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Review

The oncogenic and tumour suppressive roles of microRNAs in cancer and apoptosis

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ABSTRACT

MicroRNAs (miRNAs) are small, non-coding, endogenous RNAs that regulate gene expression at the post-transcriptional level. MiRNAs play important roles in regulating a variety of biological process such as proliferation, differentiation and apoptosis. It has been demonstrated that miRNAs have a crucial function in oncogenesis by regulating cell proliferation and apoptosis as oncogenes or tumour suppressors. As several reports have underlined the possible contribution of miRNAs to promote or evade apoptosis, it seems that the dysregulation of miRNAs involved in apoptosis may provide a mechanism for cancer development. Given emerging evidence that points to oncogenic and tumour suppressive roles of miRNAs in cancer and apoptosis, it is thought that manipulating miRNA expression level may be a potential therapeutic strategy for curing cancer.

1. Introduction to microRNA; biogenesis, processing and function

MicroRNAs (miRNAs), small RNA molecules of approximately 22 nucleotides, are a novel class of endogenously encoded non-coding RNAs that control gene expression by targeting specific mRNAs bearing partially complementary target sequences for degradation and/or translational repression.[1,2] Since the discovery of small non-coding RNAs lin-4 and let-7 (now known to be miRNAs) in *Caenorhabditis elegans*,[3,4] hundreds of miRNA sequences have been so far identified in a wide range of organisms from plants to humans. In the human genome, it is currently estimated that there may be 1000 miRNA genes which could account for approximately 1% of the genome and up to one third of human genome may be regulated by miRNAs. Each miRNA can target approximately 200 transcripts directly or indirectly, whereas a single protein coding gene target could be regulated by more than one miRNA (http://www.sanger.ac.uk/Software/Rfam/mirna/).

MiRNAs are transcribed by RNA polymerase II or III as short RNA hairpin structures which are subsequently processed by the nuclear and cytoplasmic RNase III-type enzymes. Primary microRNA transcripts (pri-miRNA) are processed in the nucleus by the RNase III endonuclease Drosha and DGCR8 (the 'microprocessor complex') to form intermediate stem-loop structures approximately 70 nucleotides long called 'precursor miRNAs' (pre-miRNAs).[5,6] The process is followed by exportin-5-mediated export of pre-miRNA to the cytoplasm. Further processing facilitated by the cytoplasmic RNase III endonuclease Dicer complex appears coupled to the assembly of the mature single strand miRNA (often designated miR) into the RNA-induced

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silencing complex (RISC), which is the effector of RNA interference (RNAi) pathways.[7] The subsequent mechanisms by which miRNAs regulate gene targets depend on the degree of complementarity between miRNAs and its target molecules. Perfect (or near perfect) complementarity allows Argonaute (Ago)-catalysed cleavage of mRNA, whereas imperfect complementarity promotes repression of translation[8] (Fig. 1).

MiRNAs bind to the 3′-untranslated regions (UTR) of the target mRNA by imperfect base pairing, although miRNA may also target coding regions of mRNA, at least in animals. Sequence complementarity between nucleotides 2–8 of miRNA, named 'seed sequence', is important for target sequence recognition because only this sequence of the approximately 22 nucleotides aligns perfectly with the 3′-UTR of target mRNA.[9] Owing to a perfect complementarity between miRNAs and their target mRNAs which almost never exists in mammals, the direct prediction of relevant downstream targets of a miRNA could be challenging. Bioinformatics approaches and several software programs such as miRanda,[10] PicTar,[11] and TargetScan[12] have been developed to predict putative miRNA targets through analysis of the miRNA seed sequences. However, due to the focus of the approach on identifying conserved targets in the 3′-UTR of an mRNA, many non-conserved targets could be missed. Therefore, the efficacy of proposed miRNA:mRNA interactions should be assayed and validated *in vitro* or *in vivo*.

MiRNAs have been shown to become involved in a variety of biological processes, including developmental timing, tissue differentiation, cellular proliferation, organ development, maintenance of stem cell potency and apoptosis.[4,13,14] Aberrant expressions of miRNAs are connected to human cancers and a growing body of evidence point to their important roles, as onco/tumour suppressor miRNAs, in the development of various human malignancies.[15–18]

2. MicroRNAs as a novel class of oncogenes and tumour suppressor genes in cancer

Aberrant expression patterns of miRNAs are associated with several examples of human tumourigenesis, suggesting that miRNAs may play a critical role as a novel class of oncogenes or tumour suppressor genes. The expression levels of oncogenic miRNAs, called 'oncomiRs', are increased in different cancers. These miRNAs usually promote tumour development by negatively inhibiting tumour suppressor genes and/or genes that control cell differentiation or apoptosis. However, there are many miRNAs which may be considered as tumour suppressors because their expression is decreased in malignant cells. These miRNAs may function by negatively

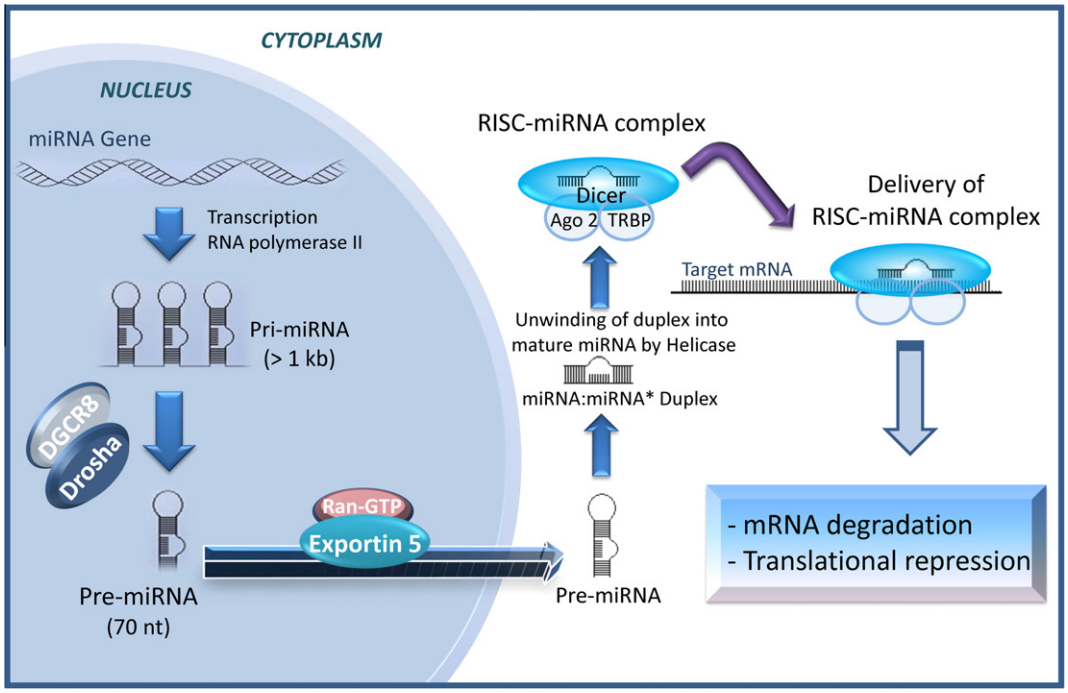

Fig. 1 – Schematic representation of biogenesis, processing and function of miRNA: RNA polymerase II primarily facilitates transcription of the miRNA gene in the nucleus. The resultant pri-miRNA transcript is processed by Drosha, producing a characteristic stem loop precursor, pre-miRNA. The pre-miRNA is then exported into the cytoplasm by exportin-5 and Ran-GTP. In the cytoplasm, final processing mediated by Dicer removes loop structures of pre-miRNAs, producing a duplex molecule containing the single stranded mature miRNA and a miRNA* fragment. The miRNA:miRNA* duplex is unwind by Helicase; the miRNA* fragment is degraded, whereas the mature miRNA molecule binds to an Argonaute (Ago) protein and incorporates into the RISC. The RISC–miRNA complex can then target mRNAs bearing a perfectly complementary target site for degradation or can repress the translation of an mRNA that shows imperfect complementarity with the small RNA. Primary miRNA, pri-miRNA; precursor miRNA, pre-miRNA; Drosha, RNase III endonuclease; DGCR8, DiGeorge syndrome critical region 8; Dicer, RNase III endonuclease; RISC, RNA-induced silencing complex.

inhibiting oncogenes and/or genes that inhibit cell differentiation or apoptosis.[15–17]

MiRNAs functioning as tumour suppressor genes such as the let-7, which negatively regulates Ras[19] and high mobility group A2 (HMGA2);[20,21] mir-15a and mir-16-1, which negatively regulate BCL2;[22] as well as the miR-34, that is induced by DNA damage and oncogenic stress in a p53-dependent manner which leads to apoptosis or cellular senescence.[23] MiRNAs functioning as oncogenes such as miR-21, targets the tumour suppressors tropomyosin 1[24] and programmed cell death 4 (PDCD4) in breast cancer cells[25] and also phosphatase and tensin homologue detected on chromosome ten (PTEN) in hepatocellular carcinomas.[26,27] The miR-17-92 cluster can be regarded as a family of oncogenes, directly targeting many genes involved in apoptotic pathways.[28] Thus, miRNAs can act both as oncogenes and tumour suppressors, depending on the particular miRNA and the cell type.

2.1. MiR-17-92 cluster, a family of oncogenic miRNAs

One of the first and well-studied oncogenic miRNAs identified is the miR-17-92 cluster, containing seven homologous miRNAs (miR-17-3p, miR-17-5p, miR-18a, miR-20a, miR-19a, miR-19b-1 and miR-92a-1), with genomic positions on chromosome X, 7 and 13. The cluster located on chromosome 13 seems to be frequently overexpressed in a range of haematopoietic malignancies, particularly B-cell lymphomas.[29] Because the miR-17-92 cluster targets many genes involved in apoptotic pathways, it seems that the combination of suppressing many target mRNAs is responsible for the anti-apoptotic effect.[28,30] The proto-oncogene c-Myc is a helix-loop-helix leucin zipper transcription factor that regulates cell proliferation, growth and apoptosis by targeting about 10–15% of the human genes.[31] The proto-oncogene c-Myc induces the expression of miR-17-92 cluster through direct binding to the locus at chromosome 13q31. This binding has been confirmed via chromatin immunoprecipitation assays (CHIPs).[32] The miR-17-92 cluster decrease Myc-induced apoptosis, possibly by targeting apoptotic factors activated in response to Myc overexpression.[28] Interaction between the cluster and c-Myc modulate the expression of the E2F transcription factor family members (E2F1, 2 and 3).[32] The E2F1, a transcription factor promoting cell cycle progression, is induced by c-Myc and creates a reciprocal positive feedback loop by inducing c-Myc expression. Owing to the miR-17-5p and miR-20a which directly target (the 3′UTR of) the E2F1 in a negative feedback loop of transcriptional regulation, it seems that c-Myc simultaneously promotes E2F1 transcription and represses following translation, indicating a tightly controlled cell cycle progressive signal. The miR-17-92 cluster represses the activity of E2F1, thus reducing Myc-induced cell proliferation. Given that excessive E2F1 induces apoptosis, the cluster might be activated by Myc to counter the apoptotic activity of E2F1, allowing Myc-mediated proliferation. This finding would suggest a tumour suppressor role for the miR-17-92 cluster, which contrasts with the hypothesised oncogenic role seen in B-cell lymphoma.[32]

The oncogenic activity of mir-17-92 cluster in malignant lymphomas was further investigated *in vivo*, in the Eu-Myc transgenic mouse model of human B cell lymphoma. In this model, transgenic mouse contain immunoglobulin heavy chain enhancer (Eu) driven c-Myc oncogene (Eu-Myc) and overexpression of the c-Myc oncogene leads to lymphoma development.[28] He et al.[28] demonstrated that overexpression of the miR-17-92 cluster not only accelerates c-Myc-induced lymphoma development, but also resulted in a more aggressive tumour in these lymphoma models, indicating oncogenic roles of miR-17-92 in B-cell cancer development and progression. Tumours resulting from combined c-Myc and miR-17-92 expression showed a low degree of apoptosis when compared to tumours lacking the cluster, suggesting a mechanism for evasion of apoptosis.

On the basis of bioinformatics studies, there are a wide variety of targets for miRNA members of the cluster: more than 600 for miR-19a and miR-20, two members of miR-17-92 cluster.[11,33] Yu et al.[34] showed the ability of cyclin D1 to induce expression of miR-17-5p and miR-20a. Cyclin D1 can bind to promoter regulatory region of the miR-17-92 cluster and induce expression of miR-17-5p and miR-20a, in turn the miRNAs bind to the complementary site (in the 3′UTR) of cyclin D1 mRNA, leading to inhibition of proliferation in breast cancer cells. The possible role of miR-17-92 cluster for evading normal apoptotic responses has been further strengthened by the validation of the pro-apoptotic gene Bim as a direct target. The pro-apoptotic gene Bim is a crucial regulator of B-cell survival and a tumour suppressor in the Eu-Myc model of B-cell lymphoma. Negative regulation of Bim by the miR-17-92 cluster may provide a mechanism for evasion of apoptosis. The postulated mechanism is that miR-17-92 may have anti-apoptotic function in B cells and thus abrogates Myc-induced apoptosis in this lymphoma model.[35]

Another two predicted targets of the miR-17-92 cluster seems to be the tumour suppressor genes PTEN and RB2.[12] PTEN promotes apoptosis through the P13K-Akt-PKB pathway.[36,37] Akt, as a major cell survival pathway which plays a key role in resistance to apoptosis, was confirmed to be the target of PTEN.[38] MiR-19 has been demonstrated to down-regulate the tumour suppressor PTEN and thereby would increase flux through the PI3K-Akt signalling pathway and promote cell survival.[36]

Inomata et al.[39] revealed that silencing of two miR-17-92 encoded miRNAs (miR-17 and miR-20a) in mantle cell lymphoma cells leads to up-regulation of cyclin-dependent kinase (CDK) inhibitor p21, suggesting that p21 is an essential target of miR-17-92 during B cell lymphomagenesis. Consequently, up-regulation of p21 results in G1–S arrest and decreased cell growth. It appears that miR-17-92 cluster down-regulates expression of distinct targets in different B-cell lymphoma subtypes.

2.2. The let-7 family of miRNAs, a family of tumour suppressive miRNAs

The human let-7 family of miRNAs is a highly conserved group comprising 12 closely related members (let-7-a-1, a-2, a-3, b, c, d, e, f-1, f-2, g, i and miR-98) organised in eight distinct clusters.[40] These 12 family members represent nine distinct let-7 sequences with identical seed sequences and, probably, overlapping sets of targets. The let-7 genes are lo-

cated at fragile sites associated with human cancers, suggesting a possible role in human cancer.[41] This is further strengthened by the validation of a common tumour suppressor role for let-7 in a variety of human tissues, particularly in the lung, by negatively regulating the expression of multiple oncogenes including RAS and MYC as well as other cell cycle progression genes.[19,42]

Johnson et al.[19] showed that lung tumour tissues of patients with both adenocarcinoma and squamous cell carcinoma exhibit significantly decreased levels of let-7 and significantly increased levels of RAS protein relative to normal lung tissue, suggesting that let-7 regulation of RAS is a possible mechanism for let-7 to function as a tumour suppressor gene in lung oncogenesis. Let-7 is complementary to multiple sites in the 3′UTR of human RAS genes, allowing let-7 to repress the expression of K-RAS and N-RAS in tissue culture. In lung squamous cell carcinoma, down-regulation of let-7 miRNA in association with over-expression of RAS oncogene has been reported,[19] consistent with let-7 negatively regulating RAS protein levels *in vivo*. The inverse correlation between the expression of let-7 and RAS protein level in the lung tumour suggests that the level of expression of the miRNA might be an important factor in limiting oncogenesis. Furthermore, poor expression level of let-7 may be a powerful diagnostic and even prognostic marker for lung tumour.[43]

Let-7 directly targets a few key cell cycle proto-oncogenes such as CDC25A and CDK6 in addition to RAS, thus directly represses cell proliferation by reducing flux through the pathways promoting the G1 to S transition. Johnson et al.[44] analysed the expression of two cell cycle regulators, CDK6 and CDC25A, in cells transfected with pre-miRs. They found that protein levels of both CDK6 and CDC25A decrease in cells transfected with pre-let-7 compared with cells transfected with a control pre-miRNA. Pre-let-7 transfection resulted in approximately a 50% reduction of protein compared with the normal levels of CDK6 and CDC25A. Owing to close correlation between CDK6 and cyclin D to promote the G1 to S transition and the fact that CCND2 (encoding cyclin D) is the highest scoring cell cycle gene predicted as a let-7 target by PicTar,[45] the CCND2 3′UTR was also tested in the same assay. Interestingly, a similar result to CDK6 was found, implying that CCND2 is also a direct target of let-7. It seems that the poor let-7 expression and/or aberrant let-7-mediated regulation of key cell cycle progression proteins (i.e. Ras, CDC25a, CDK6, cyclin D and CCND2) which are frequently observed in cancers, allows for the up-regulation of these oncogenic proteins, resulting in unregulated cell cycle progression and ultimately transformation.[46]

High mobility group A2 (HMGA2) oncogene, which is frequently mutated in several cancers, is also another target of let-7.[20,21] The HMGA2 3′UTR was shown to harbour seven let-7 binding sites and disruption of these sites reduce miRNA-mediated HMGA2 regulation and consequently enhances oncogenic transformation.[20,21] Importantly, overexpression of HMGA2 devoid of let-7 binding sites decreased the tumour suppressor function of let-7 in lung cancer cells. Finally, there seems to be an inverse correlation between let-7 and HMGA2 expression and ectopic overexpression of HMGA2 promotes cellular proliferation in the presence of let-7.[20] Although the above findings propose a tumour suppressor role for the let-7 family of miRNAs, there are no convincing evidences that let-7 miRNA can suppress tumourigenesis *in vivo*. Moreover, the regulation of individual let-7 targets on tumourigenesis *in vivo* needs to be further investigated.[47]

Apart from the tumour suppressor role of let-7 miRNA, it also modulates cell proliferation which is frequently dysregulated in cancer cells.[44] Meng et al.[48] has been reported that let-7a, one of the let-7 family miRNAs, modulates interleukin-6-dependent STAT-3 survival signalling in human malignant cholangiocytes by targeting the tumour suppressor gene NF2. In this study, let-7a was confirmed to be involved in the signalling for cancer survival. Given that the increased expression of interleukin-6 is associated with poor outcomes in cancer therapy was commonly observed, it is thought that let-7a may directly regulate the cellular response to therapeutic drugs. It seems that the outcome of cancer therapy by using chemotherapeutic drugs is determined by apoptosis.[49]

Tsang et al.[49] revealed that let-7a regulates the drug-induced apoptosis in human cancer cells by targeting caspase-3, a major executioner caspase in apoptosis. This study demonstrated that caspase-3 is a direct target of let-7a as ectopic expression of let-7a reduced the luciferase activity of a reporter construct containing the 3′UTR of caspase-3 and at the same time repressed the enzyme expression in human squamous carcinoma A431 cells and hepatocellular carcinoma HepG2 cells. By targeting caspase 3, Let-7a is confirmed to be an important factor in the regulation of the drug-induced apoptosis in A431 cells and HepG2 cells. This is supported as down-regulation of let-7a through the anti-let-7a inhibitor increased the drug-induced apoptosis in A431 parent cells, A10A cells (the drug resistant subline of A431 cells) and HepG2 cells while the increase was suppressed by caspase-3 inhibitor. Both anti-let-7a inhibitor and caspase-3 inhibitor, however, failed to affect the drug-induced apoptosis in human breast cancer MCF7 cells, the cells that do not express caspase-3. Taken together, caspase-3 was confirmed to be the target of let-7a and the miRNA plays a regulatory role in therapeutic drug-induced apoptosis of the cancer cells expressing caspase-3. MiRNA-mediated regulation of apoptosis is discussed below.

3. MicroRNA-mediated regulation of apoptosis

Apoptosis is programmed cell death, generally characterised by distinct morphological/cellular characteristics and energy-dependent biochemical mechanisms. Apoptosis is essential for normal development and homeostasis and serves to remove excess, damaged or harmful cells in multicellular organism(s). Apoptotic cell death is triggered by the extrinsic and the intrinsic (regulated by mitochondria) signalling pathways, that are closely related via protein interactions.[50] Deregulated apoptotic pathways have been shown to be involved in the pathogenesis of cancer.[51] The roles of miRNAs in apoptotic signalling pathways have not yet completely determined; however, a number of studies have highlighted pivotal regulatory roles of miRNAs in this programmed process.

3.1. MiR-21, an miRNA with proliferative and anti-apoptotic functions, targets a network of tumour suppressive pathways

In a number of studies, miR-21 was confirmed to be a regulatory miRNA that has been linked to tumour aggression and carcinogenesis, in part, by preventing apoptosis. MiR-21 is overexpressed in multiple malignancies such as lung,[43] breast,[52] pancreatic,[53,54] oesophageal,[55] cervical[56] and colon[57] cancers.

The highly malignant human brain tumour, glioblastoma, robustly overexpresses a specific miRNA, miR-21. Chan et al.[13] observed that miR-21 was strongly overexpressed (5- to 100-fold) in highly malignant human glioblastoma tumour tissues, early-passage glioblastoma cultures, and in six established glioblastoma cell lines compared with its expression in non-neoplastic controls. Their results also indicated that knockdown of miR-21 in cultured glioblastoma cells triggers activation of caspases and leads to increased apoptotic cell death, indication a role in down-regulation of apoptosis-related genes. On the base of the observations, they suggested that miR-21 is an anti-apoptotic factor in human glioblastoma and its aberrant expression may contribute to the malignant phenotype through blocking expression of critical apoptosis-related genes. Paradoxically, knockdown of miR-21 led to increased cell growth in HeLa cells,[14] indicating the opposite roles of an miRNA, as a tumour suppressor or an oncogene, in the control of cell proliferation in distinct cancers. This function discrepancy might attribute to the type of individual miR-21 targets driving tumourigenesis and the differences in the expression pattern of them. However, some methodological differences between the contradictory reports could not be ruled out.[58]

Papagiannakopoulos et al.[59] were able to show that miR-21 targets a network of p53, transforming growth factor-β (TGF-β) and mitochondrial apoptosis tumour suppressor genes in glioblastoma cells. They suggested that miR-21 up-regulation may be a key step leading to oncogenesis in glioblastoma. This study also demonstrated that down-regulation of miR-21 in glioblastoma cells led to derepression of the p53 pathway. Derepression of the p53 pathway in response to miR-21 down-regulation may assist in the derepression of the cytostatic response of TGF-β signalling, leading to repression of growth, increased apoptosis and cell cycle arrest. The phenotypic effects observed upon down-regulation of miR-21 in two established glioblastoma cell lines, U251 and U87, reflect the significant repression of multiple components of the p53, TGF-β and apoptotic pathways by miR-21. Furthermore, the results showed that down-regulation of miR-21 was accompanied by up-regulation of p21, a protein that is known to regulate cell cycle checkpoints in response to its transactivation by p53. The functional relationship between miR-21 targets suggests that altered expression levels of miR-21 modulate the robustness of a highly interconnected tumour suppressive network. This consequently leads to global regulation or dysregulation of the network functions.

One study reported that inhibition of miR-21 in MCF-7 breast cancer cells caused reduced cell growth.[25] The expression levels of miR-21 have been shown to link with the p53 tumour suppressor protein. Apart from previously identified targets for miR-21 which include the tumour suppressors tropomyosin 1 in breast cancer cells[24] and PTEN in hepatocellular carcinomas,[26,27] this study showed that the tumour suppressor PDCD4 was regulated by miR-21 in breast cancer cells.[25] Since PDCD4 seems to be involved in inhibiting AP-1-mediated trans-activation[60] and inducing expression of the CDK inhibitor p21,[61] down-regulation of PDCD4 confers growth advantages to the cells. Consequently, this increased growth capacity, which is achieved by several means, facilitates the development of cancer.[25]

The miRNA regulation of PDCD4 was demonstrated to occur at the translational level and to be mediated through direct interaction at the seed region in MCF-7 breast cancer cells. On the basis of the study, PDCD4 which is a tumour suppressor known to be up-regulated during apoptosis[62] and down-regulated in several cancer forms[63–65] could be an important mediator of the biological effects of miR-21 in breast cancer cells.[25] It seems the dysregulation of miR-21 could be responsible for cancer initiation and progression in breast cancer through the miRNA regulation of PDCD4.

3.2. Down-regulation of BCL-2 expression by the miR-15/16 cluster induce intrinsic pathway of apoptosis

The Bcl-2 family of apoptotic proteins contains pro-apoptotic and anti-apoptotic members, all of which function as crucial regulators of the intrinsic apoptotic pathway.[66] The anti-apoptotic BCL-2 which frequently overexpressed in a number of human cancers such as breast,[67] Hodgkin's lymphoma[68] and B-cell lymphoma,[69] blocks the mitochondrial release of cytochrome c and inhibits the activation of caspase 9 through imperfectly sequence complementary to the target mRNA. The miR-15a and miR-16-1 are clustered on human chromosome 13q14 which is frequently lost or down-regulated in B-cell chronic lymphocytic leukaemia (CLL) and several solid tumours. This indicates that the anti-apoptotic *BCL-2* overexpression in CLL might be due to the loss or down-regulation of the miR-15/16 cluster.[70]

It was proposed that both miR-15a and miR-16-1 promote the normal apoptotic response by direct targeting the anti-apoptotic gene BCL-2, indicating the probable tumour suppressive function of these two miRNAs in tumourigenesis.[22,46] In fact, the 3′UTR region of BCL2 contains one potential binding site for both miRNAs. The interaction between miR-15a and miR-16-1 and anti-apoptotic gene BCL2 leads to cleavage of pro-caspase 9 and poly ADP-ribose polymerase (PARP), and activation of the intrinsic apoptosis pathway. Therefore, miR-15 and miR-16 are natural antisense Bcl2 interactors that could be used for therapy of Bcl2-overexpressing tumours.[22]

Furthermore, it was recently revealed that in non-small cell lung carcinoma (NSCLC) cells, cyclin D1, cyclin D2 and cyclin E1 are directly regulated by physiological concentrations of miR-15a and miR-16. MiRNA-mediated down-regulation of these cyclins, which control the progression of cells through the cell cycle by activating CDK enzymes, leads to inhibition of cell proliferation.[71] In the study led by Bandi et al.[72], G1 cyclins were confirmed to be the major targets of miR-15a/miR-16 in NSCLC. They showed that overexpression of this couple of miRNAs induced cell cycle arrest in an Rb-dependent manner in G1–G0.

3.3. MiR-29 regulates Mcl-1 protein expression and apoptosis

Mcl-1 is a potent multi-domain anti-apoptotic protein of the Bcl-2 family which specifically binds to pro-apoptotic members Bim and Bid preventing TRAIL (tumour necrosis factor-related apoptosis-inducing ligand)-induced cell death.[73] Mcl-1 overexpression is frequently observed in cancers including cholangiocarcinoma.[74,75] In cholangiocarcinoma, overexpression of Mcl-1 prevents TRAIL-induced apoptosis, whereas its suppression sensitises cells to apoptosis.[75] The study led by Mott et al.[76] showed the ability of miR-29 to regulate Mcl-1 protein expression. In silico analysis identified a putative target site in the 3′UTR of Mcl-1 mRNA for the miR-29. Inverse correlation between miR-29b and Mcl-1 expression was demonstrated in malignant KMCH cholangiocarcinoma and non-malignant H69 cholangiocytes cell lines. Non-malignant H69 cholangiocytes cell line showed abundant expression of miR-29 accompanying reduced Mcl-1 protein levels, while miR-29 expression was reduced in cholangiocarcinoma cell line which strongly expressed Mcl-1 protein. Owing to KMCH cholangiocarcinoma cells are resistant to TRAIL-induced apoptosis compared to non-malignant H69 cells due to Mcl-1 expression, overexpression of miR-29 sensitised the cancer cells to TRAIL cytotoxicity, consistent with miR-29 negatively regulating Mcl-1 protein levels.

Garzon et al.[77] recently showed that miR-29 expression reduced cell growth and induced apoptosis in cell lines and primary acute myeloid leukaemia (AML) samples. The analysis of primary AML samples revealed an inverse correlation between miR-29b and Mcl-1 expression. Another recent work led by this author has shown that over-expressing miR-29b in AML cell lines and primary AML blasts down-regulates the expression of DNA methyltransferases isoforms; DNMT1, DNMT3A and 3B. MiR-29b induces global DNA hypomethylation and re-expression of tumour suppressor genes such as the CDK inhibitor $p15^{INK4b}$ and the oestrogen receptor ESR1 genes by targeting directly DNMT3A and 3B and indirectly DNMT1 through its activator Sp1.[78] Nevertheless, overexpression of DNA methyltransferases isoforms may mediate aberrant hypermethylation and epigenetic silencing of tumour suppressor genes.[79]

MiR-29 is also down-regulated in other malignancies such as CLL,[80] colon[81] and breast[52] cancers. On the basis of these studies, reduced expression of mir-29b helps cells evade apoptosis, a common characteristic of cancer cells. Therefore, approaches to enhance miR-29 expression would be of value to reduce Mcl-1 protein levels and induce TRAIL-mediated apoptosis. These findings strongly point towards a potential role of miR-29 in cancer therapy.

3.4. Mir-34 and the p53 tumour suppressor network

P53 is the most broadly studied tumour suppressor with pleiotropic functions. It operates as a transcription factor in response to diverse cellular stresses, regulating target genes involved in DNA repair machinery, cell cycle arrest at G1/S checkpoint and apoptosis.[82] The p53 serves as a transactivator or transrepressor for many different downstream genes to trigger apoptotic response. The p53-mediated transactivation of apoptosis-related genes includes pro-apoptotic Bcl-2 family members (e.g. Bax, Puma, Noxa and BH3-only member Bid) leading to the intrinsic apoptotic pathway; apoptotic protease activating factor-1 (APAF-1); and Fas/CD95, death receptor 4 (DR4) and DR5, components of the extrinsic apoptotic pathways.[82,83] The p53 can also function by repressing anti-apoptotic proteins such as Bcl-2 and Bcl-xL, leading to the release of cytochrome C from mitochondria, the initiation of caspase cascade, and resultant DNA fragmentation. Dysfunction of the p53 pathway is a common characteristic of most human cancers.[84]

In mammalians, the miR-34 family of miRNAs (miR-34a, b and c) comprises three processed miRNAs that are encoded by two different genes. MiR-34a is encoded by its own primary transcript, whereas miR-34b and miR-34c share a common primary transcript.[23] It is demonstrated that the miR-34 family as direct, conserved p53 target genes, presumably induce apoptosis, cell cycle arrest and senescence.[85,86] P53 occupied an evolutionarily conserved binding site proximal to the first non-coding exon of miR-34a. Several studies have identified that the highly conserved miR-34 family to be involved in p53 mediated cell apoptosis; however, miR-34a is also regulated independent of p53 during oncogene-induced senescence.[87] He et al.[88] have highlighted that overexpression of miR-34 leads to G1 cell-cycle arrest and apoptosis in various cancer cell lines, whereas reduction of miR-34 expression attenuates p53-mediated apoptosis showing that miRNAs affecting tumour suppressor pathways can suppress tumour cell proliferation.

Chang et al.[86] were able to show that p53-induced transactivation of miR-34 promotes apoptosis and leads to dramatic reprogramming of gene expression, in particular the genes regulating the cell cycle progression, apoptosis, DNA repair and angiogenesis. Apoptotic cell death measurement following transient transfection of miR-34a into HCT116 $p53^{WT}$ and $p53^{-/-}$ cells revealed low levels of apoptosis in cells transfected with control oligonucleotide ($p53^{WT}$, 6.6 ± 3.4% apoptotic cells; $p53^{-/-}$, 4.1 ± 1.6% apoptotic cells). On the contrary, transfection of $p53^{WT}$ cells with synthetic miR-34a potently induced apoptosis (24.2 ± 3.8% apoptotic cells). Interestingly, apoptosis was considerably decreased but not completely stopped following transfection of miR-34a into $p53^{-/-}$ cells (9.3 ± 2.5% apoptotic cells), suggesting both p53-dependent and p53-independent mechanisms of miR-34a-induced apoptosis. These data give more emphasis to the significance of the miR-34a expression to promote apoptosis triggered by p53 activation. They also reported that pancreatic cancer cells which frequently exhibit p53 loss of function, showed a reduction in miR-34a expression as compared to the expression in HPNE and HPDE cells, two non-transformed pancreatic ductal epithelial cell lines. In general, the genomic region encompassing miR-34a was found to be lost in several human cancers, in particular pancreatic cancer. Taken together, these findings suggest an important role for miR-34a in mediating p53 tumour suppressor function.

A genome-wide screening for p53-regulated miRNAs conducted by Tarasov et al.[89] show that the most pronounced increase in miRNA abundance after p53 activation was observed for miR-34a. Further analysis of this miRNA in this study showed that the addition of the DNA-damaging agent

Table 1 – Some miRNA involved in various human cancers with altered expression level.

Cancer type	Increased expression	Decreased expression	Reference(s)
Breast	miR-21, miR-22, miR-23, miR-29b-2, miR-96, miR-155, miR-191, miR-181, miR-182, miR-27a, miR-210	miR-205, miR-143, miR-145, miR-10b, miR-125a/b, miR-155, miR-17-5p, miR-27b, miR-9-3, miR-31, miR-34 family, let-7	52,71,93,100
Ovary	miR-200a/b/c, miR-141, miR-18a, miR-93, miR-429	miR-199a, miR-140, miR-145, miR-125a,b, let7	93,101,102
Colorectal	miR-18, miR-224, miR-10a, miR-17-92 cluster, miR-21, miR-24-1, miR-29b-2, miR-31, miR-96, miR-135b, miR-183	miR-143, miR-145, let-7, miR30-3p, miR-124a, miR-129, miR-133b, miR328	58,100,103
Lung	miR-17-92 cluster, miR-21, miR-155, miR-191, miR-205, miR-210	let-7, miR-34 family, miR-143, miR-145, miR-124a	19,43,93,100,103,104
Glioblastoma	miR-221, miR-222, miR-21	miR-181a, miR-181b, miR-181c, miR-125a, miR-125b	93,100
Oesophagus	miR-194, miR-192, miR-200c, miR-21	miR-203, miR-205	55
Gastrointestinal	miR-106b-25	miR-15b, miR-16	105,106
Thyroid	miR-146b, miR-221, miR-222, miR-181b, miR-155, miR-197, miR-224, miR-346	miR-30d, miR-125b, miR-26a, miR-30a-5p	100,107,108
Pancreas	miR-221, miR-376a, miR301, miR-21, miR-24-2, miR-100, miR-103, miR-107, miR-125b-1, miR-155, miR-181, miR-106, miR-363, miR-301, miR-376a, miR-212, miR-34a	miR-375, let-7, miR-200, miR-200b	54,71,100,103,109
Prostate	let-7d, miR-195, miR-203, miR-21, miR-181, miR-106, miR-363, miR-221	miR-128a, miR-101, miR-125a/b, miR-15a, miR-16-1, miR-143, miR-145, miR-23a/b, miR-200, miR-330, miR-331	58,71,93,100
Bladder	miR-17, miR-23a,b, miR-26b, miR-103-1, miR-185, miR-203, miR-205, miR-221, miR-223	miR-29c, miR-26a, miR-30c, miR-30e-5p, miR-145, miR-30a-3p, miR-133a/b, miR-195, miR-125b, miR-199a	110,111
AML	miR-191, miR-199, miR155, miR-221, miR-222, miR-125a/b	miR-124a, miR-148a, miR-181a, miR-204, miR-223	112–114
APL	miR-15a, miR-15b, miR-16-1, let-7a-3, let-7c, let-7d, miR-223, miR-342, miR-107	miR-181b	114
ALL	miR-17-92 cluster, miR-125b-1, miR-128a, miR-128b, miR-204, miR-218, miR-331, miR-181a, miR-181b, miR-181c, miR-142-3p, miR-142-5p, miR-150, miR-193a, miR-196b, miR30e-5p, miR-34b, miR-365, miR-582, miR-708	let-7b, miR-223, miR-100, miR-125b, miR-151-5p, miR-99a	114,115
CML	miR-17-92 cluster, miR-17-5p, miR-17-3p, miR-18a, miR-19a, miR-19b-1, miR-20a, miR-92a-1	miR-10a	114,116
CLL	miR-21, miR-23b, miR-24-1, miR-146, miR-155, miR-106b, miR-195 miR-221, miR-222	miR-15a, miR16-1, miR-29, miR-143, miR-45, miR-30d, let-7a, miR-181a/b, miR-223, miR-92, miR-150	22,70,80,103, 114,117–119
Endometrioid adenocarcinoma	miR-205, miR-449, miR-429	miR-193a, miR-204, miR-99b	120
Hepatocellular	miR-18, miR-21, miR-33, miR-130b, miR-135a, miR-221, miR-224, miR-301	miR-199a/b, miR-195, miR-200a/b, miR-214, miR-223, miR-125a, miR-122a, miR-101, miR-139, miR-150, miR-26a, miR-101	71,100,103,121

Abbreviations: APL, acute promyelocytic leukaemia; AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; CML, chronic myeloid leukaemia; CLL, chronic lymphocytic leukaemia.

etoposide induced the expression of the miR-34a in the cell line MCF-7 which expresses wild-type p53. In fact, a dramatic induction of the miR-34a primary transcript after p53 activation was detected, indicating p53-dependent induction of miR-34a after DNA damage. Furthermore, the function of p53-induced miR-34a in apoptosis was determined by trans-

fection of H1299 cells with duplex siRNAs corresponding to processed miR-34a. DNA content analysis by flow cytometry revealed an increase in the fractions of cells with a sub-G1 DNA content, which is indicative of apoptosis, whereas the number of cells in S-phase and G2/M-phase was decreased. Therefore, this study proposed that activation of miR-34a by p53 may contribute to induction of apoptosis.

The anti-apoptotic proto-oncogene Bcl2 is down-regulated by miR-15a and miR-16, two other p53-induced microRNAs with tumour suppressive activity.[22] Interestingly, the overexpression of miR-34 leads to a mild decrease in Bcl2 protein level.[90] It is thought that miR-34a can act together with other miRNAs such as miR-15 and miR-16[22] to suppress efficiently anti-apoptotic Bcl2. MiR-34a is a potent suppressor of cell proliferation through modulation of E2F signalling pathway. Thus, expression of miR-34a causes reprogramming of genes involved in p53 mediated cell apoptosis. In addition, ectopic expression of miR-34a reduces the levels of E2F3 by targeting its mRNA.[89,91] Other targets of the p53-induced miRNAs are the NOTCH1 receptor and its ligand DLL1, which were confirmed to be the targets for down-regulation by miR-34a.[12]

4. Potential use of microRNAs in cancer therapy

As stated above, it has been demonstrated that miRNAs are differentially expressed in human cancers and contribute to cancer development (Table 1). However, it remains to be further elucidated whether this altered expression of miRNAs occurs as a consequence of the pathological state of cancer or whether the cancer is a direct cause of this altered expression. Nonetheless, given that cancer cells often exhibit a distinctive pattern of miRNA expression and many functionality validated miRNA targets are oncogenes and tumour suppressors,[92,93] it is thought that manipulating of miRNA expression levels could be a potential therapeutic strategy for developing efficient therapies against cancer. The feasibility of manipulating miRNA expression levels have been demonstrated by antisense oligonucleotide targeting experiments. For oncogenic miRNAs, potential anti-miRNA therapeutics include the locked nucleic acid (LNA)-modified oligonucleotides,[94] the anti-miRNA oligonucleotides (AMOs)[95] and the 'antagomirs'.[96] For miRNAs which function as tumour suppressor, restoring the expression levels of these miRNAs via overexpression of them, in the purpose of consequent miRNA-mediated targeting of cancer oncogene, may be a potential therapeutic strategy.[27,92] However, the efficacy of the antisense oligonucleotide targeting strategy needs to be further evaluated by future *in vivo* studies of miRNA transgenics and knockouts.[92] Two examples about the potential use of miRNA in cancer therapy are briefly described below.

Galardi et al.[97] showed that ectopic overexpression of miR-221 and miR-222 enhanced the growth potential of slowly growing prostate carcinoma LNCaP cells and induced a progression to the S phase of cell cycle by targeting CDK inhibitor p27, a dosage-dependent tumour suppressor in prostate cancer. This induced increase of the colony-forming potential of LNCaP cells in soft agar. MiR-221 and miR-222 knockdown through LNA antisense oligonucleotides increases the levels of CDK inhibitor $p27^{Kip1}$ in aggressive prostate carcinoma PC3 cells, and considerably reduces *in vitro* clonogenicity feature of the cells. This study suggests that the overexpression of miR-221 and miR-222 may contribute to the oncogenesis and progression of prostate carcinoma, at least in part through down-regulation of the tumour suppressor $p27^{Kip1}$. Therefore, miR-221 and miR-222 silencing based strategy would be of value against prostate cancer.

Kefas et al.[98] demonstrated that miR-326, a neuronally-expressed microRNA, acts in a feedback loop with Notch signalling pathway in glioblastomas. Given the finding that Notch and miR-326 each suppress the other, they speculate that this Notch/miR-326 axis is shifted towards high Notch activity and low miR-326 activity. Their results showed that efficient delivery of miR-326 has therapeutic potential against both glioma stem-like cells and established glioma lines.

5. Conclusion and perspectives

Emerging evidence suggests that miRNAs play important roles in human cancers and act as onco/tumour suppressor miRNAs. MiRNAs may be involved in cancer development by controlling cell differentiation and apoptosis or targeting cancer oncogenes and/or tumour suppressors. Intriguingly, due to significantly altered expression profile of miRNA in different types of cancer, miRNA profiling could be used for classification of human tumours,[16] indicating the diagnostic and even prognostic value of miRNA profiling.[99] There is no doubt that uncovering the crucial roles of miRNAs in cancer and the potential of miRNA-based therapeutics opens up new opportunities in the future of cancer therapy. However, the basic biology of cancer/miRNA pathways is not completely elucidated and further studies, establishing the oncogenic or tumour suppressive roles of miRNAs using *in vivo* experimental models, are needed to gain more insights into basic mechanisms of miRNA functions in cancer.

Conflict of interest statement

None declared.

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