J. Cell. Mol. Med. Vol 13, No 1, 2009 pp. 12-23

Guest Editor: M. Ivan

MicroRNA and cancer – focus on apoptosis

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Received: July 30, 2008; Accepted: September 11, 2008

Abstract

MicroRNAs (miRs) are small non-coding RNAs regulating gene expression at the post-transcriptional and/or translational levels. miRs play important roles in diverse biological processes, including development, cell differentiation, proliferation and apoptosis. Recent evidence has shown that miR loci frequently map to cancer-associated genomic regions and deregulated miR expression profiles are associated with many cancer types, implicating miRs in crucial processes that lead to tumourigenesis. Here, we review the current findings about miRs and tumourigenesis, focusing on their involvement in the apoptosis pathway. A significant observation is that greater than one-quarter of all known human miRs were reported to be deregulated in at least one cancer type. The expression of a subset of miRs (*e.g.* miR-21 and miR-155) was found to be consistently up-regulated, whereas another subset of miRs (*e.g.* miR-143 and miR-145) was consistently down-regulated across different cancer types suggesting their involvement in regulating common cellular processes whose deregulation may lead to tumourigenesis. Several miRs were implicated to play roles in cell proliferation and apoptosis. Some miRs, such as miR-29b and miR-15-16, influence only the apoptotic pathway, whereas others including let-7/miR-98 and miR-17-92 may play roles in both the apoptotic and cell-proliferation pathways. In conclusion, although our current understanding of the functions of miRs is still fragmentary, taken together, this review highlights the complex and intricate roles that miRs play in the regulation of cellular processes. Perturbation of the expression of miRs may thus lead to tumourigenesis.

Keywords: microRNAs • tumourigenesis • apoptosis

Introduction

microRNAs (miRs) are a class of small non-coding RNAs whose mature products are ~22 nucleotides long. They negatively regulate gene expression at the post-transcriptional and/or translational level. They were first discovered by Ambros and colleagues in 1993 [1] in *C.elegans* and were shown to be abundantly expressed in viruses [2], plants [3] and animals [4]. To date, there are a total of 6396 miRs (miRBase Release 11. http://microrna.sanger.ac.uk/sequences/), of which, 678 miRs are found in human beings [5–7]. Many miRs show sequence and function conservation between distantly related organisms, suggesting that this class of small RNAs is an integral part of essential cellular processes [8]. For example, Lethal-7 (Let-7) was initially discovered to be responsible for the developmental transition of L4 lavae to the adult cell fates [9] in *C.elegans*. It was later found to be evolutionarily conserved, regulating development in *Drosophila*, zebrafish, annelids, mollusks [8] and mouse [10] and possibly

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doi:10.1111/j.1582-4934.2008.00510.x

human beings, which comprised 12 members of the Let-7 family. Their strong evolutionary conservation suggests that they are likely to have an ancient origin [11] although they were identified only recently. Their discovery has opened up a new dimension in our understanding of gene regulation.

miR biogenesis

microRNAs are encoded in the genome and transcribed by RNA polymerase II as primary transcripts that are called pri-miRs. Pri-miRs are typically 3 to 4 kilobases long single-stranded RNAs with 5′cap, 3′ poly(A) tail and complicated secondary structure [12, 13]. The Pri-miRs are processed in the nucleus into one or more precursor-miRs (pre-miRs) of ~70-nucleotide by microprocessor complex comprising the nuclear RNase III, Drosha, and the double-stranded RNA binding protein, Pasha/DGCR8 [13–15]. Pre-miRs are then actively exported to the cytoplasm through exportin-5 in association with RAN-GTPase [16, 17]. In the cytoplasm, another RNase III, known as Dicer, further processes the pre-miR into ~22-nucleotide mature miR, which is double-stranded (miR duplex). The miR duplex [18, 19] comprises a strand (miR strand), which is incorporated into the multi-protein RNA-induced silencing complex (miRISC) and a complementary strand (miR* strand), which is degraded. Thermodynamic stability of the strand probably determines the choice of strand to be incorporated into miRISC complex [20]. In mammalian system, the functional miRISC carrying the mature miR can bind to the 3′untranslated region (3′UTR) of its target gene mRNA to result in either mRNA degradation (for nearly perfect complementary base-paring) or protein translation inhibition (for imperfect complementary base-paring). The mechanism of inhibition will depend on the miR sequence, the target mRNA sequence and the exact composition of the miRISC protein complex [21, 22].

miR and cancer

The importance of microRNAs in cancer is highlighted by the observation that ~50% of miRNA genes are located in cancer-associated genomic regions or fragile sites [23, 24], which are frequently amplified or deleted in tumourigenesis. Global repression of microRNA processing machinery (Drosha, Pasha/DGCR8 and Dicer1) promotes cellular transformation and miRNA processing-impaired cells formed tumours with accelerated kinetics in mouse model, implicating the role of mature miRs in cancer-related processes [25]. Large-scale microRNA expression profiling of human cancers have revealed that miRNA deregulation is frequently associated with many cancer types including those originating from the blood [26–31], brain [32–34], thyroid [35–37], breast [38], lung [39–41], tongue [42], nose and pharynx [43], liver [44–47], the gastro-intestinal system (esophageal [48], gastric [49], pancreatic [50, 51] and colorectal cancers [52, 53]) as well as the genitourinary system (cervical [54], ovarian [55, 56] and prostate [57, 58] cancers).

Table 1 summarizes our current knowledge on the profile of miR expression in various human cancers. In these studies, miR expression in tumours is compared against paired non-tumourous tissues from cancer patients and significantly up- and down-regulated miRs are indicated with red-box/up-arrow and green-box/down-arrow, respectively. More than one-quarter of known human miRs (175 out of 678 miRs) have been reported to be significantly deregulated in at least one cancer type. However, this may be a gross underestimation of the actual numbers of deregulated miRs as the majority of the known miRs were only identified in the previous 2 years and were not included in earlier miR expression profiling studies. Nonetheless, this observation suggests that microRNAs may represent one of the largest classes of gene regulators implicated in cancer-related processes although very little are known about them. Table 1 also highlights some interesting patterns of miR expression profiles in cancers. Of the cancer-implicated miRs, miR-21 is the most commonly up-regulated miR in both solid and haematological tumours, consistent with the report of Volinia *et al.* [58]. Besides miR-21, other miRs including miR-155, miR-181b, miR-221 and miR-222 are also frequently up-regulated in cancers of the blood, brain, thyroid and the gastro-intestinal (GI) systems, and to a lesser extent in liver cancer, lung cancer and breast cancer. In contrast, the let-7/miR-98 cluster is commonly down-regulated in tumours of the thyroid, breast, lung, upper GI and the genitourinary system. Similarly, miR-143 and miR-145 are frequently down-regulated in the haematological tumours and solid tumours of the breast, lung, prostate and the lower GI system. Such common deregulation of miR expressions across various tumour types suggests that these miRs may be involved in crucial cellular pathways that are commonly deregulated in cancer development. Indeed, functional studies have demonstrated that let-7/miR-98 negatively regulate RAS [59] and v-myc myelocytomatosis viral oncogene homologue (MYC) [60] whilst miR-21 negatively regulate phosphatase and tensin homologue (PTEN) [44] and programmed cell death 4 (PDCD4) [61], which are proto-oncogenes or tumour suppressors that regulate important cellular processes, including cell growth, proliferation and apoptosis whose deregulation leads to tumourigenesis. In addition to miRs that are commonly deregulated across different cancers, there are also miRs that seem to be deregulated in only specific tumour types (Table 1). For example, the miR-17-92 cluster and miR-93 are frequently reported to be up-regulated mainly in cancers of the GI system. Interestingly, a very large proportion of miRs (~81%) were found to be up-regulated in thyroid tumours, whereas a high percentage of miRs (~70%) were reported to be down-regulated in prostate cancers. These observations suggest that some tumour-specific mechanisms may be in place to favour particular miR profiles depending on the tumour micro-environment. It is also worth noting that miR-105, miR-144, miR-193 and miR-199b are seldom reported to be deregulated in cancer, despite their relatively early discovery, suggesting that these miRs probably play a role in cellular

Table 1: miRs that are significantly differentially expressed in human cancers and their validated *in vivo* targets.

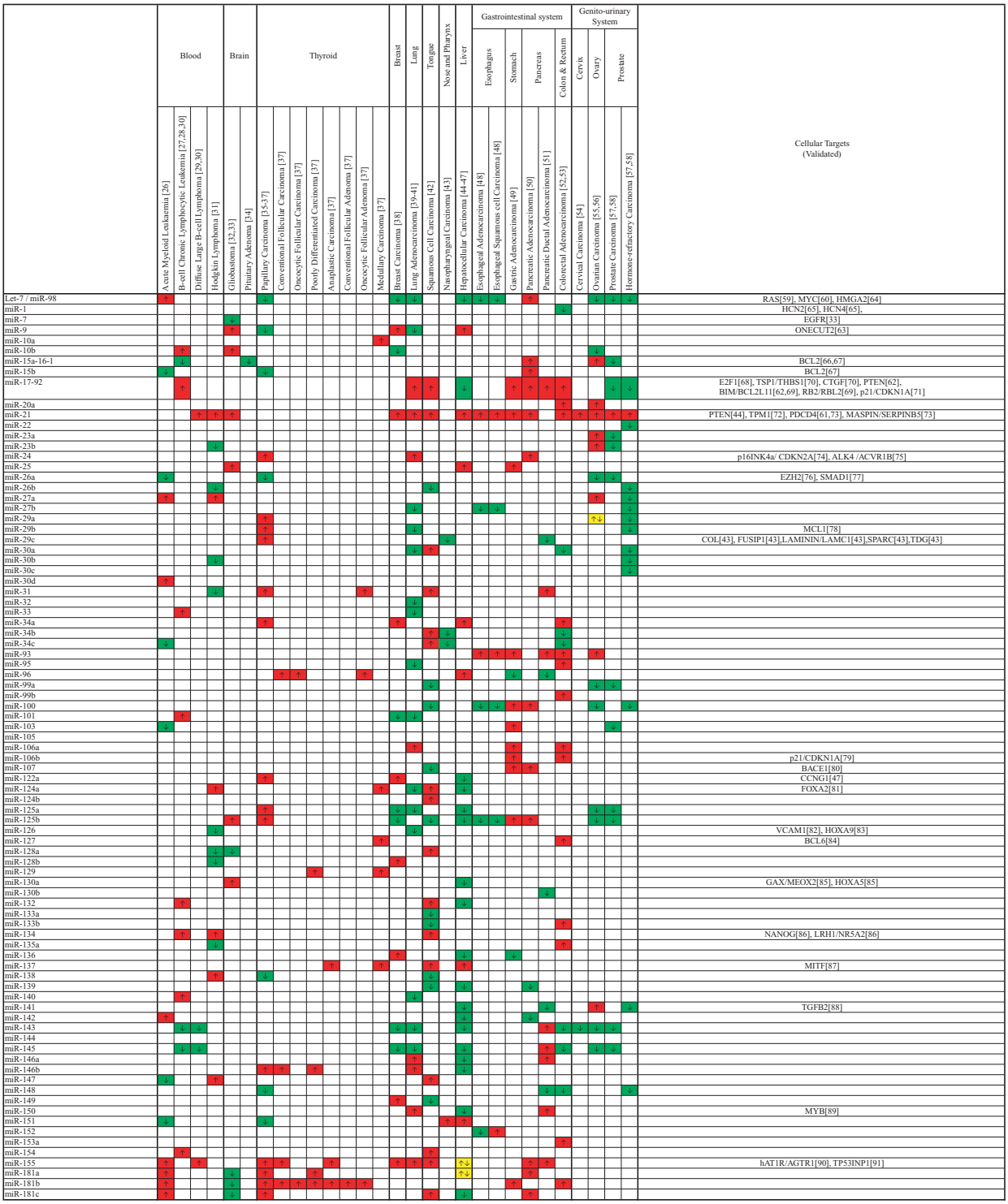

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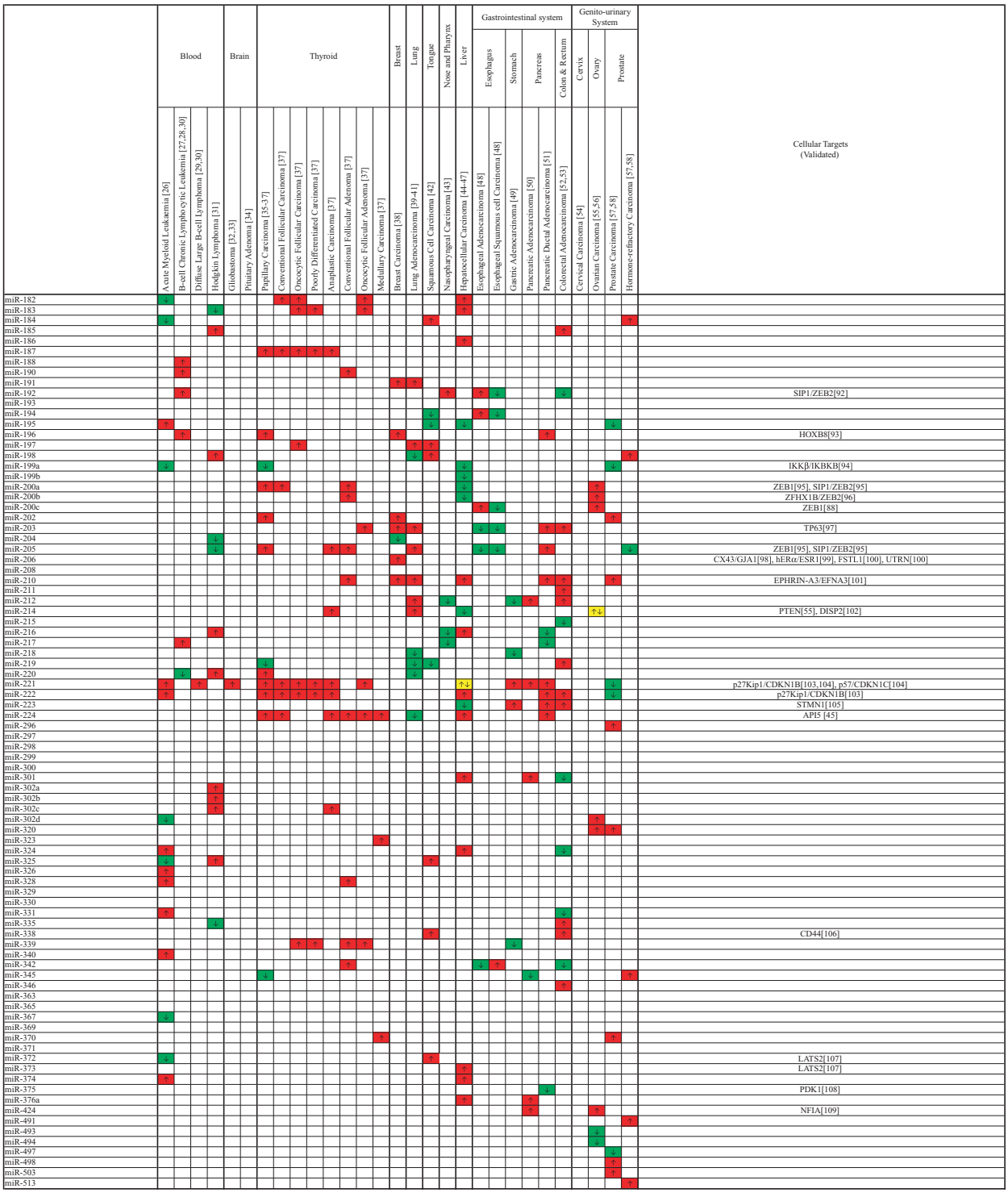
Table 1: Continued



Upward pointing arrows in red boxes indicate that the miR is up-regulated in the cancer-type while downward pointing arrows in green boxes indicate down-regulated miRs. Yellow boxes with both Upward- &downward-pointing arrows indicate that miR was reported to be up-regulated in one study but down-regulated in another study.

house-keeping processes and are less likely to be involved in oncogenesis.

Although much is known about the aberrant miR expression pattern associated with various cancers, much less is known about the functional relevance of such miR deregulation or the *in vivo* miR targets. Table 1 also summarizes a total of 65 non-overlapping experimentally validated direct cellular targets of miRs that are reported to date [33, 43–45, 47, 55, 59–109]. Table S1 annotates these validated targets based on Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. These 65 validated miR target genes show a significant enrichment in the classical cancer-associated pathways such as transcription, cell–cell adhesion and signalling, cell-cycle regulation, cell proliferation and apoptosis, strongly suggesting that the deregulation of these miR target genes may play significant roles in carcinogenesis. However, as predicted by miR target prediction algorithms (miRanda [6], PicTar [110] and TargetScan [111]), each individual miR can potentially regulate hundreds of cellular gene targets. But reports of the identification and characterization of these *in vivo* miR targets remain few, which prevent our comprehensive understanding of the miR-regulated networks that significantly impact cell differentiation, cell proliferation and apoptosis [112]. Current knowledge on a limited number of miRs or miR clusters has revealed the complexity of miR-regulatory networks, and in this review, we will discuss the role of a few well-studied miRs in tumourigenesis with a focus on its impact on the apoptotic pathway.

miR and apoptosis

Apoptosis is the intrinsic cellular mechanism to eliminate cells that are damaged or transformed. Deregulation of apoptosis is an important step in cancer as it allows the genetically unstable cells to survive and accumulate further mutations that eventually lead to tumourigenesis. As cancer cells are mostly characterized by increased cell proliferation and decreased cell death, cancer-implicated genes have conventionally been classified into two groups. One group, the oncogenes, up-regulates proliferation and down-regulates apoptosis, whereas the other group, the tumour suppressor genes, performs just the opposite function. Indeed, pro-apoptotic genes such as p53 are frequently inactivated whilst anti-apoptotic genes such as B-cell CLL/lymphoma 2 (BCL2) are frequently over-activated in cancer progression. However, recent evidence has shown that up-regulation of MYC and E2F oncogenes can increase both cell proliferation and apoptosis [113, 114], suggesting the classification of cancer-related genes into oncogenes or tumour suppressors may be an over-simplification. Figure 1 summarizes our current knowledge of miRs implicated in cell-proliferation and apoptosis, revealing that the miR-regulatory network is just as complicated as its protein-coding counterparts. Some miRs, such as miR-29b and miR-15-16, were found to affect only the apoptotic pathway, whereas others including let-7/miR-98 and miR-17-92 play roles in both the apoptotic and cell-proliferation pathways (see review [114, 115]). In the following discussion, we will discuss some pro-apoptotic miRs, anti-apoptotic miRs and miRs that regulate both proliferation and apoptosis.

The pro-apoptotic miRs targeting the BCL2 family of genes

The miR-15-16 cluster induces apoptosis by targeting the important anti-apoptotic factor BCL2 at the post-transcriptional level [66]. It was proposed to function as a tumour suppressor by keeping cell growth in check under normal physiological conditions. Like many tumour suppressors, this miR cluster is found to be frequently deleted in B-cell chronic lymphocytic leukaemia (CLL), resulting in its down-regulation in more than 68% of the CLL cases [28]. The miR-15-16 cluter is also reported to be down-regulated in pituitary adenoma [34] and prostate carcinoma [57]. Hence in these cancers, miR-15-16 expression is preferentially down-regulated to favour cancer development by inhibiting apoptosis (Fig.1). In a recent study, which utilized expression microarray to investigate the effects of miR-15a and miR-16-1 on the transcriptome and proteome of MEG-01 leukaemic cells, genes (*e.g.* MCL1, ETS1 and JUN) that directly or indirectly play a role in apoptosis and cell-cycle were found to be significantly differentially expressed in these cells. Another miR, miR-29b, which is down-regulated in lung and prostate cancers (Table 1), was reported to also target myeloid cell leukaemia sequence 1 (MCL1) [78], a member of the BCL2 family, implying that the function of miR-29b may be similar to that of miR-15-16. Curiously, in some cancers, the expression of these miRs (miR-15-16 and miR-29b) was reported to be up-regulated instead. One possible explanation to these seemingly contradictory observations may perhaps be that these miRs may deregulate other cellular processes in addition to apoptosis in these specific cancers.

The anti-apoptotic miR-21 targets PTEN and PDCD4

miR-21 is the most consistently up-regulated miR across many cancer types (Table 1). miR-21 was first implicated as an anti-apoptotic factor by the observation that knock-down of miR-21 increased apoptotic cell death in human glioblastoma cells [116] and in the mouse model [117]. miR-21 directly targets PTEN whose down-regulation will release its inhibition on protein kinase B (PKB) resulting in significantly reduced apoptosis in cancer cells (Fig.1). miR-21 also targets PDCD4 [61], a pro-apoptotic gene frequently down-regulated in hepatocellular carcinoma(HCC) [118]. Interestingly, miR-21 was also reported to be up-regulated in HCC (Table 1). This suggests that miR-21 can inhibit apoptosis through both PTEN and PDCD4. Recently, miR-21

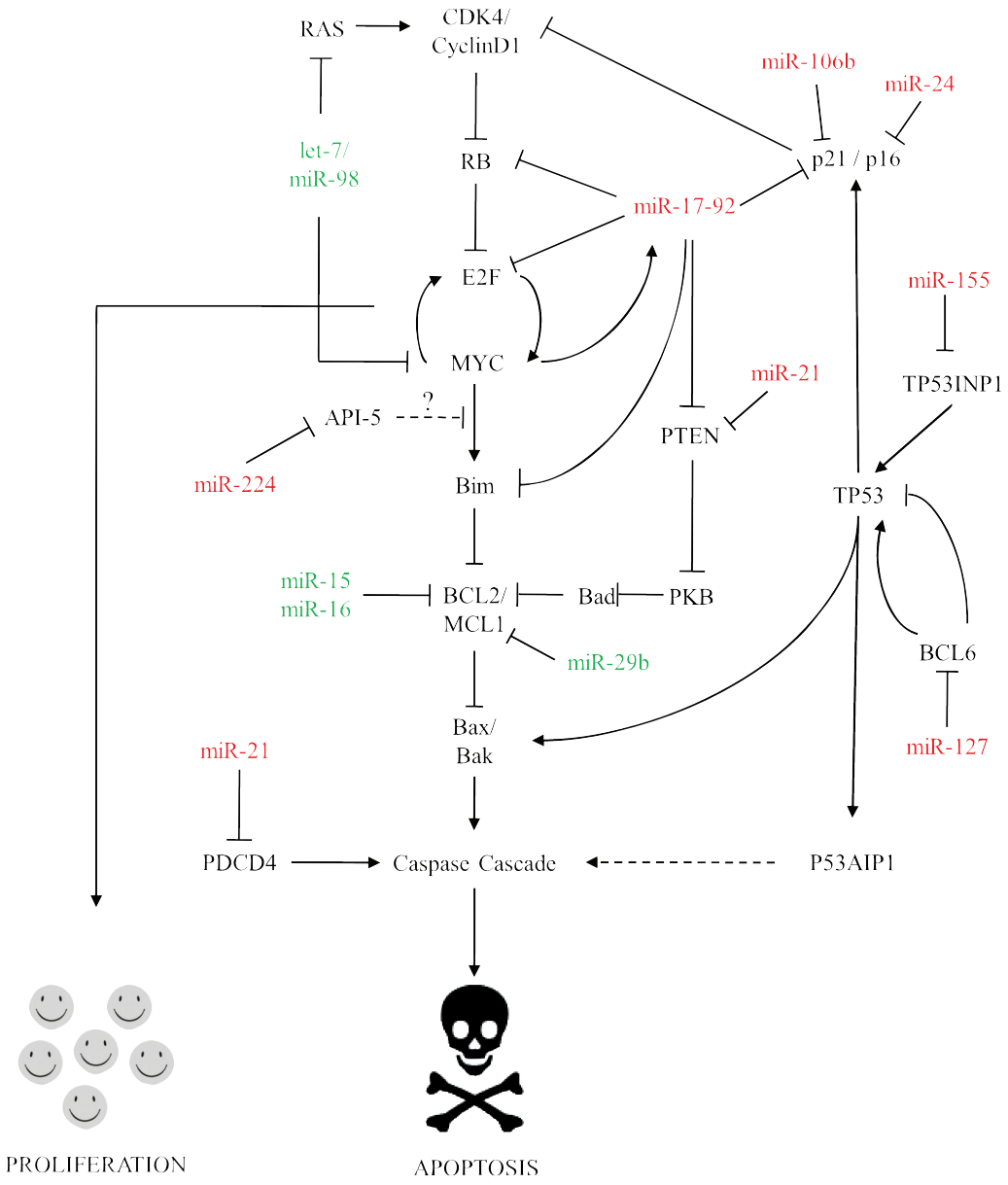


Fig. 1 Diagrammatic representation of the roles of miRs in the regulation of cell proliferation and apoptosis. Red colour indicates a general up-regulation of miRs in cancers and green colour indicates a general down-regulation of miRs in cancer.

was reported to target important tumour suppressor genes including tropomyosin 1 (TPM1) [72] and serpin peptidase inhibitor, clade B (ovalbumin), member 5 (SERPINB5) [73] suggesting that miR-21 may also play a role in tumour invasion and metastasis. Hence, the oncogenic potential of miR-21 lies in its ability to regulate multiple cancer-associated pathways probably *via* multiple cellular targets, which may partially explain its frequent up-regulation in cancer.

miR-210 decreases proapoptotic signalling in a hypoxic environment

Hypoxia-regulated microRNAs such as miR-210 is induced in response to low oxygen and play a role in cell survival by decreasing caspase activation, the central components of apoptotic signalling [119]. As hypoxia is an important feature of tumour microenvironment, it is of interest to note that miR-210 is also

over-expressed in many major tumour types (Table 1), suggesting that hypoxia may represent a contributing factor for microRNA deregulation in certain cancers. A recent study by Camps *et al.* has demonstrated that miR-210 is a good prognostic marker for breast cancer [120].

Let-7/ miR-98 family and possible co-operation with miR-21

Our understanding of the role of let-7/miR-98 family in cancer development was facilitated by the identification of two proto-oncogenes regulating cell proliferation and apoptosis, RAS [59] and MYC [60], as direct targets of let-7/miR-98. Under normal physiological conditions, Let-7 regulates cellular proliferation by inhibiting RAS and MYC expression. However, in tumours, let-7/miR-98 are frequently down-regulated resulting in increased expression of cellular RAS and MYC and subsequent elevation of cell proliferation as well as MYC-induced apoptosis [114] (Fig.1). Increased apoptosis and proliferation seem to contradict the conventional wisdom that apoptosis is reduced during carcinogenesis. However, oncogenic changes that promote apoptosis are thought to provide the selective pressure for the cells to override apoptosis during the multistage process of carcinogenesis [121], resulting in the final cell population that retain high proliferative but reduced apoptotic potential. It is important to note that expression of miR-21 is frequently up-regulated in let-7/miR-98 down-regulated tumours of the thyroid, breast, lung, liver, esophagus and prostate (Table 1). This suggests that miR-21 or other cellular factors may counter-balance MYC-induced apoptosis in tumours in which let-7/miR-98 expression is down-regulated, whereas still maintaining a high rate of cell proliferation.

miR-17-92 cluster highlights the complexity of miR regulatory networks

The miR-17-92 cluster, which modulates E2F1 expression, is positively regulated by MYC [122]. Under normal physiological conditions, miR-17-92 facilitates the tight regulation of MYC-mediated cellular proliferation by inhibiting the MYC-induced E2F1 expression. However, when miR-17-92 is over-expressed as in the case of GI cancers, it can potentially become a very potent oncogene targeting multiple cellular pathways to favour tumourigenesis by enhancing cell proliferation and inhibiting apoptosis. As illustrated in Fig.1, miR-17-92 can increase MYC-enhanced proliferation by targeting p21 [71] and consequently activating the CyclinD1/CDK4 complex to release retinoblastoma (RB)'s inhibition on E2F. In addition, miR-17-92 is also capable of down-regulating RB [69] directly to drive cell proliferation. On the other hand, miR-17-92 is also capable of minimizing MYC-induced apoptosis by targeting BCL2-like 11 (BIM) and PTEN [62] to increase the level of anti-apoptotic BCL2. Hence, miR-17-92 is truly worthy of its reputation as the first non-coding oncogene, oncomiR-1 [123]. This miR demonstrates the complexity of miR regulatory network.

miR-224, the double-edged sword

miR-224 is up-regulated in HCC, pancreatic ductal adenoma and various types of thyroid cancers (Table 1). We have demonstrated that over-expression of miR-224 sensitizes cells to apoptosis through API-5, an apoptosis inhibitor, and increase cell proliferation through yet an unknown mechanism [45]. Sassen *et al.* has previously proposed that a single miR can potentially regulate opposing cellular activities such as cell proliferation and apoptosis [124] and miR-224 represents the first such miR identified. Similar to the MYC oncogene, which regulates both cell-proliferation and apoptosis, the dual role of miR-224 to influence both cell proliferation and apoptosis can potentially hasten the selective process favouring cells that accumulate sufficient heritable genetic mutations to override apoptosis during the multistage of carcinogenesis.

Other miRs implicated in apoptosis

There are a number of other miRs that may potentially play roles in regulating apoptosis in cancer. For example, miR-155 is frequently over-expressed in many cancers and targets the tumour protein p53 inducible nuclear protein 1 (TP53INP1) [91]. TP53INP1 was reported to be a positive regulator of p53-dependent apoptosis by enhancing Ser46 phosphorylation of p53 which in turn induced p53-regulated apoptosis-inducing protein 1(p53AIP1) expression and subsequent apoptotic cell death [125]. Hence, over-expression of miR-155 in cancers will inhibit TP53INP1 expression and attenuate apoptotic cell death induced by TP53INP1. In contrast, miR-127 was reported to target B-cell CLL/lymphoma 6 (BCL6) [84] to potentially increase TP53-dependent apoptosis by disrupting the negative regulatory feedback loop between BCL6 and TP53 [126, 127]. However, our understanding of the rationale behind this deregulation in cancer remains unclear.

Conclusion

One of the hallmarks of cancer is defects in the regulatory circuits that control normal cell proliferation and homeostasis. Previously, great efforts were focused on understanding the roles of protein-coding genes in cancer. As discussed above, emerging research are implicating miRNAs as a novel class of non-coding tumour suppressors and oncogenes that play important roles in tumourigenesis. As we review the roles of miRNAs in apoptosis and

cancer, we begin to appreciate that miR's role in tumourigenesis is not merely either pro- or anti-apoptosis. Rather, it is likely that coordination and perhaps synergism between several deregulated miRs and their protein-coding counterparts facilitate a favourable environment for cancer formation. Although current knowledge of miR function and targets is incomplete, it underscores the complexity of the roles of RNA in the regulation of cellular pathways. Continued effort in the detailed characterization of miR target and function is necessary to improve our understanding of the role of miRs in tumourigenesis and facilitates the design of appropriate therapies targeting this novel group of molecules.

Acknowledgements

This work is supported by grants from the National Medical Research Council (NMRC) (NMRC/1131/2007) of Singapore and the Singapore Millennium Foundation (SMF) to Caroline Lee.

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