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REVIEWS

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ROLE OF COMPONENTS OF microRNA MACHINERY IN CARCINOGENESIS

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MicroRNAs (miRNAs) are a broad class of non-coding RNAs nearly 21 nucleotides length, which play crucial functions in post-transcriptional gene regulation. These molecules are associated with many developmental and cellular processes in eukaryotic organisms. Current investigation has reported major factors contributing to miRNA biogenesis and has constituted basic principles of miRNA function. More recently, it was confirmed that various miRNAs are clearly implicated in human malignancies, such as lung, breast, ovarian, bladder, colon cancer and other kinds of carcinoma. In addition, dysregulation in the miRNA machinery elements such as Dicer, Drosha, DGCR8, Argonaut, and TRBP could be involved in the progress of many tumor types. The purpose of the current review was to compile growing information besides how miRNA biogenesis and gene silencing are modified to develop cancer.
Key Words: **DGCR8, miRNA machinery components, miR, cancer, regulator.**

Several types of short non-coding RNAs including small interfering RNAs (siRNAs), Piwi-interacting RNAs (piRNAs) and microRNAs (miRNAs) have a principal and crucial regulatory functions of development processes in all eukaryotes [1]. Formerly, a finding revealed the first miRNAs in *Caenorhabditis elegans* [2]. miRNAs are extremely protected from species and supposedly may regulate up to 30% of all genes in the human genome [3, 4]. miRNAs are small noncoding RNAs of 18–24 nt in length that controls gene expression post-transcriptionally [5] via negative regulation through binding to the 3'-untranslated region (3'-UTR) of mRNA transcripts, stimulating translational suppression or break of the target [6, 7]. Multiple thousands of miRNAs have been classified in humans and they are evolutionarily conserved. Lately, it has been uncovered that variable expression of special miRNA genes could be increased during the beginning and improvement of cancer [8]. Accordingly, cancer-associated alterations in miRNA expression models are emerging as promising diagnostic markers often compared with disease improvement and survival rates, thus offering a new window for treatment of another cancer types [9]. In general, miRNAs can control several processes, including metabolism, cell differentiation, proliferation and survival, inflammation, genome stability, tumor invasion and angiogenesis [10]. Appearing in the cell nucleus, miRNA operation on the regulation of gene expression continues in the cytoplasm by binding to its supplementary base-sequence of the targeted mRNA molecule. Subsequently, gene silencing through degradation of target mRNA or inhibition with the translation process takes its place [11]. Presently hundreds of miRNAs have been distinguished in different species [12]. Notwithstanding, most miRNA research is focused on the growth and progress of stem cells, differentiation, tumorigenesis and other pathological processes [13].

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Abbreviations used: 3'-UTR – 3'-untranslated region; BC – breast cancer; BCC – basal cell carcinoma; DGCR8 – DiGeorge syndrome critical region 8; HCC – hepatocellular carcinoma; miRNAs – microRNAs; PACT – PKR activating protein; P-bodies – processing bodies; PCMM – primary cutaneous malignant melanoma; piRNAs – Piwi-interacting RNAs; pre-miRNAs – precursor miRNAs; pri-miRNAs – primary miRNAs; RISC – RNA-induced silencing complex; SCC – squamous cell carcinoma; siRNAs – small interfering RNAs; TRBP – transactivation-responsive RNA-binding protein; XPO5 – exportin-5.

miRNA BIOGENESIS AND MECHANISM OF ACTION

Mainly, miRNAs are encoded within the genome and are transcribed by RNA polymerase II (Pol II) or RNA polymerase III (Pol III) as long precursor transcripts, known as primary miRNAs (pri-miRNAs) of several kilobases (kb) in length [14]. Mature miRNAs are produced from pri-miRNAs by sequential processing strategies. Conventionally animal pri-miRNAs possess nearly 33 bp stem and have a terminal loop structure with flanking segments [15]. Precursor miRNAs (pre-miRNAs) are commonly the microprocessor system in the nucleus, whose core components are the RNase III enzyme Drosha and its binding partner DiGeorge syndrome critical region 8 (DGCR8) [16]. Approximately 31% of miRNAs are prepared from introns of protein-coding genes, whereas many other miRNAs are expressed from committing miRNA gene loci. An own pri-miRNA can either produce a single miRNA or contain clusters of two or more miRNAs that are changed from a regular primary transcript [17]. However, these long pri-miRNAs are cleaved by Microprocessor, comprising the double-stranded RNase III enzyme Drosha and its crucial cofactor, the double-stranded RNA binding protein DGCR8 [18]. In this pathway, the nuclear splicing machinery supplies pre-miRNA from introns. miRNAs originated from this process are appropriately termed mirtrons. These molecules enclose a short class of miRNAs, but are identified in multiple organisms [19]. Preliminary, the nuclear microprocessor complex identifies the miRNA hairpins in the main transcript and cleaves each hairpin roughly 11 nucleotides from its base [20]. Recent manifestation suggests that some miRNAs that survive

within introns, the so-called mirtrons, bypass Drosha break and depend on the proceeding of the pre-mRNA splicing machinery to originate an approximately 60 nt pre-miRNA hairpin [21]. Regardless of whether the pre-miRNA hairpin is excised from the initial transcript by canonical Drosha break or through the mirtron pathway, the next stage in the miRNA biogenesis is recognition of the nearly 60 nt pre-miRNA by exportin-5 (XPO5) and export to the cytoplasm in a ran guanine triphosphatase-dependent manner [22]. Mirtrons encompass a small cluster of miRNAs, but are found in numerous organisms. Pre-miRNAs are transported from the nucleus to the cytoplasm by the exportin-5/RanGTPheterocomplex [23]. Complex in cytoplasm compromised of Dicer RNase III endonuclease, transactivation-responsive RNA-binding protein (TRBP), and protein kinase Re-activating. Protein further cleaves the pre-miRNA generating a short double-stranded miRNA:miRNA complex intermediate [24]. In the cytoplasm, pre-miRNAs are processed by RNase III enzyme Dicer. Dicer is thought to react with dsRBDs-containing partner proteins, HIV TRBP and/or PKR activating protein (PACT) [25]. Then, Dicer cleaves pre-miRNAs into 21–25 nt long miRNA/miRNA duplexes, each strand of which bears 5' monophosphate, 3' hydroxyl group and a 3' 2-nt overhang. It preferentially incorporates one of the duplex strands into the RNA-induced silencing complex (RISC). Of a miRNA/miRNA duplex, only one strand, designated the miRNA strand, is selected as the guide of mature RISC, whereas the other strand, the miRNA strand, is discarded during RISC assembly [26]. Such biased strand selection depends on the stability of at least three properties of a miRNA/miRNA duplex: the structure; the 5' nucleotide identity; and the thermodynamic asymmetry [27]. These findings propose miRNA processing by Dicer, during RISC loading, and target RNA cleavage by argonaute 2 (Ago2) is coupled [28]. The translational repression mechanism by miRNAs has been poorly understood. Recently, it was declared that the target mRNAs binding to RISC through partial base pairing, are accumulated in the cytoplasmic foci referred to as processing bodies (P-bodies). P-bodies, in which the mRNAs are stored or degraded by the decapping enzymes and exonucleases, do not contain the translational machinery [29] (Figure).

DICER

Dicer, a critical RNase III endonuclease of the miRNA processing, performs a role in carcinogenesis and various types of human malignancies [30]. Dicer is a large multi-domain protein with a principal function for the last step of miRNA and short-interfering RNA biogenesis. In human and mouse cell lines, Dicer is considered to act in the nuclear clearance of dsRNA as well as the foundation of chromatin agreements [31]. Dicer is supposed to play an essential function in the biogenesis of eukaryotic small RNAs/miRNAs; Dicer target transcripts have not been directly mapped. Interestingly, mainly Dicer-binding sites

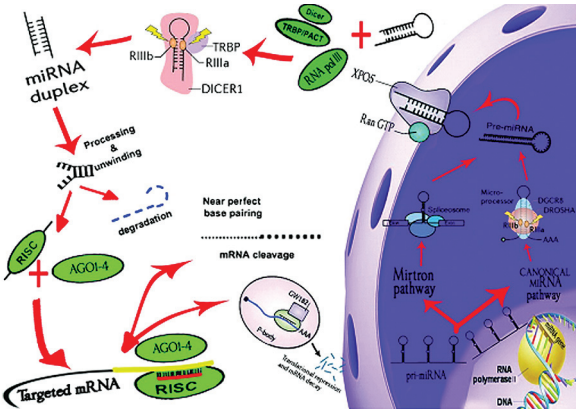

Figure. Schematic presentation of the miRNA biogenesis and functional mechanisms. The miRNA genes are transcribed by RNA polymerase II (Pol II) as long precursor transcripts (pri-miRNAs). These long pri-miRNAs are cleaved by RNase III enzyme Drosha and DGCR8. Pre-miRNAs are exported from the nucleus to the cytoplasm by the XPO5/RanGTP heterocomplex. Complex in cytoplasm compromised of Dicer RNase III endonuclease and TRBP. Protein further cleaves the pre-miRNA generating a short double-stranded miRNA:miRNA complex intermediate. miRNA/miRNA duplex, only one strand,designated the miRNA strand, is selected as the guide of mature RISC and target RNA cleavage by AGO2 is coupled

remain on mRNAs/lncRNAs and are not substantially prepared into miniature RNAs. These passive sites normally harbor short, Dicer-bound hairpins with entire transcripts and regularly maintain target expression [32]. Dicer, single of the proteins implicated in the synthesis miRNA, is implicated in the biogenesis of miRNAs and is influential in carcinogenesis and cancer progress and progression [33]. Low expression of Dicer gene and protein linked to poor prognosis and recurrence of cervical cancer.

In addition, the investigation revealed that decreased Dicer gene expression and protein levels are associated with metastasis relapse and tumor stage. The patients with decreased Dicer miRNA and protein expression displayed a shorter 5-year disease-free survival and overall survival. Moreover, low expression of Dicer befits to be a significant prognostic factor for cervical malignancy and other tumor types [34]. Previous studies have shown that inhibition of Dicer in von Hippel — Lindau deficient clear cell renal cell carcinoma contributed to the high levels of the hypoxia-inducible factors-2α and a cancer phenotype, which suggests Dicer could be a useful therapeutic target for managing this disease [35]. miR-200a and miR-31 are targeted for Dicer and are involved in the carcinogenesis, cell migration, and behavior of castration-resistant prostate cancer, showing that they could be possible biomarkers for monitoring of prostate cancer progression [36]. The Dicer protein expression is significantly associated with hormone receptor status and cancer subtype in breast tumors [37]. Drosha and Dicer1 mutations impair expression of tumor-suppressing miRNAs, including the let-7 family, significant regulators of MYCN, LIN28 and other Wilms tumor oncogenes. Current findings explored the mechanisms through which mutations in miRNA biogenesis components reprogrammed miRNA expres-

sion in human malignancies and proposed that these defects clarify a distinct subclass of Wilms tumors [38]. However, several investigations have revealed that dysregulation of Dicer gene are observed in many disorders, including upregulation in Dicer1 gene expression with tumor stages and progression of prostate cancer and smooth muscle tumors [39]. In addition, preliminary investigations revealed that upregulation of Dicer1 is correlated with gastric tumor subtype and advanced tumor stages in gastric cancer and serous ovarian cancer [40, 41]. However, the other studies showed that the increased Dicer1 expression levels are required for proliferation of oral cancer cells [42]. Faber *et al.* [43] evaluated Dicer1 expression levels in colorectal cancer, and revealed an increased level, which is associated with tumor stage and poor survival of the patients. Chiosea *et al*. [44] disclosed that upregulation of Dicer1 expression levels are associated with histological subtypes and stages of lung cancer. Ma *et al.* [45] showed that up-regulated Dicer1 expression levels correlated with clinical stage of cutaneous melanoma. In general, Dicer expression levels are different in various cancer types; Witkowski *et al.* [46] revealed that downregulation of Dicer1 expression levels is associated with the altered miRNA profile in patients with bladder cancer. Pampalakis *et al*. [47] revealed that decreased Dicer1 expression levels are associated with advanced tumor stage and poor survival of ovarian cancer patients. Contradictory to the other findings, Torres *et al.* [48] showed that downregulation of Dicer1 gene is not associated with histological grade in patients with endometrial cancer. Guo *et al.* [49] observed the decreased expression of Dicer1, which correlated with lower survival of patients with nasopharyngeal carcinoma. In addition, Lin *et al*. [50] revealed that Dicer1 expression levels were significantly lower and associated with total downregulation of miRNAs and poor outcome of patients with neuroblastoma. Khoshnaw *et al*. [51] revealed that decreased Dicer1 expression levels are associated with breast cancer (BC) progression and recurrence. Furthermore, investigations displayed that decrease of Dicer1 levels are correlated with metastasis, invasion and poor prognosis and shortened survival in the patients with gallbladder adenocarcinoma and non-small cell lung cancer [52, 53]. Wu *et al*. [54] revealed that downregulation of Dicer1 is not associated with clinical characteristics of hepatocellular carcinoma (HCC). Findings in other studies revealed that decreased Dicer1 expression levels are associated with progression, tumor stage, prognosis and shorter survival of patients with chronic lymphocytic leukemia, colorectal cancer, BC, and papillary thyroid carcinoma (Table) [55–59].

DROSHA

Drosha involves two RNase III domains, which performs a vital role in miRNA biogenesis; Drosha and its double-stranded RNA-binding partner protein Pasha/DGCR8 likely identify and cut miRNA precursor RNAs or pri-miRNA hairpins co-transcriptionally [60]. Long pri-miRNAs are cleaved by microprocessor complex comprising of Drosha, a type III ribonuclease (RNase III), and an RNA-binding protein DGCR8, is so called because a Drosha/DGCR8 complex is essential and sufficient to process a pri-miRNA into a pre-miRNA hairpin *in vitro*. Dicer then cleaves the pre-miRNA hairpin at the loop to form the mature miRNA. Correct Drosha cleavage in the pri-miRNA is critical to the production of a functional miRNA [61]. Drosha is an important factor for miRNA biogenesis and as such obligatory for cellular homeostasis and developmental processes. Together with its cofactor DGCR8, it changes the pri-miRNA into the pre-miRNA in the nucleus. Whilst the middle and the C-terminal domain are important for pri-miRNA processing and DGCR8 binding, the activity of the N-terminus remains mysterious. Different investigations have linked this region to the subcellular localization of Drosha, stabilization, and response to tension [62]. The repression occurs solely in mature miRNAs and not in pri-miRNA transcripts, evidencing that the Drosha E1147K mutation affects processing of pri-miRNAs. The pivotal role of the miRNA biogenesis pathway is shown in Wilms tumor development, particularly the major miRNA processing gene Drosha [63]. Decreased expression of Drosha was found in melanoma. Furthermore, the irregular subcellular location of Drosha reveals potential deregulation in the procedures responsible for its proper localization in the nucleus [64]. The new results show that cytoplasmic Drosha potentially plays a role in blocking carcinogenesis and progression of gastric cancer and may serve as an independent prognostic marker [65]. The copy number discrepancy of Dicer1 and Drosha correlates well with their expression levels and survival of patients with non-small cell lung cancer and other cancer types. The increased expression of Drosha and Dicer1 are associated with decreased and increased survival, respectively. As a result, copy number variation may be a significant mechanism of upregulation/downregulation of miRNAs in malignancy and propose an oncogenic role for Drosha [66]. Muralidhar *et al*. [39] revealed that upregulation of Drosha expression levels could alter miRNA profile associated with neoplastic progression in cervical squamous cell carcinoma (SCC). In addition, Sugito *et al*. [67] have shown that increased Drosha expression was associated with poor survival of patients with esophageal cancer. The findings of several investigations have shown that upregulation of Drosha was associated with tumor progression, advanced tumor stages and poor prognosis in the patients with various cancer types [37–39, 51]. Moreover, Sand *et al*. [68] revealed that upregulation of Drosha expression levels was not determined in SCC and basal cell carcinoma (BCC). Avery-Kiejda *et al.* [69] revealed that upregulation of Drosha expression levels had no significant clinical correlation in triple-negative BC. Similarly, in others investigations, findings showed that downregulation of Drosha expression levels could

alter miRNA profile and correlated with poor patient survival, histological grade, metastasis, invasion and poor prognosis in many cancer types [48–50, 52, 64, 70, 71]. Findings revealed that altered Drosha expression levels are not associated with clinical features in colorectal cancer, BC, and papillary thyroid carcinoma (see Table) [57–59].

DGCR8

Mirtrons pathways include a small class of miRNAs; although miRNAs are synthesized via the miRtron pathway rather than by Drosha, the synthesis of main miRNAs looks to be Drosha dependent. The prominent stem-loop in pri-miRNAs is identified through Drosha together with its partner Pasha/DGCR8. Indeed, Pasha/DGCR8 is thought to bind preferentially at the junction between the stem and the more inflexible loop, and this process can be co-transcriptional. This binding then positions Drosha midway up to the stem so that it is correctly positioned to make a pair of staggered breaks to generate the nearly 70 bp pre-miRNA [72]. The microprocessor complex mediates intranuclear biogenesis of pre-miRNAs from the pri-miRNA transcript. Extranuclear, mature miRNAs are merged into the RISC before interaction with completing target mRNA that leads to repression of translation

Table. Patterns of miRNA expression in different tumor types

Genes	Increase or decrease	Cancer type	Refs
Drosha	Altered miRNA profile; associated with neoplastic progression	Cervical SCC	[39]
	Regulates cell proliferation; associated with poor patient survival	Oesophageal cancer	[67]
	Associated with pathological characteristics and patient survival	Gastric cancer	[40]
	Associated with advanced tumor stages	Serous ovarian carcinoma	[41]
	Associated with poor prognosis	Non-small cell lung cancer	[53]
	Not determined	SCC and BCC	[68]
	No clinical correlation	Triple-negative BC	[69]
	Altered miRNA profile	Bladder cancer	[70]
	Associated with poor patient survival	Ovarian cancer	[71]
	Correlated with histological grade	Endometrial cancer	[48]
	Correlated with shorter patient survival	Nasopharyngeal carcinoma	[49]
	Correlated with metastasis, invasion and poor prognosis	Gallbladder adenocarcinoma	[50]
	Correlated with total downregulation of miRNAs and poor outcome	Neuroblastoma	[52]
	Associated with cancer progression and poor survival	Cutaneous melanoma	[64]
	Down-regulated	BC	[58]
	Significantly upregulated, not associated with clinical characteristics	Colorectal cancer	[57]
	Significantly lower expressed, not associated with clinical characteristics	Papillary thyroid carcinoma	[59]
Dicer	Correlated with a gastric tumor subtype	Gastric cancer	[40]
	Associated with advanced tumor stages	Serous ovarian carcinoma	[41]
	Required for proliferation	Oral cancer	[42]
	Correlated with tumor stage and associated with poor survival	Colorectal cancer	[43]
	Associated with histological subtypes and stages	Precursor lesions of lung adenocarcinoma	[44]
	Correlated with clinical stage	Cutaneous melanoma	[45]
	Altered miRNA profile	Bladder cancer	[46]
	Associated with advanced tumor stage and poor patient survival	Ovarian cancer	[47]
	No association with histological grade detected	Endometrial cancer	[48]
	Correlated with shorter patient survival	Nasopharyngeal carcinoma	[49]
	Associated with a total down regulation of miRNAs and poor outcome	Neuroblastoma	[50]
	Associated with cancer progression and recurrence	BC	[51]
	Correlated with metastasis, invasion and poor prognosis	Gallbladder adenocarcinoma	[52]
	Low levels of DICER1 expression correlate with shortened survival	Non-small cell lung cancer	[53]
	Not associated with clinical characteristics	HCC	[54]
	Associated with progression and prognosis	Chronic lymphocytic leukemia	[55]
	Associated with tumor stage and shorter survival	Colorectal cancer	[56]
	Significantly upregulated, not associated with clinical characteristics	Colorectal cancer	[57]
	Significantly altered gene expression, not associated with clinical characteristics	Papillary thyroid carcinoma	[59]
	Down-regulated	BC	[58]
DGCR8	Associated with poor patient survival	Oesophageal cancer	[67]
	Altered miRNA profile	Bladder cancer	[70]
	Not determined	SCC and BCC	[68]
	Associated with dysregulated miRNA	Prostate cancer	[64]
	Not associated with any clinical parameters	Colorectal carcinoma	[73]
	Required for cell proliferation, migration and invasion	Ovarian cancer	[75]
	Significantly altered gene expression, not associated with clinical characteristics	Papillary thyroid carcinoma	[59]
XPO5	Associated with an altered miRNA profile	Bladder cancer	[70]
AGO1/AGO2	Not determined	Actinic keratoses, SCC and BCC	[68]
	Associated with advanced tumor stages	Serous ovarian carcinoma	[41]
	Correlated with advanced tumor stages and associated with shorter survival		
	Significantly lower expressed, not associated with clinical characteristics	Papillary thyroid carcinoma	[59]
TRBP	Amplified in BC	BC	[85]
	Significantly up-regulated, associated with clinical characteristics	BC	[87]
	Significantly up-regulated	Malignant melanoma	[88]
	Significantly up-regulated	Diffuse large B cell lymphomas	[96]
	Significantly up-regulated, not associated with histopathological and clinical parameters	Adrenocortical carcinoma	[97]
	Altered miRNA profile	Ewing sarcoma	[98]

Notes: AGO – Argonaute; BC – breast cancer; BCC – basal cell carcinoma; DGCR8 – DiGeorge syndrome critical region 8; SCC – squamous cell carcinoma; XPO5 – exportin-5; TRBP – transactivation-responsive RNA-binding protein.

or mRNA destabilization. The DGCR8 is a component of microprocessor complex crucial for miRNA maturation. The Ago2 proteins are a component of a complex protein named as RISC. A previous investigation has shown that DGCR8 mRNA expression is down-regulated in prostate cancer. Upregulated DGCR8 mRNA expression has been found in epithelial skin cancer and pleomorphic adenomas of the salivary gland. It has been documented that the Ago2 mRNA expression level is up-regulated in epithelial skin cancer [73]. Firstly, the DGCR8 mRNA expression level was up-regulated in colorectal cancer, evidencing on its role in the pathobiology of the colorectal carcinogenesis [74]. DGCR8 was expressed in ovarian cancer. MiR-27b was detected as significantly down-regulated miRNA in DGCR8-knockdown cells and endorsed cell proliferation in ovarian cancer cells [75]. Preliminary studies reported that expression levels of Drosha in AGS and HepG2 cell lines were higher than in the controls, whereas, Drosha expression level in KYSE-30 cell line was lower. The Dicer expression levels in AGS and HepG2 cells were increased, whereas, its expression level in KYSE-30 cell was lower. The DGCR8 expression levels in all three cell lines were significantly higher than in the control samples [76]. In addition, Sugito *et al*. [67] revealed that upregulation of DGCR8 expression was associated with poor survival of patients with esophageal cancer. Catto *et al*. [70] showed that increased DGCR8 expression levels altered the miRNA profile in bladder cancer. Sand *et al*. [68] reported that upregulation of DGCR8 expression was not determined in SCC and bladder cancer, additionally, revealed that up-regulated DGCR8 expression was associated with dysregulated miRNA in prostate cancer. Kim *et al*. [73] revealed that upregulation of DGCR8 expression was not associated with any clinical parameters in colorectal carcinoma. Furthermore, Guo *et al.* [49] revealed that increased DGCR8 expression levels were required for cell proliferation, migration, and invasion in ovarian cancer. Findings revealed that altered DGCR8 expression is not associated with clinical features in papillary thyroid carcinoma [59].

AGO

Pre-miRNAs exported into the cytoplasm are processed by another RNase III enzyme, Dicer, into nearly 21 bp double-stranded miRNA-miRNA duplexes and moved into the groove of AGO. Afterwards the miRNA strand dissociation, mature single-stranded miRNA remains loaded into and stabilized by AGO1. AGO was subsequently separated from Dicer to bind TNRC64, an essential co-factor in the miRNA-induced silencing complex [77]. AGO2 is a major part of the RISC that can directly deteriorate mRNA through slicing AGO2 amasses in cytoplasmic processing bodies, where additional binding interactions promote translational inhibition and mRNA decay. AGO2 also couples with MVEs in structures that have been called “GW-bodies” because of the presence of GW182 but lack of other P-body components. Current reports have demonstrated that AGO2 binds to miRNAs to generate AGO2-miRNA complexes that are found in the extracellular space. Although the majority of descriptions illustrates Ago2 as being present in the extracellular space as a free protein, other investigations have shown that AGO2 and other RNA-processing proteins are present in the secreted exosomes [78]. The genetic polymorphism of AGO2 may be a risk factor for the progressive lymph node metastasis in nasopharyngeal carcinoma in the Chinese population, and AGO2 acts as an oncogene in the development of nasopharyngeal carcinoma [79]. Sand *et al.* [68] revealed that upregulation of AGO1 and AGO2 expression was not determined in actinic keratosis, SCC and BCC, while such upregulation was associated with advanced tumor stages in serous ovarian carcinoma. Findings revealed that the AGO2 expression levels was significantly lower in neoplastic tissues compared to the healthy tissues in papillary thyroid carcinoma (see Table) [59].

XPO5

XPO5 is a component of the importin-β family of proteins that consist of one major class of nucleocytoplasmic transporters. XPO5 binds directly to its pre-miRNA cargo in a Ran-GTP-dependent manner. As well, XPO5 is capable of recognizing and export structured RNAs that are unrelated to pre-miRNAs, involving viral mini-helix RNA and tRNA, along with certain other proteins, such as STAU2, ILF3, and JAZ. It has also been indicated that XPO5 is important in siRNA biogenesis and therefore, is a basic point of intersection between the siRNA and miRNA pathways [80]. XPO5 is a transporter protein regularly mediating pre-miRNAs nuclear export. Recent investigations have displayed that XPO5 may play an important role in some cancers. Anyway, little is known about XPO5 in HCC [81]. The XPO5 genetic defect traps pre-miRNAs in the nucleus decreases miRNA processing and diminishes miRNA-target inhibition [82]. XPO5 knockdown promoted HCC cell migration and decreased the expression of E-cadherin and p53. Furthermore, after treatment with DAC and TSA, the mRNA level of XPO5 was up-regulated in HCC cells, implicating that epigenetic modulation may be involved in the transcription of XPO5. Generally, these findings suggest that XPO5 functions as a potential tumor suppressor in the development and progression of HCC as well as a promising molecular target for HCC therapy [83]. Catto *et al*. [70] revealed that upregulation of XPO5 expression was associated with the altered miRNA profile in bladder cancer (see Table).

TRBP

miRNAs are transported from the nucleus to the cytoplasm via XPO5 mediated pathway, where they are bound with Dicer and the TRBP, and maturate into double-stranded miRNAs. The active strand of such mature miRNA is retained in the Dicer-TRBP complex, which then binds with the endonuclease AGO2. One strand of the mature miRNA (the guide strand)

is loaded into the RISC target mRNAs that are complementary to the miRNA [84]. Huang *et al*. [85] revealed that TRBP is overexpressed in BC. TRBP is multifunctional and mediates crosstalk between different pathways. The protein AIB3, also known as ASC-2, RAP250, PRIP, TRBP, and NCR, is a newly recognized nuclear receptor co-activator that is amplified and over-expressed in BC [86]. Lin *et al.* [87] revealed that the BC patients with cytoplasmic overexpression of TRBP2 had shorter disease free survival and overall survival. Sand *et al*. [88] showed that TRBP2 was significantly up-regulated in benign melanocytic nevi compared to primary cutaneous malignant melanoma (see Table). Earlier, we have applied a gold standard method for lymphoma diagnosis using TRBP2 gene expression analysis [89–95]. However, Caramuta *et al*. [96] revealed that the expression of TRBP2 was significantly higher in diffuse large B cell lymphomas than in lymph nodes, and also in the adrenocortical carcinomas compared with adenomas or normal adrenal cortices. Whereas, TRBP2 expression was not correlated with histopathological and clinical parameters [97]. De Vito *et al.* [98] revealed that deregulation of TRBP2 in the Ewing sarcoma.

In conclusion, the newest findings on miRNA expression patterns in cancerous tissues will allow to develop the use of these molecules as novel biomarkers for tumor diagnosis, prognosis, and therapy.

CONFLICT OF INTERESTS

The authors have declared no conflict of interests.

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