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Dysregulation of MicroRNA Biogenesis Machinery in Cancer

Akiko Hata[#] and **Risa Kashima**

Cardiovascular Research Institute, University of California, San Francisco, Box 3118, 555 Mission Bay Blvd. South, Smith Cardiovascular Research Building, Room 252T, San Francisco, CA 94143

Abstract

MicroRNAs (miRNAs) are integral to the gene regulatory network. A single miRNA is capable of controlling the expression of hundreds of protein coding genes and modulate a wide spectrum of biological functions, such as proliferation, differentiation, stress responses, DNA repair, cell adhesion, motility, inflammation, cell survival, senescence and apoptosis, all of which are fundamental to tumorigenesis. Overexpression, genetic amplification and gain-of-function mutation of oncogenic miRNAs ("onco-miRs") as well as genetic deletion and loss-of-function mutation of tumor suppressor miRNAs ("suppressor-miRs") are linked to human cancer. In addition to the dysregulation of a specific onco-miR or suppressor-miRs, changes in global miRNA levels resulting from a defective miRNA biogenesis pathway play a role in tumorigenesis. The function of individual onco-miRs and suppressor-miRs and their target genes in cancer has been described in many different articles elsewhere. In this review we primarily focus on the recent development regarding the dysregulation of the miRNA biogenesis pathway and its contribution to cancer.

Keywords

Argonaute; microRNA; pri-miRNA; pre-miRNA; post-translational modifications; Drosha; DGCR8; Dicer; TRBP; stability; processing

Introduction

MicroRNAs (miRNAs) are small noncoding RNAs (ncRNAs) of ~22-nucleotides (nt) which mediate destabilization and/or translational suppression of target mRNAs bearing partially complementary sequences(Kim et al., 2009, Siomi and Siomi, 2010). The biogenesis of miRNAs starts by transcription of the miRNA gene encoded in the genome by RNA polymerase II (Pol II). This process generates long primary (pri-miRNA) transcripts comprising a stem-loop hairpin structure (Kim et al., 2009, Siomi and Siomi, 2010) (Figure 1). Pri-miRNAs undergo stepwise processing. The first processing takes place in the nucleus and involves the RNase III enzyme Drosha and its cofactor DiGeorge syndrome critical region gene 8 (DGCR8), which compose the "Drosha microprocessor" complex. The Drosha microprocessor complex recognizes the base of the stem-loop hairpin structure, cleaves it and releases a ~60–70-nt hairpin-shaped precursor miRNA (pre-miRNA). The pre-miRNA is

[#]Correspondence should be addressed to akiko.hata@ucsf.edu.

then exported to the cytoplasm by exportin 5 (Xpo5) and undergoes the second processing by the RNase III enzyme Dicer and the cofactor transactivation-responsive RNA-binding protein (TRBP, also known as TARBP2), which generates a ~22-nt miRNA duplex(Kim et al., 2009, Siomi and Siomi, 2010) (Figure 1). As exceptions to this general pathway, some miRNAs are generated by Drosha-independent or Dicer-independent mechanisms, including splicing of miRNA-containing introns, which are known as miRtrons (Yang and Lai, 2011). The miRNA duplex is loaded into an Argonaute (Ago) protein, which preferentially ejects one strand ("passenger strand") and retains the mature miRNA ("guide strand") (Meister, 2013). Ago proteins and the GW182/TNRC6 family of proteins form the miRNA-induced silencing complex (miRISC). MiRNA/Ago complexes recognize target mRNAs by paring the 5′ end of the miRNA molecule (nts 2–8), called the "seed" region, with a partially complementary sequence in the 3′-untranslated region (3′-UTR) of target mRNAs. A specific miRNA can regulate hundreds of target mRNAs simultaneously, although the degree of regulation is only 30–50%. During the last decade, numerous regulatory pathways have been shown to modulate the biogenesis (transcription and processing), stability, and silencing activity of miRNAs. Dysregulation of these processes has been implicated in the pathogenesis of human disorders, including cancer. Several review articles summarize the involvement of individual miRNAs in cancer (Hata and Lieberman, 2015, Lin and Gregory, 2015). This review mainly focuses instead on the dysregulation of "global" miRNA levels in the context of cancer as a result of aberrant expression and/or activity of molecules that control miRNA biogenesis.

Global dysregulation of miRNAs and its activity on cancer

The level of expression and activity of different components of the miRNA biogenesis pathway are often found to be dysregulated in cancer. For example, the expression of Drosha and Dicer is either increased or decreased in various types of cancers where it inversely correlates with advanced stages of tumor and poor clinical outcome (Table 1). Defects in components of the miRNA biogenesis pathway produce expression changes in the large number of cellular miRNAs indicated as "altered miRNA profile (AMP)" in Table 1. Instances of global "downregulation" of miRNAs in tumors, especially in poorly differentiated ones, have been reported (Lu et al., 2005). One explanation proposed for this widespread downregulation of miRNAs in cancer cells is that the function of many miRNAs is to define lineage specific properties and, therefore, the low abundance of miRNAs promotes the undifferentiated state of tumor cells and enhances their potential of invasion and metastasis (Lu et al., 2005). In support of a general "tumor suppressor" action of global miRNAs, knockdown or haploinsufficiency of *Dicer1* in mouse has been found to foster tumor formation and progression (Kumar et al., 2007, Kumar et al., 2009, Lambertz et al., 2010). On the other hand, although global "upregulation" of miRNAs is uncommon, elevated levels of the processing enzymes Drosha and Dicer have been found in tumor cells. Below, we discuss some examples of dysregulation of different components of the miRNA biogenesis pathway.

(a) Dysregulation of the Drosha microprocessor

Long pri-miRNAs transcribed by Pol II undergo primary processing in the nucleus by a large complex, the Drosha microprocessor, composed of various cofactors, including DGCR8. The processing of some pri-miRNAs also requires the RNA helicases (p68 and p72) (Figure 1).

Upregulation of DGCR8 has been found in various types of cancer (Table 1). Gene mutation analyses have recently identified frequent heterozygous mutations in the *Drosha* gene in the rare pediatric form kidney cancer of Wilms tumors (Rakheja et al., 2014, Torrezan et al., 2014, Walz et al., 2015, Wegert et al., 2015) (Figure 2, Table 2). More than 60% of all the *Drosha* mutations consist of a single missense change (E to K) at amino acid (aa) 1147, located in the second RNase domain (Rakheja et al., 2014, Torrezan et al., 2014, Walz et al., 2015, Wegert et al., 2015) (Figure 2, Table 2). This E1147K mutation is thought to interfere with metal binding and negatively regulate the processing function of Drosha in a dominant fashion, which is consistent with the global downregulation of miRNAs observed in Wilms tumors harboring mutated *Drosha* (Rakheja et al., 2014, Torrezan et al., 2014, Walz et al., 2015, Wegert et al., 2015). In addition to the E1147K mutation, many other missense, nonsense, and splice-site mutations of *Drosha* have been identified in Wilms tumors (Figure 2, Table 2), but their effect has yet to be assessed.

Somatic and germline mutations of *DGCR8* were also found in Wilms tumors (Torrezan et al., 2014, Walz et al., 2015, Wegert et al., 2015) (Figure 2, Table 2). The E518K missense mutation in the first double strand RNA (dsRNA) binding domain (dsRBDe) of DGCR8 is clearly a “hot-spot” and makes up for more than 70% of the *DGCR8* mutations in Wilms tumors (Torrezan et al., 2014, Walz et al., 2015, Wegert et al., 2015) (Figure 2, Table 2). The E518K mutation causes a reduction of critical miRNAs in tumors (Torrezan et al., 2014, Walz et al., 2015, Wegert et al., 2015), which is consistent with the observation that knockdown of *DGCR8* promotes tumor growth (Kumar et al., 2007).

Besides gene mutations, the expression of alternatively spliced variants of Drosha has been reported in melanoma and teratocarcinoma cells (Grund et al., 2012). The splice variants encode a Drosha protein with a truncated carboxyl (C)-terminal RNase domain (see Figure 2). Its dsRBD fails to interact with DGCR8, and therefore is functionally compromised and potentially acts as dominant negative (Grund et al., 2012). Conversely, an increased copy number of the *Drosha* gene or overexpression of Drosha protein, which in turn lead to a global change in miRNA levels, have been found in more than half of the advanced cervical squamous cell carcinomas (Muralidhar et al., 2011). Further study is required to uncover why *Drosha* mutations are frequently found in Wilms tumors, and why Drosha levels are regulated in opposite directions depending on the tumor type.

Various post-translational modifications (PTMs) of Drosha and DGCR8 that are able to affect miRNA processing have been identified. For example, phosphorylation of serine residues by glycogen synthase kinase 3β (GSK3β) is required for nuclear localization of Drosha (Tang et al., 2011, Tang et al., 2010); acetylation of Drosha by p300, CBP and GCN5 prevents ubiquitin-mediated degradation and stabilizes Drosha (Tang et al., 2013); deacetylation of DGCR8 by histone deacetylase 1 (HDAC1) increases the affinity of

DGCR8 for pri-miRNAs (Wada et al., 2012), while phosphorylation of DGCR8 by Erk stabilizes DGCR8 protein and promotes miRNA production (Herbert et al., 2013). It is not clear whether changes in miRNA production that result from these Drosha PTMs play an important role in cancer. However, GSK3β and HDAC1 are often dysregulated in cancer, therefore it is possible that different Drosha PTMs may be found in tumor cells versus non-tumor cells.

Drosha activity is regulated by different nuclear proteins, in a manner that generally affects the biogenesis of only a small subset of miRNAs. One such nuclear protein is adenosine deaminase acting on RNA (ADAR). ADAR is a RNA editing enzyme that converts adenosine (A) to inosine (I) in double-stranded RNAs. ADAR1 forms a complex with DGCR8 and inhibits Drosha activity (Nemlich et al., 2013). In metastatic melanoma in which ADAR1 level is reduced, two miRNAs (miR-17 and miR-432) are overproduced and promote tumor growth (Nemlich et al., 2013). Smad proteins, the signal transducers of the transforming growth factor-β (TGF-β)–superfamily of growth factors, also modify Drosha activity in the nucleus. Although primarily cytoplasmic at steady state, Smad proteins translocate to the nucleus upon ligand activation. Smads are bona fide transcription factors with DNA binding and transcription activating domains (Massague, 2012). However, they are also recruited to the Drosha microprocessor complex through physical interaction with the RNA helicase p68 (also known as DDX5), where they promote pri- to pre-miRNA processing of about 20 miRNAs (Davis et al., 2008), including miR-21 and miR-199. miR-21 is one of the most commonly upregulated onco-miRs in nearly all tumor samples and is known to inhibit a large group of tumor suppressor genes, including *PTEN*, *PDCD4*, *TPM1*, *SPRY1/2* and *TP53BP2*(Di Leva et al., 2014). In addition, miR-21 can drive tumorigenesis by inhibiting negative regulators of the Ras/MEK/Erk pathway(Hatley et al., 2010). The specificity of R-Smad-mediated regulation of pri-miRNA processing is achieved by direct association of R-Smads with a sequence element in the stem region of pri-miR-21 (Davis et al., 2010). Like Smads, p53 induces the expression of a group of suppressor-miRs (miR-15/16, miR-143, miR-145 and miR-203) by associating with the Drosha microprocessor complex via p68 and facilitating pri-miRNA processing (Suzuki et al., 2009). Genotoxic stimuli, which induce acetylation of K120 in the DNA binding domain of p53, do not affect the transcription activity of p53, but prompt the association of p53 with the Drosha microprocessor complex and elevate miR-203 levels to promote apoptosis instead of cell cycle arrest (Chang et al., 2013). It is yet unclear how regulation of processing of a specific subset of miRNA is accomplished by p53, and how TGF-β-mediated activation of Smads can affect p53-dependent regulation of miRNA processing.

A recent study also identified Yes-associated protein (YAP), a signal transducer of the Hippo pathway that controls organ size by sensing cell density, as a regulator of Drosha microprocessor activity (Mori et al., 2014). When Hippo signaling is inactive, such as in cancer cells or in normal cells at low cell density, YAP accumulates in the nucleus and binds the RNA helicase p72 (also known as DDX17), sequestering it from the Drosha microprocessor and thus inhibiting processing of a subset of miRNAs that targets *Myc* (Mori et al., 2014). It is plausible that this YAP/p72-dependent inhibition of Drosha may play a significant role in the widespread downregulation of a large number of miRNAs in cancer.

The single strand RNA binding factor KH-type splicing regulatory protein (KSRP, also known as FUBP2)(Trabucchi et al., 2009) binds the terminal loop of a small subset of pri-miRNAs, including onco-miRs in the let-7 family, miR-21 and miR-125, and promotes processing by Drosha through an unknown mechanism (Trabucchi et al., 2009, Briata et al., 2012). When the PI3K/Akt signaling pathway is activated, KSRP is phosphorylated at S274 and S670, which triggers enhanced binding to pri-miRNAs and stimulates their Drosha-dependent processing (Briata et al., 2012). KSRP-enhanced miRNA biogenesis is also activated by DNA damage (Zhang et al., 2011). KSRP is highly expressed in chronic myeloid leukemia (CML) acute phase/blast crisis, compared with the chronic phase disease. It remains unclear, however, whether altered expression or function of KSRP contributes to leukemogenesis as a result of dysregulation of miRNA processing (Radich et al., 2006). A few other RNA binding proteins, such as hnRNP A1, serine/arginine-rich splicing factor 1 (SRSF1) and FUS (also known as TLS), have been found to interact with pri-miRNAs and facilitate Drosha processing, but there is yet no evidence of a direct link to tumorigenesis.

(b) Alteration of pre-miRNA export

Following Drosha processing and synthesis in the nucleus, pre-miRNAs are exported to the cytoplasm where they undergo secondary processing by Dicer (Figure 1). Xpo5 and Ran-GTP form a nuclear complex that transports and releases pre-miRNAs into the cytoplasm. Xpo5 protein is rapidly induced during cell cycle entry by a PI3K-dependent post-transcriptional mechanism, leading to an overall increase in mature miRNAs in proliferating cells (Iwasaki et al., 2013). Nuclear export of pre-miRNAs is also induced upon DNA damage. Ataxia telangiectasia mutated (ATM) activates Akt kinase to phosphorylate Nup153, a key component of the nucleopore, leading to enhanced interaction between Nup153 and Xpo5 and more efficient nuclear export of pre-miRNAs (Wan et al., 2013). The PI3K-dependent mechanism underlying increased Xpo5 protein and pre-miRNA nuclear export has not been defined, but could potentially also be mediated by Akt, which is activated by PI3K.

Somatic and germline heterozygous mutations in *Xpo5* occur in gastric, endometrial, and colon tumors with microsatellite instability (Melo et al., 2010) (Figure 2, Table 2). *Xpo5* mutations impair the export of pre-miRNAs, causing their accumulation in the nucleus, consequent reduction of mature miRNAs, and possibly tumorigenesis (Melo et al., 2010). Genetic and epigenetic association studies reveal a link between variations in *Xpo5* and the risk of developing breast cancer (Leaderer et al., 2011), adding support to a role for dysregulation of miRNA biogenesis and transport in cancer. Upregulation of Xpo5 and dysregulation of miRNA expression profile have also been reported in bladder cancer (Table 1).

(c) Dysregulation of the Dicer/TRBP processing complex

Pre-miRNAs exported from the nucleus via Xpo5 undergo secondary processing by Dicer and its cofactor TRBP in the cytoplasm to finally give rise to ~22-nt mature miRNAs (Figure 1). Unlike Drosha processing, which is not required for the processing of a subset of miRNAs known as “miRtrons” that are encoded in the intronic region of other genes and processed by the splicing machinery, Dicer processing is essential for the biogenesis of all

miRNAs, with the sole exception miR-451, whose pre-miRNA is processed by Ago2 (Yang and Lai, 2010, Yang et al., 2010). Germline mutations in the *Dicer1* gene have been identified in a broader spectrum of inherited tumors compared to *Drosha* mutations (Figure 2, Table 2). Heterozygous germline *Dicer1* mutations were first reported in a rare pediatric lung cancer, pleuropulmonary blastoma (PPB)(Hill et al., 2009). Currently, both germline and somatic *Dicer1* mutations have been associated not only with PPB (de Kock et al., 2013, Hill et al., 2009, Pugh et al., 2014, Schultz et al., 2011, Seki et al., 2014, Wagh et al., 2014) but also with other tumors, including Wilms tumor (Foulkes et al., 2014, Torrezan et al., 2014, Wu et al., 2013, Heravi-Moussavi et al., 2012, Schultz et al., 2011, Witkowski et al., 2013), nonepithelial ovarian cancer (Heravi-Moussavi et al., 2012, Schultz et al., 2011, Witkowski et al., 2013), pituitary blastoma (de Kock et al., 2013), cystic nephroma (Doros et al., 2014), and embryonal rhabdomyosarcoma (ERMS) (Doros et al., 2012). As the carriers of *Dicer1* mutations exhibit a predisposition to distinctive inheritable tumors, this condition has been defined “the *Dicer1* syndrome” (OMIM #601200)(de Kock et al., 2015). For many of these mutations, the predicted effect is a reduction of Dicer protein level and/or processing activity and a resulting global reduction of miRNA levels and their tumor suppressor activities (Foulkes et al., 2014). Loss of heterozygosity (LOH) of *Dicer* has not been found in human cancer.

Beside gene mutations, both increase and decrease of Dicer expression have been reported in different types of tumors (Table 1). For example, Dicer expression is positively regulated transcriptionally by the p53 homolog TAp63 (Su et al., 2010). The induction of Dicer is critical for the ability of TAp63 to suppress tumor formation and inhibit metastasis (Su et al., 2010). Also, tumor hypoxia causes reduction of Dicer. This occurs through an epigenetic mechanism that involves inhibition of oxygen-dependent Histone H3K27me3 KDM6A/B and silencing of the *Dicer* promoter (van den Beucken et al., 2014).

Expression of an alternative splice variant of *Dicer1* which lacks the dsRBD is detected in neuroblastoma cells but not in normal tissues(Potenza et al., 2010). It is intriguing to speculate that this splice variant might act as a dominant negative mutant that promotes tumorigenesis by downregulating global miRNA levels.

Several proteins regulate the activity of Dicer. For example, association of KSRP to pre-miRNA promotes Xpo5-mediated export of the pre-miRNA, while its binding to Dicer facilitates pre-miRNA processing when the PI3K/Akt signaling pathway is activated (Briata et al., 2012). Also, the Dicer cofactor TRBP stabilizes Dicer and enhances pre-miRNA processing. Mutations in the TARBP2 gene encoding TRBP are found in sporadic and hereditary carcinoma with microsatellite instability (Figure 2, Table 2). The TRBP expression is reduced in the cancer stem cell (CSC) population of Ewing sarcoma family tumor (ESFT)(De Vito et al., 2012). Restoration of TARBP2 activity inhibits ESFT CSC clonogenicity and tumor growth, suggesting that Dicer/TRBP-mediated miRNA processing plays a tumor suppressor function(De Vito et al., 2012). The TRBP activity is post-translationally regulated. The MAPK/Erk pathway mediates TRBP phosphorylation, which enhances global miRNA biogenesis by increasing Dicer levels and facilitating Dicer/TRBP miRNA processing (Paroo et al., 2009). Expression of phosphomimetic TRBP enhances growth-promoting miRNAs and increases cell proliferation (Paroo et al., 2009).

(d) Post-translational modifications of Ago proteins

Ago proteins are the critical downstream effectors of miRNA-mediated gene silencing (Figure 1). There are four Ago proteins (Ago1–4) in humans. They associate with cofactors of the GW182/TNRC6 family in the RISC to guide miRNAs to target specific transcripts and mediate gene silencing (Meister, 2013). Perfectly paired siRNA or miRNA duplexes are cleaved by catalytically active Ago2, while Ago1, Ago3, and Ago4 are catalytically inactive and unable to cleave RNAs (Meister, 2013). Binding to Ago proteins stabilizes mature miRNAs, possibly for weeks to months under basal conditions. In the absence of Ago2 in the cell, miRNAs are unstable, with half-lives of approximately 8 hours, and global miRNA levels are markedly reduced (Winter and Diederichs, 2011). Thus, a pathological change in the level of Ago proteins and/or their activity could result in major impairments in global miRNA stability and/or silencing. Indeed, upregulation of Ago1 and Ago2 has been reported in various types of cancer (Table 1). Currently, there is no report of mutations in the genes encoding Ago1–4. However, different PTMs found in Ago proteins can affect their stability and/or activity and critically modulate global miRNA levels (Figure 1). Prolyl-hydroxylation of Ago proteins by type I collagen prolyl-4-hydroxylase I [C-P4H(I)] was the first of these critical PTMs to be reported (Qi et al., 2008). C-P4H(I) hydroxylates multiple human Ago proteins, and especially Ago2 at P700, which stabilizes the protein and augments siRNA-mediated silencing. Hypoxia potently induces C-P4H(I) expression and promotes Ago2 hydroxylation, resulting in a rapid rise in the silencing activity of miRNAs (Wu et al., 2011a). In response to mitogens and growth factor signaling, Ago1 and Ago2 are also phosphorylated. Phosphorylation of the serine residue S387 of Ago2, mediated by the p38 mitogen-activated protein kinase (MAPK) pathway and Akt3, promotes its association with the TNRC6 cofactor and localization to the processing bodies (P-bodies)(Zeng et al., 2008), where translational repression by miRNAs is enhanced (Horman et al., 2013). It is currently unclear whether phosphorylation of S387 in Ago2 is augmented in human tumors. Another phosphorylation event, at the Ago2 tyrosine Y393, is induced by activation of epidermal growth factor receptor (EGFR) signaling and causes a reduction of the Ago2 association with Dicer and its cofactor TRBP (Shen et al., 2013). Hypoxia, which upregulates EGFR and increases its activity, consequently also enhances P-Y393 Ago2. The P-Y393 Ago2 modification suppresses the maturation of a subset of growth-repressing pre-miRNAs with longer terminal loops and leads to increased survival in response to hypoxia. P-Y393 Ago2 is elevated in hypoxic areas of human breast tumors. Moreover, higher levels of P-Y393 Ago2 correlate with poor overall survival in breast cancer patients (Shen et al., 2013). The effect of the P-Y393 Ago2 modification on the RISC activity, miRNA stability or subcellular localization of miRNAs remains to be investigated in the future. The RISC activity of Ago proteins can also be modulated by ubiquitination and poly(ADP-ribosyl)ation (PARylation). Ubiquitination of Ago leads to its proteosomal degradation and to global downregulation of miRNAs. This occurs rapidly during T cell activation when the miRNA repertoire is suddenly altered to allow T cell clonal expansion and activated T cell effector functions (Bronevetsky et al., 2013). PARylation of Ago2 complexes occurs rapidly during infection with viruses, such as the Herpes simplex viruses, that trigger innate immune alarms and interferons via an unknown mechanism that involves PARP13 (Seo et al., 2013). PARylation inhibits RISC activity and the silencing activity of miRNAs. This has the net effect of derepressing antiviral interferon-stimulated genes, which would be otherwise inhibited by

cellular miRNAs, and enhancing the cell's ability to withstand viral infection (Seo et al., 2013). Whether the Ago2 amino acid residues targeted by PTMs are mutated in human tumors remains the subject of future investigations.

Conclusions

Considerable evidence points to a significant role of miRNAs in human disease. The evolutionary conservation of miRNAs and their biogenesis pathway, the highly tuned regulation of their tissue- and developmental stage-specific expression, and the reproducible detection of their biological activity in vitro altogether predict a key biological function. Yet, the functional significance of miRNAs, especially in the context of human development and physiology, has been questioned because the knockdown of a single miRNA, unlike a protein-coding gene, rarely gives rise to a phenotype in an organism. However, the recent discovery of germline and somatic mutations in the genes encoding core components of the miRNA biogenesis pathway in human cancer illuminate the significance of the proper production and action of miRNAs in order to maintain homeostasis. During the last decade of miRNA research, the focus of the investigators in the cancer field has yielded the identification of onco-miRs and suppressor-miRs, many of which are expressed strictly in tumor type-specific and stage-specific manner. Some of these studies led to the identification of a biomarker that can properly diagnose a specific type or stage of cancer. However, modulation by antisense oligonucleotides or miRNA mimicry of a single onco-miR or suppressor-miR, or even a few of them, has not been successful in treating cancer in humans. The recent work that we have summarized in this review raises a new hope. Future research may uncover novel gene mutations and PTMs of the components of the miRNA biogenesis pathway in cancer and help us understand the activities they disrupt, which might lead to the discovery of regulatory factors amenable to pharmacological intervention. These tools will enable us to modulate globally the expression and activity of miRNAs in tumor cells or in the tumor environment, and could be applied to cancer prevention and treatment.

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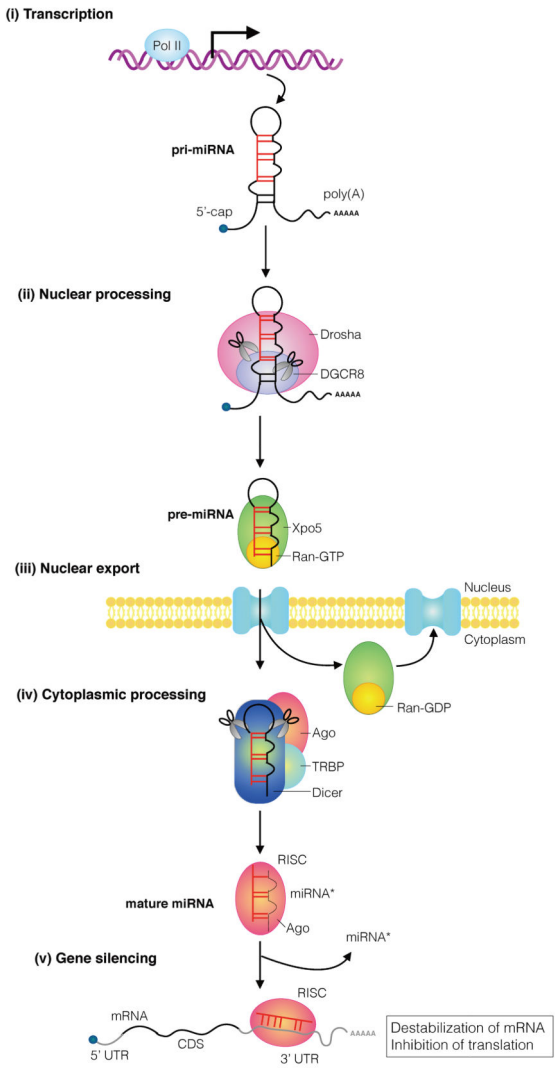

Figure 1. Overview of miRNA biogenesis pathway

The biogenesis of an miRNA is a stepwise process that includes (i) transcription of a primary transcript (pri-miRNA), (ii) nuclear cropping to produce the pre-miRNA, (iii) export to the cytoplasm, and (iv) cytoplasmic cropping to a ds miRNA precursor. miRNA genes are generally transcribed into long, 5′-capped, and 3′-polyadenylated transcripts (pri-miRNAs) by RNA polymerase II (Pol II) and subjected to a primary processing step by a nuclear enzyme of the RNase III family, Drosha in the microprocessor complex. The primary processing products, hairpin-loop RNAs known as precursor-miRNAs (pre-miRNAs) are recognized by the Exportin-5 (Xpo5)/Ran-GTP transporter and exported to the cytoplasm, where an another enzyme of the RNase III family, Dicer, catalyzes the secondary processing to produce miRNA/miRNA* duplexes. Dicer, TRBP, and Argonaute (Ago) proteins mediate the processing of pre-miRNAs and the assembly of the RNA-induced silencing complex (RISC) in humans. In most cases, one strand of each duplex remains on the Ago protein as the mature miRNA; the other strand (miRNA*) is degraded. Ago associates with Dicer in both the cropping and RISC assembly steps. Every molecule that plays a role in miRNA

biogenesis and RISC assembly can be regulated in response to environmental changes or physiological stimuli. Mutations and dysregulation of the expression of miRNA pathway molecules can contribute to human cancer. 5′ cap: 5′-7-Methylguanosine cap; poly(A): polyadenylation; TRBP: transactivation-responsive RNA-binding protein; CDS: coding sequence, UTR: untranslated region;

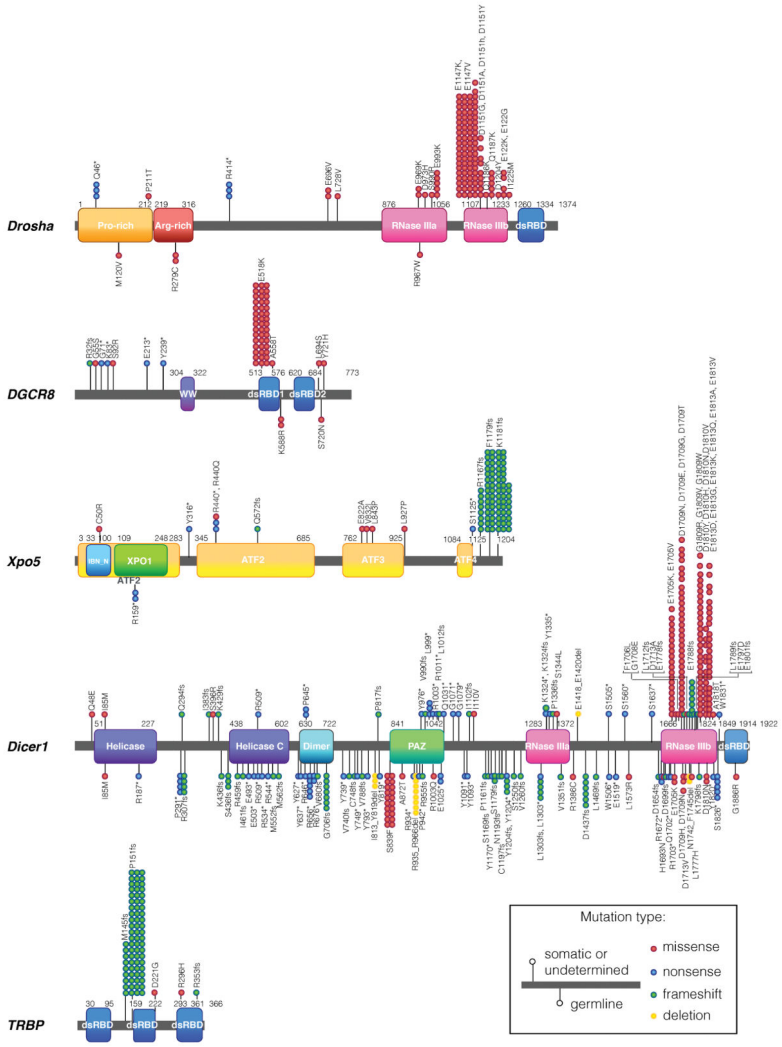

Figure 2. The miRNA biogenesis pathway genes mutated in cancer

Schematic representation of somatic and germline mutations of the miRNA biogenesis pathway genes (*Drosha, DGCR8, Xpo5, Dicer1,* and *TRBP*) identified in cancer. The location and the types of mutation are indicated. Somatic and germline mutations are plotted above and below the protein structure, respectively. Undermined mutations are plotted together with somatic mutations. ATF: armadillo-type fold; 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/importinβ-like domain; Xpo5: exportin 5.

Table 1

Dysregulation of proteins in the miRNA biogenesis pathway in various cancers.

Dysregulated Protein	Role in tumorigenesis	Type of Cancer	Clinical Relevance	References
Drosha	Oncogene	BCC	NO	(Sand et al., 2010)
		Cervical SCC	AMP; AAS	(Muralidhar et al., 2007, Muralidhar et al., 2011)
		Esophageal cancer	APP	(Sugito et al., 2006)
		Gastric cancer	APP	(Tchernitsa et al., 2010)
		Non-small cell lung cancer	APP	(Diaz-Garcia et al., 2013)
		SCC	NO	(Sand et al., 2010)
		Serous ovarian carcinoma	AAS	(Vaksman et al., 2012)
		Smooth muscle tumours	AAS	(Papachristou et al., 2012)
		Triple-negative breast cancer	NO	(Passon et al., 2012, Avery-Kiejda et al., 2014)
	Tumor suppressor	Bladder cancer	AMP	(Catto et al., 2009)
		Breast cancer	NO	(Yan et al., 2012)
		Cutaneous melanoma	APP	(Jafarnejad et al., 2013)
		Endometrial cancer	AAS	(Torres et al., 2011)
		Gallbladder adenocarcinoma	APP	(Shu et al., 2012)
		Nasopharyngeal carcinoma	APP	(Guo et al., 2012)
		Neuroblastoma	AMP;APP	(Lin et al., 2010)
		Ovarian cancer	APP	(Merritt et al., 2008)
DGCR8	Oncogene	Bladder cancer	AMP	(Catto et al., 2009)
		Colorectal carcinoma	NO	(Kim et al., 2014)
		Esophageal cancer	APP	(Sugito et al., 2006)
		Ovarian cancer	APP	(Guo et al., 2015)
		Prostate cancer	AMP	(Ambs et al., 2008)
		SCC and BCC	NO	(Sand et al., 2012)
Dicer1	Oncogene	Colorectal cancer	ASS; APP	(Faber et al., 2011, Stratmann et al., 2011, Papachristou et al., 2011)
		Cutaneous melanoma	AAS	(Ma et al., 2011)
		Gastric cancer	Correlated with tumor subtype	(Tchernitsa et al., 2010)
		Oral cancer	NO	(Jakymiw et al., 2010)
		Precursor lesions of lung adenocarcinoma	AAS	(Chiosea et al., 2007)
		Prostate cancer	AMP; AAS	(Ambs et al., 2008, Chiosea et al., 2006, Vaksman et al., 2012)
		Serous ovarian carcinoma	AAS	(Vaksman et al., 2012)
		Smooth muscle tumours	AAS	(Papachristou et al., 2012)
	Tumor suppressor	BCC	NO	(Sand et al., 2010)

Dysregulated Protein	Role in tumorigenesis	Type of Cancer	Clinical Relevance	References
		Bladder cancer	AMP	(Catto et al., 2009, Wu et al., 2012)
		Breast cancer	APP	(Yan et al., 2012, Khoshnaw et al., 2012)
		Chronic lymphocytic leukemia	APP	(Zhu et al., 2012)
		Colorectal cancer	APP	(Faggad et al., 2012)
		Endometrial cancer	NO	(Torres et al., 2011)
		Gallbladder adenocarcinoma	APP	(Shu et al., 2012)
		Hepatocellular carcinoma	NO	(Wu et al., 2011b)
		Nasopharyngeal carcinoma	APP	(Guo et al., 2012)
		Neuroblastoma	AMP;APP	(Lin et al., 2010)
		Non-small cell lung cancer	APP	(Diaz-Garcia et al., 2013, Karube et al., 2005)
		Ovarian cancer	AAS; APP	(Merritt et al., 2008, Pampalakis et al., 2010, Faggad et al., 2010)
		Triple-negative breast cancer	NO	(Avery-Kiejda et al., 2014, Dedes et al., 2011)
Xpo5	Oncogene	AK, SCC and BCC	NO	(Sand et al., 2012)
Ago1	Oncogene	Bladder cancer	AMP	(Catto et al., 2009)
		Serous ovarian carcinoma	AAS	(Vaksman et al., 2012)
Ago2	Oncogene	AK, SCC and BCC	NO	(Sand et al., 2012)
		Serous ovarian carcinoma	AAS; APP	(Vaksman et al., 2012)

AAS, associated with advanced stages; Ago, Argonaute; AK, actinic keratoses; AMP, altered miRNA profile; APP, associated with poor prognosis; BCC, basal cell carcinoma; DGCR8, DiGeorge syndrome critical region 8; miRNA, microRNA; NO, not determined or no clinical correlation; SCC, squamous cell carcinoma; Xpo5, exportin 5.

Table 2

Somatic and germline mutations associated with molecules in the miRNA biogenesis pathway in various cancers.

Gene	Location	Mutation	Type of mutation	Type of cancer	References
Drosha	Q46*	Nonsense	Somatic	Wilms tumors	(Torrezan et al., 2014, Walz et al., 2015)
	M120V	Missense	Gemline	Wilms tumors	(Rakheja et al., 2014)
	P211T	Missense	Somatic	Wilms tumors	(Torrezan et al., 2014)
	R279C	Missense	Gemline	Wilms tumors	(Wegert et al., 2015)
	R414*	Nonsense	Somatic	Wilms tumors	(Torrezan et al., 2014, Walz et al., 2015)
	E696V	Missense	Somatic	Wilms tumors	(Walz et al., 2015)
	L728V	Missense	Somatic	Wilms tumors	(Rakheja et al., 2014)
	R967W	Missense	Gemline	Wilms tumors	(Rakheja et al., 2014)
	E969K	Missense	Somatic	Wilms tumors	(Walz et al., 2015)
	D973H	Missense	Somatic	Wilms tumors	(Wegert et al., 2015)
	S990R	Missense	Somatic	Wilms tumors	(Wegert et al., 2015)
	E993K	Missense	Somatic	Wilms tumors	(Walz et al., 2015, Wegert et al., 2015)
	E1147K	Missense	Somatic	Wilms tumors	(Torrezan et al., 2014, Rakheja et al., 2014, Walz et al., 2015, Wegert et al., 2015)
	E1147V	Missense	Somatic	Wilms tumors	(Walz et al., 2015)
	D1151Y	Missense	Somatic	Wilms tumors	(Rakheja et al., 2014)
	D1151A	Missense	Somatic	Wilms tumors	(Walz et al., 2015)
	D1151H	Missense	Somatic	Wilms tumors	(Walz et al., 2015)
	D1151G	Missense	Somatic	Wilms tumors	(Torrezan et al., 2014, Walz et al., 2015)
	Q1186K	Missense	Somatic	Wilms tumors	(Wegert et al., 2015)
	Q1187K	Missense	Somatic	Wilms tumors	(Walz et al., 2015)
	D1204Y	Missense	Somatic	Wilms tumors	(Wegert et al., 2015)
	E1222K	Missense	Somatic	Wilms tumors	(Walz et al., 2015, Wegert et al., 2015)
	E1222G	Missense	Somatic	Wilms tumors	(Walz et al., 2015)
	I1225M	Missense	Somatic	Wilms tumors	(Wegert et al., 2015)
DGCR8	R32fs	Frameshift	Somatic	Wilms tumors	(Torrezan et al., 2014)
	G55S	Missense	Somatic	Wilms tumors	(Torrezan et al., 2014)
	G71*	Nonsense	Somatic	Wilms tumors	(Walz et al., 2015)

Gene	Location	Mutation	Type of mutation	Type of cancer	References
	K82*	Nonsense	Somatic	Wilms tumors	(Wegert et al., 2015)
	S92R	Missense	Somatic	Wilms tumors	(Torrezan et al., 2014)
	E213*	Nonsense	Somatic	Wilms tumors	(Wegert et al., 2015)
	Y239*	Nonsense	Somatic	Wilms tumors	(Walz et al., 2015)
	E518K	Missense	Somatic	Wilms tumors	(Torrezan et al., 2014, Wegert et al., 2015, Walz et al., 2015)
	A558T	Missense	Somatic	Wilms tumors	(Torrezan et al., 2014)
	K588R	Missense	Gemline	Wilms tumors	(Wegert et al., 2015)
	L694S	Missense	Somatic	Wilms tumors	(Walz et al., 2015)
	S720N	Missense	Gemline	Wilms tumors	(Rakheja et al., 2014)
	Y721H	Missense	Somatic	Wilms tumors	(Torrezan et al., 2014)
Xpo5	C50R	Missense	Somatic	Wilms tumors	(Walz et al., 2015)
	R159*	Nonsense	Germline	Wilms tumors	(Walz et al., 2015)
	Y316*	Nonsense	Somatic	Wilms tumors	(Walz et al., 2015)
	R440*	Nonsense	Somatic	Wilms tumors	(Walz et al., 2015)
	R440Q	Missense	Somatic	Wilms tumors	(Walz et al., 2015)
	Q572fs	Frameshift	Somatic	Wilms tumors	(Walz et al., 2015)
	E822A	Missense	Somatic	Wilms tumors	(Walz et al., 2015)
	V832I	Missense	Somatic	Wilms tumors	(Torrezan et al., 2014)
	L843P	Missense	Somatic	Wilms tumors	(Walz et al., 2015)
	L927P	Missense	Somatic	Wilms tumors	(Walz et al., 2015)
	S1125*	Nonsense	Somatic	Wilms tumors	(Walz et al., 2015)
	R1167fs	Frameshift	ND	Mix tumors	(Melo et al., 2010)
	F1179fs	Frameshift	ND	Mix tumors	(Melo et al., 2010)
	K1181fs	Frameshift	ND	Mix tumors	(Melo et al., 2010)
TRBP	M145fs	Frameshift	ND	Mix tumors	(Melo et al., 2009)
	P151fs	Frameshift	ND	Mix tumors	(Melo et al., 2009)
	D221G	Missense	Somatic	Wilms tumors	(Rakheja et al., 2014)
	R296H	Missense	Somatic	Wilms tumors	(Torrezan et al., 2014)
	R353fs	Frameshift	Somatic	Wilms tumors	(Torrezan et al., 2014)

Gene	Location	Mutation	Type of mutation	Type of cancer	References
Dicer1	R187*	Nonsense	Germline	Pleuropulmonary blastoma	(Pugh et al., 2014)
	I383fs	Frameshift	ND	Pleuropulmonary blastoma	(Seki et al., 2014)
	S436fs	Frameshift	Gemline	Pleuropulmonary blastoma	(Foulkes et al., 2011)
	R459fs	Frameshift	Germline	Pleuropulmonary blastoma	(Pugh et al., 2014)
	I461fs	Frameshift	Germline	Pleuropulmonary blastoma	(Seki et al., 2014)
	E493*	Nonsense	Gemline	Pleuropulmonary blastoma	(Hill et al., 2009)
	R534*	Nonsense	Gemline	Pleuropulmonary blastoma	(Hill et al., 2009)
	R544*	Nonsense	Germline	Pleuropulmonary blastoma	(Pugh et al., 2014)
	M552fs	Frameshift	Gemline	Pleuropulmonary blastoma	(Hill et al., 2009)
	M562fs	Frameshift	Germline	Pleuropulmonary blastoma	(Pugh et al., 2014)
	Y627*	Nonsense	Gemline	Pleuropulmonary blastoma	(Hill et al., 2009)
	R646*	Nonsense	Gemline	Pleuropulmonary blastoma	(Hill et al., 2009)
	R656*	Nonsense	Germline	Pleuropulmonary blastoma	(Pugh et al., 2014)
	V680fs	Frameshift	Germline	Pleuropulmonary blastoma	(Pugh et al., 2014)
	Y739*	Nonsense	Gemline	Pleuropulmonary blastoma	(Hill et al., 2009)
	P740fs	Frameshift	Gemline	Pleuropulmonary blastoma	(Hill et al., 2009)
	T788fs	Frameshift	Gemline	Pleuropulmonary blastoma	(Hill et al., 2009)
	R934*	Nonsense	Gemline	Pleuropulmonary blastoma	(Hill et al., 2009)
	T955fs	Frameshift	Germline	Pleuropulmonary blastoma	(Seki et al., 2014)
	R1003*	Nonsense	ND	Pleuropulmonary blastoma	(Seki et al., 2014)
	Y1091*	Nonsense	Germline	Pleuropulmonary blastoma	(Pugh et al., 2014)
	P1161fs	Frameshift	Germline	Pleuropulmonary blastoma	(Seki et al., 2014)
	Y1170*	Nonsense	Gemline	Pleuropulmonary blastoma	(Hill et al., 2009)
	C1197fs	Frameshift	Germline	Pleuropulmonary blastoma	(Pugh et al., 2014)
	S1250fs	Frameshift	Germline	Pleuropulmonary blastoma	(Seki et al., 2014)
	L1469fs	Frameshift	Germline	Pleuropulmonary blastoma	(Pugh et al., 2014)
	W1506*	Nonsense	Germline	Pleuropulmonary blastoma	(Pugh et al., 2014)
	E1519*	Nonsense	Germline	Pleuropulmonary blastoma	(de Kock et al., 2013)
	L1573R	Missense	Gemline	Pleuropulmonary blastoma	(Hill et al., 2009)

Gene	Location	Mutation	Type of mutation	Type of cancer	References
	S1637*	Nonsense	ND	Pleuropulmonary blastoma	(Seki et al., 2014)
	D1654fs	Frameshift	Germline	Pleuropulmonary blastoma	(Pugh et al., 2014)
	E1705K	Missense	Somatic	Pleuropulmonary blastoma	(Pugh et al., 2014)
	E1705V	Missense	ND	Pleuropulmonary blastoma	(Seki et al., 2014)
	G1708E	Missense	Somatic	Pleuropulmonary blastoma	(Pugh et al., 2014)
	D1709N	Missense	Somatic	Pleuropulmonary blastoma	(Pugh et al., 2014, Seki et al., 2014)
	K1798fs	Frameshift	Germline	Pleuropulmonary blastoma	(Pugh et al., 2014)
	G1809R	Missense	Somatic	Pleuropulmonary blastoma	(Pugh et al., 2014, Seki et al., 2014)
	D1810Y	Missense	Somatic	Pleuropulmonary blastoma	(Pugh et al., 2014, Seki et al., 2014)
	E1813D	Missense	Somatic	Pleuropulmonary blastoma	(Pugh et al., 2014)
	E1813G	Missense	Somatic	Pleuropulmonary blastoma	(Pugh et al., 2014, Seki et al., 2014, de Kock et al., 2013)
	E1813K	Missense	Somatic	Pleuropulmonary blastoma	(Pugh et al., 2014)
	E1813Q	Missense	Somatic	Pleuropulmonary blastoma	(Pugh et al., 2014)
	Y1820*	Nonsense	Germline	Pleuropulmonary blastoma	(Seki et al., 2014)
	E503*	Nonsense	Germline	Nonepithelial ovarian cancers	(Schultz et al., 2011)
	R459fs	Frameshift	Germline	Nonepithelial ovarian cancers	(Schultz et al., 2011)
	C748fs	Frameshift	Germline	Nonepithelial ovarian cancers	(Schultz et al., 2011)
	Y819*	Nonsense	Gemline	Nonepithelial ovarian cancers	(Heravi-Moussavi et al., 2012)
	P942*	Nonsense	Gemline	Nonepithelial ovarian cancers	(Heravi-Moussavi et al., 2012)
	E1025*	Nonsense	Germline	Nonepithelial ovarian cancers	(Schultz et al., 2011)
	G1079*	Nonsense	Somatic	Nonepithelial ovarian cancers	(Heravi-Moussavi et al., 2012)
	Y1204*	Nonsense	Gemline	Nonepithelial ovarian cancers	(Heravi-Moussavi et al., 2012)
	V1260fs	Frameshift	Germline	Nonepithelial ovarian cancers	(Schultz et al., 2011)
	D1699fs	Frameshift	Germline	Nonepithelial ovarian cancers	(Schultz et al., 2011)
	R1703*	Nonsense	Gemline	Nonepithelial ovarian cancers	(Heravi-Moussavi et al., 2012)
	E1705K	Missense	Somatic	Nonepithelial ovarian cancers	(Heravi-Moussavi et al., 2012, Witkowski et al., 2013)
	D1709N	Missense	Somatic	Nonepithelial ovarian cancers	(Heravi-Moussavi et al., 2012, Witkowski et al., 2013)
	D1709N	Missense	ND	Nonepithelial ovarian cancers	(Schultz et al., 2011)
	D1709E	Missense	Somatic	Nonepithelial ovarian cancers	(Heravi-Moussavi et al., 2012)

Gene	Location	Mutation	Type of mutation	Type of cancer	References
	D1709G	Missense	Somatic	Nonepithelial ovarian cancers	(Heravi-Moussavi et al., 2012)
	E1788fs	Frameshift	Somatic	Nonepithelial ovarian cancers	(Witkowski et al., 2013)
	D1810H	Missense	Somatic	Nonepithelial ovarian cancers	(Heravi-Moussavi et al., 2012)
	D1810N	Missense	Somatic	Nonepithelial ovarian cancers	(Heravi-Moussavi et al., 2012)
	D1810Y	Missense	Somatic	Nonepithelial ovarian cancers	(Heravi-Moussavi et al., 2012, Witkowski et al., 2013)
	D1810V	Missense	Somatic	Nonepithelial ovarian cancers	(Witkowski et al., 2013)
	E1813A	Missense	ND	Nonepithelial ovarian cancers	(Schultz et al., 2011)
	E1813D	Missense	Somatic	Nonepithelial ovarian cancers	(Witkowski et al., 2013)
	E1813K	Missense	Somatic	Nonepithelial ovarian cancers	(Heravi-Moussavi et al., 2012, Witkowski et al., 2013)
	E1813G	Missense	Somatic	Nonepithelial ovarian cancers	(Heravi-Moussavi et al., 2012)
	E1813Q	Missense	Somatic	Nonepithelial ovarian cancers	(Heravi-Moussavi et al., 2012, Witkowski et al., 2013)
	W1831*	Nonsense	Somatic	Nonepithelial ovarian cancers	(Heravi-Moussavi et al., 2012)
	Q48E	Missense	Somatic	Wilms tumors	(Torrezan et al., 2014)
	I85M	Missense	Somatic	Wilms tumors	(Torrezan et al., 2014)
	I85M	Missense	Gemline	Wilms tumors	(Walz et al., 2015)
	R307fs	Frameshift	Germline	Wilms tumors	(Foulkes et al., 2011, Wu et al., 2013)
	S436fs	Frameshift	Germline	Wilms tumors	(Foulkes et al., 2011, Wu et al., 2013)
	P645*	Nonsense	Somatic	Wilms tumors	(Wu et al., 2013)
	G706fs	Frameshift	Germline	Wilms tumors	(Foulkes et al., 2011, Wu et al., 2013)
	A872T	Missense	Germline	Wilms tumors	(Wu et al., 2013)
	L999*	Nonsense	Somatic	Wilms tumors	(Wu et al., 2013)
	R1003Q	Missense	Gemline	Wilms tumors	(Walz et al., 2015)
	A1011*	Nonsense	Somatic	Wilms tumors	(Wu et al., 2013)
	R1071*	Nonsense	Somatic	Wilms tumors	(Wu et al., 2013)
	I1102fs	Frameshift	Somatic	Wilms tumors	(Rakheja et al., 2014)
	I1110V	Missense	ND	Wilms tumors	(Wu et al., 2013)
	K1324*	Nonsense	Somatic	Wilms tumors	(Wu et al., 2013)
	S1344L	Missense	Somatic	Wilms tumors	(Wu et al., 2013)
	R1386C	Missense	Gemline	Wilms tumors	(Walz et al., 2015)

Gene	Location	Mutation	Type of mutation	Type of cancer	References
	S1505*	Nonsense	Somatic	Wilms tumors	(Wu et al., 2013)
	A1560*	Nonsense	Somatic	Wilms tumors	(Wu et al., 2013)
	H1693N	Missense	Gemline	Wilms tumors	(Walz et al., 2015)
	E1705K	Missense	Gemline	Wilms tumors	(Walz et al., 2015)
	D1709N	Missense	Gemline	Wilms tumors	(Walz et al., 2015)
	D1713A	Missense	Somatic	Wilms tumors	(Wu et al., 2013)
	D1713V	Missense	Gemline	Wilms tumors	(Walz et al., 2015)
	L1777H	Missense	Germline	Wilms tumors	(Wu et al., 2013)
	E1788fs	Frameshift	Somatic	Wilms tumors	(Wu et al., 2013)
	G1809R	Missense	Somatic	Wilms tumors	(Rakheja et al., 2014)
	G1809V	Missense	Somatic	Wilms tumors	(Rakheja et al., 2014)
	D1810N	Missense	Somatic	Wilms tumors	(Torrezan et al., 2014)
	D1810N	Missense	Gemline	Wilms tumors	(Walz et al., 2015)
	A1818T	Missense	Somatic	Wilms tumors	(Wu et al., 2013)
	G1886R	Missense	Gemline	Wilms tumors	(Wegert et al., 2015)
	K429fs	Frameshift	Gemline	Pituitary blastoma	(de Kock et al., 2014a)
	R509*	Nonsense	Gemline	Pituitary blastoma	(de Kock et al., 2014a)
	R676*	Nonsense	Gemline	Pituitary blastoma	(de Kock et al., 2014a)
	Y793*	Nonsense	Gemline	Pituitary blastoma	(de Kock et al., 2014a)
	N1093*	Nonsense	Gemline	Pituitary blastoma	(de Kock et al., 2014a)
	S1179fs	Frameshift	Gemline	Pituitary blastoma	(de Kock et al., 2014a)
	D1437fs	Frameshift	Gemline	Pituitary blastoma	(de Kock et al., 2014a)
	D1709H	Missense	Gemline	Pituitary blastoma	(de Kock et al., 2014a)
	D1709T	Missense	Somatic	Pituitary blastoma	(de Kock et al., 2014a)
	D1709N	Missense	Somatic	Pituitary blastoma	(de Kock et al., 2014a)
	G1809W	Missense	Somatic	Pituitary blastoma	(de Kock et al., 2014a)
	E1813D	Missense	Somatic	Pituitary blastoma	(de Kock et al., 2014a)
	E1813V	Missense	Somatic	Pituitary blastoma	(de Kock et al., 2014a)
	E1813K	Missense	Somatic	Pituitary blastoma	(de Kock et al., 2014a)

Gene	Location	Mutation	Type of mutation	Type of cancer	References
	Y793*	Nonsense	Germline	Differentiated thyroid carcinoma	(de Kock et al., 2014b)
	S1169fs	Frameshift	Germline	Differentiated thyroid carcinoma	(de Kock et al., 2014b)
	N1193fs	Frameshift	Germline	Differentiated thyroid carcinoma	(de Kock et al., 2014b)
	E1705K	Missense	Somatic	Differentiated thyroid carcinoma	(de Kock et al., 2014b)
	E1813D	Missense	Somatic	Differentiated thyroid carcinoma	(de Kock et al., 2014b)
	E1813G	Missense	Somatic	Differentiated thyroid carcinoma	(de Kock et al., 2014b)
	Q249fs	Frameshift	ND	Childhood cystic nephroma	(Doros et al., 2014)
	S396R	Missense	ND	Childhood cystic nephroma	(Doros et al., 2014)
	K429fs	Frameshift	ND	Childhood cystic nephroma	(Doros et al., 2014)
	R509*	Nonsense	ND	Childhood cystic nephroma	(Doros et al., 2014)
	P817fs	Frameshift	ND	Childhood cystic nephroma	(Doros et al., 2014)
	Y976*	Nonsense	ND	Childhood cystic nephroma	(Doros et al., 2014)
	V990fs	Frameshift	ND	Childhood cystic nephroma	(Doros et al., 2014)
	L1012fs	Frameshift	ND	Childhood cystic nephroma	(Doros et al., 2014)
	Q1031*	Nonsense	ND	Childhood cystic nephroma	(Doros et al., 2014)
	K1324fs	Frameshift	ND	Childhood cystic nephroma	(Doros et al., 2014)
	Y1335*	Nonsense	ND	Childhood cystic nephroma	(Doros et al., 2014)
	P1336fs	Frameshift	ND	Childhood cystic nephroma	(Doros et al., 2014)
	D1437fs	Frameshift	Germline	Familial cystic nephroma	(Bahubeshi et al., 2010)
	E1705K	Missense	ND	Childhood cystic nephroma	(Doros et al., 2014)
	D1709N	Missense	ND	Childhood cystic nephroma	(Doros et al., 2014)
	E1778fs	Frameshift	ND	Childhood cystic nephroma	(Doros et al., 2014)
	G1809R	Missense	ND	Childhood cystic nephroma	(Doros et al., 2014)
	D1810H	Missense	ND	Childhood cystic nephroma	(Doros et al., 2014)
	E1813D	Missense	ND	Childhood cystic nephroma	(Doros et al., 2014)
	E1813G	Missense	ND	Childhood cystic nephroma	(Doros et al., 2014)
	E1813K	Missense	Childhood	Childhood cystic nephroma	(Doros et al., 2014)
	S1826*	Nonsense	Germline	Familial cystic nephroma	(Bahubeshi et al., 2010)
	Y637*	Nonsense	Germline	Embryonal rhabdomyosarcoma (DICER1 syndrome)	(Doros et al., 2012)

Gene	Location	Mutation	Type of mutation	Type of cancer	References
	G706fs	Frameshift	Germline	Cervix embryonal rhabdomyosarcoma	(Foulkes et al., 2011)
	Y749*	Nonsense	Germline	Embryonal rhabdomyosarcoma (ERMS)	(Torres et al., 2012)
	S1179fs	Frameshift	Germline	Embryonal rhabdomyosarcoma (ERMS)	(Darrat et al., 2014)
	Y1204fs	Frameshift	Germline	Cervix embryonal rhabdomyosarcoma	(Foulkes et al., 2011)
	L1303*	Nonsense	Germline	Rhabdomyosarcoma	(Heravi-Moussavi et al., 2012)
	L1303fs	Frameshift	Germline	Cervix embryonal rhabdomyosarcoma	(Foulkes et al., 2011)
	E1418_E1420del	Deletion	Somatic	Embryonal rhabdomyosarcoma (ERMS)	(Torres et al., 2012)
	D1437fs	Frameshift	Germline	Embryonal rhabdomyosarcoma (ERMS)	(Torres et al., 2012)
	Q1702*	Nonsense	Germline	Embryonal rhabdomyosarcoma (ERMS)	(Torres et al., 2012)
	E1705K	Missense	Somatic	Rhabdomyosarcoma	(Heravi-Moussavi et al., 2012)
	L1789fs	Frameshift	Somatic	Embryonal rhabdomyosarcoma (ERMS)	(Torres et al., 2012)
	E1813K	Missense	Somatic	Embryonal rhabdomyosarcoma (ERMS)	(Darrat et al., 2014)
	P291*	Nonsense	Germline	Nontoxic multinodular goiter	(Rio Frio et al., 2011)
	R509*	Nonsense	ND	Multinodular goiter	(Darrat et al., 2013)
	S839F	Missense	Germline	Nontoxic multinodular goiter	(Rio Frio et al., 2011)
	I813_Y819del	Deletion	Germline	Nontoxic multinodular goiter	(Rio Frio et al., 2011)
	R935_R996del	Deletion	Germline	Nontoxic multinodular goiter	(Rio Frio et al., 2011)
	R1672*	Nonsense	Germline	Nontoxic multinodular goiter	(Rio Frio et al., 2011)
	N1742_F1745del	Deletion	Germline	Multinodular goiter	(Rath et al., 2014)
	R656*	Nonsense	Germline	Pulmonary sequestration	(Foulkes et al., 2011)
	V1351fs	Frameshift	Germline	Primitive neuroectodermal tumor	(Foulkes et al., 2011)
	F1706L	Missense	ND	Non-small cell lung cancers	(Kim et al., 2013)
	L1712fs	Frameshift	ND	Gastric carcinoma	(Kim et al., 2013)
	E1801fs	Frameshift	ND	Prostate carcinoma	(Kim et al., 2013)
	E1797D	Missense	ND	Soft tissue sarcoma	(Kim et al., 2013)

DGCR8, DiGeorge syndrome critical region 8; ND, not determined; TRBP: transactivation-responsive RNA-binding protein; Xpo5, exportin 5