**MicroRNA and cancer – focus on apoptosis**

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**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 gene 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 larvae 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
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 transported 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-pairing) or protein translation inhibition (for imperfect complementary base-pairing). 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 microRNA genes are located in cancer-associated genomic regions or fragile sites [23, 24], which are frequently amplified or deleted in tumorigenesis. Global repression of microRNA processing machinery (Drosha, Pasha/DGCR8 and Dicer) promotes cellular transformation and microRNA 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 has 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 favourable 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.

| miR | Blood | Brain | Thyroid | Breast | Lung | Prostate | Gastrointestinal system | Genito-urinary System | Nasopharynx | Liver | Pancreas | Esophagus | Tongue |
|-----|-------|-------|---------|--------|------|----------|------------------------|----------------------|-------------|-------|---------|----------|--------|
| miR-1 | +     | +     | +       | -      | -    | -        | -                      | -                    | -           | -     | -       | -        | -      |
| miR-2 | +     | +     | +       | -      | -    | -        | -                      | -                    | -           | -     | -       | -        | -      |
| miR-3 | +     | +     | +       | -      | -    | -        | -                      | -                    | -           | -     | -       | -        | -      |

Cellular Targets (Validated)

- Blood Brain Thyroid
- Breast Lung Prostate
- Gastrointestinal system Genito-urinary System
- Nasopharynx Liver Pancreas
- Esophagus Tongue

Continued
Table 1: Continued

| Cellular Targets | Esophagus | Pancreas | Prostate | Blood Brain Thyroid | Breast | Lung | Tongue | Nose and Pharynx | Liver | Gastrointestinal system | Genito-urinary System |
|-----------------|-----------|----------|----------|---------------------|--------|------|--------|------------------|-------|------------------------|----------------------|
| Acute Myeloid Leukaemia | [19] | | | | | | | | | | |
| B-cell Chronic Lymphocytic Leukaemia | [27, 28, 30] | | | | | | | | | | |
| Diffuse Large B-cell Lymphoma | [29, 30] | | | | | | | | | | |
| Hodgkin Lymphoma | [31] | | | | | | | | | | |
| Glioblastoma | [32, 33] | | | | | | | | | | |
| Pituitary Adenoma | [34] | | | | | | | | | | |
| Papillary Carcinoma | [35-37] | | | | | | | | | | |
| Conventional Follicular Carcinoma | [37] | | | | | | | | | | |
| Oncocytic Follicular Carcinoma | [37] | | | | | | | | | | |
| Poorly Differentiated Carcinoma | | | | | | | | | | | |
| Anaplastic Carcinoma | | | | | | | | | | | |
| Conventional Follicular Adenoma | | | | | | | | | | | |
| Oncocytic Follicular Adenoma | | | | | | | | | | | |
| Medullary Carcinoma | | | | | | | | | | | |
| Breast Carcinoma | [38] | | | | | | | | | | |
| Lung Adenocarcinoma | [39-41] | | | | | | | | | | |
| Squamous Cell Carcinoma | [42] | | | | | | | | | | |
| Nasopharyngeal Carcinoma | [43] | | | | | | | | | | |
| Hepatocellular Carcinoma | [44-47] | | | | | | | | | | |
| Esophageal Adenocarcinoma | [48] | | | | | | | | | | |
| Esophageal Squamous cell Carcinoma | | | | | | | | | | | |
| Gastric Adenocarcinoma | [49] | | | | | | | | | | |
| Pancreatic Adenocarcinoma | [50] | | | | | | | | | | |
| Pancreatic Ductal Adenocarcinoma | [51] | | | | | | | | | | |
| Colorectal Adenocarcinoma | [52, 53] | | | | | | | | | | |
| Cervical Carcinoma | [54] | | | | | | | | | | |
| Ovarian Carcinoma | [55, 56] | | | | | | | | | | |
| Prostate Carcinoma | [57, 58] | | | | | | | | | | |
| Hormone-refractory Carcinoma | | | | | | | | | | | |

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

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References

1. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993; 75: 843–54.
2. Sullivan CS, Grundhoff A, Tevethia S, Treisman R, Pipsas JM, Ganem D. Expression and function of microRNAs in viruses great and small. Cold Spring Harb Symp Quant Biol. 2006; 71: 351–6.
3. Mallory AC, Vaucheret H. Functions of microRNAs and related small RNAs in plants. Nat Genet. 2006; 38 Suppl: S31–6.
4. Stefanì G, Slack FJ. Small non-coding RNAs in animal development. Nat Rev Mol Cell Biol. 2008; 9: 219–30.
5. Griffiths-Jones S. The microRNA Registry. Nucleic Acids Res. 2004; 32: D109–11.
6. Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ. miRBase: microRNA sequences, targets and gene nomenclature. Nucleic Acids Res. 2006; 34: D140–4.
7. Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ. miRBase: tools for microRNA genomics. Nucleic Acids Res. 2008; 36: D154–8.
8. Pasquinelli AE, Reinhart BJ, Slack F, Martindale MQ, Kuroda MI, Maller B, Hayward DC, Ball EE, Degnan B, Muller P, Spring J, Srinivasan A, Fishman M, Finnerty J, Corbo J, Levine M, Leahy P, Davidson E, Ruvkun G. Non-conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. Nature. 2000; 408: 96–9.
9. Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G. The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. Nature. 2000; 403: 901–6.
10. Lagos-Quintana M, Rauhut R, Yalcin A, Meyer J, Lendeckel W, Tuschl T. Identification of tissue-specific microRNAs from mouse. Curr Biol. 2002; 12: 735–9.
genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci USA. 2002; 99: 15524–9.

29. Akao Y, Nakagawa Y, Kitade Y, Kinoshita T, Naoe T. Downregulation of microRNAs-143 and -145 in B-cell malignancies. Cancer Sci. 2007; 98: 1194–7.

30. Lawrie CH, Soneji S, Marafioti T, Cooper CD, Palazzo S, Paterson JC, Cattan H, Enver T, Mager R, Boulwood J, Wainscoat JS, Hatton CS. MicroRNA expression distinguishes between germinal center B cell-like and activated B cell-like subtypes of diffuse large B cell lymphoma. Int J Cancer. 2007; 121: 1156–61.

31. Navarro A, Gaya A, Martínez A, Urbano-Ispizua A, Pons A, Balague O, Gel B, Abrissi P, Lopez-Guillermo A, Artells R, Montserrat E, Monzo M. MicroRNA expression profiling in classic Hodgkin lymphoma. Blood. 2008; 111: 2825–32.

32. Cifre SA, Galardi S, Mangiola A, Ferracin M, Liu CG, Sabatino G, Negrini M, Maira G, Croce CM, Farace MG. Extensive modulation of a set of microRNAs in primary glioblastoma. Biochim Biophys Acta. 2005; 1734: 1351–8.

33. Kefas B, Godlewski J, Comeau L, Li Y, Abounader R, Hawkinson M, Lee J, Fine H, Chiocca EA, Lawler S, Purow B. microRNA-7 inhibits the epidermal growth factor receptor and the Akt pathway and is down-regulated in glioblastoma. Cancer Res. 2008; 68: 3566–72.

34. Bottoni A, Piccin D, Tagliati F, Luchin A, Zatelli MC, degli Uberti EC, miR-15a and miR-16–1 down-regulation in pituitary adenomas. J Cell Physiol. 2005; 204: 280–5.

35. Pallante P, Visone R, Ferracin M, Ferraro A, Berlingeri MT, Troncone G, Chiappa G, Liu CG, Santoro M, Negrini M, Croce CM, Fusco A. MicroRNA deregulation in human thyroid papillary carcinomas. Endocr Relat Cancer. 2006; 13: 497–508.

36. He H, Jadziewski K, Li W, Liyanarachchi S, Nagy R, Volinia S, Calin GA, Liu CG, Franssila K, Suster S, Klaus RT, Croce CM, de la Chapelle A. The role of microRNA genes in papillary thyroid carcinoma. Proc Natl Acad Sci USA. 2005; 102: 19075–80.

37. Nikiforova MN, Tseng GC, Steward D, Diorio D, Nikiforov YE. MicroRNA expression profiling of thyroid tumors: biological significance and diagnostic utility. J Clin Endocrinol Metab. 2008; 93: 1600–8.

38. Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, Magri E, Pedriali M, Fabbri M, Campiglio M, Menard S, Palazzo JP, Rosenberg A, Musi P, Volinia S, Nenci I, Calin GA, Querzoli P, Negrini M, Croce CM. MicroRNA gene expression deregulation in human breast cancer. Cancer Res. 2005; 65: 7065–70.

39. Hayashi Y, Osada H, Tatematsu Y, Yamada H, Yanagisawa K, Tomida S, Yatabe Y, Kawaihara K, Sekido Y, Takahashi T. A polycistronic microRNA cluster, miR-17–92, is overexpressed in human lung cancers and enhances cell proliferation. Cancer Res. 2005; 65: 9628–32.

40. Yanalyhara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, Stephens RM, Okamoto A, Yokota J, Tanaka T, Calin GA, Liu CG, Croce CM, Harris CC. Unique microRNA molecules in lung cancer diagnosis and prognosis. Cancer Cell. 2006; 9: 189–98.

41. Takamizawa J, Konishi H, Yanagisawa K, Tomida S, Osada H, Endoh H, Harano T, Yatabe Y, Nagino M, Nimura Y, Mitsudomi T, Takahashi T. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. Cancer Res. 2004; 64: 3753–6.

42. Wong TS, Liu XB, Wong BY, Ng RW, Yuen AP, Wei WI. Mature miR-184 as potential oncogenic microRNA of squamous cell carcinoma of the tongue. J Cancer Res. 2004; 64: 2565–70.

43. Sengupta S, den Boon JA, Chen IH, Newton MA, Stanhope SA, Cheng YJ,Imai M, Zhang H, Chen KG, Kenney JD, Hahn SA, Bernards R, Lai EC. miR-143 and -145 in B-cell malignancies. Blood. 2006; 108: 2179–86.

44. Wang Y, Lee AT, Ma JZ, Wang J, Ren J, Yang Y, Tang J, Lei X, Oei LL, Tan P, Lee CG. Profiling microRNA expression in hepatocellular carcinoma reveals microRNA-224 up-regulation and apoptosis inhibitor-5 as a microRNA-224-specific target. J Biol Chem. 2008; 283: 13205–15.

45. Murakami Y, Yasuda T, Saigo K, Urashima T, Toyoda H, Okanoue T, Shimotohno K. Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues. Oncogene. 2006; 25: 2537–45.

46. Gramantieri L, Ferracin M, Fornari F, Veronese A, Sabbioni S, Liu CG, Calin GA, Giovannini C, Ferrazzi E, Grazi GL, Croce CM, Bolondi L, Negrini M. Cyclin G1 is a target of miR-122a, a microRNA frequently down-regulated in human hepatocellular carcinoma. Cancer Res. 2007; 67: 6092–9.

47. Feber A, Xi L, Luketich JD, Pennathur A, Landreneau RJ, Wu M, Swanson SJ, Godfrey TE, Lilfe VR. MicroRNA expression profiles of esophageal cancer. J Thorac Cardiovasc Surg. 2008; 135: 255–60.

48. Petrocca F, Visone R, Onelli MR, Shah MH, Nicoloso MS, de Martino I, Ilipooulos D, Pilozzi E, Liu CG, Negrini M, Cavazzini L, Volinia S, Alder H, Ruco L, Baldassarre G, Croce CM, Vecchione A. E2F1-regulated microRNAs impair TGFbeta-dependent cell-cycle arrest and apoptosis in gastric cancer. Cancer Cell. 2008; 13: 272–86.

49. Lee EJ, Gusev Y, Jiang J, Nuovo GJ, Lerner MR, Frankel WL, Morgan DL, Postler RG, Brackett DJ, Schmittgen TD. Expression profiling identifies microRNA signature in pancreatic cancer. Int J Cancer. 2007; 120: 1046–54.

50. Szulwarska AE, Davidson TS, John J, Cannon T, Sipos B, Maghnouj A, Labourier E, Hahn SA. MicroRNA expression alterations are linked to tumorigenesis and non-neoplastic processes in pancreatic ductal adenocarcinoma. Oncogene. 2007; 26: 4452–52.

51. Michael MZ, SM OC, van Holst Pellekaan NG, Young GP, James RJ. Reduced accumulation of specific microRNAs in colorectal neoplasia. Mol Cancer Res. 2003; 1: 882–91.

52. Schetter AJ, Leung SY, Sohn JJ, Zanetti KA, Bowman ED, Yanaihara N, Yuen ST, Chan TL, Kwong DL, Au GK, Liu CG, Calin GA, Croce CM, Harris CC. MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. JAMA. 2008; 299: 425–36.

53. Lui WD, Pourmand N, Patterson BK, Fire A. Patterns of known and novel small RNAs in human cervical cancer. Cancer Res. 2007; 67: 6031–43.

54. Yang H, Kong W, He L, Zhao JJ, O’Donnell JD, Wang J, Wenham RM, Coppola D, Kruk PA, Nicosia SV, Cheng JQ. MicroRNA expression profiling in human ovarian cancer: miR-214 induces
cell survival and cisplatin resistance by targeting PTEN. Cancer Res. 2008; 68: 425–33.
56. Nam EJ, Yoon H, Kim SW, Kim H, Kim YT, Kim JH, Kim JW, Kim S. MicroRNA expression profiles in serous ovarian carcinoma. Clin Cancer Res. 2008; 14: 2690–5.
57. Porkka KP, Pfeiffer MJ, Waltering KK, Vessella RL, Tammela TL, Visakorpi T. MicroRNA expression profiling in prostate cancer. Cancer Res. 2007; 67: 6130–5.
58. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarp A, Vecchione A, Negrini M, Harris CC, Croce CM. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci USA. 2006; 103: 2257–61.
59. Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, Labourier E, Reintert KL, Brown D, Slack FJ. RAS is regulated by the let-7 microRNA family. Cell. 2005; 120: 635–47.
60. Sampson VB, Rong NH, Han J, Yang Q, Aris V, Sotopoulous P, Petrelli NJ, Dunn SP, Krueger LJ. MicroRNA let-7a down-regulates MYC and reverts MYC-induced growth in Burkitt lymphoma cells. Cancer Res. 2007; 67: 9762–70.
61. Lu Z, Liu M, Stibrinskas V, Klinge CM, Ramos KS, Colburn NH, Li Y. MicroRNA-21 promotes cell transformation by targeting the programmed cell death 4 gene. Oncogene. 2008; 27: 4373–9.
62. Xiao C, Srinivasan L, Calado DP, Patterson HC, Zhang B, Wang J, Henderson JM, Kikut JL, Rajewsky K. Lymphoproliferative disease and autoimmunity in mice with increased miR-17–92 expression profiles in serous ovarian carcinoma. J Biol Chem. 2008; 283: 9836–43.
63. Piaiasance V, Abderrahmane A, Perret-Menoud V, Jacquetim P, Lemaigre F, Regazzi R. MicroRNA-9 controls the expression of Granulphin/SIP4 and the secretory response of insulin-producing cells. J Biol Chem. 2006; 281: 26992–42.
64. Peng Y, Laser J, Shi G, Mittal K, Melamed J, Lee F, Wei JJ. Antiproliferative effects by let-7 repression of high-mobility group K2 in uterine leiomyoma. Mol Cancer Res. 2008; 6: 663–73.
65. Luo X, Lin H, Pan Z, Xiao J, Zhang Y, Lu Y, Yang B, Wang Z. Down-regulation of MI RNA-1/MIRNA-133 contributes to re-expression of pacemaker channel genes HCN2 and HCN4 in hypertrophic heart. J Biol Chem. 2008; 283: 20045–52.
66. Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferrarin R, Shimizu M, Wojcik SE, Aqeilan RI, Zupo S, Dono M, Rassenti L, Alder H, Volinia S, Liu CG, Kripp TJ, Negrini M, Croce CM. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci USA. 2005; 102: 1394–9.
67. Xia L, Zhang D, Du R, Pan Y, Zhao L, Sun H, Hong L, Liu J, Fan D. miR-15b and miR-16 modulate multidrug resistance by targeting BCL2 in human gastric cancer cells. Int J Cancer. 2008; 123: 372–9.
68. Sylvestre Y, De Gaire V, Querido E, Mukhopadhyay UK, Bourdeau V, Major F, Ferbeyre G, Chartrand P. An E2F/miR-20a autoregulatory feedback loop. J Biol Chem. 2007; 282: 2135–43.
69. Wang Q, Li YC, Wang J, Kong J, Qi Y, Quigg RJ, Li X. miR-17–92 cluster accelerates adipocyte differentiation by negatively regulating tumor-suppressor Rb2/p130. Proc Natl Acad Sci USA. 2008; 105: 2889–94.
70. Dews M, Homayouni A, Yu D, Murphy D, Sevignani C, Wentzel E, Furth EE, Lee WM, Enders GH, Mendell JT, Thomas-Tikhonenko A. Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster. Nat Genet. 2006; 38: 1060–5.
71. Fontana L, Fiori ME, Albini S, Cifaldi L, Giacomini P, Donfrancesco A, Federici V, Giacomini P, Peschle C, Fruci D. Antagonism-17p5 abolishes the growth of therapy-resistant neuroblastoma through p21 and BIM. PLoS ONE. 2008; 3: e2236.
72. Zhu S, Si ML, Wu H, Mo YY. MicroRNA-21 targets the tumor suppressor gene tropomysin 1 (TPM1). J Biol Chem. 2007; 282: 14328–36.
73. Zhu S, Wu H, Wu F, Nie D, Sheng S, Mo YY. MicroRNA-21 targets tumor suppressor genes in invasion and metastasis. Cell Res. 2008; 18: 350–9.
74. Lal A, Kim HH, Abdemohsen K, Kuyano Y, Pullman R Jr, Srikantan S, Subrahmanym R, Martinlde JL, Yang X, Ahmed F, Navarro F, Dykhoom D, Lieberman J, Gorospe M. p16(INK4a) translation suppressed by miR-24. PLoS ONE. 2008; 3: e1864.
75. Wang Q, Huang Z, Xue H, Jin C, Ju XL, Han JD, Chen YG. MicroRNA miR-24 inhibits erythropoiesis by targeting activin type I receptor ALK4. Blood. 2008; 111: 588–95.
76. Wong CF, Tellam RL. MicroRNA-26a targets the histone methyltransferase Enhancer of Zeste homolog 2 during myogenesis. J Biol Chem. 2008; 283: 9836–43.
77. Luzi E, Marini F, Sala SC, Tognarini I, Galli G, Brandi ML. Osteogenic differentiation of human adipose tissue-derived stem cells is modulated by the miR-26a targeting of the SMAD1 transcription factor. J Bone Miner Res. 2008; 23: 287–95.
78. Mott JL, Kobayashi S, Bronk SF, Gores GJ. miR-29 regulates Mcl-1 protein expression and apoptosis. Oncogene. 2007; 26: 6139–40.
79. Ivanovska I, Ball AS, Diaz RL, Magnus JF, Kikukawa M, Schelter JM, Kobayashi SV, Lim L, Burchard J, Jackson AL, Linsley PS, Cleary MA. MicroRNAs in the miR-106b family regulate p21/CDKN1A and promote cell cycle progression. Mol Cell Biol. 2008; 28: 2167–74.
80. Wang WX, Rajeev BW, Stromberg AJ, Ren N, Tang G, Huang Q, Rigoutsos I, Nelson PT. The expression of microRNA miR-107 decreases early in Alzheimer’s disease and may accelerate disease progression through regulation of beta-si amyloid precursor protein-cleaving enzyme 1. J Neurosci. 2008; 28: 1213–23.
81. Baroukh N, Ravier MA, Loder MK, Hill EV, Bounacar A, Scharffmar R, Rutter GA, Van Obberghen E. MicroRNA-124a regulates Foxa2 expression and intracellular signaling in pancreatic beta-cell lines. J Biol Chem. 2007; 282: 19575–88.
82. Harris TA, Yamakuchi M, Ferlito M, Mendell JT, Lowenstein CJ. MicroRNA-126 regulates endothelial expression of vascular cell adhesion molecule 1. Proc Natl Acad Sci USA. 2008; 105: 1516–21.
83. Shen WF, Hu YL, Uttarwar L, Passaghe E, Largman C. MicroRNA-126 regulates HOXA9 by binding to the homeobox. Mol Cell Biol. 2008; 28: 4609–19.
84. Saito Y, Liang G, Egger G, Friedman JM, Chuang JC, Coetzee GA, Jones PA. Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. Cancer Cell. 2006; 9: 435–43.
85. Chen Y, Gorski DH. Regulation of angiogenesis through a microRNA (miR-130a) that down-regulates antiangiogenic homeobox genes GAX and HOX5. Blood. 2008; 111: 1217–26.
86. Tay YM, Tam WL, Ang SY, Gaughwin PM, Yang H, Wang W, Liu R, George J, Ng HH, Perera RJ, Lufkin T, Rigoutsos I, Thomson AM, Lim B. MicroRNA-134 modulates the differentiation of mouse
enlymphoma by targeting ZEB1 and SIP1. Nat Cell Biol. 2010; 10: 593–601.

96. Christoffersen NR, Silaharakoolu A, Orom UA, Kauppinen S, Lund AH. miR-200b mediates post-transcriptional repression of ZFHX1B. RNA. 2007; 13: 1172–8.

97. Lena AM, Shalom-Feuerstein R, di Val Cervo PR, Abd erman D, Knight RA, Melino G, Candi E. miR-203 represses ‘stemness’ by repressing DeltaNp63. Cell Death Differ. 2008; 15: 1187–95.

98. Anderson C, Catone H, Werner R. MiR-206 regulates con nexitin43 expression during skeletal muscle development. Nucleic Acids Res. 2006; 34: 5863–71.

99. Adams BD, Far neaux H, White BA. The micro-ribonucleic acid (miRNA) miR-206 targets the human estrogen receptor-alpha (ERalpha) and represses ERalpha messenger RNA and protein expression in breast cancer cell lines. Mol Endocrinol. 2007; 21: 1132–47.

100. Rosenberg MI, Georges SA, As wachaicharn A, Analau E, Taps cott SJ. MyoD inhibits Fstl1 and Utrn expression by inducing transcription of miR-206. J Cell Biol. 2006; 175: 77–85.

101. Fasanaro P, D’ Alessandra Y, Di Stefano V, Melch iunonna C, Romeni S, Pomp ilio G, Capogrossi M C, Martelli F. MicroRNA-210 modulates endothelial cell response to hypoxia and inhibits the receptor tyrosine kinase ligand Ephrin-A3. J Biol Chem. 2008; 283: 15878–93.

102. Li N, Flynt AS, Kim HR, Sol nica-Krezel L, Patton JG. Dispatched Homolog 2 is targeted by miR-214 through a combination of three weak microRNA recognition sites. Nucleic Acids Res. 2008; 36: 4277–85.

103. Galardi S, Mercatelli N, Giorda E, Massal ini S, Frajese GV, Ci afre SA, Farace MG. miR-221 and miR-222 expression affects the proliferation potential of human prostate carcinoma cell lines by targeting p27Kip1. J Biol Chem. 2007; 282: 23716–24.

104. Fornari F, Gramantieri L, Ferracin M, Rosenberg MI, Georges SA, Stabile BL, Karran E, Rajewsky N. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Gastroenterology. 2008; 135: 257–69.

105. Wong QW, Lung RW, Law PT, Lai PB, Chan KY, To KF, Wong N. MicroRNA-223 is commonly repressed in hepatocellular carcinoma and potentiates expression of Stathmin1. Gastroenterology. 2008; 135: 257–69.

106. Wang CH, Lee DY, Deng Z, Jeyapalan Z, Lee SC, Kahi s H, Lu WY, Zhang Y, Yang BB. MicroRNA miR-328 regulates zonation morphogenesis by targeting CD44 expression. PLoS ONE. 2008; 3: e2420.
in human hepatocellular carcinoma. 
Oncogene. 2006; 25: 6101–12.

119. Kulshreshtha R, Ferracin M, Wojcik SE,
Garzon R, Alder H, Agosto-Perez FJ,
Davuluri R, Liu CG, Croce CM, Negrini M,
Calin GA, Ivan M. A microRNA signature of
hypoxia. Mol Cell Biol. 2007; 27: 1859–67.

120. Camps C, Butta FM, Colella S, Moore J,
Sotiriou C, Sheldon H, Harris AL, Gleadle
JM, Raguosssis J. hsa-miR-210 is induced
by hypoxia and is an independent prog-
nostic factor in breast cancer. Clin Cancer
Res. 2008; 14: 1340–8.

121. Lowe SW, Lin AW. Apoptosis in cancer.
Carcinogenesis. 2000; 21: 485–95.

122. O’Donnell KA, Wentzel EA, Zeller KI,
Dang CV, Mendell JT. c-Myc-regulated
microRNAs modulate E2F1 expression.
Nature. 2005; 435: 839–43.

123. He L, Thomson JM, Hemann MT,
Hernando-Monge E, Mu D, Goodson S,
Powers S, Cordon-Cardo C, Lowe SW,
Hannon GJ, Hammond SM. A microRNA
polycistron as a potential human onco-
gene. Nature. 2005; 435: 828–33.

124. Sassen S, Miska EA, Caldas C.
MicroRNA: implications for cancer.
Virchows Arch. 2008; 452: 1–10.

125. Okamura S, Arakawa H, Tanaka T,
Nakanishi H, Ng CC, Taya Y, Monden M,
Nakamura Y. p53DINP1, a p53-inducible
gene, regulates p53-dependent apoptosis.
Mol Cell. 2001; 8: 85–94.

126. Phan RT, Dalla-Favera R. The BCL6
proto-oncogene suppresses p53 expres-
sion in germinal-centre B cells. Nature.
2004; 432: 635–9.

127. Margalit O, Amram H, Amariglio N,
Simon AJ, Shklai S, Granot G, Minsky
N, Shimoni A, Harmelin A, Givol D,
Shohat M, Oren M, Rechavi G. BCL6 is
regulated by p53 through a response ele-
ment frequently disrupted in B-cell non-
Hodgkin lymphoma. Blood. 2006; 107:1599–607.