA is degraded to repress translational process [47]. Some miRs of animals bind to their targets via imperfect base-pairing and involves in the repression of translation slightly than cleavage and degradation.

MiRNAs in cancer and it mechanism

According to central dogma of life, RNAs work as messenger to transport the genetic information from DNA to protein. On the other hand, controversies were arised about

the central dogma after the discovery of non-coding RNA (micro RNA), they involved in the regulation of gene expression at post-transcriptional level. Short non coding microRNAs involves in the repression of gene expression, it imperfectly binds to the 3'UTR region of target mRNAs and impair the protein synthesis. Metastasis is a complex process which takes place in a multistep. These steps are-

Step 1: Cancer cells cuts off from the primary tumor and merge into basement membrane to other tissue which is present at their nearby site.

Step 2: Intravasation of cancer cells.

Step 3: survival of cancer cells in circulation.

Step 4: Extravasation of cancer cells (cancer cells leave circulatory system).

Step 5: In new environment cancer cells start to colonize, and formation of metastatic tumor [49-52].

In various data, it have been confirmed that 90% cancer mortality is due to metastasis. Even though the deep knowledge about metastasis, there is no targeted therapeutic have found for this complex process. Some novel biomarkers such as microRNAs have been considered as predictive biomarker in metastasis patients. Calin *et al*., reported about deregulation of miR in B cell chronic lymphocytic leukemia of human by microarray containing miRNA probes [53]. After that many studies revealed the fact that miRs involve in different cellular processes such as diabetes [54, 55], cardiovascular diseases [56, 57], hepatic viral infection [58, 59] and Alzheimer's disease [60, 61]. Additionally circulating miRs are known to act as diagnostic and prognostic biomarker including cancers [62-64].

Many studies have concluded about the relation of microRNA with metastasis. A wide variety of miRs have also been considered as novel tool for treatment of primary tumor as well as therapeutic involvement in metastasis. These miRs have been implicated in various steps of metastasis like EMT, intravasation/extravasation, migration/Invasion, anoikis survival, distant organ colonization.

miRs in EMT/MET processes

EMT is generally known as cellular transition process which initiates early phase of cancer metastasis, Epithelial mesenchymal transition (EMT) involves in the morphological changes of epithelial cells. In the process of EMT, when epithelial cells start to convert into mesenchymal cells, the interaction between one cell to other cell become lose, cancer cell become movable and invade to their neighbour tissues and similarily to organs at distant site [65, 66].

When metastatic cancer cells grow at their higher rate at distant distribution, they revert the EMT process and again convert into epithelial forms [67-69].The main characteristic of EMT is suppression of E-cadherin in cancerous cell of epithelium. There are many molecules such as TGF-β, Twist, Snail, Slug, ZEB act as inhibitor of E-Cadherin [70-76]. Manyreports have evidenced that miR-200 family target ZEB to inhibit cell migration and invasion processes in many cancers like bladder, breast and ovarian cancers [77-80]. Suppression of miR-200 promotes inhibition of E-cadherin whereas activating expression of vimentin and induces the movement of cells [77].

Furthermore, it found there is a mutual relationship of repression between miR-200 and ZEB, where miR-200 target ZEB, induced ZEB to repress the expression of miR-200 [81]. So, in many studies (Fig a). It have been concluded that miR-200/ZEB acts as an essential role player in the EMT/MET processes. Some investigators have also mentioned that EMT induced by TGF-β, blocked by the ectopic expression of miR-200 family [76, 77, 82]. In other study, in the cancer cells of liver and non –small cell lung cancerous cells miR-30a had been shown to inhibit EMT by their target Snail, by this means, it promotes expression pattern of E-cadherin [83, 84]. Similarly, miR-30a was found to inhibit the cell motility by targeting their target vimentin in cancerous cell of gastric cancer [85]. In the pigment of retinal epithelium, miR-204/211 and other miR-3 in cancerous cells of liver and non-small cell lung carcinoma have noticed for the repression of EMT via maintaining the epithelial structure by targeting transcriptional growth factor such as TGF-β2 and slug [86]. In a study, Siemens and colleagues mentioned about ectopically expressed P53 regulator miR-34a, have been seen to be involved in down-regulation of Snai1 for inhibition of EMT. Furthermore miR-34a not only regulate EMT process but also acts as tumor suppressor by increasing the activity of apoptosis and cell cycle arrest via targeting many molecules that was supporting tumor growth like Bcl-2, HDAC1, E2F3, CDK4/6, MET (Fig b).The first oncogenic miRs was identified in the promotion of EMT named as miR-21. Treatment of antagomir MDA-MB-231 of miR-21 for the inhibition of miR-21 in cancerous cells of breast was known to reverse the process of EMT via the up-regulation of tumor suppressor PTEN by the inactivation of AKT/ERK signaling pathway [87]. Myc induces miR, Mir-9 was shown to directly target E-cadherin to promote

metastatic stage of breast cancer [88]. MiR biogenesis involves RNase III enzyme (Dicer) had been shown to be linked with EMT. In a report of Dicer targeting miR-103/107 involved in the induction of EMT, progressed to decreased expression of miR-200 [89]. An opposite process of EMT, MET play an important role in colony formation of metastatic cells at distant site of organs. There are many reports which have supported the roles of miRs regulation in transition process of MET. Chen *et al*. mentioned in his report on miR-103/107 induced metastasis by targeting MET inducer factors such as KLF4 and DAPK [90]. These results show that miRs play central role in MET processes of cancer cells.

miR-10b was firstly characterized to act as an inducer in metastasis of cancer. Ma and colleagues have noticed up-regulation of miR-10b in cancerous cells of breast cancer. This miR was known to promote invasion and distant metastasis via targeting a transcriptional repressor- HOXD10 by targeting many genes such as MTA-MMP (MMP-14), RHOC, α3, and integrin [91]. Similarly, an eminent class of inducer of EMT, Twist regulates the expression of miR-10b in positive manner [91]. Additionally, the regulation process of miR-10b, HOXD10 and their downstream effectors has been identified to mediate invasiveness of cancer cells of glioblastoma [92]. Another miRNAs which involve in invasion/metastasis inducing miRs like miR-373, as an oncomir that targets LATS2, a tumor suppressor in tumors of testicular germ-cells [93]. Apart from some pro-metastatic miRs, many studies reported that a large number of miRs involve in repression of migration/invasion and metastasis.miR-31 involves in the inhibition of different staging processes of metastasis comprising of anoikis, local invasion, extravastion, resistance and metastatic colonization. In breast cancer cells, miR-31 have been known to target many pro-metastatic genes such as radixin, RHOA, α5 integrin [94]. Recent studies mentioned about the scaffold protein for focal adhesion complexes, GIT1 (G protein coupled receptor kinase interacting ArfGAP1), it plays a central role in migration /invasion and metastasis of cancer cells. MiR-149 and miR-491-5p was noticed to target GIT1in many perspective of cancerous cells [95, 96]. Moreover, Chan and colleagues (year) identified that target of miR-149 is GIT1,and it get down-regulated by this miR which finally results in instability of paxillin and α5β1 integrins. Thus it have been concluded that MiR-149 involves in the repression of

migration/invasion and metastasis. Apart from regulation of gene expression by miRs throughout the metastatic processes, recent studies unmask the transcriptional control of metastatic related miRs. In these studies, one report have mentioned about breast cancer metastasis suppressor, 1(BRMS1), it involves in transcription of many miRs related to metastasis. It has been involved in the suppression of many metastasis inducing miRs like miR-373, miR-10b, miR-520c and also played the central role as an activator for metastasis repressing miRs such as miR-21,miR-335 and miR-146a/b [97] (Fig c). Many studies have reported that activation of NFκB induces the expression of miR-146 [98]. A consequent research confirmed that miR-146a/b reduces the activity of NFκB via targeting IRAK1 and TRAF6 to suppress metastasis of breast cancer [99] (Fig c).

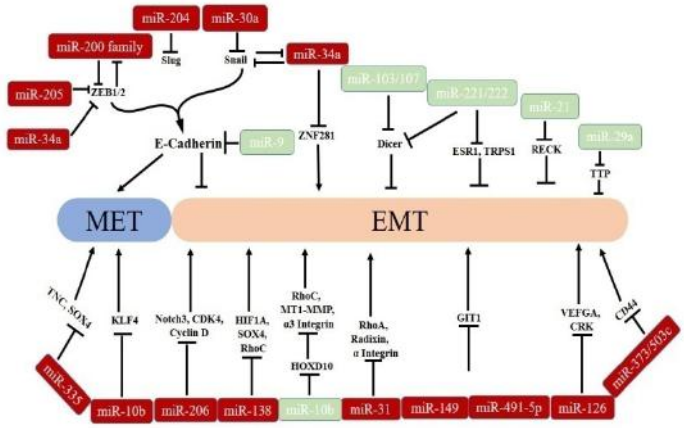

Fig a: Regulation of many miRs in MET and EMT processes of Metastasis

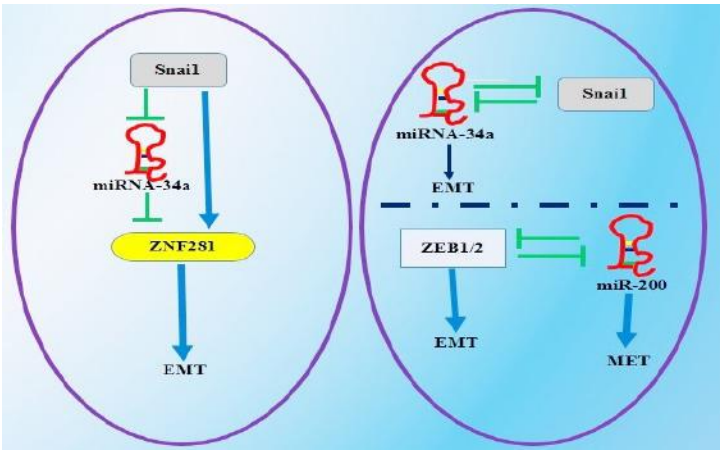

Fig b: Feedback inhibition of genes by miRNA in EMT and MET miRNAs in regulation of migration, invasion and metastasis

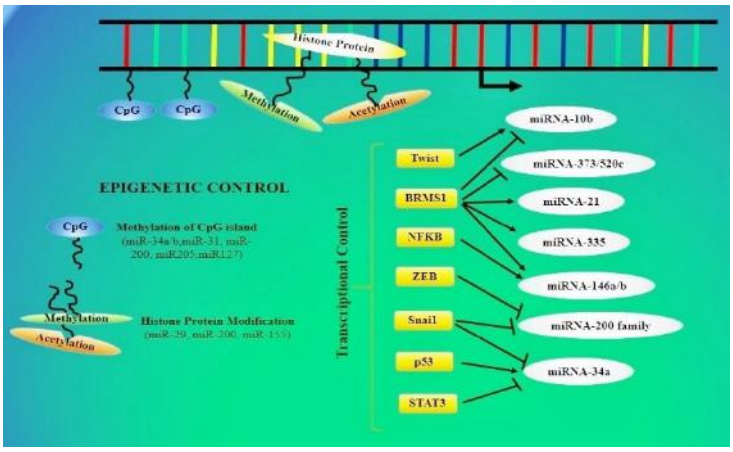

Fig c: Impact of miRNA on different signaling molecules in different signaling pathways.

CONCLUSION

Over the past few decades, investigators have done many studies on miRs mechanisms, expression pattern and explain about the fundamental roles in progression of cancer metastasis. Recent findings have suggested that miRs act as therapeutic target for intervention of progression of cancers and their metastatic processes.

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