INTRODUCTION
MicroRNAs (miRNAs) are non-coding RNAs ~22 nt long that bind to target mRNAs, resulting in mRNA degradation or inhibition of miRNA expression (1, 2), and play a key role in post-transcriptional gene regulation in up to 30–60% of all human genes (3). miRNA targets mRNA by specific base-pairing interactions between the seed region of the miRNA and the 5′-untranslated regions of the mRNA (4–6). miRNAs can be grouped into families on the basis of their seed sequences, and members of one family usually effect the same mRNAs. A small number of miRNAs outside the seed sequences have also been reported (7, 8).

MicroRNAs can be produced from long RNA transcripts. Primary miRNAs (pri-miRNAs), which are 1–2 kb long and contain one or more 70-nt hairpin precursor miRNAs (pre-miRNAs), are excised to pre-miRNAs by ribonuclease III (RNase III) and DiGeorge critical region 8 (DGCR8) excised to pre-miRNAs by ribonuclease III (RNase III) and DiGeorge critical region 8 (DGCR8), which plays a crucial role in the repression or degradation of miRNA maturation. Drosha–DGCR8 complex, known as a microprocessor, is essential for miRNA maturation. Drosha, as the catalytic subunit, has been shown to cleave pri-miRNA-like hairpins harbored within the 5′-untranslated region of the mRNA encoding the DGCR8 protein (12, 13). Drosha is a member of the RNase III family and can convert pri-miRNAs into pre-miRNAs (11), which are exported from the nucleus into the cytoplasm by an exportin-5 (XPO5)/Ran–GTP complex (14–16). In the cytoplasm, the endoribonuclease Dicer complex catalyzes these pre-RNAs to form miRNAs (17). The mature miRNAs are loaded into an argonaute 2 (AGO2) protein, which associates with a TAR RNA-binding protein (TRBP) and forms an RNA-induced silencing complex (RISC) (18, 19), which plays a crucial role in the repression or degradation of mRNAs.

miRNA MACHINERY GENES
The miRNA machinery genes include Drosha, DGCR8, Dicer1, XPO5, TRBP, and AGO2, which synthesize proteins to regulate the processing of miRNAs and influence different fields in vivo. Drosha, a nuclear RNase III enzyme, has two RNase III catalytic sites with a double-stranded RNA-binding domain (dsRBD) at the C terminus and a proline-rich domain and arginine/serine-rich domains at the N terminus (11). Drosha recognizes the stem-loop structure and cleaves both arms of the stem-loop through the tandem RNase III domains. The RNase III family of enzymes, which are found in all eubacteria and eukaryotes (20), is divided into three classes based on their structure. Of these classes, Drosha class II and Dicer class III have crucial effects on miRNA processing. The long pri-miRNA, which is typically generated by RNA polymerase II, contains a short stem-loop structure (11). DGCR8 can stabilize the Drosha protein through protein–protein interaction (12) and is an essential miRNA processing factor that includes an N-terminal region for nuclear localization, a heme-binding domain, two dsRBDs, and a C-terminal tail (21, 22). DGCR8 binds to the base of the long primary transcript pri-miRNA hairpin, positioning Drosha to cleave the pri-miRNA stem at a distance of 11
base pairs from the junction between the double-stranded RNA (dsRNA) stem and the flanking single-stranded RNA regions (23).

XPO5 is a nuclear receptor that transports pre-miRNA from the nucleus to the cytoplasm (24, 25). Once in the cytoplasm, pre-miRNA is cleaved by Dicer in complex with another dsRNA-binding protein, TRBP (19, 25, 26). As a key protein in the cleaving process of pri-miRNA, Dicer has two RNase III domains, a less-conserved ATPase/DExD helicase domain and a Piwi–Argonaute–Zwille (PAZ) domain (27, 28). The key regions for miRNA maturation, these domains have different effects. The RNase IIIA domain of Dicer1 is essential for generating small RNAs embedded in the 3′ stem of exogenous hairpin-like RNAs (29). Inactivation of this domain results in complete loss of 3p-derived mature miRNAs but only partial reduction in 5p-derived mature miRNAs (30). In contrast, inactivation of the RNase IIIB domain by mutation of D1709 results in complete loss of 5p-derived mature miRNAs but only partial reduction in 3p-derived mature miRNAs. Mutation of the PAZ domain in Dicer results in global reduction of miRNA processing (30).

Argonaute proteins are core components of RISCs and are highly conserved between species. Many organisms encode multiple members of this protein family, which have essential roles in RNA-mediated gene silencing (31). AGO2 protein contains four major domains, N-terminal, PAZ domain, MID domain, and PIWI domain (32, 33), as well as two structured linker domains, L1 and L2 (34). The PAZ domain, like Dicer, binds to the 3′ end of guide RNA (35). The MID domain of the eukaryotic AGO protein QDE-2 adopts a Rossmann-like fold and recognizes the 50-nt terminal of a guide RNA in a manner similar to its prokaryotic counterparts, for which the 50-nt-binding site shares common residues with a second, adjacent ligand-binding site (36).

TAR RNA-binding protein is a dsRNA-binding protein that includes two dsRBDs and a C4 domain (37). The two dsRBDs together express a much higher affinity for binding dsRNA than either one alone, confirming that the two domains cooperate for dsRNA binding (38, 39). However, a KR-helix motif in dsRBD2 gives it a stronger dsRNA-binding efficacy than dsRBD1 has (38). The C-terminal domain in TRBP binds to the tumor suppressor Merlin, the RNase III Dicer, and PKR activator (PACT) to create the Medipal domain (40). The C4 domain has a major influence on the reactions of TRBP–PACT and TRBP–Dicer.

**Figure 1** clarifies the molecular mechanisms underlying the miRNA processing machinery and the three-dimensional structures of the relevant proteins. Two RNase III domains, IIIA and IIIB are, common to Drosha and Dicer1. The PAZ domain is common to Dicer1 and AGO2 (23).

**FUNCTIONS OF miRNA MACHINERY GENES**

Because of their core functions in miRNA processing, the genes Drosha, DGCR8, Dicer1, XPO5, AGO2, and TRBP are important in several aspects. Drosha can recognize and cleave the stem-loop structures in mRNAs, leading to their dysfunction, which occurs
mostly in stem or progenitor cell populations (41–44). Due to the fact that the transcription factor Neurogenin 2 has a conserved hairpin-like pri-miRNAs do, Drosha can regulate the expression of this transcription factor (41). Drosha knockout mice are infertile due to oligotermatozoospermia or azoospermia, which suggests that Drosha-mediated miRNA production is important in male fertility (45). Drosha can affect the proliferation of human mesenchymal stem cells by regulating rRNA processing (46). Inhibition of Drosha also affects rRNA processing in HeLa cells (47).

DGCR8 is also a part of a microprocessor, the Drosha–DGCR8 complex, with an important function in miRNA maturation. This complex cleaves the hairpin structures in DGCR8 mRNA (12). Deficiency of DGCR8 results in altered short-term plasticity in the prefrontal cortex, affects dendritic spines and complexity, and alters brain miRNA biogenesis (48, 49). Moreover, inactivation of DGCR8 in cardiac neural crest cells results in malformations and increased apoptosis (50). In addition, the loss of DGCR8 in vascular smooth muscle cells results in liver hemorrhage, dilated blood vessels, and disarrayed vascular architecture in murine models, implying that the DGCR8 gene plays an important role in vascular development by regulating the apoptosis and differentiation of these cells (51).

As an RNase III endonuclease, Dicer is a core enzyme which cleaves pre-miRNAs into 21- to 25-nucleotide species in miRNA processing. Dicer has many important roles in the morphogenesis of developing tissues. For example, it plays an essential role in neuron polarity and neuronal development (52) and represses neuronal genes during endocrine cell maturation (53). Loss of Dicer results in gross abnormalities in cell number and function in the cortex and hippocampus (54), and deletion of Dicer in the early pancreatic lineage in Pdx1-Cre mouse models results in early pancreatic bud development and pancreas agenesis (55). On the other hand, Dicer is required for maintaining adult pancreas, and morphologic abnormalities in Dicer1-hypomorphic mice can be detected after 4 weeks of age (56). Deregulation of Dicer1 in β-cells leads to progressive reduction in insulin secretion, glucose tolerance, and development of diabetes and impaired islet architecture (57, 58). Moreover, loss of Dicer results in significant reductions of testis mass and sperm number in germ cell knockout mouse as well as impaired meiotic progression (59). Finally, inactivation of Dicer in developing mouse lymphocytes can impair cell proliferation and survival and alter the repertoires of antigen receptors (60).

Mature RISC consists of a single-stranded small RNA bound to an AGO protein. AGO proteins can bind small interfering RNAs as well as miRNAs and mediate the repression of specific target RNAs either by degrading RNA or by inhibiting translation. Members of the AGO protein family have been implicated in both transcriptional and post-transcriptional gene silencing (31, 61). As a highly specialized member of the AGO family, AGO2 has an essential non-redundant Slicer-independent function within the mammalian miRNA pathway. AGO2 is also a key regulator of B lymphoid and erythroid development and function. However, deficiency in AGO2 impairs miRNA biogenesis from pre-miRNAs and reduces miRNA expression levels (62). On the other hand, AGO protein-associated small RNAs repress mitogen-induced transcripts, and stabilized and stored mature miRNAs can be activated to regulate the mitogenic responses (63). Interestingly, AGO2 in dopamine 2 receptor-expressing neurons regulates cocaine addiction (64), and nuclear AGO2 has been reported to regulate voltage-gated potassium channels in adipose tissue-derived stromal cells with crucial functions in the self-renewal and cell de-aging processes (65). Furthermore, the interaction between the epidermal growth factor receptor gene (EGFR), a novel upstream regulator of the RISC-loading complex, and AGO2 increases under hypoxia stress, which leads to elevated AGO2-Y393 phosphorylation and inhibition of the transition of pre-miRNAs into mature miRNAs (66).

As a steroid receptor RNA activator-binding nuclear receptor coregulator, TRBP targets steroid-responsive promoters and regulates nuclear receptor activity and downstream gene expression (67). TRBP contributes to HIV-1 gene expression by inhibiting the activation of the dsRNA-dependent protein kinase R (PKR) (68). Knockdown of TRBP can reduce the accumulation of hepatitis C virus RNA (69) and TRBP has been proposed as a target for antiviral therapies (68, 70, 71). The structures of TRBP and the PKR activator (PACT) are highly homologous (72). TRBP can control the PACT activation of PKR and the expression of the HIV-1 gene (73). The interaction between TRBP and PACT may influence other cellular processes as well. TRBP can bind to the small-molecule enoxacin and express tumor suppressors in human cell cultures and mouse cancer models (74).

XPO5 protein directly binds and mediates the nuclear export of dsRNA, including pre-miRNAs, viral hairpin RNAs, and tRNAs (16, 75). Inhibition of XPO5 results in down-regulation of Dicer (76), global miRNA elevation disorder, and delayed G1/S transition (77), indicating that XPO5 is a critical component in miRNA biogenesis, regulates global miRNA expression, and is associated with cell-cycle control. Because aberrant expression of XPO5 increases the risk of cancer (78), it is a potential target for drug intervention.

miRNA MACHINERY GENES IN CANCER

Alterations in the miRNA machinery play important roles in the carcinogenesis of a variety of tumors (79). Preliminary analysis of data from The Cancer Genome Atlas consortium of multiple types of cancer through cBioPortal (80, 81) has shown a significant incidence of alterations in miRNA machinery genes (Table 1), especially the AGO2 gene, which has a high incidence of gene alterations across cancer types, including breast invasive carcinoma (23.30%), colon and rectum adenocarcinoma (12.3%), bladder urothelial carcinoma (20.8%), and prostate adenocarcinoma (20.7%). This evidence supports prior reports linking miRNA-related alterations to cancers. The incidence of alterations mutation, copy number variation, and/or deregulated miRNA expression for these cancer types was 80.6%, 95.4%, 96.0%, and 80.5%, respectively.

Since the incidence of alteration of the AGO2 gene was highest in breast invasive carcinoma, we analyzed the miRNA machinery genes in breast invasive carcinoma datasets to identify patterns of mutual genetic alterations and driver genes. A strong tendency of mutual exclusivity was noted for genetic alterations in the miRNA machinery gene TRBP with the driver genes PIK3R1 (p = 0.03) and KMT2C (p = 0.0019) (Table 2). We also noted several incidences of co-occurrences in Table 2. Our analysis suggested that
alterations in miRNA machinery genes interact with driver genes in at least a subset of tumors. Considering the regulatory role of miRNAs, the underlying mechanisms and cellular consequences of these interactions may be critical for understanding cancer pathology.

miRNA MACHINERY GENES AS BIOMARKERS FOR CANCERS

Although the mechanism of microprocessor activity has been intensively investigated and dysregulation of miRNA machinery genes plays a pivotal role in the initiation and progression of malignancies, it remains largely unknown how miRNA machinery genes

Table 1 | The Cancer Genome Atlas consortium data on the incidence of genetic alterations in microRNA machinery genes and driver genes, by cancer type.

| Gene symbol | Breast invasive carcinoma \( (n = 463) \) | Colon and rectum adenocarcinoma \( (n = 195) \) | Bladder urothelial carcinoma \( (n = 125) \) | Prostate adenocarcinoma \( (n = 82) \) |
|-------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
|             | \( n \) | %    | \( n \) | %    | \( n \) | %    | \( n \) | %    |
| AGO2        | 108   | 23.3 | 24   | 12.3 | 26   | 20.8 | 17   | 20.7 |
| APC         | 40    | 8.6  | 153  | 78.5 | 10   | 8    | 6    | 7.3  |
| CCND1       | 99    | 21.4 | 9    | 4.6  | 21   | 17   | 0    | 0    |
| CCNE1       | 42    | 9.1  | 11   | 5.6  | 29   | 23.2 | 8    | 9.8  |
| CDKN2A      | 61    | 13.2 | 14   | 7.2  | 56   | 44.8 | 6    | 7.3  |
| CHD1        | 30    | 6.5  | 15   | 7.7  | 12   | 9.6  | 11   | 13.4 |
| CTCF        | 41    | 8.9  | 17   | 8.7  | 11   | 8.8  | 7    | 8.5  |
| DEFB135     | 15    | 3.2  | 6    | 3.1  | 12   | 9.6  | 8    | 9.8  |
| Dicer1      | 23    | 5    | 9    | 4.6  | 13   | 10.4 | 2    | 2.4  |
| Dicer1      | 32    | 6.9  | 14   | 7.2  | 42   | 33.6 | 6    | 7.3  |
| Dicer1      | 32    | 6.9  | 14   | 7.2  | 42   | 33.6 | 6    | 7.3  |
| Dicer1      | 32    | 6.9  | 14   | 7.2  | 42   | 33.6 | 6    | 7.3  |
| Dicer1      | 32    | 6.9  | 14   | 7.2  | 42   | 33.6 | 6    | 7.3  |
| Dicer1      | 32    | 6.9  | 14   | 7.2  | 42   | 33.6 | 6    | 7.3  |
| Dicer1      | 32    | 6.9  | 14   | 7.2  | 42   | 33.6 | 6    | 7.3  |
| Dicer1      | 32    | 6.9  | 14   | 7.2  | 42   | 33.6 | 6    | 7.3  |
| Dicer1      | 32    | 6.9  | 14   | 7.2  | 42   | 33.6 | 6    | 7.3  |
| Dicer1      | 32    | 6.9  | 14   | 7.2  | 42   | 33.6 | 6    | 7.3  |
| Dicer1      | 32    | 6.9  | 14   | 7.2  | 42   | 33.6 | 6    | 7.3  |
| Dicer1      | 32    | 6.9  | 14   | 7.2  | 42   | 33.6 | 6    | 7.3  |
| Dicer1      | 32    | 6.9  | 14   | 7.2  | 42   | 33.6 | 6    | 7.3  |
| Dicer1      | 32    | 6.9  | 14   | 7.2  | 42   | 33.6 | 6    | 7.3  |
| Dicer1      | 32    | 6.9  | 14   | 7.2  | 42   | 33.6 | 6    | 7.3  |
| Dicer1      | 32    | 6.9  | 14   | 7.2  | 42   | 33.6 | 6    | 7.3  |

\[ a \] Genetic alterations comprise of mutations and/or CNV and/or mRNA expression deregulation.
\[ b \] miRNA machinery genes are in bold font.
\[ c \] Driver genes are those with the highest incidence of alterations in a given dataset.
are regulated and whether they can serve as biomarkers for cancers. Abnormal expression of miRNA machinery genes has been found in a variety of human tumors (Table 3). The expression levels of Drosha, DGC8R8, Dicer, XPO5, AGO2, and TRBP have all been associated with several cancers.

The expression level of Drosha is up-regulated in basal cell carcinoma and squamous cell carcinoma (SCC) (82, 83), and elevated levels of Drosha are observed in smooth muscle neoplasms compared with smooth muscle, indicating that this enzyme is involved in smooth muscle neoplasms (85). Down-regulation of Drosha is associated with patient outcome in ovarian cancer (85), outcomes and risk groups in neuroblastoma (86), occurs in endometrial cancer (87), correlates with nasopharyngeal carcinoma and the patient outcomes (88), is associated with the specific subgroups of breast cancer (89), and is associated with metastasis, invasion, and poor prognosis in gallbladder adenocarcinoma (90).

DGC8R8 expression levels are over-expressed in basal cell carcinoma (110), SCC (110), colorectal cancer (CRC) (91), gastrointestinal cancer (92), and ovarian cancer (93). Knockdown of DGC8R8 in ovarian cancer cells disturbs their proliferation, migration, and invasion and increases their sensitivity to the chemotherapeutic drug cisplatin (93), which suggests that an elevated level of DGC8R8 is associated with carcinogenesis.

Dicer is down-regulated in many tumors, such as transitional cell carcinoma of the urinary bladder (94), neuroblastoma (86), nasopharyngeal carcinoma (88), endometrial cancer (87), breast cancer (102), lung cancer (101, 111), gastric cancer (GC) (112), ovarian cancer (113), and gallbladder adenocarcinoma (90). Repression of Dicer is associated with poor prognosis for patients with lung cancer (101), ovarian cancer (114), chronic lymphocytic leukemia (115), or colorectal CRC (116), and it promotes cell proliferation in A2780 and SKOV3 ovarian cancer cells (117). Conversely, compared with normal tissue, the expression of Dicer is higher in cutaneous SCC (82), salivary gland pleomorphic adenoma (118), acute myeloid leukemia (119), smooth muscle neoplasm (85), and prostate cancer (100). Overexpression of Dicer has been shown to lead to poor survival in patients with soft tissue sarcoma (84). Loss of Dicer expression suppresses the growth and oncogenicity of human prostate cancer cell lines but enhances migratory capacity in some prostate cancer cell lines (120). Dicer is increased in human prostate cancer specimens, but lower Dicer expression predicts faster cancer recurrence (120). Complete ablation and hemizygous loss of Dicer reduced tumor growth. Hemizygous loss also resulted in an invasive phenotype and causes seminal vesicle obstruction, which indicated that the regulation of Dicer depends on dosage and context (120).

The expression of AGO2 is up-regulated in GC (103), epithelial skin cancer (110), prostate cancer (100), and hepatocellular carcinoma (104). AGO2 binds to the tumor metastasis factor focal adhesion kinase promoter and triggers its transcription, which suggests a new function of AGO2 in tumor progression (104). Repression of AGO2 protein has been found in human lung adenocarcinomas (105) and in melanoma, for which the mRNA level of AGO2 did not change (106). Overexpression of AGO2 has been shown to inhibit cancer cell proliferation and migration in mice models (105). The stability of AGO2 protein is essential, as is the frame shift mutation of the AGO2 gene in GC and CRC with high microsatellite instability (MSI-H), which suggests that these alternations are risk factors for GC and CRC (97). Single-nucleotide polymorphisms of AGO2 have been associated with the outcome of breast cancer patients (107).

Compared with in lymph nodes, TRBP is over-expressed in prostate cancer (116). Similarly, TRBP is over-expressed in diffuse large B-cell lymphoma and is associated with a poor chemotherapy response (108). Both TRBP mRNA and TRBP protein levels are higher in adenocortical carcinomas than in adenomas or adrenal cortices (109). Knockdown of TRBP decreases cell proliferation and induces cell apoptosis in diffuse large B-cell lymphoma cells (108) and adenocortical carcinomas cells (109). However, the expression levels of TRBP are not significantly different between patients with epithelial skin cancer and persons who do not (110). Melo et al. found that the presence of inactivating mutations in TRBP gene in human cancer cell lines and primary tumors with MSI-H impaired miRNA processing and enhanced cellular transformation and the loss of TRBP led to a secondary defect in Dicer1 activity. These results further confirmed the role of loss of function events in the regulation of miRNA processing machinery during tumorigenesis (121).

Dysfunction of XPO5 can also result in carcinogenesis. The expression level of XPO5 is up-regulated in urothelial carcinoma of the bladder (95) and breast cancer (96) and is positively correlated with tumor development and invasion (95). The XPO5 mutant rs11077 increases the risk of renal cell carcinoma (79), is associated with chemotherapy response and survival of patients with advanced non-small-cell lung cancer (24), and is associated with the outcomes of patients with multiple myeloma undergoing autologous stem cell transplantation (99). The discoveries of a mutation in a CRC patient (97) and two CRC cell lines, HCT-15 and DLD-1 (98), with MSI-H imply that the XPO5-inactivating mutant results in pre-miRNA accumulating in the nucleus. The restoration of XPO5 repairs the impaired export and expresses tumor suppressor features (98).
Table 3 | Expression levels of microRNA machinery genes in human tumors

| miRNA machinery gene | Alteration type | Cancer type (reference) |
|----------------------|----------------|------------------------|
| Drosha               | Up-regulation  | BCC (62), SCC (62, 63), smooth muscle neoplasm (64) |
|                      | Down-regulation| Ovarian cancer (65), neuroblastoma (66), endometrial cancer (67), NPC (68), breast cancer (69), gallbladder adenocarcinoma (90) |
| DGCR8                | Up-regulation  | BCC (91), SCC (91), CRC (92), gastrointestinal cancer (93), ovarian cancer (94) |
| XPO5                 | Up-regulation  | Urothelial carcinoma (95), breast cancer (96) |
|                      | Mutant         | Non-small-cell lung cancer (101), renal cell carcinoma (79), CRC (97, 98), multiple myeloma (99) |
| Dicer                | Up-regulation  | SCC (67), prostate cancer (100), smooth muscle neoplasm (104) |
|                      | Down-regulation| Neuroblastoma (68), breast cancer (101), endometrial cancer (67), NPC (68), transitional cell carcinoma (102), gallbladder adenocarcinoma (90) |
| AGO2                 | Up-regulation  | Prostate cancer (100), epithelial skin cancer (91), GC (103), hepatocellular carcinoma (104) |
|                      | Down-regulation| Lung adenocarcinoma (105), melanoma (106) |
|                      | Mutant         | GC (97), CRC (97), breast cancer (107) |
| TRBP                 | Up-regulation  | Prostate cancer (100), diffuse large B-cell lymphoma (108), adrenocortical carcinoma (109) |
|                      | Mutant         | CRC cells (105), endometrial cancer cells (105) |

BCC, basal cell carcinoma; GC, gastric cancer; NPC, nasopharyngeal carcinoma; SCC, squamous cell carcinoma.

Additional analysis of the expression levels of these miRNA machinery genes and alterations and their interactions with their driver genes in tumors could discriminate cancer patients from healthy controls and be associated with the outcomes of cancer patients.

FUTURE PERSPECTIVES
Along with conducting intensive studies of tumor-associated miRNAs and miRNA machinery genes, which play crucial roles in tumorigenesis, scientists are focusing on the miRNA machinery genes Drosha, DGCR8, XPO5, Dicer, AGO2, and TRBP for their potential as cancer biomarkers. The mechanisms involved in miRNA maturation still need to be explored, and new functions of some known genes in miRNA maturation need be uncovered, such as the EGFR gene was induced miRNAs mature as a regulator of AGO2 (71) and ADAR1 formed a complex with Dicer through direct interaction and regulated miRNA processing (122). The dysregulation of miRNA machinery genes (mutation, up-regulation, or down-regulation) can result in oncogenicity and poor patient outcomes. The functions of miRNA machinery genes will be difficult to comprehend because the same gene can have different functions in different types of cancers, and these functions may be not only dosage-dependent but also tissue-dependent (118). Finally, scientists need to explore the different roles of miRNA machinery genes in the physiology and pathology of tumorigenesis. Understanding these roles will help us to use miRNA to develop cancer biomarkers, experimental tools, and antitumor therapy.

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