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MicroRNA deregulation and cancer and medicinal plants as microRNA regulator

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ABSTRACT

MircroRNAs (miRNAs) are short non-coding RNAs with a length of approximately 20-22 nucleotides, which interact with their target mRNAs at 3'-untranslated region by partial pairing. The miRNA-mRNA interaction leads to induction of mRNA degradation and eventually translational inhibition. Thus, miRNAs play an important role in virtually all cellular processes, especially differentiation, proliferation, migration, and apoptosis. The deregulation of miRNAs may lead to serious diseases including cancer. There is mounting evidence demonstrating the participation of miRNA regulation during carcinogenesis. In this review, we discuss an updated miRNA biogenesis, mechanisms involved in their deregulation, and their role in cancer development. This review also summarizes updated information on potential medicinal plants which regulate miRNA expression as a promising molecular miRNA therapeutic approach for cancers.

KEYWORDS: MiRNA; Biogenesis; Cancer; Medicinal plants

1. Introduction

Small endogenous RNA molecules can be classified into several types, including transfer RNA (tRNA), ribosomal RNA (rRNA), small nucleolar RNA (snoRNA), small interfering RNA (siRNA) and micro RNA (miRNA). The endogenous small miRNA molecules which are approximately 20-22 nucleotides long are derived from the double stranded RNA precursor molecules[1]. The breakthrough of miRNA was first discovered in *Caenorhabditis elegans* and the disclosure of small non-coding lin-4 transcript from *Caenorhabditis elegans* which was 22 nucleotides long found to downregulate LIN-14 protein expression *via* sequence complementary binding to 3'-untranslated region (UTR) of lin-14 mRNA[2]. Since then, miRNA has attained great attention and led to detailed investigation of miRNA biogenesis and function in the advancement of molecular biology. Contemporarily, 38 589 mature miRNAs from 271 species have been identified[3−7]. This arising principal class of regulatory genes have been identified by bioinformatics prediction approaches and validated through several experimental methods. The involvement of miRNA in the negative regulation of gene expression at post transcriptional level and subsequent protein translational repression[8] clearly substantiates the major role of miRNA in diverse biological processes such as cell death[9], cell proliferation[10], cell development[11], cell differentiation[12], stress resistance[13], haematopoiesis[14], fat metabolism[15,16] and insulin secretion[17]. Hence, the evolution of miRNA has exposed a novel and attractive therapeutic target and diagnostic tool for various diseases including cancer.

2. MiRNA biogenesis

In like manner of precursor mRNA synthesis, miRNAs are also generated by RNA polymerase Ⅱ by initially producing a lengthy transcript called the primary miRNA (pri-miRNA)[8,18]. The pri-miRNA transcripts have been evidently validated to possess 5' cap and poly (A) tail at 3' end as any other typical mRNA[19,20]. Previous studies suggest that the length of pri-miRNA transcript can be approximately 1 000 nucleotide[19,21]. Considering the length of pri-miRNA is pretty long with complementary bases within the transcript, it is legitimate to form a partially paired stem-loop structure[22]. This structure acts as substrate for RNase Ⅲ class of enzymes, namely Drosha and DiGeorge Syndrome critical region gene 8 (*DGCR8*) which eventually recognises the hairpin-loop structure of pri-miRNA and catalyzes it into a short precursor miRNA (pre-miRNA)[23−25]. This first cleavage process is initiated by the binding of the microprocessor complex (complex of Drosha and *DGCR8*) to the open-ended part of the stem-looped miRNA and finally the double-stranded cleavage produces a concise hair-pin shaped RNA molecule with a two nucleotide over hang at the 3' end[26,27]. The double stranded stem-loop structure of pre-miRNA has been identified to be approximately 70-100 bp long[22].

Subsequently, the transportation of pre-miRNA from nucleus to

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the cytoplasm is mediated by the nuclear export receptor, known as the Exportin 5[28,29]. Previous studies demonstrated that the Exportin 5 performs its role as nuclear cargo with the aid of RanGTP in which stable complexes of pre-miRNA•Exportin 5•RanGTP are productively exported to cytoplasm down the RanGTP gradient across the nuclear envelope and pre-miRNA and Exportin 5 are dissociated upon the hydrolysis of RanGTP to RanGDP in cytoplasm[30]. The free Exportin 5 is then returned back to the nucleus to mediate new pre-miRNA exportation.

Instantaneously, the second cleavage in the biogenesis process of miRNA takes place in the cytoplasm by RNase Ⅲ enzyme called the Dicer[31,32]. Dicer incorporates PAZ (Piwi, Argonaute and Zwille) domain that binds to the two nucleotide 3' overhangs and anchors the pre-miRNA in position while placing the stem loop terminal at the positively charged catalytic domain of the Dicer[33,34]. This arrangement enables the Dicer to act as a molecular ruler, thereby assisting the cleavage to occur efficiently at approximately 65 angstrom (Å) from PAZ domain and cleaves off the loop from the pre-miRNA[1,33,35]. The subsequent shorter double stranded RNA of about 20-25 nucleotides in length, with two nucleotide 3' overhangs at both terminals is known as miRNA duplex or miRNA/miRNA*[36].

miRNA duplex is then loaded into the miRNA-Induced Silencing Complex (miRISC) and releases one of the strands while selectively binds to one strand in order to generate an active complex[8]. The strand which is integrated into the miRISC is termed as the guide strand (miRNA) while the strand which is released and degraded is termed as the passenger strand (miRNA*). The Argonaute protein, being the major component of RISC, acts as the capital for catalytic process. The Argonaute protein comprises two essential domains, namely PAZ and PIWI. The PAZ domain has been demonstrated to bind to the backbone of the guide strand[37,38] while the PIWI domain acts as the RNase H which breaks down the passenger strand[39-41]. Figure 1 shows an overview of the miRNA biogenesis process.

3. MiRNA and cancer

Ever since the exploration of miRNA and its correlation with the widespread biological processes mainly including apoptosis and cell proliferation, the fundamental significance of miRNA in tumorigenesis is strongly postulated. Henceforth, the miRNA-mediated molecular mechanism in cancer biology has unfastened a novel dimension for cancer therapeutic targets as well as cancer biomarkers. The miRNA binds to its target mRNA by partial complementary binding, thus silences the gene expression and represses the post translational activity. The means of function of miRNA *via* alteration of gene expression and consecutive translational expression, points out that miRNAs can act as tumor suppresser genes or oncogenes depending on their target genes[42,43]. For instance, up-regulation of specific miRNA targeting the tumor suppressor genes which eventually promotes cell growth and cancer initiation acts as oncogenes. On the other hand, up-regulation of specific miRNA targeting genes responsible for oncogenic activities which ultimately lead to cancer inhibition or repression acts as tumor suppressor genes[44]. However, the increasing investigations on miRNA have uncovered the dual role of miRNA in cancer, in which various evidence supports the concept that a same individual miRNA can act as both oncogene and tumor-suppressor gene depending on the cellular environment[45-47]. Based on the literature, extensive studies have reported the correlation between miRNAs and cancer to date.

3.1. Mechanisms involved in miRNA deregulation in cancer

The dysregulation of miRNAs in cancer occurs through numerous overlapping mechanisms including chromosomal abnormalities, transcriptional control alterations, epigenetic modulation and disruption in the miRNA processing machinery[48]. For instance,

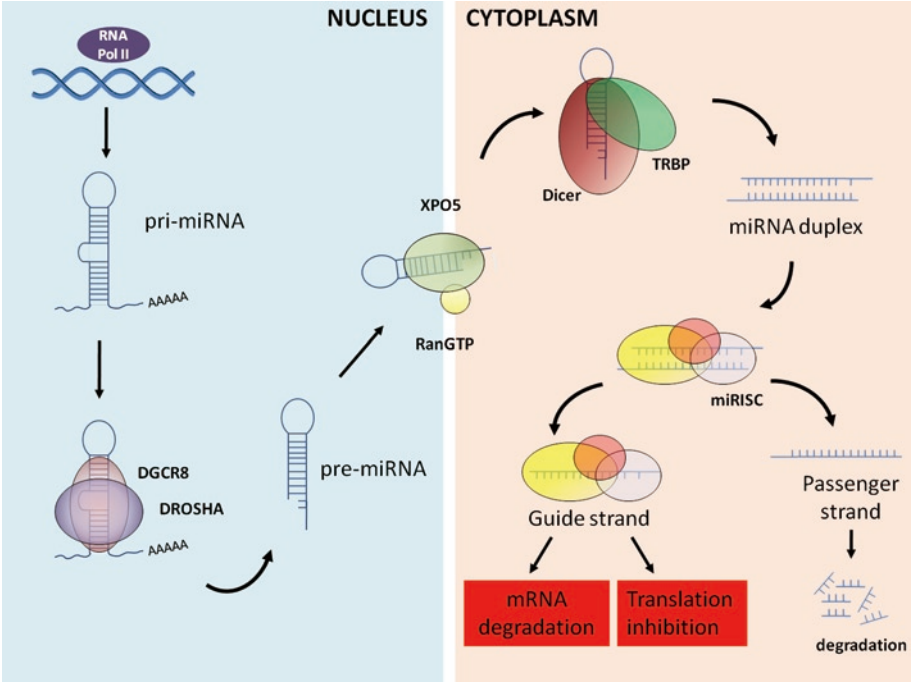

Figure 1. Overview of miRNA biogenesis process.

chromosomal alterations may occur due to amplification of a chromosome site harbouring a specific miRNA, leading to an over-expression of the particular miRNA[49,50] while deletion of the chromosome site may result in down-regulation of the specific miRNA[51,52].

Other than that, various transcriptional factors have been evidently reported to control the expression of miRNAs such as c-Myc[51,53,54], p53[55,56], myeloid transcription factors PU.1 and C/EBPs[57] and transcription factors NFI-A and C/EBP α [58]. Besides, miRNAs have also been reported to undergo epigenetic changes through CpG methylation[59], DNA methylation with histone acetylation inhibitors[60], and hypermethylation[61,62].

Finally, dysregulation or mutation of any proteins involved in miRNA biogenesis process such as Drosha[63], DGCR8[64], Dicer[65,66], Argonaute proteins[67,68], TRBP[69] and Exportin 5[70] leads to miRNA dysregulation.

3.2. Pathways involved in miRNA regulation in cancer

The current chemotherapy targeting miRNA is attaining great interest due to their important participation in cancer pathway. Numerous miRNAs were also evidently shown to regulate apoptosis pathway induced by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Based on the research conducted by Yang *et al*.[71], over-expression of miR-145 was shown to down-regulate ZEB2 expression, which causes an increase in TRAIL-induced apoptosis in LX-2 cells through NF- κ B signaling pathway. The up-regulation of miR-221 and miR-222 was also demonstrated to be over-expressed, leading to the down-regulation of tumor suppressor $p27^{kip}$ in prostate carcinoma[72] and melanoma[73].

Besides, interactions between miR-203a with ITGA4, miR-6071 with ITGAV, and miR-375 with THBS2 were reported to be associated with the dysregulation of PI3K/Akt-signaling pathway in colorectal cancer[74]. Recently, miRNA-146b was found to regulate the PI3K/Akt/NF- κ B signaling pathway to mediate vascular inflammation and apoptosis in myocardial infarction by phosphatase and tensin homologue (PTEN)[75]. Another example of miRNA which participates in Fas-mediated apoptotic pathway is MicroRNA-181c which was shown to hinder apoptosis by targeting FAS receptor in Ewing's sarcoma cells[76]. Another cancer pathway, namely PTEN pathway, was also shown to be regulated by the expression of miRNAs. For instance, many miRNAs are reported to target and suppress the expression of *PTEN* which is one of the prominent tumor suppressor genes such as *miR-17-5p*[77], *miR-19305p*[77], *miR-2127*[74] and *miR-221* and *miR-222*[78].

There are various miRNAs which have been reported to regulate the cell cycle regulatory pathway, in which oncogenic miRNAs tend to expedite cell cycle progression while the miRNAs with tumor suppressor effect tend to facilitate cell cycle arrest. Exemplary oncogenic miRNAs include miR-106b and miR-17-92 families which have been reported to be over-expressed in various cancers that are known to target one of the important inducer of G_1 arrest, namely p21 from the Cip/Kip family of CDK inhibitors[79,80]. Other studies have also experimentally validated other miRNAs to target other genes involved in cell cycle which eventually regulate the RAS/RAF/MAPK pathway as well as the p53 pathway[81]. Furthermore, miRNAs are also very well known to target numerous genes involved in DNA damage response in cancer cells. For instance, miR-421 was reported to be highly over-expressed in neuroblastoma and B-cell lymphoma cell lines and was shown to target the apical damage sensor Ataxia-Telangiectasia Mutated kinase[82].

4. MiRNAs regulated by medicinal plants in human cancer cells

Medicinal plants rich in bioactive phytochemicals are well utilized to treat various diseases including cancer by regulating diverse signaling pathway. Mechanistic studies revealed that plants exert their biologic effects, especially anti-cancer properties by inducing apoptosis in cancer cells through the regulation of miRNA. Plant-derived chemotherapy has recently attained vast interest as the natural secondary metabolites exert lower toxic side effects compared to that of chemically synthesised anti-cancer drugs. Based on the extensive literature, various medicinal plants have been evidently reported to regulate a diversified range of miRNAs to date.

Various medicinal plants exhibit pharmacological properties by up-regulating specific miRNAs in humans. For instance, the bioactive compound isolated from the root of *Astragalus membranaceus* (Fisch.) Bunge was reported to exhibit anti-cancer property on human osteosarcoma MG63 cells by inducing apoptosis through the up-regulation of miR-133a[83]. Furthermore, Western blotting analysis revealed that the over-expression of miR-133a induced the down-regulation of proteins such as p-JNK and p-c-Jun, which eventually inactivates the c-Jun *N*-terminal protein kinase (JNK) pathway[83]. Another well-known medicinal plant, *Salvia miltiorrhiza* (*S. miltiorrhiza*) was previously reported to induce caspase-dependent intrinsic apoptosis in multiple myeloma and myeloid leukemia[84-86]. Recent study also revealed the molecular mechanism underlying the induction of apoptosis by *S. miltiorrhiza*, in which up-regulation of tumor suppressor gene, *miR-216b* was reported in *S. miltiorrhiza* treated U266 and U937 cells in comparison with the untreated cells[87]. Interestingly, the target protein of miR-216b, namely c-Jun protein, was shown to be down-regulated in *S. miltiorrhiza* treated cells[87].

Saponin-rich tuber extract from *Cyclamen pseudibericum* was reported to inhibit cell proliferation in A549 non-small cell lung carcinoma cells through the up-regulation of miR-200c[88]. Western blotting analysis showed that the over-expression of miR-200c inhibits its target protein, namely the zinc-finger E-box binding homeobox 1[88]. Similarly, various studies demonstrated the anti-cancer properties of saponin content from American ginseng (*Panax quinquefolius*)[89,90]. Recently, hexane fraction of *Panax quinquefolius* was demonstrated to exhibit anti-proliferative activity in human colon cancer cell lines by up-regulating the miR-29b expression as compared to that of vehicle control cells, and subsequent Western blotting analysis confirmed the repression of its target protein matrix metalloproteinase-2 protein[91].

Pterostilbene, one of the bioactive components isolated from the blueberries, was reported to promote anti-cancer activity in breast cancer cells by up-regulating the expression of miR-448, which eventually suppresses the expression of NF κ B[92]. Another

such phytochemical, namely sulforaphane which can be found in cruciferous plants, like broccoli sprouts, kale, and carrots, was shown to have anti-cancer property[93]. Sulforaphane exhibits anti-cancer property in human gastric carcinoma cell lines, MGC803 and BGC823 *via* up-regulation of miR-124, which directly targets and suppresses the expression of interleukin-6/IL-6 receptor/signal transducer and activator of transcription 3 signalling[94]. Oxymatrine, another bioactive compound which is found in various medicinal plants of the genus *Sophora*, was reported to inhibit cell proliferation and to induce apoptosis in various cancer types, including gallbladder cancer[95], breast cancer[96], melanoma[97] and prostate cancer[98]. Further investigation on the molecular mechanism underlying the pharmacology effect of oxymatrine revealed the up-regulation of miR-29b in oxymatrine-treated ovarian carcinoma OVCAR-3 cells, which led to reduction on the matrix metalloproteinase-2 expression, in order to inhibit proliferation and to induce apoptosis[99].

Moreover, medicinal plants, along with their isolated phytochemicals, have been evidently reported to exhibit anti-cancer activity *via* down-regulation of specific miRNAs in human cancer cells. One such medicinal plant is *Cnidium officinale* Makino, which has been previously reported to exert anti-cancer effect on various cancers, such as liver cancer[100], colorectal cancer[101] and oral cancer[102]. In recent study, *Cnidium officinale* showed its anticancerous effect through the down-regulation of miR-211 in multiple myeloma U266 cells and lymphoma U937 cells, which caused the ROS generation/CHOP activation to induce apoptosis[103].

One of the bioactive compounds, namely icariin, which is mainly found in the traditional Chinese medicinal plant *Epimedium*[104], has been well-documented to exhibit various pharmacological activities including anti-cancer[105,106]. In ovarian cancer A2780 cells, icariin induced caspase-dependent apoptosis through the down-regulation of miR-21 expression, which was then revealed to increase the expression levels of its target proteins, namely PTEN and RECK, in Western blotting assay[107]. Another bioactive compound responsible for anti-cancer activity is mistletoe lectin-Ⅰ (ML-Ⅰ) which was isolated from a medicinal plant called the mistletoe (*Viscum album*)[108]. The *in vitro* and *in vivo* experiments revealed the anti-cancer effect of ML-Ⅰ in colorectal cancer cells, through MTT assay and nude mouse xenograft models, respectively[109]. Further investigation by miRNA expression array indicated the down-regulation of miR-135a&b expressions in ML-Ⅰ treated colorectal cancer cells as compared to that of control cells. In addition, Western blotting analysis showed up-regulation of target proteins of miR-135a&b, namely adenomatous polyposis coli[109].

Interestingly, curcumin (diferuloylmethane) which is a flavonoid isolated from the rhizome of *Curcuma longa* has been reported to show anti-cancer activity in pancreatic cancer cell line through the regulation of miRNAs. The miRNA microarray revealed a significant up-regulation of miR-22 and down-regulation of miR-199a* in curcumin treated BxPC-3 human pancreatic carcinoma cell line as compared to the untreated cell line. The over-expression of miR-22 was shown to significantly down-regulate the expression levels of SP1 transcription factor and estrogen receptor 1 proteins, corresponding to the prediction of target genes of miR-22 through PicTar and TargetScan bioinformatics tools[110].

5. Conclusion and future prospects

Cancer which is one of the deadly diseases globally demands for more effective, cheap and less toxic therapies. Ever since miRNAs play an important role in regulating important biological processes including cell proliferation and apoptosis, gene therapy targeting miRNAs has been well established. Medicinal plants as the reservoir of various bioactive components responsible for anti-cancer properties through the regulation of miRNAs have been considered as a promising candidate for cancer chemotherapy. In this review, the possible pathways of miRNA regulation in cancer as well as the contribution of medicinal plants to regulating miRNAs are presented. To sum up, plant-derived anti-cancer drugs are highly recommended to treat cancers due to their effective miRNA targeting approach.

Conflict of interest statement

The authors declare that there is no conflict of interests.

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Authors' contributions

SS conceived the original idea and supervised the project. NS and SS wrote the manuscript.

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