HHS Public Access

Author manuscript

Nat Rev Cancer. Author manuscript; available in PMC 2016 June 01.

Published in final edited form as:

Nat Rev Cancer. 2015 June ; 15(6): 321–333. doi:10.1038/nrc3932.

MicroRNA biogenesis pathways in cancer

Shuibin Lin[1,2] and **Richard I. Gregory**[1,2,3,4]

[1]Stem Cell Program, Boston Children's Hospital, Boston, Massachusetts 02115, USA

[2]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115, USA

[3]Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02115, USA

[4]Harvard Stem Cell Institute, Boston, Massachusetts 02115, USA

Abstract

MicroRNAs (miRNAs) are critical regulators of gene expression. Amplification and overexpression of individual 'oncomiRs' or genetic loss of tumour suppressor miRNAs are associated with human cancer and are sufficient to drive tumorigenesis in mouse models. Furthermore, global miRNA depletion caused by genetic and epigenetic alterations in components of the miRNA biogenesis machinery is oncogenic. This, together with the recent identification of novel miRNA regulatory factors and pathways, highlights the importance of miRNA dysregulation in cancer.

MicroRNAs (miRNAs) repress gene expression by binding to complementary sequences in the 3′ untranslated region (3′ UTR) of mRNAs to target them for degradation and thereby prevent their translation[1]. Considering that more than 1,000 individual miRNA genes have been identified, that an individual miRNA can target hundreds or thousands of different mRNAs, and that an individual mRNA can be coordinately suppressed by multiple different miRNAs, the miRNA biogenesis pathway therefore has an important role in gene regulatory networks. Over the past decade, it has emerged that miRNAs have crucial roles in cancer. Propelled by the original publication that described the deletion of the *miR-15* and *miR-16* loci in the majority of samples from patients with B cell chronic lymphocytic leukaemia (B-CLL), a plethora of subsequent publications described altered miRNA expression in diverse types of cancer[2,3]. Functionally, it has been shown through both loss-of-function and gain-of-function experiments in human cancer cells, mouse xenografts, transgenic mouse models and knockout mouse models that miRNAs have key roles in cancer initiation, progression and metastasis[4,5]. The first example was provided by enforced expression of the miR-17~92 cluster, the so-called oncomiR-1, that acted with MYC to accelerate tumour development in a mouse model of B cell lymphoma[6]. Certain other miRNAs can function as tumour suppressors: for example, the let-7 family of miRNAs targets important oncogenes such as *MYC*, RAS family members (*HRAS*, *KRAS* and *NRAS*) and high-mobility group AT-hook

Correspondence to R.I.G. rgregory@enders.tch.harvard.edu.

Competing interests statement

The authors declare no competing interests.

2 (*HMGA2*) to suppress tumour growth[7–9]. Therefore, cancer-associated changes in miRNA expression patterns are emerging as promising diagnostic markers that often correlate with disease progression and patient survival. This pathway might also represent a new therapeutic target for multiple types of cancer[2]. Mechanistically, miRNAs can control cell proliferation, differentiation, survival, metabolism, genome stability, inflammation, invasion and angiogenesis to affect tumour development.

Although individual miRNAs can have either oncogenic or tumour-suppressive function, several studies have shown that miRNA expression is globally suppressed in tumour cells compared with normal tissue, suggesting that miRNA biogenesis might be impaired in cancer[10,11]. Indeed, the expression levels of miRNAprocessing machinery components such as the ribonuclease III (RNase III) DROSHA and DICER1 are decreased in some cancers, such as lung cancer, ovarian cancer and neuroblastoma[12–14]. Additionally, low DROSHA or DICER1 expression levels are associated with advanced tumour stage and poor clinical outcome in patients with neuroblastoma and patients with ovarian cancer[13,14]. Support that this global suppression can have a causative role in cancer was initially provided by the demonstration that genetic deficiency of components of the miRNA biogenesis pathway can accelerate tumour growth in a mouse model of lung cancer[15]. Although this work provided proof-of-concept that the miRNA biogenesis pathway can have an important role in cancer progression, it is the recently reported mutations in and dysregulation of miRNA biogenesis pathway components that highlight the pathophysiological relevance of the miRNA biogenesis machinery in human tumours[16–24]. Moreover, the recent discovery of certain molecular and cellular mechanisms that control miRNA biogenesis provided compelling evidence that disruption of this pathway is crucially important for a wide variety of paediatric and adult cancers.

In this Review, we discuss what is known about dysregulation of the miRNA biogenesis pathway in cancer, summarize the growing evidence that germline mutations and somatic mutations in core components of the miRNA biogenesis machinery promote oncogenesis, and provide specific examples of how certain RNA-binding proteins and cell signalling pathways contribute to cancer through their control of miRNA expression. With these examples, we aim to highlight emerging themes and the relevance of the miRNA biogenesis pathway in cancer.

miRNAs and their biogenesis

miRNAs are a group of short non-coding RNAs that mediate post-transcriptional gene silencing. The first miRNA was reported in *Caenorhabditis elegans* in 1993 (REF. [25]); however, the general regulatory function of miRNAs was not well appreciated until 2001 (REFS 26–28). Since then, thousands of miRNAs have been identified in various species[29]. Binding of the ~22-nucleo tide miRNA to target mRNA mediates mRNA degradation and blocks translation[30]. The majority of miRNA genes are transcribed by RNA polymerase II (Pol II) in the nucleus, and the primary miRNAs (pri-miRNAs) are capped, spliced and polyadenylated[31]. Approximately 30% of miRNAs are processed from introns of proteincoding genes, whereas most other miRNAs are expressed from dedicated miRNA gene loci. An individual primiRNA can either produce a single miRNA or contain clusters of

two or more miRNAs that are processed from a common primary transcript. Nonetheless, these long pri-miRNAs are cleaved by Microprocessor, which comprises the double-stranded RNase III enzyme DROSHA and its essential cofactor, the double-stranded RNA (dsRNA)-binding protein DiGeorge syndrome critical region 8 (DGCR8)[32,33]. DROSHA contains two RNase III domains, each of which cleaves one strand of the dsRNA towards the base of stem–loop secondary structures contained within pri-miRNAs to liberate ~60–70-nucleotide hairpin-shaped precursor miRNAs (pre-miRNAs)[32–35]. Microprocessor recognizes the single-stranded RNA (ssRNA)–stem junction as well as the distance from the terminal loop region. It specifically cleaves the dsRNA ~11 bp from the junction with the flanking ssRNA to produce hairpin-shaped pre-miRNAs with an overhang at the 3′ end of either 2 nucleotides (group I miRNAs) or 1 nucleotide (group II miRNAs)[36–39]. Although the core components, DROSHA and DGCR8, are required for the biogenesis of almost all miRNAs in the cell, and Microprocessor activity can be reconstituted *in vitro* with recombinant DROSHA and DGCR8 proteins[32,35], numerous accessory factors are known to have a role in pri-miRNA processing in cells (discussed in more detail below). The pre-miRNAs are then exported from the nucleus to the cytoplasm by exportin 5 (XPO5)[40–42] and further processed by DICER1, an RNase III enzyme that measures from the 5′ and 3′ ends of the pre-miRNA[43]. DICER1 binding to the end of the pre-miRNA positions its two catalytic RNase III domains so that asymmetrical cleavage of the dsRNA stem, close to the terminal loop sequence, produces the mature ~22-nucleotide miRNA duplex with 2-nucleotide 3′ overhangs[44]. DICER1 associates with transactivation-responsive RNA-binding protein (TRBP; also known as TARBP2), which binds to dsRNA[45]. Although it is not required for pre-miRNA processing by DICER1, TRBP enhances the fidelity of DICER1-mediated cleavage of a subset of pre-miRNAs in a structure-dependent manner and alters miRNA guide-strand selection by triggering the formation of isomiRNAs, which are 1 nucleotide longer than the regular miRNAs[46,47]. TRBP also physically bridges DICER1 with the Argonaute proteins (AGO1, AGO2, AGO3 or AGO4) to participate in the assembly of the miRNAinduced silencing complex (miRISC)[45]. One strand of the mature miRNA (the guide strand) is bound by an Argonaute protein and retained in the miRISC to guide the complex, together with members of the GW182 family of proteins, to complementary target mRNAs for post-transcriptional gene silencing. This occurs in processing bodies (P-bodies), which are the cytoplasmic foci that are induced by mRNA silencing and decay but are not necessarily required for miRNA-mediated gene silencing[48–50] (FIG. 1).

Pri-miRNA transcription in cancer

miRNA biogenesis initiates with the transcription of the pri-miRNA, and this step is dysregulated in multiple human cancers. A considerable number of human miRNA genes are located at fragile sites or in genomic regions that are deleted, amplified or translocated in cancer[51]. These genomic variations alter pri-miRNA transcription and miRNA expression, which leads to the aberrant expression of downstream target mRNAs that can promote cancer initiation and progression[51,52]. For example, the locus including *miR-15* and *miR-16* on chromosome 13q14 is frequently deleted in B-CLL, resulting in the loss or reduced expression of these two miRNAs in ~70% of B-CLLs[3]. miR-15 and miR-16 normally control apoptosis by targeting *BCL-2* mRNAs[53]. In another example, a point mutation in the

miR-128b (also known as *miR-128-2*) gene blocks the processing of pri-miR-128b and reduces the levels of mature miR-128b, thus leading to glucocorticoid resistance in acute lymphoblastic leukaemia (ALL) cells with the mixed-lineage leukaemia (*MLL*)–*AF4* (also known as *KMT2A–AFF1*) translocation[54].

In addition to genomic alterations, dysregulated miRNA expression can arise from alterations in tumour suppressor or oncogenic factors that function as transcriptional activators or repressors to control pri-miRNA transcription. For example, expression of the miR-34 family of miRNAs is driven by p53 and reflects the status of p53 in human cancers[55–59]. The miR-34a, miR-34b and miR-34c miRNAs repress growth-promoting genes and coordinate with other members of the p53 tumour-suppressive network to inhibit uncontrolled cell proliferation and to promote apoptosis[55–59]. In addition, the proto-oncoprotein MYC activates expression of oncogenic miRNAs, including the miR-17~92 cluster, in cancer[60,61]. These MYC-target miRNAs promote cancer progression by controlling the expression of *E2F1*, thrombospondin 1 (*THBS1*), connective tissue growth factor (*CTGF*) and other target mRNAs to regulate cell cycle progression and angiogenesis[60,61]. MYC can also contribute to the widespread repression of tumour-suppressive miRNAs in B cell lymphoma[62]. Expression of the miR-200 family (miR-200a, miR-200b and miR-200c) is frequently suppressed in human tumours. These miRNAs are known to directly target the mRNAs encoding the zinc-finger E-box-binding homeobox (ZEB) transcription factors, ZEB1 and ZEB2, which suppress the expression of epithelial genes to promote the epithelial–mesenchymal transition (EMT)[63]. Interestingly, ZEB1 and ZEB2 directly bind to a regulatory element at the *miR-200* promoter to repress transcription of *miR-200* as part of a negative regulatory feedback loop that promotes EMT[64]. Many other cancer-associated transcription factors also aberrantly regulate miRNA transcription in cancer. Therefore, transcriptional dysregulation — through either genetic loss of miRNA genes or aberrant transcription factor activity — is an important mechanism for altered miRNA expression in cancer.

Epigenetic modification of histone proteins and DNA controls local chromatin structure and has an important role in the regulation of both coding and non-coding gene expression. Indeed, epigenetic alteration is a common feature of cancer pathogenesis that drives the dysregulation of miRNA expression. The CpG islands at the gene promoters of tumour-suppressive miRNAs are frequently hypermethylated in cancer, thereby leading to the epigenetic silencing of these miRNAs. Treatment of cancer cells with DNA-demethylating agents can reactivate the expression of tumour-suppressive miRNAs, such as *miR-148a*, *miR-34b*, *miR-34c* and *miR-9*, that inhibit tumour growth and metastasis[65]. In addition to DNA methylation, histone modifications have important roles in chromatin remodelling and cooperate with DNA methylation to suppress miRNA expression in cancer[66]. Overall, epigenetic silencing is an important mechanism underlying miRNA repression in cancer.

Defective Microprocessor in cancer

The nascent pri-miRNA generated by Pol II forms a typical secondary structure consisting of a stem–loop hairpin flanked by ssRNA that is a substrate for cleavage by Microprocessor to generate pre-miRNA intermediates. A negative feedback mechanism involving the

Microprocessor-mediated cleavage and destabilization of *DGCR8* mRNA operates to help to control the relative DGCR8 expression level and to maintain the homeostatic control of miRNA biogenesis in cells[67–69]. The expression and function of the Microprocessor components are often dysregulated in cancer. For example, copy-number gain or overexpression of *DROSHA* occurs in more than 50% of advanced cervical squamous cell carcinomas[70]. In addition, DROSHA expression levels are upregulated in multiple types of cancer (TABLE 1). The increased expression of DROSHA alters the global miRNA expression profile and promotes cell proliferation, migration and invasion, which contributes to cancer progression[70,71]. Conversely, DROSHA expression levels have been shown to be downregulated in many other types of cancer. DROSHA downregulation results in decreased miRNA expression[13] and is correlated with metastasis, invasion[72] and poor patient survival[13,14,73,74] (TABLE 1). Knockdown of DROSHA in lung adenocarcinoma cells results in increased proliferation and tumour growth *in vitro* and *in vivo*[15], suggesting that DROSHA can function as a tumour suppressor to inhibit cancer progression in some contexts. Why DROSHA is upregulated in certain types of cancer but downregulated in others is not well understood, but one possibility is that different cancers have different genetic or epigenetic mechanisms controlling *DROSHA* expression, thus resulting in the abnormal expression of oncogenic or tumour-suppressive miRNAs in a given cancer type.

Mutational analysis revealed that *DROSHA* is frequently mutated in Wilms tumour samples[21–24] (FIG. 2; see Supplementary information S1 (table)). More than 70% of the DROSHA mutations occur at E1147, a metalbinding residue in the RNase IIIb domain. The recurrent somatic missense mutation E1147K interferes with metal binding and therefore affects the function of DROSHA in the processing of pri-miRNAs through a dominantnegative mechanism[21–24]. As a result, mature miRNAs are globally downregulated in *DROSHA*-mutated Wilms tumours[21–24]. Several missense mutations and a splicesite mutation of the *DROSHA* gene have been found in ovarian cancer; however, these mutations do not affect DROSHA expression levels. Therefore, it remains to be characterized whether the functions of DROSHA are affected by these mutations[14]. In addition, *DROSHA* was found to be alternatively spliced in melanoma and teratocarcinoma cells[75]. The splice variants encode carboxy-terminal-truncated DROSHA proteins that partially lack the RNase IIIb domain and the dsRNAbinding domain (dsRBD). These truncated proteins fail to interact with DGCR8 and are deficient in pri-miRNA processing *in vitro*. However, the splice variants have little effect on mature miRNA expression, which might be due to the relatively low expression level of the splice variants in the cells[75].

DGCR8 expression is also dysregulated in cancer (TABLE 1). In addition, mutations of DGCR8 were reported in Wilms tumours: a recurrent mutation (E518K) in dsRBD1 results in the reduced expression of crucial miRNAs in the tumours[22–24] (FIG. 2; see Supplementary information S1 (table)). Similar to knockdown of DROSHA, knockdown of DGCR8 also promotes cellular transformation and tumour growth[15], further confirming the important role of Microprocessor in cancer.

Pre-miRNA export in cancer

Pre-miRNAs are exported into the cytoplasm to be processed into mature miRNAs. The export of pre-miRNAs is mediated by XPO5 and its cofactor, RanGTP[41]. Three recurrent heterozygous *XPO5*-inactivating mutations were identified in sporadic colon, gastric and endometrial tumours with microsatellite instability[76] (FIG. 2; see Supplementary information S1 (table)). These *XPO5* mutations impair pre-miRNA export and result in an accumulation of pre-miRNAs in the nucleus, leading to defects in miRNA biogenesis. In addition, genetic and epigenetic association studies revealed that *XPO5* genetic variation and expression level are associated with the risk of breast cancer[77]. Therefore, XPO5 dysregulation contributes to miRNA processing defects and tumorigenesis.

Pre-miRNA processing in cancer

DICER1 mutations

After being exported to the cytoplasm, pre-miRNAs are then processed by DICER1 to form ~22-nucleotide mature miRNAs[78]. DICER1 is a large multi-domain nuclease that contains two helicase domains, a dimerization domain, a Piwi–Argonaute–Zwille (PAZ) domain, two RNase III domains (RNase IIIa and RNase IIIb) and a dsRBD (FIG. 2; see Supplementary information S1 (table)). In addition to its function in pre-miRNA cleavage, DICER1 is required for the assembly of the minimal miRISC that executes miRNA function in repressing target gene expression[48]. Depletion of DICER1 in cancer cells or mouse models promotes cell growth and tumorigenesis, indicating the important function of DICER1 in oncogenesis[15,79]. Furthermore, Dicer is considered a haploinsufficient tumour suppressor gene, as loss of a single *Dicer1* allele reduces survival in a mouse model of lung cancer[79].

Heterozygous germline *DICER1* mutations were first identified to be responsible for pleuropulmonary blastoma (PPB), a rare paediatric lung tumour that arises during fetal lung development and is often part of an inherited cancer syndrome (Online Mendelian Inheritance in Man (OMIM) #601200)[16]. Germline frameshift or nonsense mutations mainly affect *DICER1* upstream of the region encoding RNase III domains (FIG. 2), resulting in truncated DICER1 proteins lacking the C-terminal catalytic domains. *DICER1* loss of heterozygosity (LOH) is almost never observed in human tumours, and homozygous *Dicer1* loss is generally selected against in mouse cancer models[79]. Although more than 50% of heterozygous germline *DICER1* mutation carriers are clinically unaffected, the tumours that develop in PPB patients are typically associated with another important group of DICER1 mutations: recurrent somatic mutations in the RNase IIIb domain[18,80]. The mutation hot spots of the RNase IIIb domain occur in the metal-binding residues (E1705, D1709, G1809, D1810 and E1813)[18] (FIG. 2); this domain is responsible for the cleavage of the 3′ end of the miRNAs derived from the 5′ side of the pre-miRNA hairpin called 5p miRNAs. These mutations do not change DICER1 protein expression but instead cause defects in the function of the RNase IIIb domain. As a result, the maturation of 5p miRNAs is specifically blocked, while the processing of 3p miRNAs (miRNAs derived from the 3′ side of the pre-miRNA hairpin) remains unaffected, leading to the global loss of 5p miRNAs in cancer[17,18]. Particularly, DICER1 RNase IIIb mutations strongly reduce the expression of the members of the let-7 tumour-suppressive miRNA family (that are all 5′ derived), which probably helps

to explain the selective pressures that give rise to this specific mutation spectrum in cancers. Interestingly, modelling of PPB in mice supports the idea that *Dicer1* deletion in the distal airway epithelium causes non-cellautonomous tumour initiation, whereby *Dicer1* loss in the epithelium causes the underlying mesenchymal cells to be malignantly transformed[81]. *DICER1* mutations are frequently found in different types of inherited tumours: PPB[16,80–84], non-epithelial ovarian cancer[18,84,85], Wilms tumour[22,86,87], pituitary blastoma[88], cystic nephroma[89], rhabdomyosarcoma[90] and others[91] (see Supplementary information S1 (table)). As a result, patients harbouring these *DICER1* mutations have reduced DICER1 expression and/or impaired DICER1 function, which cause the abnormal expression of miRNAs and contribute to the pathogenesis of cancer. As such, *DICER1* mutation is considered a tumour predisposition syndrome known as DICER1 syndrome[20]. This topic has recently been reviewed in detail[19].

In addition to genetic mutations of *DICER1*, DICER1 expression is often dysregulated in cancer. Similar to that of DROSHA, DICER1 expression can be increased or decreased in cancer, depending on the cancer type (TABLE 1). Many oncoproteins and dysregulated tumour suppressors regulate cancer progression by targeting DICER1 expression. For example, the p53 family member TAp63 directly binds to the promoters of *DICER1* and *miR-130b* and drives their expression to suppress tumorigenesis and metastasis[92]. Overall, both genetic mutation and dysregulation of DICER1 can result in aberrant miRNA expression and tumorigenesis.

TRBP mutations

Impaired function of TRBP also contributes to miRNA dysregulation in cancer. Sequencing of the genes encoding the miRNA processing machinery revealed two frameshift mutations of *TRBP* in sporadic and hereditary carcinomas with microsatellite instability[93,94] (FIG. 2; see Supplementary information S1 (table)). These mutations cause reduced TRBP and DICER1 expression as well as defective processing of pre-miRNAs. Re-introduction of wild-type *TRBP* in the mutated cell lines rescued TRBP and DICER1 expression, restored miRNA processing and suppressed cancer cell growth *in vitro* and *in vivo*[93]. Interestingly, the expression of TRBP is repressed in the cancer stem cell (CSC) population of Ewing sarcoma family tumour (ESFT), which results in the miRNA profile of ESFT CSCs that is required for CSCassociated self-renewal and tumour growth[95]. Therefore, TRBP-mediated miRNA processing has an important tumour-suppressive role in normal cells.

Other miRNA regulators in cancer

Aberrant expression of or mutations in the genes encoding key components of the miRNA biogenesis pathway contributes to the global repression of miRNAs in cancer. However, a widespread suppression of miRNA expression has been observed in cancers with normal expression of the miRNA biogenesis machinery. This suggests that other pathways regulating miRNA processing are dysregulated in cancer. We highlight below recent discoveries of selected cancer-relevant pathways involved in the regulation of miRNA biogenesis.

Regulators of Microprocessor

The original characterization of a large DROSHA-containing complex identified multiple classes of RNA-binding proteins, including the DEAD (Asp-Glu-Ala-Asp) box helicases DDX5 (also known as p68) and DDX17 (also known as p72), Ewing sarcoma family proteins and heterogeneous nuclear ribonucleoproteins (hnRNPs)[32]. These Microprocessorassociated proteins can directly affect Microprocessor activity, and alterations in this regulation can result in aberrant miRNA biogenesis in cancer[96]. Other factors might also regulate Microprocessor activity in cancer: for example, the tumour suppressor BRCA1 interacts with multiple Microprocessor regulators to facilitate miRNA biogenesis[97]. Moreover, RNA-binding proteins such as KH type-splicing regulatory protein (KSRP; also known as FUBP2)[98], serine/arginine-rich splicing factor 1 (SRSF1)[99], hnRNP A1 (REFS [100,101]) and FUS (also known as TLS)[102] bind to certain regions of primiRNAs (stem or terminal loop) and facilitate DROSHA recruitment and function (FIG. 3).

In addition to regulating Microprocessor activity, DDX5 and DDX17 function as bridging factors for important oncoproteins or tumour suppressors to regulate miRNA biogenesis in cancer. For example, the tumour suppressor protein p53 regulates miRNA biogenesis through association with DDX5 and DDX17. In response to DNA damage, the level of p53 expression increases, which enhances the expression levels of tumour-suppressive miRNAs including miR-34a, miR-16-1, miR-143 and miR-145 (REF. 103). In contrast to *miR-34a*, which is a transcriptional target of p53 (REF. 55), the other miRNAs are post-transcriptionally regulated by p53. Mediated by DDX5 and DDX17, p53 interacts with the DROSHA complex and promotes the processing of tumour-suppressive pri-miRNAs. Accordingly, miRNA processing is hindered in p53-mutant cells[103]. Given that p53 is frequently mutated in human cancer, dysregulation of miRNA biogenesis by p53 mutation might account for the widespread miRNA repression in cancer (FIG. 3).

Cell signalling control

Cell signalling pathways also modulate Microprocessor activity to dynamically control pri-miRNA processing and miRNA expression in cancer[96] (FIG. 3). For example, SMADs — which transduce transforming growth factor-β (TGFβ) and bone morphogenetic protein (BMP) signalling — associate with DDX5 and promote miRNA processing by binding to a consensus sequence in the stem region of primiRNAs[104,105]. Moreover, the core biogenesis machinery components, including DROSHA, DGCR8, DICER1 and TRBP, are subject to post-translational control such as phosphorylation and/or acetylation (reviewed in REFS [106,107]). The effect of these protein modifications, and their possible dysregulation in cancer, remains to be determined.

It was recently found that the Hippo pathway controls Microprocessor activity[108]. The Hippo pathway controls organ size by regulating cell proliferation and differentiation in response to cell density[109]. Given its key role in regulating organ size and cell proliferation, it is perhaps not surprising that the Hippo signalling pathway is frequently perturbed in a variety of human cancers[109]. miRNA biogenesis is activated by cell–cell contact and Hippo signalling[108,110]. Mechanistically, it was found that the Hippo downstream effector Yes-associated protein 1 (YAP1) post-transcriptionally regulates miRNA biogenesis by targeting

DDX17. In *in vitro* cell culture systems, at low cell density, the growth-suppressive Hippo pathway is inactive, and nuclear YAP1 binds to and sequesters DDX17 to suppress pri-miRNA processing, whereas at high cell densities, the Hippo pathway is active, which leads to YAP1 phosphorylation and its retention in the cytoplasm. When YAP1 is cytoplasmic, DDX17 is able to bind to a specific sequence motif in pri-miRNA, associate with Microprocessor and enhance miRNA biogenesis. Accordingly, inactivation of the Hippo pathway or constitutive activation of YAP1, which occurs in cancer cells, results in widespread miRNA suppression both in human cancer cell lines and in mouse tumour models[108]. It will be interesting to explore whether Hippo signalling is responsible for the widespread repression of miRNA expression in cancer.

Stress response

Rapidly growing tumours often experience hypoxia owing to the limited oxygen supply in the tumour microenvironment. Interestingly, miRNA expression and function are dynamically regulated under stress conditions[111]. Oncogenic epidermal growth factor receptor (EGFR) signalling is activated by hypoxia to promote cell growth and oncogenesis[112]. Identification of the EGFR protein complex in serum-starved EGFtreated HeLa cells revealed that EGFR interacts with AGO2 (REF. 113). In response to hypoxia, EGFR induces the phosphorylation of AGO2 at Y393, which inhibits the interaction between DICER1 and AGO2 and blocks miRNA accumulation. Furthermore, EGFR-mediated AGO2-Y393 phosphorylation is required for cell survival and invasion under hypoxic conditions and is associated with poor survival rates in patients with breast cancer[113]. In addition, recent studies uncovered the important role of hypoxia in suppressing DROSHA and DICER1 expression in cancer cells, which results in aberrant miRNA biogenesis and promotes tumour progression[114,115]. These studies provide an interesting link between hypoxia and miRNA repression in cancer and uncover a novel oncogenic role of hypoxia in regulating miRNA biogenesis during tumorigenesis[113–115] (FIG. 3).

LIN28-mediated blockade of let-7

The let-7 miRNA family members function as tumour suppressors in multiple cancer types by inhibiting expression of oncogenes and key regulators of mitogenic pathways[116–118]. In humans, there are 12 let-7 family members (*let-7a-1*, *let-7a-2*, *let-7a-3; let-7b; let-7c; let-7d; let-7e; let-7f-1*, *let-7f-2; let-7g; let-7i; miR-98*) located at 8 unlinked chromosomal loci. The let-7 miRNAs are downregulated in numerous cancer types, and low let-7 expression levels correlate with poor prognosis[119–122]. The expression of the let-7 miRNA family is coordinately regulated by the paralogous RNA-binding proteins LIN28A and LIN28B during early embryonic development[123–126]. Reactivation of this embryonic pathway in adult cells by expression of LIN28A and LIN28B is sufficient to promote cellular transformation and tumorigenesis *in vitro* and *in vivo*[127–130]. Of note, expression of LIN28B is sufficient to drive neuroblastoma, T cell lymphoma, intestinal adenocarcinoma, Wilms tumour (nephroblastoma) and hepatocellular carcinoma in mouse models[128,130–133]. LIN28 proteins block cell differentiation, promote cell proliferation and alter cellular metabolism to promote tumorigenesis[134,135]. The repression of the let-7 family in these contexts is crucial, as tumour formation is suppressed by enforced expression of let-7g, and genetic deletion of a let-7 locus (*let7c2* and *let7b*) recapitulated the effects of LIN28B overexpression in the

intestine[127–129,133]. Depletion of LIN28A or LIN28B in human cancer cell lines results in decreased cell proliferation, cell invasion and tumorigenicity[129,136], and withdrawal of LIN28B expression can revert liver tumorigenesis in mice[130]. At least 15% of all human cancer samples investigated are characterized by reactivation of either LIN28A or LIN28B, with a corresponding reduction in let-7 levels[129]. Moreover, elevated LIN28A or LIN28B expression correlates with poor prognosis and decreased patient survival[129,131,137–140]. Considering also that LIN28A and LIN28B expression may characterize distinct tumorigenic subpopulations of cells within the tumour, known as tumour-initiating cells or CSCs[141], these studies underscore the importance of the LIN28 proteins in promoting and characterizing various human malignancies and suggest that this pathway represents an important new target for effective cancer therapies.

Mechanistically, LIN28 proteins selectively bind to the terminal loop region of pre-let-7 through RNA–protein interactions through its `cold-shock domain` and tandem `Cys-Cys-His-Cys (CCHC)-type zinc-fingers`[142,143]. LIN28 proteins recruit two alternative 3′ `terminal uridylyltransferases` (TUTases), ZCCHC11 (also known as TUT4) and ZCCHC6 (also known as TUT7), to pre-let-7 RNA[144–146]. These TUTases are key mediators in the LIN28 blockade of let-7 biogenesis, in which they catalyse the addition of an oligouridine tail to pre-let-7. Uridylated pre-let-7 is resistant to DICER1 processing and is rapidly degraded to prevent let-7 biogenesis in LIN28Aor LIN28B-expressing cells[125]. The enzyme responsible for this decay pathway was recently identified as DIS3L2, a novel 3′–5′ exonuclease that selectively degrades 3′ oligouridylated (>12 uridines) RNA[147–149] (FIG. 3). Intriguingly, *DIS3L2* is a tumour suppressor gene that is deleted in Perlman syndrome, which is characterized by fetal overgrowth and cancer predisposition, as well as in ~30% of sporadic Wilms tumours analysed[150]. Considering the strong links between *DROSHA* and *DICER1* mutations in Wilms tumours, the demonstrated ability of LIN28A and LIN28B to promote tumorigenesis as well as the tumour-suppressive role of DIS3L2, it is perhaps likely that loss of let-7 expression and/or function is a unifying driver of Wilms tumours and of other types of cancer. This let-7 loss might be accomplished by any of the aforementioned mechanisms as well as by the possible titration of let-7 function via the considerable overexpression of mRNAs containing let-7 binding sites, as was recently suggested for *HMGA2* (REF. 151). Another possible mechanism involves mutations in the let-7 binding sites of key downstream targets, thus relieving these mRNAs from let-7 regulation. In support of this, a single-nucleotide polymorphism (SNP) in a let-7 binding site in the 3′ UTR of the *KRAS* mRNA has been genetically associated with an increased risk of cancer[152] (FIG. 3).

Conclusions and perspectives

Discoveries over the past 15 years have provided substantial insights into the mechanisms controlling miRNA biogenesis. The identification and characterization of the core miRNA biogenesis machinery provided the framework for recent developments that uncovered cancer-causing mutations in miRNA biogenesis components as well as for the identification of cellular signalling and regulatory pathways that control different subsets of miRNAs. Although clear examples of individual miRNAs with oncogenic function have been described, the net effect of widespread miRNA depletion is to promote tumorigenesis. This

was first demonstrated in human cancer cells and mouse models and is strongly supported by the mutations recently identified in core miRNA biogenesis genes.

Analogous to the defective differentiation phenotype of miRNA-deficient embryonic stem cells, it seems that also in the context of cancer the dominant function of miRNAs is to help to maintain differentiated cells in a particular cell state or lineage[153,154]. In this model, loss of miRNAs facilitates epigenetic reprogramming, loss of differentiated cell identity and adoption of an undifferentiated cancer phenotype. Indeed, DGCR8 depletion is sufficient to reprogramme human primary keratinocytes to induced pluripotent-like cells[155]. Furthermore, miRNA expression is globally elevated in confluent cells, which is consistent with their roles in suppressing cell proliferation and in coordinating the altered metabolic demands of less-proliferative cells and tissues[108,110]. Presumably this is how widespread miRNA depletion — through loss of components of the biogenesis machinery or loss of growth-suppressive signalling pathways (for example, the Hippo pathway) — contributes to rapid cancer cell proliferation and tumour growth. In this way, widespread loss of miRNAs functionally cooperates with other cancer hallmarks to regulate cancer progression[156]. Is loss of any particular miRNA or miRNA family responsible for these tumorigenic effects? One good candidate is the let-7 family. The let-7 family is required in adult fibroblasts to suppress the expression of a mid-gestation embryonic gene signature that is enriched with oncofetal genes[157]. Conversely, antagonizing let-7 with antisense oligonucleotides can enhance reprogramming to induced pluripotent stem cells, suggesting that let-7 has a dominant role in stem cell differentiation[158]. Indeed, re-introduction of let-7 into miRNA-deficient mouse embryonic stem cells rescued the stem cell differentiation phenotype[158]; similarly, restoration of let-7 expression was shown to effectively inhibit growth of lung and breast cancer cells, as well as in mouse models of hepatocellular carcinoma and Wilms tumours[118,159,160]. Thus, let-7 emerges as a key regulator in stem cell biology and tumorigenesis and, as outlined in this Review, there are multiple mechanisms by which cancer cells inactivate this miRNA ‘guardian’ of differentiation, proliferation and metabolic reprogramming.

Future work promises to illuminate the most relevant miRNAs in the context of different cancer types and will probably uncover additional pathways that control the expression of individual miRNAs or of miRNA subsets. Studies in this area will be facilitated by the recent advances in genome engineering using CRISPR–Cas9 (clustered regularly interspaced short palindromic repeat–CRISPR-associated protein 9) technology, in mouse modelling and in the use of organoid culture systems to model cancer[161], as well as by the application of high-throughput sequencing technologies that will uncover cancer-causing mutations in patients and that can be applied in the laboratory to examine the effects of possible regulators on global miRNA expression profiles[21]. With this powerful toolkit in hand, the next several years promise exciting discoveries that will help to unlock the secrets of miRNA dysregulation in cancer. Understanding the molecular and cellular pathways controlling miRNA biogenesis and how these mechanisms go awry in cancer will identify promising therapeutic targets that might be readily manipulated by small pharmacological agents to allow restoration of miRNA expression profiles and to bypass the challenges associated with delivering synthetic miRNA mimics or antagomiRs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

S.L. is a Damon Runyon-Sohn Pediatric Cancer Research Fellow supported by the Damon Runyon Cancer Research Foundation (DRSG-7-13). R.I.G. is supported by grants from the US National Cancer Institute (NCI) (R01CA163467) and the American Cancer Society (121635-RSG-11-175-01-RMC).

Glossary

3′ untranslated region	(3′ UTR). The non-coding region of mRNA between the translation termination codon and the poly(A) tail. The 3′ UTR often contains regulatory elements, such as miRNA binding sites, for post-transcriptional regulation of gene expression
Ribonuclease III	(RNase III). Enzymes that can specifically recognize and cleave double-stranded RNA with their ribonuclease III domains
Germline mutations	Heritable gene mutations that occur in germline tissues
Somatic mutations	Gene mutations that occur in non-germline tissues that are not inherited
Post-transcriptional gene silencing	A gene-silencing effect that controls gene expression after transcription, often mediated by small non-coding RNAs such as small interfering RNAs (siRNAs) and microRNAs (miRNAs)
Epithelial–mesenchymal transition	(EMT). A process that occurs during development or cancer progression in which the epithelial cells lose their cell polarity and cell–cell adhesion to become mesenchymal cells with migratory and invasive characteristics
CpG islands	Genetic regions with high CpG content, often located at the gene promoter, that have important functions in regulating gene expression
Microsatellite	Short (2–5 bp) tandem repeat of DNA that can be used as a genetic marker
Loss of heterozygosity	(LOH). Deletion or mutation of the normal allele of a gene, of which the other allele is already deleted or inactivated, resulting in loss of both alleles of the gene
Cold-shock domain	A protein domain of ~70 amino acids that is often found in DNA- or RNA-binding proteins and that functions to protect cells during cold temperatures

Cys-Cys-His-Cys (CCHC)-type zinc-fingers	Protein domains that are found in RNA-binding proteins or single-stranded DNA-binding proteins
Terminal uridylyltransferases	(TUTases). Enzymes that catalyse the addition of one or more uridine monophosphate (UMP) molecules to the 3′ end of RNA
Oncofetal genes	Genes that are typically highly expressed during fetal development and repressed in adult life, and reactivated in cancers

References

1. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009; 136:215–233. [PubMed: 19167326]
2. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nature Rev Cancer. 2006; 6:857–866. [PubMed: 17060945]
3. Calin GA, et al. Frequent deletions and downregulation of micro-RNA genes *miR15* and *miR16* at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci USA. 2002; 99:15524–15529. [PubMed: 12434020]
4. Di Leva G, Croce CM. Roles of small RNAs in tumor formation. Trends Mol Med. 2010; 16:257–267. [PubMed: 20493775]
5. Mendell JT, Olson EN. MicroRNAs in stress signaling and human disease. Cell. 2012; 148:1172–1187. [PubMed: 22424228]
6. He L, et al. A microRNA polycistron as a potential human oncogene. Nature. 2005; 435:828–833. This paper was the first to reveal that genes in the miR-17~92 cluster function as potential human oncogenes. [PubMed: 15944707]
7. Kim HH, et al. HuR recruits let-7/RISC to repress c-Myc expression. Genes Dev. 2009; 23:1743–1748. [PubMed: 19574298]
8. Johnson SM, et al. *RAS* is regulated by the *let-7* microRNA family. Cell. 2005; 120:635–647. This paper was the first to show that members of the let-7 family of miRNAs function as tumour suppressors by targeting RAS. [PubMed: 15766527]
9. Kumar MS, et al. Suppression of non-small cell lung tumor development by the *let-7* microRNA family. Proc Natl Acad Sci USA. 2008; 105:3903–3908. [PubMed: 18308936]
10. Lu J, et al. MicroRNA expression profiles classify human cancers. Nature. 2005; 435:834–838. This paper was the first to report that miRNAs are globally downregulated in cancers. [PubMed: 15944708]
11. Thomson JM, et al. Extensive post-transcriptional regulation of microRNAs and its implications for cancer. Genes Dev. 2006; 20:2202–2207. [PubMed: 16882971]
12. Karube Y, et al. Reduced expression of *Dicer* associated with poor prognosis in lung cancer patients. Cancer Sci. 2005; 96:111–115. [PubMed: 15723655]
13. Lin RJ, et al. microRNA signature and expression of *Dicer* and *Drosha* can predict prognosis and delineate risk groups in neuroblastoma. Cancer Res. 2010; 70:7841–7850. [PubMed: 20805302]
14. Merritt WM, et al. Dicer, Drosha, and outcomes in patients with ovarian cancer. N Engl J Med. 2008; 359:2641–2650. This paper reveals that the expression levels of DICER1 and DROSHA are associated with clinical outcomes in patients with ovarian cancer. [PubMed: 19092150]
15. Kumar MS, Lu J, Mercer KL, Golub TR, Jacks T. Impaired microRNA processing enhances cellular transformation and tumorigenesis. Nature Genet. 2007; 39:673–677. This paper shows that impaired miRNA biogenesis promotes oncogenesis. [PubMed: 17401365]

16. Hill DA, et al. *DICER1* mutations in familial pleuropulmonary blastoma. Science. 2009; 325:965. This study was the first to identify the germline mutations of *DICER1* in patients with familial PPB. [PubMed: 19556464]
17. Anglesio MS, et al. Cancer-associated somatic *DICER1* hotspot mutations cause defective miRNA processing and reverse-strand expression bias to predominantly mature 3p strands through loss of 5p strand cleavage. J Pathol. 2013; 229:400–409. [PubMed: 23132766]
18. Heravi-Moussavi A, et al. Recurrent somatic *DICER1* mutations in nonepithelial ovarian cancers. N Engl J Med. 2012; 366:234–242. This study identified the recurrent somatic mutations encoding the RNase IIIb catalytic domain of *DICER1* that affect the processing of 5′ derived miRNAs. [PubMed: 22187960]
19. Foulkes WD, Priest JR, Duchaine TF. *DICER1*: mutations, microRNAs and mechanisms. Nature Rev Cancer. 2014; 14:662–672. [PubMed: 25176334]
20. Slade I, et al. *DICER1* syndrome: clarifying the diagnosis, clinical features and management implications of a pleiotropic tumour predisposition syndrome. J Med Genet. 2011; 48:273–278. [PubMed: 21266384]
21. Rakheja D, et al. Somatic mutations in *DROSHA* and *DICER1* impair microRNA biogenesis through distinct mechanisms in Wilms tumours. Nature Commun. 2014; 2:4802. [PubMed: 25190313]
22. Torrezan GT, et al. Recurrent somatic mutation in *DROSHA* induces microRNA profile changes in Wilms tumour. Nature Commun. 2014; 5:4039. [PubMed: 24909261]
23. Wegert J, et al. Mutations in the SIX1/2 pathway and the DROSHA/DGCR8 miRNA microprocessor complex underlie high-risk blastemal type Wilms tumors. Cancer Cell. 2015; 27:298–311. [PubMed: 25670083]
24. Walz AL, et al. Recurrent *DGCR8, DROSHA*, and *SIX* homeodomain mutations in favorable histology Wilms tumors. Cancer Cell. 2015; 27:286–297. References [21–24] identified the recurrent somatic mutation of *DROSHA* and *DGCR8* in Wilms tumours. [PubMed: 25670082]
25. Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. Cell. 1993; 75:843–854. This study was the first to identify miRNA. [PubMed: 8252621]
26. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. Science. 2001; 294:853–858. [PubMed: 11679670]
27. Lau NC, Lim LP, Weinstein EG, Bartel DP. An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. Science. 2001; 294:858–862. [PubMed: 11679671]
28. Lee RC, Ambros V. An extensive class of small RNAs in *Caenorhabditis elegans*. Science. 2001; 294:862–864. [PubMed: 11679672]
29. Kozomara A, Griffiths-Jones S. miRBase: integrating microRNA annotation and deep-sequencing data. Nucleic Acids Res. 2011; 39:D152–D157. [PubMed: 21037258]
30. Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nature Rev Genet. 2008; 9:102–114. [PubMed: 18197166]
31. Lee Y, et al. MicroRNA genes are transcribed by RNA polymerase II. EMBO J. 2004; 23:4051–4060. [PubMed: 15372072]
32. Gregory RI, et al. The Microprocessor complex mediates the genesis of microRNAs. Nature. 2004; 432:235–240. [PubMed: 15531877]
33. Denli AM, Tops BB, Plasterk RH, Ketting RF, Hannon GJ. Processing of primary microRNAs by the Microprocessor complex. Nature. 2004; 432:231–235. [PubMed: 15531879]
34. Lee Y, et al. The nuclear RNase III Drosha initiates microRNA processing. Nature. 2003; 425:415–419. [PubMed: 14508493]
35. Han J, et al. The Drosha–DGCR8 complex in primary microRNA processing. Genes Dev. 2004; 18:3016–3027. [PubMed: 15574589]
36. Han J, et al. Molecular basis for the recognition of primary microRNAs by the Drosha–DGCR8 complex. Cell. 2006; 125:887–901. [PubMed: 16751099]
37. Zeng Y, Yi R, Cullen BR. Recognition and cleavage of primary microRNA precursors by the nuclear processing enzyme Drosha. EMBO J. 2005; 24:138–148. [PubMed: 15565168]

38. Burke JM, Kelenis DP, Kincaid RP, Sullivan CS. A central role for the primary microRNA stem in guiding the position and efficiency of Drosha processing of a viral pri-miRNA. RNA. 2014; 20:1068–1077. [PubMed: 24854622]
39. Heo I, et al. Mono-uridylation of pre-microRNA as a key step in the biogenesis of group II let-7 microRNAs. Cell. 2012; 151:521–532. [PubMed: 23063654]
40. Yi R, Qin Y, Macara IG, Cullen BR. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes Dev. 2003; 17:3011–3016. [PubMed: 14681208]
41. Lund E, Guttinger S, Calado A, Dahlberg JE, Kutay U. Nuclear export of microRNA precursors. Science. 2004; 303:95–98. [PubMed: 14631048]
42. Bohnsack MT, Czaplinski K, Gorlich D. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. RNA. 2004; 10:185–191. [PubMed: 14730017]
43. Park JE, et al. Dicer recognizes the 5′ end of RNA for efficient and accurate processing. Nature. 2011; 475:201–205. [PubMed: 21753850]
44. Bernstein E, Caudy AA, Hammond SM, Hannon GJ. Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature. 2001; 409:363–366. [PubMed: 11201747]
45. Chendrimada TP, et al. TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. Nature. 2005; 436:740–744. [PubMed: 15973356]
46. Lee HY, Doudna JA. TRBP alters human precursor microRNA processing *in vitro*. RNA. 2012; 18:2012–2019. [PubMed: 23006623]
47. Kim Y, et al. Deletion of human *tarbp2* reveals cellular microRNA targets and cell-cycle function of TRBP. Cell Rep. 2014; 9:1061–1074. [PubMed: 25437560]
48. Gregory RI, Chendrimada TP, Cooch N, Shiekhattar R. Human RISC couples microRNA biogenesis and posttranscriptional gene silencing. Cell. 2005; 123:631–640. [PubMed: 16271387]
49. Liu J, Valencia-Sanchez MA, Hannon GJ, Parker R. MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies. Nature Cell Biol. 2005; 7:719–723. [PubMed: 15937477]
50. Eulalio A, Behm-Ansmant I, Schweizer D, Izaurralde E. P-body formation is a consequence, not the cause, of RNA-mediated gene silencing. Mol Cell Biol. 2007; 27:3970–3981. [PubMed: 17403906]
51. Calin GA, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci USA. 2004; 101:2999–3004. [PubMed: 14973191]
52. Zhang L, et al. microRNAs exhibit high frequency genomic alterations in human cancer. Proc Natl Acad Sci USA. 2006; 103:9136–9141. [PubMed: 16754881]
53. Cimmino A, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci USA. 2005; 102:13944–13949. [PubMed: 16166262]
54. Kotani A, et al. A novel mutation in the *miR-128b* gene reduces miRNA processing and leads to glucocorticoid resistance of MLL–AF4 acute lymphocytic leukemia cells. Cell Cycle. 2010; 9:1037–1042. [PubMed: 20237425]
55. He L, et al. A microRNA component of the p53 tumour suppressor network. Nature. 2007; 447:1130–1134. [PubMed: 17554337]
56. Raver-Shapira N, et al. Transcriptional activation of miR-34a contributes to p53-mediated apoptosis. Mol Cell. 2007; 26:731–743. [PubMed: 17540598]
57. Chang TC, et al. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. Mol Cell. 2007; 26:745–752. [PubMed: 17540599]
58. Bommer GT, et al. p53-mediated activation of miRNA34 candidate tumor-suppressor genes. Curr Biol. 2007; 17:1298–1307. [PubMed: 17656095]
59. Tarasov V, et al. Differential regulation of microRNAs by p53 revealed by massively parallel sequencing: miR-34a is a p53 target that induces apoptosis and G1-arrest. Cell Cycle. 2007; 6:1586–1593. [PubMed: 17554199]
60. O'Donnell KA, Wentzel EA, Zeller KI, Dang CV, Mendell JT. c-Myc-regulated microRNAs modulate E2F1 expression. Nature. 2005; 435:839–843. [PubMed: 15944709]

61. Dews M, et al. Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster. Nature Genet. 2006; 38:1060–1065. [PubMed: 16878133]
62. Chang TC, et al. Widespread microRNA repression by Myc contributes to tumorigenesis. Nature Genet. 2008; 40:43–50. [PubMed: 18066065]
63. Gregory PA, et al. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nature Cell Biol. 2008; 10:593–601. [PubMed: 18376396]
64. Bracken CP, et al. A double-negative feedback loop between ZEB1–SIP1 and the microRNA-200 family regulates epithelial–mesenchymal transition. Cancer Res. 2008; 68:7846–7854. [PubMed: 18829540]
65. Lujambio A, et al. A microRNA DNA methylation signature for human cancer metastasis. Proc Natl Acad Sci USA. 2008; 105:13556–13561. [PubMed: 18768788]
66. Guil S, Esteller M. DNA methylomes, histone codes and miRNAs: tying it all together. Int J Biochem Cell Biol. 2009; 41:87–95. [PubMed: 18834952]
67. Han J, et al. Posttranscriptional crossregulation between Drosha and DGCR8. Cell. 2009; 136:75–84. [PubMed: 19135890]
68. Triboulet R, Chang HM, Lapierre RJ, Gregory RI. Post-transcriptional control of DGCR8 expression by the Microprocessor. RNA. 2009; 15:1005–1011. [PubMed: 19383765]
69. Kadener S, et al. Genome-wide identification of targets of the Drosha–Pasha/DGCR8 complex. RNA. 2009; 15:537–545. [PubMed: 19223442]
70. Muralidhar B, et al. Functional evidence that Drosha overexpression in cervical squamous cell carcinoma affects cell phenotype and microRNA profiles. J Pathol. 2011; 224:496–507. [PubMed: 21590768]
71. Sugito N, et al. RNASEN regulates cell proliferation and affects survival in esophageal cancer patients. Clin Cancer Res. 2006; 12:7322–7328. [PubMed: 17121874]
72. Shu GS, Yang ZL, Liu DC. Immunohistochemical study of Dicer and Drosha expression in the benign and malignant lesions of gallbladder and their clinicopathological significances. Pathol Res Pract. 2012; 208:392–397. [PubMed: 22658478]
73. Guo X, et al. The microRNA-processing enzymes: Drosha and Dicer can predict prognosis of nasopharyngeal carcinoma. J Cancer Res Clin Oncol. 2012; 138:49–56. [PubMed: 21953080]
74. Jafarnejad SM, Sjoestroem C, Martinka M, Li G. Expression of the RNase III enzyme DROSHA is reduced during progression of human cutaneous melanoma. Mod Pathol. 2013; 26:902–910. [PubMed: 23370771]
75. Grund SE, Polycarpou-Schwarz M, Luo C, Eichmuller SB, Diederichs S. Rare Drosha splice variants are deficient in microRNA processing but do not affect general microRNA expression in cancer cells. Neoplasia. 2012; 14:238–248. [PubMed: 22496623]
76. Melo SA, et al. A genetic defect in exportin-5 traps precursor microRNAs in the nucleus of cancer cells. Cancer Cell. 2010; 18:303–315. [PubMed: 20951941]
77. Leaderer D, et al. Genetic and epigenetic association studies suggest a role of microRNA biogenesis gene exportin-5 (*XPO5*) in breast tumorigenesis. Int J Mol Epidemiol Genet. 2011; 2:9–18. [PubMed: 21552306]
78. Hutvagner G, et al. A cellular function for the RNA-interference enzyme Dicer in the maturation of the *let-7* small temporal RNA. Science. 2001; 293:834–838. [PubMed: 11452083]
79. Kumar MS, et al. *Dicer1* functions as a haploinsufficient tumor suppressor. Genes Dev. 2009; 23:2700–2704. [PubMed: 19903759]
80. Pugh TJ, et al. Exome sequencing of pleuropulmonary blastoma reveals frequent biallelic loss of *TP53* and two hits in *DICER1* resulting in retention of 5p-derived miRNA hairpin loop sequences. Oncogene. 2014; 33:5295–5302. [PubMed: 24909177]
81. Wagh PK, et al. Cell- and developmental stage-specific *Dicer1* ablation in the lung epithelium models cystic pleuropulmonary blastoma. J Pathol. 2014; 236:41–52. [PubMed: 25500911]
82. de Kock L, et al. Germ-line and somatic *DICER1* mutations in a pleuropulmonary blastoma. Pediatr Blood Cancer. 2013; 60:2091–2092. [PubMed: 23868280]
83. Seki M, et al. Biallelic *DICER1* mutations in sporadic pleuropulmonary blastoma. Cancer Res. 2014; 74:2742–2749. [PubMed: 24675358]

84. Schultz KA, et al. Ovarian sex cord-stromal tumors, pleuropulmonary blastoma and *DICER1* mutations: a report from the International Pleuropulmonary Blastoma Registry. Gynecol Oncol. 2011; 122:246–250. [PubMed: 21501861]
85. Witkowski L, et al. *DICER1* hotspot mutations in non-epithelial gonadal tumours. Br J Cancer. 2013; 109:2744–2750. [PubMed: 24136150]
86. Foulkes WD, et al. Extending the phenotypes associated with *DICER1* mutations. Hum Mutat. 2011; 32:1381–1384. [PubMed: 21882293]
87. Wu MK, et al. Biallelic *DICER1* mutations occur in Wilms tumours. J Pathol. 2013; 230:154–164. [PubMed: 23620094]
88. de Kock L, et al. Pituitary blastoma: a pathognomonic feature of germ-line *DICER1* mutations. Acta Neuropathol. 2014; 128:111–122. [PubMed: 24839956]
89. Doros LA, et al. *DICER1* mutations in childhood cystic nephroma and its relationship to *DICER1*-renal sarcoma. Mod Pathol. 2014; 27:1267–1280. [PubMed: 24481001]
90. Doros L, et al. *DICER1* mutations in embryonal rhabdomyosarcomas from children with and without familial PPB-tumor predisposition syndrome. Pediatr Blood Cancer. 2012; 59:558–560. [PubMed: 22180160]
91. Schultze-Florey RE, et al. *DICER1* syndrome: a new cancer syndrome. Klin Padiatr. 2013; 225:177–178. [PubMed: 23625684]
92. Su X, et al. TAp63 suppresses metastasis through coordinate regulation of Dicer and miRNAs. Nature. 2010; 467:986–990. [PubMed: 20962848]
93. Melo SA, et al. A *TARBP2* mutation in human cancer impairs microRNA processing and DICER1 function. Nature Genet. 2009; 41:365–370. [PubMed: 19219043]
94. Garre P, Perez-Segura P, Diaz-Rubio E, Caldes T, de la Hoya M. Reassessing the *TARBP2* mutation rate in hereditary nonpolyposis colorectal cancer. Nature Genet. 2010; 42:817–818. [PubMed: 20877318]
95. De Vito C, et al. A TARBP2-dependent miRNA expression profile underlies cancer stem cell properties and provides candidate therapeutic reagents in Ewing sarcoma. Cancer Cell. 2012; 21:807–821. [PubMed: 22698405]
96. van Kouwenhove M, Kedde M, Agami R. MicroRNA regulation by RNA-binding proteins and its implications for cancer. Nature Rev Cancer. 2011; 11:644–656. [PubMed: 21822212]
97. Kawai S, Amano A. BRCA1 regulates microRNA biogenesis via the DROSHA microprocessor complex. J Cell Biol. 2012; 197:201–208. [PubMed: 22492723]
98. Trabucchi M, et al. The RNA-binding protein KSRP promotes the biogenesis of a subset of microRNAs. Nature. 2009; 459:1010–1014. [PubMed: 19458619]
99. Wu H, et al. A splicing-independent function of SF2/ASF in microRNA processing. Mol Cell. 2010; 38:67–77. [PubMed: 20385090]
100. Guil S, Caceres JF. The multifunctional RNA-binding protein hnRNP A1 is required for processing of miR-18a. Nature Struct Mol Biol. 2007; 14:591–596. [PubMed: 17558416]
101. Michlewski G, Guil S, Semple CA, Caceres JF. Posttranscriptional regulation of miRNAs harboring conserved terminal loops. Mol Cell. 2008; 32:383–393. [PubMed: 18995836]
102. Morlando M, et al. FUS stimulates microRNA biogenesis by facilitating co-transcriptional Drosha recruitment. EMBO J. 2012; 31:4502–4510. [PubMed: 23232809]
103. Suzuki HI, et al. Modulation of microRNA processing by p53. Nature. 2009; 460:529–533. [PubMed: 19626115]
104. Davis BN, Hilyard AC, Lagna G, Hata A. SMAD proteins control DROSHA-mediated microRNA maturation. Nature. 2008; 454:56–61. [PubMed: 18548003]
105. Davis BN, Hilyard AC, Nguyen PH, Lagna G, Hata A. Smad proteins bind a conserved RNA sequence to promote microRNA maturation by Drosha. Mol Cell. 2010; 39:373–384. [PubMed: 20705240]
106. Ha M, Kim VN. Regulation of microRNA biogenesis. Nature Rev Mol Cell Biol. 2014; 15:509–524. [PubMed: 25027649]
107. Drake M, et al. A requirement for ERK-dependent dicer phosphorylation in coordinating oocyte-to-embryo transition in *C. elegans*. Dev Cell. 2014; 31:614–628. [PubMed: 25490268]

108. Mori M, et al. Hippo signaling regulates Microprocessor and links cell-density-dependent miRNA biogenesis to cancer. Cell. 2014; 156:893–906. [PubMed: 24581491]
109. Harvey KF, Zhang X, Thomas DM. The Hippo pathway and human cancer. Nature Rev Cancer. 2013; 13:246–257. [PubMed: 23467301]
110. Hwang HW, Wentzel EA, Mendell JT. Cell–cell contact globally activates microRNA biogenesis. Proc Natl Acad Sci USA. 2009; 106:7016–7021. [PubMed: 19359480]
111. Leung AK, Sharp PA. MicroRNA functions in stress responses. Mol Cell. 2010; 40:205–215. [PubMed: 20965416]
112. Franovic A, et al. Translational up-regulation of the EGFR by tumor hypoxia provides a nonmutational explanation for its overexpression in human cancer. Proc Natl Acad Sci USA. 2007; 104:13092–13097. [PubMed: 17670948]
113. Shen J, et al. EGFR modulates microRNA maturation in response to hypoxia through phosphorylation of AGO2. Nature. 2013; 497:383–387. [PubMed: 23636329]
114. Rupaimoole R, et al. Hypoxia-mediated downregulation of miRNA biogenesis promotes tumour progression. Nature Commun. 2014; 5:5202. [PubMed: 25351346]
115. van den Beucken T, et al. Hypoxia promotes stem cell phenotypes and poor prognosis through epigenetic regulation of DICER. Nature Commun. 2014; 5:5203. [PubMed: 25351418]
116. Peter ME. Let-7 and miR-200 microRNAs: guardians against pluripotency and cancer progression. Cell Cycle. 2009; 8:843–852. [PubMed: 19221491]
117. Barh D, Malhotra R, Ravi B, Sindhurani P. MicroRNA let-7: an emerging next-generation cancer therapeutic. Curr Oncol. 2010; 17:70–80. [PubMed: 20179807]
118. Mayr C, Hemann MT, Bartel DP. Disrupting the pairing between *let-7* and *Hmga2* enhances oncogenic transformation. Science. 2007; 315:1576–1579. [PubMed: 17322030]
119. Akao Y, Nakagawa Y, Naoe T. *let-7* microRNA functions as a potential growth suppressor in human colon cancer cells. Biol Pharm Bull. 2006; 29:903–906. [PubMed: 16651716]
120. Boyerinas B, Park SM, Hau A, Murmann AE, Peter ME. The role of let-7 in cell differentiation and cancer. Endocr Relat Cancer. 2010; 17:F19–F36. [PubMed: 19779035]
121. Bussing I, Slack FJ, Grosshans H. *let-7* microRNAs in development, stem cells and cancer. Trends Mol Med. 2008; 14:400–409. [PubMed: 18674967]
122. Droge P, Davey CA. Do cells let-7 determine stemness? Cell Stem Cell. 2008; 2:8–9. [PubMed: 18371414]
123. Rybak A, et al. A feedback loop comprising lin-28 and let-7 controls pre-let-7 maturation during neural stem-cell commitment. Nature Cell Biol. 2008; 10:987–993. [PubMed: 18604195]
124. Viswanathan SR, Daley GQ, Gregory RI. Selective blockade of microRNA processing by Lin28. Science. 2008; 320:97–100. [PubMed: 18292307]
125. Heo I, et al. Lin28 mediates the terminal uridylation of let-7 precursor MicroRNA. Mol Cell. 2008; 32:276–284. References [124–126] reveal that LIN28A and LIN28B selectively inhibit let-7 miRNA biogenesis. [PubMed: 18951094]
126. Newman MA, Thomson JM, Hammond SM. Lin-28 interaction with the Let-7 precursor loop mediates regulated microRNA processing. RNA. 2008; 14:1539–1549. [PubMed: 18566191]
127. Madison BB, et al. LIN28B promotes growth and tumorigenesis of the intestinal epithelium via Let-7. Genes Dev. 2013; 27:2233–2245. [PubMed: 24142874]
128. Urbach A, et al. Lin28 sustains early renal progenitors and induces Wilms tumor. Genes Dev. 2014; 28:971–982. [PubMed: 24732380]
129. Viswanathan SR, et al. Lin28 promotes transformation and is associated with advanced human malignancies. Nature Genet. 2009; 41:843–848. This paper reveals the roles of the LIN28–let-7 pathway in the regulation of oncogenesis. [PubMed: 19483683]
130. Nguyen LH, et al. Lin28b is sufficient to drive liver cancer and necessary for its maintenance in murine models. Cancer Cell. 2014; 26:248–261. [PubMed: 25117712]
131. Molenaar JJ, et al. LIN28B induces neuroblastoma and enhances MYCN levels via let-7 suppression. Nature Genet. 2012; 44:1199–1206. [PubMed: 23042116]

132. Beachy SH, et al. Enforced expression of Lin28b leads to impaired T-cell development, release of inflammatory cytokines, and peripheral T-cell lymphoma. Blood. 2012; 120:1048–1059. [PubMed: 22723554]
133. King CE, et al. LIN28B fosters colon cancer migration, invasion and transformation through let-7-dependent and -independent mechanisms. Oncogene. 2011; 30:4185–4193. [PubMed: 21625210]
134. Thornton JE, Gregory RI. How does Lin28 let-7 control development and disease? Trends Cell Biol. 2012; 22:474–482. [PubMed: 22784697]
135. Zhu H, et al. The Lin28/let-7 axis regulates glucose metabolism. Cell. 2011; 147:81–94. [PubMed: 21962509]
136. Iliopoulos D, Hirsch HA, Struhl K. An epigenetic switch involving NF-κB, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. Cell. 2009; 139:693–706. [PubMed: 19878981]
137. Hamano R, et al. High expression of Lin28 is associated with tumour aggressiveness and poor prognosis of patients in oesophagus cancer. Br J Cancer. 2012; 106:1415–1423. [PubMed: 22433967]
138. Picard D, et al. Markers of survival and metastatic potential in childhood CNS primitive neuro-ectodermal brain tumours: an integrative genomic analysis. Lancet Oncol. 2012; 13:838–848. [PubMed: 22691720]
139. Diskin SJ, et al. Common variation at 6q16 within *HACE1* and *LIN28B* influences susceptibility to neuroblastoma. Nature Genet. 2012; 44:1126–1130. [PubMed: 22941191]
140. Hovestadt V, et al. Decoding the regulatory landscape of medulloblastoma using DNA methylation sequencing. Nature. 2014; 510:537–541. [PubMed: 24847876]
141. Zhang WC, et al. Glycine decarboxylase activity drives non-small cell lung cancer tumor-initiating cells and tumorigenesis. Cell. 2012; 148:259–272. [PubMed: 22225612]
142. Piskounova E, et al. Determinants of microRNA processing inhibition by the developmentally regulated RNA-binding protein Lin28. J Biol Chem. 2008; 283:21310–21314. [PubMed: 18550544]
143. Nam Y, Chen C, Gregory RI, Chou JJ, Sliz P. Molecular basis for interaction of let-7 microRNAs with Lin28. Cell. 2011; 147:1080–1091. [PubMed: 22078496]
144. Hagan JP, Piskounova E, Gregory RI. Lin28 recruits the TUTase Zcchc11 to inhibit let-7 maturation in mouse embryonic stem cells. Nature Struct Mol Biol. 2009; 16:1021–1025. [PubMed: 19713958]
145. Heo I, et al. TUT4 in concert with Lin28 suppresses microRNA biogenesis through pre-microRNA uridylation. Cell. 2009; 138:696–708. [PubMed: 19703396]
146. Thornton JE, Chang HM, Piskounova E, Gregory RI. Lin28-mediated control of let-7 microRNA expression by alternative TUTases Zcchc11 (TUT4) and Zcchc6 (TUT7). RNA. 2012; 18:1875–1885. [PubMed: 22898984]
147. Chang HM, Triboulet R, Thornton JE, Gregory RI. A role for the Perlman syndrome exonuclease Dis3l2 in the Lin28–let-7 pathway. Nature. 2013; 497:244–248. [PubMed: 23594738]
148. Faehnle CR, Walleshauser J, Joshua-Tor L. Mechanism of Dis3l2 substrate recognition in the Lin28–let-7 pathway. Nature. 2014; 514:252–256. [PubMed: 25119025]
149. Ustianenko D, et al. Mammalian DIS3L2 exoribonuclease targets the uridylated precursors of let-7 miRNAs. RNA. 2013; 19:1632–1638. [PubMed: 24141620]
150. Astuti D, et al. Germline mutations in *DIS3L2* cause the Perlman syndrome of overgrowth and Wilms tumor susceptibility. Nature Genet. 2012; 44:277–284. [PubMed: 22306653]
151. Kumar MS, et al. HMGA2 functions as a competing endogenous RNA to promote lung cancer progression. Nature. 2014; 505:212–217. [PubMed: 24305048]
152. Chin LJ, et al. A SNP in a *let-7* microRNA complementary site in the *KRAS* 3′ untranslated region increases non-small cell lung cancer risk. Cancer Res. 2008; 68:8535–8540. [PubMed: 18922928]
153. Kanellopoulou C, et al. Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. Genes Dev. 2005; 19:489–501. [PubMed: 15713842]

154. Wang Y, Medvid R, Melton C, Jaenisch R, Blelloch R. DGCR8 is essential for microRNA biogenesis and silencing of embryonic stem cell self-renewal. Nature Genet. 2007; 39:380–385. [PubMed: 17259983]
155. Chakravarti D, et al. Induced multipotency in adult keratinocytes through down-regulation of Δ*Np63* or *DGCR8*. Proc Natl Acad Sci USA. 2014; 111:E572–E581. [PubMed: 24449888]
156. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144:646–674. [PubMed: 21376230]
157. Gurtan AM, et al. Let-7 represses *Nr6a1* and a mid-gestation developmental program in adult fibroblasts. Genes Dev. 2013; 27:941–954. [PubMed: 23630078]
158. Melton C, Judson RL, Blelloch R. Opposing microRNA families regulate self-renewal in mouse embryonic stem cells. Nature. 2010; 463:621–626. [PubMed: 20054295]
159. Trang P, et al. Regression of murine lung tumors by the *let-7* microRNA. Oncogene. 2009; 29:1580–1587. [PubMed: 19966857]
160. Yu F, et al. *let-7* regulates self renewal and tumorigenicity of breast cancer cells. Cell. 2007; 131:1109–1123. [PubMed: 18083101]
161. Li X, et al. Oncogenic transformation of diverse gastrointestinal tissues in primary organoid culture. Nature Med. 2014; 20:769–777. [PubMed: 24859528]
162. Muralidhar B, et al. Global microRNA profiles in cervical squamous cell carcinoma depend on Drosha expression levels. J Pathol. 2007; 212:368–377. [PubMed: 17471471]
163. Sand M, et al. Expression levels of the microRNA processing enzymes Drosha and dicer in epithelial skin cancer. Cancer Invest. 2010; 28:649–653. [PubMed: 20210522]
164. Passon N, et al. Expression of Dicer and Drosha in triple-negative breast cancer. J Clin Pathol. 2012; 65:320–326. [PubMed: 22259182]
165. Avery-Kiejda KA, Braye SG, Forbes JF, Scott RJ. The expression of Dicer and Drosha in matched normal tissues, tumours and lymph node metastases in triple negative breast cancer. BMC Cancer. 2014; 14:253. [PubMed: 24725360]
166. Papachristou DJ, et al. Immunohistochemical analysis of the endoribonucleases Drosha, Dicer and Ago2 in smooth muscle tumours of soft tissues. Histopathology. 2012; 60:E28–E36. [PubMed: 22394132]
167. Tchernitsa O, et al. Systematic evaluation of the miRNA-ome and its downstream effects on mRNA expression identifies gastric cancer progression. J Pathol. 2010; 222:310–319. [PubMed: 20726036]
168. Vaksman O, Hetland TE, Trope CG, Reich R, Davidson B. Argonaute, Dicer, and Drosha are up-regulated along tumor progression in serous ovarian carcinoma. Hum Pathol. 2012; 43:2062–2069. [PubMed: 22647351]
169. Diaz-Garcia CV, et al. DICER1, DROSHA and miRNAs in patients with non-small cell lung cancer: implications for outcomes and histologic classification. Carcinogenesis. 2013; 34:1031–1038. [PubMed: 23349018]
170. Catto JW, et al. Distinct microRNA alterations characterize high- and low-grade bladder cancer. Cancer Res. 2009; 69:8472–8481. [PubMed: 19843843]
171. Torres A, et al. Major regulators of microRNAs biogenesis Dicer and Drosha are down-regulated in endometrial cancer. Tumour Biol. 2011; 32:769–776. [PubMed: 21559780]
172. Yan M, et al. Dysregulated expression of dicer and drosha in breast cancer. Pathol Oncol Res. 2012; 18:343–348. [PubMed: 21898071]
173. Sand M, et al. Expression levels of the microRNA maturing microprocessor complex component DGCR8 and the RNA-induced silencing complex (RISC) components argonaute-1, argonaute-2, PACT, TARBP1, and TARBP2 in epithelial skin cancer. Mol Carcinog. 2011; 51:916–922. [PubMed: 22025453]
174. Ambs S, et al. Genomic profiling of microRNA and messenger RNA reveals deregulated microRNA expression in prostate cancer. Cancer Res. 2008; 68:6162–6170. [PubMed: 18676839]
175. Kim B, et al. An essential microRNA maturing microprocessor complex component DGCR8 is up-regulated in colorectal carcinomas. Clin Exp Med. 2013; 14:331–336. [PubMed: 23775303]

176. Guo Y, et al. Silencing the double-stranded RNA binding protein DGCR8 inhibits ovarian cancer cell proliferation, migration, and invasion. Pharm Res. 2013; 32:769–778. [PubMed: 25823356]
177. Chiosea S, et al. Up-regulation of dicer, a component of the microRNA machinery, in prostate adenocarcinoma. Am J Pathol. 2006; 169:1812–1820. [PubMed: 17071602]
178. Jakymiw A, et al. Overexpression of dicer as a result of reduced *let-7* microRNA levels contributes to increased cell proliferation of oral cancer cells. Genes Chromosomes Cancer. 2010; 49:549–559. [PubMed: 20232482]
179. Faber C, Horst D, Hlubek F, Kirchner T. Overexpression of Dicer predicts poor survival in colorectal cancer. Eur J Cancer. 2011; 47:1414–1419. [PubMed: 21345667]
180. Stratmann J, et al. Dicer and miRNA in relation to clinicopathological variables in colorectal cancer patients. BMC Cancer. 2011; 11:345. [PubMed: 21827717]
181. Papachristou DJ, et al. Expression of the ribonucleases Drosha, Dicer, and Ago2 in colorectal carcinomas. Virchows Arch. 2011; 459:431–440. [PubMed: 21769619]
182. Chiosea S, et al. Overexpression of Dicer in precursor lesions of lung adenocarcinoma. Cancer Res. 2007; 67:2345–2350. [PubMed: 17332367]
183. Ma Z, et al. Up-regulated Dicer expression in patients with cutaneous melanoma. PLoS ONE. 2011; 6:e20494. [PubMed: 21698147]
184. Dedes KJ, et al. Down-regulation of the miRNA master regulators Drosha and Dicer is associated with specific subgroups of breast cancer. Eur J Cancer. 2011; 47:138–150. [PubMed: 20832293]
185. Wu D, et al. Downregulation of Dicer, a component of the microRNA machinery, in bladder cancer. Mol Med Rep. 2012; 5:695–699. [PubMed: 22179432]
186. Pampalakis G, Diamandis EP, Katsaros D, Sotiropoulou G. Down-regulation of dicer expression in ovarian cancer tissues. Clin Biochem. 2010; 43:324–327. [PubMed: 19782670]
187. Faggad A, et al. Prognostic significance of Dicer expression in ovarian cancer — link to global microRNA changes and oestrogen receptor expression. J Pathol. 2010; 220:382–391. [PubMed: 19960504]
188. Khoshnaw SM, et al. Loss of Dicer expression is associated with breast cancer progression and recurrence. Breast Cancer Res Treat. 2012; 135:403–413. [PubMed: 22821364]
189. Wu JF, et al. Down-regulation of Dicer in hepatocellular carcinoma. Med Oncol. 2011; 28:804–809. [PubMed: 20405249]
190. Zhu DX, et al. Downregulated Dicer expression predicts poor prognosis in chronic lymphocytic leukemia. Cancer Sci. 2012; 103:875–881. [PubMed: 22320315]
191. Faggad A, et al. Down-regulation of the microRNA processing enzyme Dicer is a prognostic factor in human colorectal cancer. Histopathology. 2012; 61:552–561. [PubMed: 22716222]

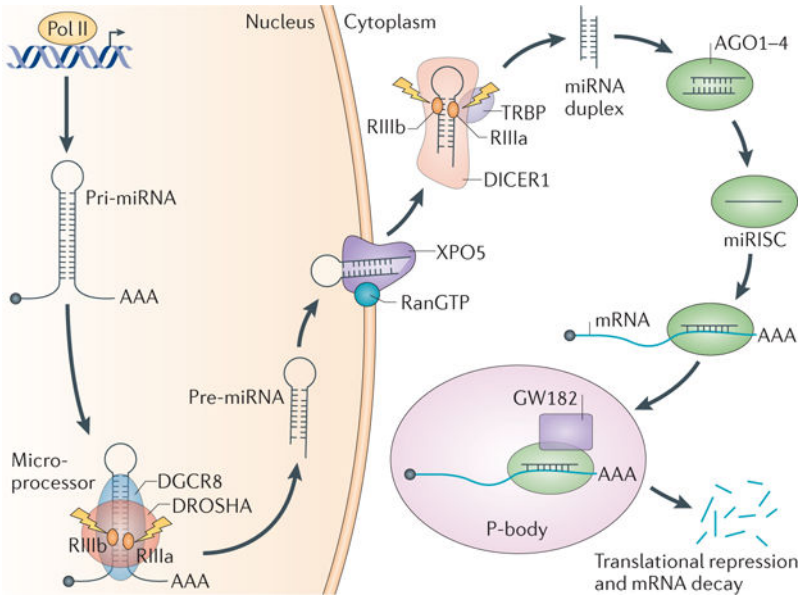

Figure 1. Overview of miRNA biogenesis pathway

MicroRNA (miRNA) genes are transcribed as primary miRNAs (pri-miRNAs) by RNA polymerase II (Pol II) in the nucleus. The long pri-miRNAs are cleaved by Microprocessor, which includes DROSHA and DiGeorge syndrome critical region 8 (DGCR8), to produce the 60–70-nucleotide precursor miRNAs (pre-miRNAs). The pre-miRNAs are then exported from the nucleus to the cytoplasm by exportin 5 (XPO5) and further processed by DICER1, a ribonuclease III (RIII) enzyme that produces the mature miRNAs. One strand of the mature miRNA (the guide strand) is loaded into the miRNA-induced silencing complex (miRISC), which contains DICER1 and Argonaute (AGO) proteins, directs the miRISC to target mRNAs by sequence complementary binding and mediates gene suppression by targeted mRNA degradation and translational repression in processing bodies (P-bodies). TRBP, transactivation-responsive RNA-binding protein.

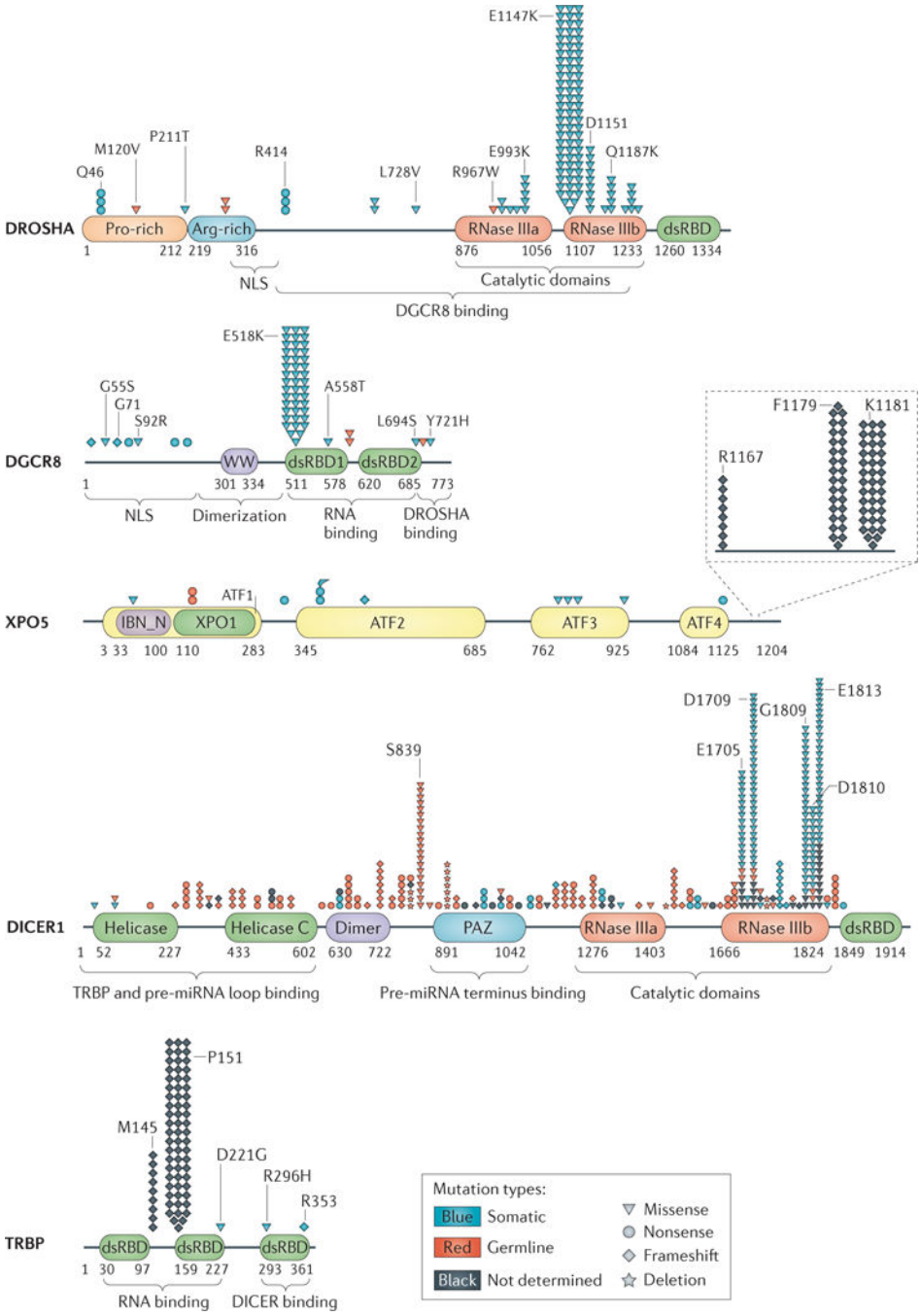

Figure 2. Mutation of the miRNA biogenesis pathway in cancer

Mutations of the microRNA (miRNA) biogenesis pathway genes identified in cancer are summarized and represented by their relative locations in the protein and the type of mutation. The detailed mutational information (mutation locations, mutation types and tumour types) is provided in Supplementary information S1 (table). ATF, armadillo-type fold; DGCR8, DiGeorge syndrome critical region 8; Dimer, dimerization domain; dsRBD, double-stranded RNA-binding domain; IBN_N, importin-β amino-terminal domain; NLS, nuclear localization signal; PAZ, Piwi–Argonaute–Zwille domain; RNase, ribonuclease; pre-

miRNA, precursor miRNA; TRBP, transactivation-responsive RNA-binding protein; WW, WW domain (also known as WWP-repeating motif); XPO1, exportin 1/importin β-like domain; XPO5, exportin 5.

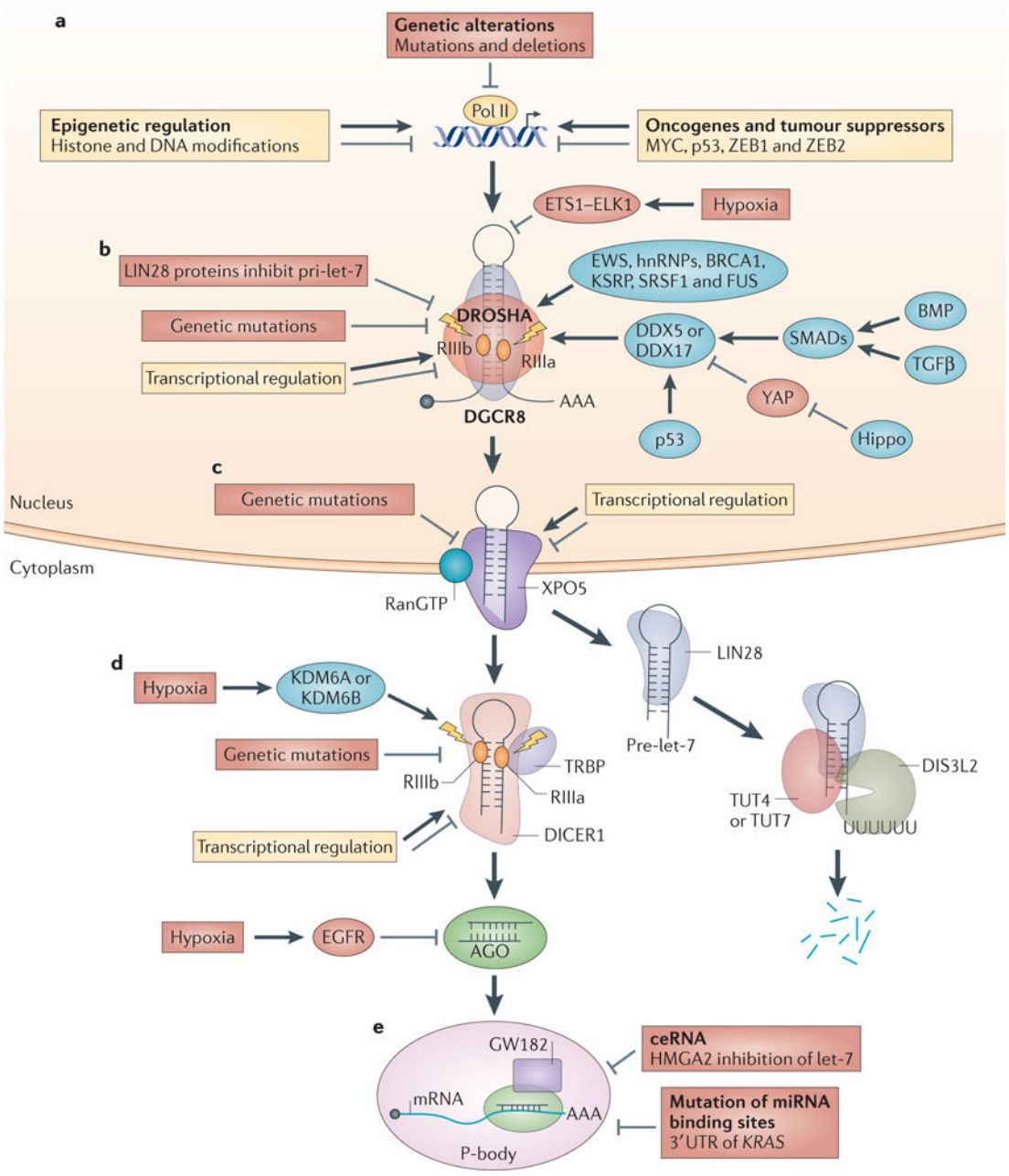

Figure 3. Dysregulated miRNA biogenesis in cancer

Aberrant microRNA (miRNA) biogenesis in cancer occurs at different steps during miRNA maturation. **a** | Genetic alterations, epigenetic modifications, oncogenes and tumour suppressors negatively or positively regulate primary miRNA (pri-miRNA) transcription in cancer. **b** | Pri-miRNA processing is regulated in the following ways: hypoxia, genetic mutations and transcriptional regulation control DROSHA and DiGeorge syndrome critical region 8 (DGCR8) expression in cancer; RNA-binding proteins such as DEAD box protein 5 (DDX5), DDX17 and BRCA1 modulate Microprocessor activity in cancer; cell signalling pathways such as Hippo and bone morphogenetic protein (BMP) regulate pri-miRNA processing; and LIN28 proteins selectively block the processing of pri-let-7. **c** | Genetic

mutations in and transcriptional regulation of exportin 5 (*XPO5*) affect XPO5-mediated precursor miRNA (pre-miRNA) export in cancer. **d** | Pre-miRNA processing in cancer is regulated in the following ways: hypoxia, genetic mutations and transcriptional regulation modulate DICER1 expression and function to control pre-miRNA cleavage in cancer; LIN28 proteins selectively bind to pre-let-7 and recruit terminal uridylyltransferase 4 (TUT4), TUT7 and DIS3-like exonuclease 2 (DIS3L2) to degrade pre-let-7; and hypoxia-induced and epidermal growth factor receptor (EGFR)-induced phosphorylation of Y393 of Argonaute 2 (AGO2) inhibits pre-miRNA processing. **e** | miRNA function is regulated in the following ways: competing endogenous RNA (ceRNA) inhibits miRNA function in cancer (high-mobility group AT-hook 2 (HMGA2) blocks let-7 function), as do mutations of miRNA-binding sites in non-small cell lung cancer (mutation of let-7-binding site in the 3′ untranslated region (UTR) of *KRAS* mRNA). hnRNP, heterogeneous nuclear ribonucleoprotein; KDM6, lysine-specific demethylase 6; KSRP, KH-type splicing regulatory protein; Pol II, RNA polymerase II; RIII, ribonuclease III; SRSF1, serine/arginine-rich splicing factor 1; TGFβ, transforming growth factor-β; TRBP, transactivation-responsive RNA-binding protein; YAP, Yes-associated protein; ZEB, zinc-finger E-box-binding homeobox.

Table 1

Dysregulation of miRNA biogenesis machinery in cancers

Protein	Dysregulation	Cancer type	Clinical correlation	Refs
DROSHA	Upregulation	Cervical SCC	Altered miRNA profile; associated with neoplastic progression	70,162
		Oesophageal cancer	Regulates cell proliferation; associated with poor patient survival	71
		BCC	Not determined	163
		SCC	Not determined	163
		Triple-negative breast cancer	No clinical correlation	164,165
		Smooth muscle tumours	Associated with tumour progression	166
		Gastric cancer	Associated with pathological characteristics and patient survival	167
		Serous ovarian carcinoma	Associated with advanced tumour stages	168
		Non-small cell lung cancer	Associated with poor prognosis	169
	Downregulation	Bladder cancer	Altered miRNA profile	170
		Ovarian cancer	Associated with poor patient survival	14
		Endometrial cancer	Correlated with histological grade	171
		Nasopharyngeal carcinoma	Correlated with shorter patient survival	73
		Breast cancer	Not determined	172
		Gallbladder adenocarcinoma	Correlated with metastasis, invasion and poor prognosis	72
		Neuroblastoma	Correlated with global downregulation of miRNAs and poor outcome	13
		Cutaneous melanoma	Associated with cancer progression and poor survival	74
DGCR8	Upregulation	Oesophageal cancer	Associated with poor patient survival	71
		Bladder cancer	Altered miRNA profile	170
		SCC and BCC	Not determined	173
		Prostate cancer	Associated with dysregulated miRNA	174
		Colorectal carcinoma	Not associated with any clinical parameters	175
		Ovarian cancer	Required for cell proliferation, migration and invasion	176
DICER1	Upregulation	Smooth muscle tumours	Associated with high-grade disease and tumour progression	166
		Gastric cancer	Correlated with gastric tumour subtype	167
		Serous ovarian carcinoma	Associated with advanced tumour stages	168
		Prostate cancer	Dysregulated miRNA expression; correlated with tumour stage	174,177
		Oral cancer	Required for proliferation	178
		Colorectal cancer	Correlated with tumour stage and associated with poor survival	179–181
		Precursor lesions of lung adenocarcinoma	Associated with histological subtypes and stages	182
		Cutaneous melanoma	Correlated with clinical stage	183

Protein	Dysregulation	Cancer type	Clinical correlation	Refs
	Downregulation	Triple-negative breast cancer	No clinical correlation	165,184
		Bladder cancer	Altered miRNA profile	170,185
		BCC	Not determined	163
		Ovarian cancer	Associated with advanced tumour stage and poor patient survival	14,186, 187
		Endometrial cancer	No association with histological grade detected	171
		Nasopharyngeal carcinoma	Correlated with shorter patient survival	73
		Neuroblastoma	Associated with global downregulation of miRNAs and poor outcome	13
		Breast cancer	Associated with cancer progression and recurrence	172,188
		Gallbladder adenocarcinoma	Correlated with metastasis, invasion and poor prognosis	72
		Non-small cell lung cancer	Low levels of *DICER1* expression correlate with shortened survival	12,169
		Hepatocellular carcinoma	Not associated with clinical characteristics	189
		Chronic lymphocytic leukaemia	Associated with progression and prognosis	190
		Colorectal cancer	Associated with tumour stage and shorter survival	191
PACT	Upregulation	AK, SCC and BCC	Not determined	173
XPO5	Downregulation	Bladder cancer	Associated with altered miRNA profile	170
AGO1	Upregulation	AK, SCC and BCC	Not determined	173
		Serous ovarian carcinoma	Associated with advanced tumour stages	168
AGO2	Upregulation	AK, SCC and BCC	Not determined	173
		Serous ovarian carcinoma	Correlated with advanced tumour stages and associated with shorter survival	168

AGO, Argonaute; AK, actinic keratoses; BCC, basal cell carcinoma; DGCR8, DiGeorge syndrome critical region 8; miRNA, microRNA; PACT, interferon-inducible double-stranded RNA-dependent protein kinase activator A; SCC, squamous cell carcinoma; XPO5, exportin 5.