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Review

REVIEW

Wanted DEAD/H or Alive: Helicases Winding Up in Cancers

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Abstract

Cancer is one of the most studied areas of human biology over the past century. Despite having attracted much attention, hype, and investments, the search to find a cure for cancer remains an uphill battle. Recent discoveries that challenged the central dogma of molecular biology not only further increase the complexity but also demonstrate how various types of noncoding RNAs such as microRNA and long noncoding RNA, as well as their related processes such as RNA editing, are important in regulating gene expression. Parallel to this aspect, an increasing number of reports have focused on a family of proteins known as DEAD/H-box helicases involved in RNA metabolism, regulation of long and short noncoding RNAs, and novel roles as "editing helicases" and their association with cancers. This review summarizes recent findings on the roles of RNA helicases in various cancers, which are broadly classified into adult solid tumors, childhood solid tumors, leukemia, and cancer stem cells. The potential small molecule inhibitors of helicases and their therapeutic value are also discussed. In addition, analyzing next-generation sequencing data obtained from public portals and reviewing existing literature, we provide new insights on the potential of DEAD/H-box helicases to act as pharmacological drug targets in cancers.

Cancer arises from dynamic changes in the genome, exemplified by the discovery of mutations that produce oncogenes with dominant gain of functions and tumor suppressor genes with recessive loss of functions. Genomic alterations in cancer often result in widespread deregulation of gene expression profiles, leading to the production of aberrant proteins and RNA, which disrupt important processes responsible for cellular proliferation, differentiation, and death (1,2). The rationale that numerous RNA processing steps control protein expression underscores the importance of these regulatory roles. In turn, the involvement of RNA molecules is influenced by their ability to form secondary structures and interact with other RNA molecules and proteins. The modulation of RNA structures is performed by a family of proteins known as the RNA helicases (3).

The discovery of the first eukaryotic helicases was a DNA helicase isolated from the lily plant in the 1970s before human

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RNA helicases were discovered a decade later (4). Since their identification in the 1980s, RNA helicases have received substantial attention. They have been widely studied for their biological roles in RNA metabolism and have been recently implicated in cancer research involving noncoding RNAs and cancer stem cells (5–7). RNA helicases are highly conserved enzymes that contain domains regulating their NTPase and helicase activities (8). Based on the sequences and structures, DNA/RNA helicases are classified into six superfamilies with DEAD-box and DEAH-box (DEAD/H-box) helicases representing the two largest groups in the superfamily 2 (SF2) of helicases and characterized by nine conserved motifs: Q, I, Ia, Ib, II, III, IV, V, and VI. Motifs I, II, VI, and Q are needed for ATP binding and hydrolysis, while motifs Ia, Ib, III, IV, and V are involved in intramolecular rearrangements and RNA interactions. Among the nine conserved motifs, motif II (or Walker B motif) contains the distinctive amino acids D-E-A-D (asp-glu-ala-asp) or D-E-A-H (asp-glu-ala-his) (8). There is a total of 59 DEAD/H-box helicases in the superfamily, including 44 DEAD-box and 15 DEAH-box helicases (Supplementary Table 1, available online) (9). These proteins are multifunctional and participate mainly in biological processes involving RNA. These include: pre-mRNA splicing, microRNA (miRNA) processing, RNA export, RNA editing, RNA decay, ribosome biogenesis, transcription, and translational processes (3,8,10). Moreover, novel roles of DEAD/H-box helicases that involve neither RNA metabolism nor RNA itself have been identified recently. They act as transcriptional coregulators and regulators of post-translational modifications and are critical in modulating several cellular signaling pathways (11,12). Therefore, deregulation of functionally diverse DEAD/H-box helicases can have potentially deleterious effects on normal cellular homeostasis and contribute to cancer development and progression (13,14). Here, we summarize the aberrant mechanisms of DEAD/H-box helicases in cancers and discuss their potential as biomarkers for diagnosis and prognosis, or therapeutic modalities (Figure 1).

DEAD/H-Box Helicases in Adult Solid Tumors

Breast Cancer

Many DEAD-box helicases have been associated with breast cancer. DEAD/H-box helicase DDX3 expression was first found to be increased in the breast epithelial cell line MCF10A upon exposure to a cancer-causing agent, benzo(a)pyrene diol epoxide (BPDE) (15). More recently, stable overexpression of DDX3 in MCF10A cells showed an epithelial-mesenchymal transition (16) signature along with an increase in invasiveness and motility (15,17). Furthermore, DDX3 overexpression was shown to

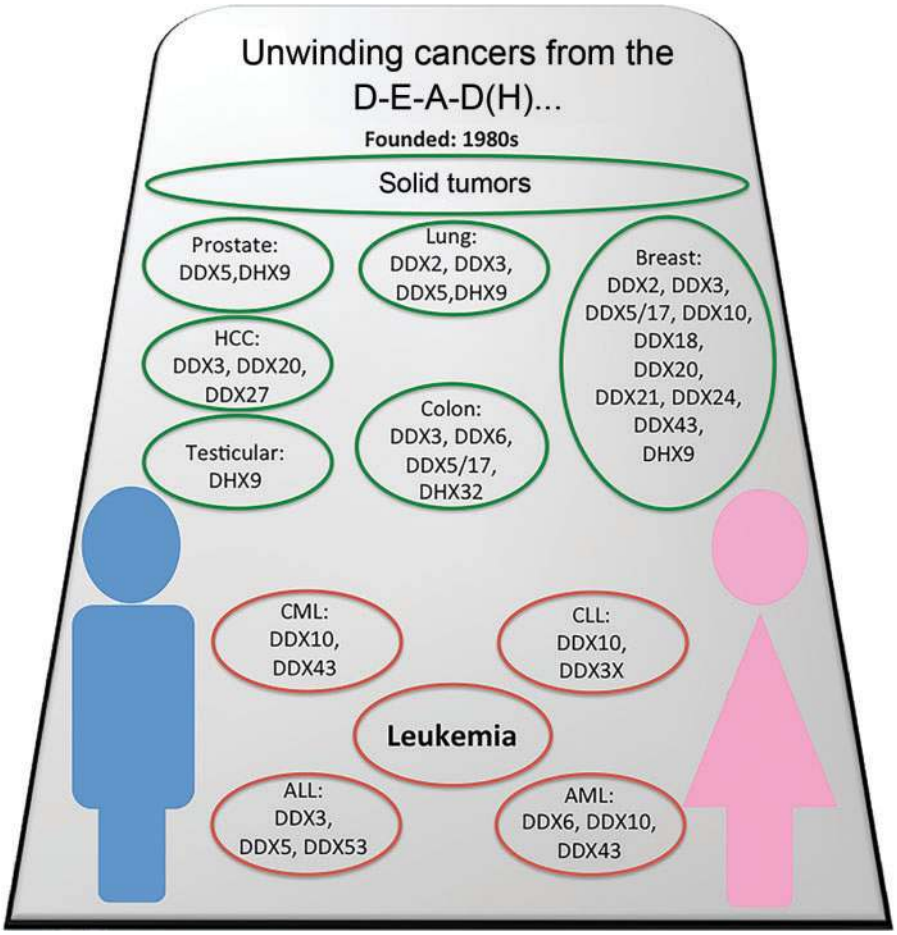


Figure 1. DEAD/H helicases associated with adult cancers. The figure shows a summary of DEAD/H-box helicases reported in various adult cancers, classified into solid tumors and leukemia. The **left side of the solid tumors group** shows DEAD/H members associated with male-dominant cancers (prostate and testicular) while **the right side** shows female-dominant cancer (breast cancer). ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; CLL = chronic lymphoblastic leukemia; CML = chronic myeloid leukemia; HCC = hepatocellular carcinoma.

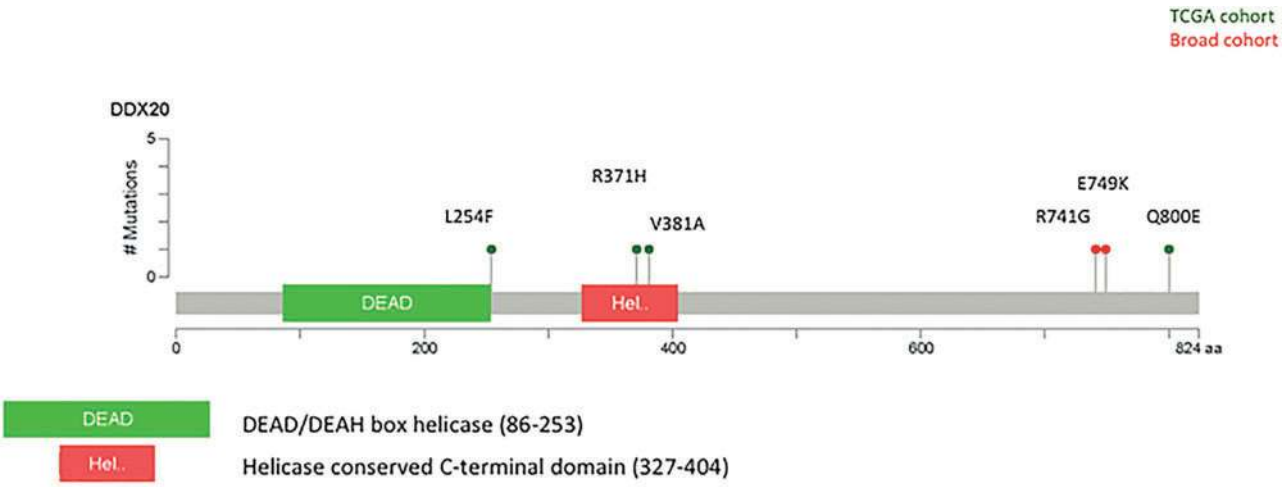
exert anti-apoptotic effects via the intrinsic and extrinsic pathway (18). (Human DDX3 includes two closely related genes, X-linked DDX3X and DDX3Y [19]. DDX3Y is a homologue of DDX3 located on the Y chromosome, sharing 92% sequence identity with DDX3 [20]. Whenever a specific DDX3 isoform is not mentioned in this article, DDX3 refers to both DDX3X and DDX3Y.)

Two paralog DEAD-box RNA helicases, DDX5 (p68) and DDX17 (p72), have been shown to be important in breast cancer development, where they act as transcriptional cofactors to tumor suppressor p53, estrogen receptor alpha (ERα), and β-catenin (21–23). Both helicases contain a consensus SUMOylation site where they undergo post-translational modifications through SUMO attachment and phosphorylation to regulate their co-activation potential (24,25). Dysregulated SUMO modification is common during breast cancer progression, resulting in enhanced cell proliferation and anti-apoptotic signals, suggesting that DDX5 and DDX17 act as potential proto-oncoproteins (26,27). Moreover, it was found that DDX5 upregulated oncomiRs miR-21 and miR-182 in cancers (28–30). DDX17, a SOX2-binding protein, has also been reported to promote stem-like properties in hormone receptor–positive breast cancer (31).

Other DEAD-box RNA helicases associated with breast cancer include DDX10, DDX18, DDX20, DDX21, DDX24, and HAGE (DDX43). Using next-generation sequencing, *DDX10* has been identified as one of the novel genes that has undergone aberrant genomic rearrangements in breast cancer, where downregulation of *DDX10* leads to increased frequency of apoptosis (32). In another study, novel genomic interactions between estrogen receptor (ER), steroid receptor coactivator-1 (SRC-1), and high mobility group box 2 (HMGB2) were enriched in endocrine-resistant breast tumors, resulting in increased DDX18 expression through transcription (33).

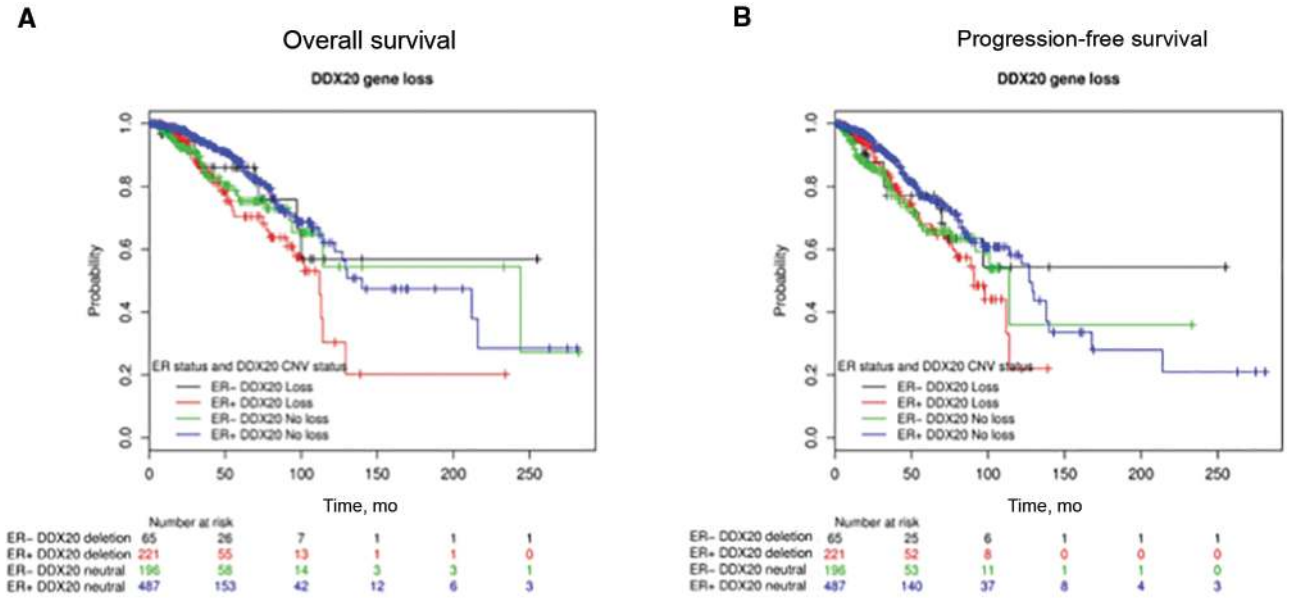
Our recent study revealed the oncogenic potential of elevated DDX20 expression in invasive triple-negative breast cancers by acting as an important cofactor for phosphorylation of TGF-β–activated kinase-1 (TAK1), a key regulator of signal transduction cascades leading to activation of NF-κB pathway (34). Outcomes of increased NF-κB activation include increases in metastasis and chemoresistance through increased expression of matrix metalloproteinase 9 (MMP9) and multidrug resistance gene 1 (MDR1) (34,35). Interestingly, our data analyses using the global The Cancer Genome Atlas and cBioPortal data sets (36,37) revealed genetic aberrations in the *DDX20* gene in multiple breast cancer subtypes (Figure 2). Furthermore, ER+ patients with loss of a single copy of the *DDX20* gene were found to have poorer overall and progression-free survival based on copy number variation (CNV) data obtained from a data set of 1097 patients on GISTIC (Figure 3) (37,38).

More recently, increased DDX21 expression was observed in breast cancer, where it was associated with increased c-Jun activity and rRNA processing, driving breast tumorigenesis (39). Another DEAD-box RNA helicase DDX24 was reported to suppress apoptosis in breast cancers through hindering p300-p53 interaction, hence reducing acetylated-p53, which is required for p53-mediated cell death (40). DDX43 was also recently found to be statistically significantly associated with aggressive clinico-pathological features in breast cancer and increased risk of reoccurrence as well as metastasis (41). Furthermore, in a



Sample	Subtype/ER?	Cohort	AA Change	Type	Copy	COSMIC	Mutation Assessor	#Mutation in sample
BR-M-055	Luminal-B	Breast (Broad)	*R741Gfs*24*	FS del	NA		Medium	91
BR-V-067	HER2	Breast (Broad)	*E749Kfs*16*	FS del	NA		Low	147
TCGA-A8-A06X-01	ER+	Breast (TCGA)	*R371H*	Missense	Diploid	2	High	142
TCGA-D8-A1JA-01	ER-	Breast (TCGA)	*L254F*	Missense	Diploid	1	Medium	694
TCGA-C8-A26Y-01	ER-	Breast (TCGA)	*Q800E*	Missense	ShallowDel	1	Low	519
TCGA-BH-A0HA-01	ER+	Breast (TCGA)	*V381A*	Missense	Diploid	1	High	228

Figure 2. Lollipop plot shows mutation frequency (**y-axis**) within domains DDX20 protein (**x-axis**) in breast cancer. Missense and deletion mutations of DDX20 are observed in breast cancer. The percentage of mutation for the Broad cohort and The Cancer Genome Atlas are 1.9% and 0.4%, respectively. AA = amino acid; COSMIC = The Catalogue Of Somatic Mutations In Cancer; ER = estrogen receptor; TCGA = The Cancer Genome Atlas.



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Samples	Total	DDX20	
		Loss	No loss
All samples	970	287	683
ER+ samples	708	221	487
ER- samples	260	65	195

Figure 3. Gene loss in DDX20 in estrogen receptor (ER)–positive breast cancer patients is associated with poorer survival outcome. Combined Kaplan-Meier plots of ER-positive/negative samples with or without DDX20 deletion. **A)** Overall survival Kaplan-Meier plot. **B)** Progression-free survival Kaplan-Meier plot. **C)** Summary of patients with DDX20 gene loss in all samples, ER+ samples and ER- samples. ER = estrogen receptor.

recent proteomic screen using Isobaric tags for relative and absolute quantitation (iTRAQ), multiple DEAD-box RNA helicases DDX1, DDX19A, DDX39, and DDX42 were found to be overexpressed and identified as potential biomarkers in breast cancer cell lines (42).

Eukaryotic initiation factor 4A or DDX2 is a crucial protein during translational initiation and is commonly overexpressed in several malignancies including breast cancer. The eIF4A is a subunit of the heterotrimeric eIF4F complex, which interacts extensively with other eukaryotic initiation factors (eIFs) for Cap-dependent ribosome recruitment during translational initiation (43). There are three paralogs of eIF4A, eIF4A1 (DDX2A), eIF4A2 (DDX2B), and eIF4A, with eIF4A1 and eIF4A2 implicated in various malignancies (Table 1) (44). eIF4A3 shares only 62% homology with eIF4A1 and eIF4A2 and is functionally distinct from eIF4A1 and eIF4A2. It does not bind to the 40S ribosome for translation initiation, but is involved in exon junction complex formation (45,46). eIF4A1 is most commonly implicated in tumorigenesis, whereby its overexpression predicts poor outcomes in breast cancer (47). Translatome analysis revealed an aberrant increase in the translation of oncogenic targets induced by eIF4A1 overexpression, including components of the oncogenic mitogen-activated protein kinases (MAPK), transforming growth factor beta (TGFβ), and receptor kinase (ERBB2, FGF) signaling pathways (47). Moreover, c-Myc and eIF4F protein complex (of which eIF4A1 protein is a member) have been interlinked in a feed forward loop. eIF4A1 mRNA was found to be regulated by c-Myc transcription factor, which in turn led to increase in c-Myc translation (48). Furthermore, eIF4A1 overexpression has also been reported in endometrial and cervical cancers, extending the importance of the protein in gynecological cancers (49,50). Although eIF4A1 and eIF4A2 share approximately 90% homology, they are functionally distinct, with contrasting functions in cancers (44). Unlike eIF4A1, eIF4A2 has been reported to possess tumor suppressor–like functions with increased expression correlated to improved survival outcome, possibly via eIF4A2-dependent antiproliferative effects through miRNA-mediated gene regulation (51). The involvement of eIF4A isoforms makes them attractive therapeutic targets (52,53) and good prognostic biomarkers to predict survival outcome for these gynecological cancers (44).

Apart from DEAD-box RNA helicases, members of the DEAH-box helicase family have been shown to be involved in breast cancer development. For example, DHX9 functions as a bridging factor that links the C-terminal domain of tumor suppressor BRCA1 to RNA polymerase II (54). Germline mutations of BRCA1 have been found to predispose to breast and ovarian cancers (55,56). Overexpression of BRCA1-binding DHX9 peptide resulted in the inhibition of endogenous BRCA1 function and induced a dominant negative phenotype with a ploidy defect and aberrant cytokinesis (57). The study showed that deregulation of DHX9 expression may affect the tumor

Table 1. Summary of DEAD/H-box specific therapeutics in development

Drug	Target DEAD/H-box helicase	Mode of action	Current status	Reference(s)
Ribavirin 5′-diphosphate/ triphosphate	HCV NTPase/helicase DDX3	ATP competitor (at low concentration) and allosteric inhibitor (at high concentration)	FDA approved	(219,220)
Ring-expanded nucleoside analogues	DDX3	ATPase/helicase inhibitor	Ex vivo culture	(171,174,221)
Hippuristanol	eIF4AI/II/III	Inhibition of RNA binding	Preclinical	(222–225)
Pateamine A (PatA)	eIF4AI/II/III	RNA-mediated sequestration of eIF4A, resulting in inhibition of translational initiation	Preclinical	(226,227)
Hypericin	eIF4AI/II Ded1p	ATPase/helicase inhibitor	Preclinical, tool compound	(228)
Rocaglamides (silvestrol)	eIF4AI/II/III	RNA-mediated sequestration of eIF4A, resulting in inhibition of translational initiation	Preclinical	(229,230)
RK-33	DDX3	Binds DDX3 and impedes helicase activity	Preclinical	(175–177,179,181)
Ketorolac salt	DDX3	Downregulates DDX3 expression andinhibits ATPase activity	Preclinical	(179)
RX-5902	DDX5	Binds p-tyr-593- DDX5 and inhibits ATPase activity	Phase I/II	(185,190–192)

suppressor activity of BRCA1 and highlighted an important role for DHX9 in breast cancer.

Lung Cancer

Lung cancers are divided into two main types: small cell lung cancer (SCLC) and non–small cell lung cancer (NSCLC) (58). Reports on the involvement of DEAD-box RNA helicases in lung cancers has increased recently. DDX3, a DEAD-box helicase reported in a number of viral-associated cancers, has been associated with human papillomavirus (HPV)–mediated lung cancer via the p53 pathway, leading to poor relapse-free survival (59). HPV E6 protein was able to inhibit p53-mediated DDX3 expression in infected cells, resulting in reduced $p21^{WAF/CIP1}$, an important modulator required for cell cycle arrest (59). In addition, loss of DDX3 by p53 inactivation led to increased MDM2/E-cadherin/slug signaling, promoting tumor malignancy and poor treatment outcome in NSCLC (60). Recently, DDX3X has been associated with epidermal growth factor receptor (EGFR)–tyrosine kinase inhibitor (TKI) resistance in lung cancer cells containing EGFR-activating mutations (19). Separately, DDX5 was also shown to play a role in NSCLC via the Wnt/β-catenin signaling pathway (61). This is not surprising as DDX5 is known to act as a transcriptional co-activator within the pathway and is regulated by β-catenin as a novel Wnt target gene (23,62). In NSCLC, eIF2A1 (DDX2) expression has also been associated with distant metastasis, together with a novel noncoding RNA MALAT-1 and thymosin beta-4, making them biomarkers with prognostic and therapeutic value (63).

Using several lung cancer cell lines representing squamous cell lung carcinoma, SCLC, NSCLC, and lung carcinoid, DHX9 was found to bind and interact with the p16INK4α promoter in a sequence-specific manner, leading to expression of the tumor suppressor p16INK4α (64). However, expression analysis of a panel of lung cancer cell lines and primary lung carcinomas revealed elevated DHX9 levels. Moreover, the extent of overexpression was greater in SCLC compared with NSCLC (65). At the genetic level, sequencing of lung cancers in nonsmokers revealed a higher expression of mutated *DHX9* in tumors compared with those without *DHX9* mutations. This upregulation of mutant *DHX9* expression was associated with poor outcome and survival (66).

Colorectal Cancer

Colorectal cancer (CRC) is the third most frequently diagnosed cancer worldwide (67). DEAD/H-box helicase DDX6 was first found overexpressed in colorectal tumors. Subsequent findings showed DDX6 to be co-expressed with *Myc* gene, driving oncogenesis (68). Recent findings discovered that DDX6 maintains the Warburg effect in colon cancer cells, a process frequently hijacked by cancer cells through increasing glycolysis during cellular respiration to promote tumor growth hypoxic conditions (69,70). DDX6 is a direct target of miRNA-124, where miRNA-124 acts as a tumor suppressor, often silenced in colon cancer cells (71). miRNA-124-mediated DDX6 overexpression results in increased c-Myc-dependent glycolysis and polypyrimidine tract-binding protein 1 (PTB1) expression and increased pyruvate kinase in muscle isoforms, giving rise to the Warburg effect (70).

DDX5/DDX17 have also been found to be involved in colorectal cancer development (72,73). At the cellular level, they are reported to be associated with aberrant β-catenin activation and transcription of downstream target genes that drive colorectal oncogenesis (23,74). Recently, DDX5 was discovered to regulate tumor suppressive activity of FOXO3a as a result of increased AKT expression (75). DDX5-NFκB and DDX5-β-catenin complexes are both responsible for regulating AKT expression (75). Reduced FOXO3a activity resulted in increased expression of pro-proliferative and metastatic genes, leading to increased lung metastasis in colorectal allografted mice (75). In another study, a nonsynonymous single-nucleotide polymorphism (SNP) in DDX20, DDX20-rs197412, was found associated with

overall survival in colorectal cancer, while predicted to have no effect on protein function (76,77).

DDX3 is also associated with colorectal cancer as either an oncogene or a tumor suppressor. As an oncogene, DDX3 was shown to promote invasion in colorectal cancer via the CK1ε/Dvl2 axis and by enhancing the transcription of oncogenic KRAS (78,79). Conversely, according to a large cohort survival analysis, DDX3 expression was inversely correlated with clinical outcome in colorectal cancer, and downregulation of DDX3 resulted in cancer progression and metastasis via Snail/E-cadherin, making it a potential prognostic marker (80). Therefore, contrasting roles of DDX3 exist between different cancers and within the same cancer, suggesting that more studies are needed to validate its functions and corresponding mechanisms.

DHX32, a DEAH-box helicase, has been reported to be upregulated in human CRC tissue samples by fluorescent mRNA differential display polymerase chain reaction (PCR) and real-time PCR techniques (81). Further studies pointed DHX32 overexpression in CRC toward a role in positively regulating metastatic and anti-apoptotic gene expression (ie, *WISP1, MMP7, VEGFA, bcl2*, and *CA9*) (82).

Hepatocellular Carcinoma

Human hepatocellular carcinoma (HCC) is a primary malignancy of the liver and the sixth most diagnosed cancer worldwide (83). HCC is the third leading cause of cancer deaths worldwide, with highest incidence in East Asia and sub-Saharan Africa, where hepatitis B and hepatitis C are endemic (84). Chronic infection of both hepatitis viruses has provided strong evidence for chronic liver disease predisposition, and subsequent development of HCC. A regulator of host genes' expression, the hepatitis C virus core protein has been shown to interact with and regulate DDX3 cellular localization, which may be essential for virus-associated HCC development (85). Another study found that DDX3 is downregulated in hepatitis B–positive HCC patients and that DDX3 knockdown led to an increase in cell growth via cyclin D1 upregulation and $p21^{WAF1}$ downregulation (86). Suppressing cancer stem cells via DDX3-dependent miRNAs revealed another tumor-suppressive function in HCC (87). While these studies implied the tumor-suppressive role of DDX3, several other studies have shown the oncogenic potential of DDX3 in other cancers, suggesting that DDX3 has dual roles in different cancers. These various mechanisms of DDX3 reported in cancers suggest its potential as a therapeutic target or a prognostic biomarker.

Downregulated DDX20 expression has been reported in HCC. It is involved in miR-140 processing, an miRNA responsible for suppressing NFκB activity in HCC. Downregulation of DDX20 in HCC resulted in elevated NFkB activity and cancer progression (88,89). DDX27 has also been reported to be statistically significantly overexpressed and may act as a prognostic marker in HCC (90).

Testicular Cancer

Embryonal carcinoma is a germ cell tumor that occurs most commonly in the testes and is classified as a nonseminomatous testicular tumor (91,92). Several studies have demonstrated the involvement of DEAH-box RNA helicase DHX9 in embryonal carcinomas. DHX9 was reported to be essential for the translation of selected mRNAs that contain a structured 5'-terminal post-transcriptional control element (PCE) through recognition and interaction with these mRNAs via the PCE (93). This facilitates polyribosome assembly and promotes translation. Thus, DHX9 serves as an effective mediator for transition from transcription to translation. Interestingly, some of these mRNAs include *JUND* growth control gene transcript, suggesting that DHX9 may play a role in growth, proliferation, senescence, and apoptosis by controlling translation of crucial genes (93). Other reports suggested that DHX9 might act as a translational component of polysomes and interact with oncoproteins such as LIN28 to promote embryonal carcinomas through translation of self-renewal and proliferative proteins such as OCT4, SOX2, and NANOG (94,95).

Prostate Cancer

Apart from breast and colon cancers, DDX5 has been identified as a proto-oncogene in prostate cancer and a novel androgen receptor (AR)–interacting protein (96). DDX5 was found to be a unique AR transcriptional co-activator, whereby overexpression in prostate cancer cell lines regulates the expression of several AR-mediated genes.

Moreover, *DHX9* maps to the major susceptibility locus for prostate cancer at chromosome 1q25, suggesting *DHX9* as a candidate gene involved in prostate cancer (97). Interestingly, *DHX9* is a target gene of SOX4, a developmental transcription factor involved in Wnt signaling regulation, signifying that prostate cancer may be linked to development and DHX9 may play a role in developmental pathways and cancer progression (98,99).

DEAD/H-Box Helicases in Childhood Solid Tumors

Ewing's Sarcoma

Ewing's sarcoma is the second most common bone malignancy in children and adolescents (100,101). Its pathogenesis is driven by potent fusion oncoproteins derived from chromosomal translocations between the *EWS* gene on chromosome 22 and the *FLI1* gene on chromosome 11. The oncoprotein EWS-FLI1 accounts for 80% to 90% of cases in patients with Ewing's sarcoma family of tumors (ESFT) (102). Mutations in the *EWSR1* gene have also been associated with Ewing's sarcoma (103). EWS is a multifunctional protein involved in several processes such as transcriptional co-activation, DNA recombination and splicing, and mRNA transport. Analysis of EWS binding revealed DDX5 and DDX17 (Figure 4) as direct interacting partners (104). DDX5/DDX17 was also shown to colocalize in the nucleus with EWS, whereby EWS links the transcriptional and splicing machineries of gene expression (105–107). In another study, Toretsky et al. reported that DHX9 was found to enhance oncogenic activity of EWS-FLI1 (108). DHX9 was shown to interact with EWS-FLI1 as a transcriptional co-activator and enhance its function in cellular transformation (108). This observation is consistent with findings from another study, which demonstrated a reduction in the growth of Ewing's sarcoma when the binding of EWS-FLI1 and DHX9 was disrupted (109). This indicates a supporting role for DHX9 in the regulation of EWS-FLI1 activity and the subsequent progression of Ewing's sarcoma (Figure 4).

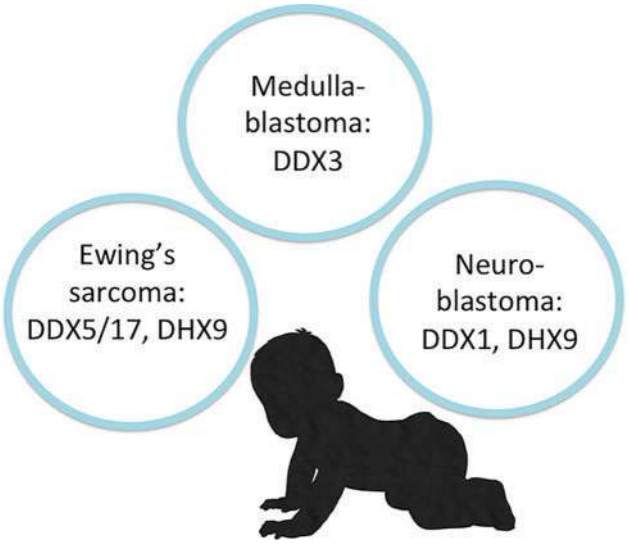

Figure 4. DEAD/H-box helicases associated with childhood cancers. DEAD/H helicases DDX1, DDX3X, DDX5/17, and DHX9 have been associated with Ewing's sarcoma, neuroblastoma, medulloblastoma, and Wilms' tumor in childhood solid tumors.

Neuroblastoma

Neuroblastoma (NB) is an extracranial solid tumor of early childhood and arises from the neural crest. Deletion of the 1p chromosome was associated with overexpression of the *MYCN* oncogene (110). *MYCN* amplification or overexpression is associated with tumorigenesis and is a poor prognostic indicator in NB (110). Intriguingly, *DDX1* was found to be co-amplified with *MYCN* in primary NB cell lines. Recent reports also demonstrated DHX9 to be required for the apoptotic function of tumor suppressor candidate KIF1Bβ in neural crest-derived tumors such as NB (Figure 4). KIF1Bβ was found to interact with DHX9 via calcineurin as an adaptor, promoting the translocation and accumulation of nuclear DHX9, followed by transactivation of its target gene *XAF1* to induce apoptosis (111). Silencing of DHX9 inhibits KIF1Bβ-dependent apoptosis, implicating DHX9 as a necessary downstream effector of KIF1Bβ-mediated apoptosis signaling (111). Furthermore, DHX9 plays a role in miRNA processing such as RNA duplex unwinding, triggering RNA-induced silencing complex (RISC) activation in miRNA pathway (112). Some of these processes are implicated in NB.

In 2012, Kawai and Amano showed that DHX9 was able to interact and colocalize with tumor suppressor BRCA1 to mediate BRCA1-induced miRNA processing (113). DHX9 associated with primary miRNAs (pri-miRNAs) let-7a-1, miR-16-1, miR-145, and miR-34a and was required for BRCA1-induced maturation of these miRNAs via the Drosha microprocessor complex (113). Interestingly, let-7a-1 belongs to the let-7 family, which is repressed in high-risk NBs. miR-16-1 is responsible for targeting oncogenes *BCL2* and *MCL1*, whereas miR-34a, a potential tumor suppressor, resides on chromosomal region 1p36, which is frequently deleted in NB (114–117). This suggests that DHX9 may play an important role in tumor suppression by interfering with miRNA processing and maturation in cooperation with tumor suppressors such as BRCA1. This is in contrast with the function of BRCA1-DHX9 interaction in breast cancer, suggesting that the role of DHX9 in cancers may be function specific and tumor dependent but indispensable.

Medulloblastoma

Medulloblastoma is the most common malignant brain tumor in children, and it has four distinct subtypes. The best-characterized Wnt subtype is driven by a stabilizing mutation in *CTNNB1* (β-catenin) that activates Wnt target genes. Whole-exome sequencing revealed that 50% of the Wnt subtype medulloblastomas consists of concurrent somatic missense mutations in *DDX3* (Figure 4) (118–120). Interestingly, DDX3 was reported to stimulate CK1ε-mediated phosphorylation of Wnt effector disheveled and activate Rac-1 translation to stabilize β-catenin, leading to enhancement of Wnt-β-catenin signaling. This suggests an implication for DDX3X mutations in Wnt subtype medulloblastoma (12,121,122). Moreover, DDX3X mutants were shown to drive the hyperassembly of stress granules in human cells, while others were revealed to have defective RNA-stimulated ATPase ability. These may lead to impairment in mRNA translation of critical cellular regulators and contribute to medulloblastoma development (120–123). Finally, the ability of DDX3X mutants to potentiate the activity of mutated β-catenin in TCF/LEF-responsive reporter assays suggests an oncogenic potential of DDX3X mutations in medulloblastoma (119).

Involvement of DEAD/H-Box Helicases in Leukemia

Leukemia is a progressive, malignant disease of the blood, bone marrow, lymphatic system, and the hematopoietic and lymphoid tissues. Early pathogenesis is characterized by development and increased proliferation of dysfunctional leukocytes, resulting in a suppressed production of normal blood cells. Leukemia may be broadly classified into four main types: acute lymphoblastic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia (Figure 1) (124–127). Other less common clinical presentations of leukemia will not be discussed in this review.

Acute Lymphoblastic Leukemia

Acute lymphoblastic leukemia (ALL) is an aggressive cancer of lymphoid T-cell and B-cell progenitors (124). ALL is characterized by a rapid overproduction of immature lymphoid progenitor cells. The pathophysiology of ALL is similar to other cancers, which include aberrant expression of proto-oncogenes, activating chromosomal translocations and resulting in fusion genes encoding active kinases and altered transcription factors.

Several DEAD-box RNA helicases have been associated with T-cell and B-cell ALL (Figure 1). Recently, DDX3X has been associated with pediatric T-cell ALL. MLLT10-DDX3 gene fusions have been identified whereby the DEAD-box domain of DDX3X is lost (128). Previously, Nucleoporin98–DDX10 (NUP98-DDX10) fusions with activation of chromosomal translocations at position 11p15q22 were also reported (129). Apart from cytogenetic aberrations, DDX5 was found to positively regulate the frequently mutated *NOTCH1* signaling pathway in T-cell ALL (130). Furthermore, DDX53 (CAGE) expression is found to be increased with B- and T-cell ALL with aggressive lymphoma cell lines displaying widespread expression of CAGE (131).

Acute Myeloid Leukemia

Acute myeloid leukemia (AML) is a heterogeneous and aggressive cancer of hematopoietic progenitor cells (125). Similar to

ALL, chromosomal rearrangements have been reported in AML. Numerous reports have shown DDX6 to be associated with chromosomal rearrangements (Figure 1) (132–136). Patients with AML harbor a complex karyotype with three or more acquired chromosomal aberrations, and DDX6 is often one of them (104). In another example, gene arrangements of *NUP98* result in expression of fusion proteins, such as NUP98-DDX10 (129,137). Through double fusion fluorescence in situ hybridization (138), a recent study identified two splicing fusions (type II and type III) involving DDX10 and NUP98 (139). Interestingly, the conserved helicase motif of DDX10 resulted in transformation and transcriptional deregulation of leukemic cells in vitro. Mutations in the ATP-binding and helicase motifs compromised the colony-forming ability and transcriptional regulation of leukemic cells (140). Apart from helicase function, NUP98-DDX10 fusion also affected chromosome region maintenance 1 (CRM1)–dependent nuclear export of transcription factors such as NFAT or NFκB (139). While many of these DEAD/H-box helicases were reported to possess oncogenic roles, a recent report suggested that epigenetic hypomethylation of DDX43 (135) led to a positive prognostic outcome in AML (141). More recently, reports of somatic mutations in DDX41 and other DEAD/H-box proteins in AML unraveled their potential tumor-suppressive roles in myeloid neoplasms (142,143).

Chronic Lymphoblastic Leukemia

Chronic lymphocytic leukemia (CLL) affects immunoglobulin-producing B-lymphocytes (126,144). Cytogenetic aberrations are common in CLL and are found in 80% of cases (145). In a particular study, a *DDX3X* mutation was reported in CLL, although its function remains to be determined (Figure 1) (146). Recently, exome sequencing of an untreated CLL patient's cohort discovered high prevalence of mutations in a group of genes including *DDX3X*, where a recurrent truncated mutation was observed (147,148). In addition, *DDX3X* mutations are also found in batural killer/T-cell lymphoma, a cancer prevalent in Asian and South American populations (148). Similar to CLL patients, recurrent *DDX3X* mutations have also been reported that resulted in activation of the NFκB and MAPK cell cycle pathways (148).

Chronic Myeloid Leukemia

Chronic myeloid leukemia (CML) affects the myelogenous precursors, specifically the granulocytes (127). The molecular pathogenesis of CML has been well characterized through confirmation of cytogenetic aberrations at the Philadelphia chromosome, resulting in translocation of chromosomes 9 and 11 (t(9;22)(q34;q11)), although the exact mechanisms remain unclear. Among the gene fusions in the Philadelphia chromosome, the *DDX10-NUP90* gene fusion was found to be located in positions where translocations take place, resulting in the development of CML (149,150). More recently, frequent epigenetic deregulation of DDX43 has also been reported in Philadelphia-positive CML, whereby hypomethylation resulted in DDX43 overexpression (151). Subsequently, this led to poor prognosis and treatment resistance against tyrosine kinase inhibitor imatinib (152).

DEAD/H-Box Helicases in Cancer Stem Cells: Unwinding the Root of Cancer

Cancer stem cells (CSCs) are a group of de-differentiated, self-initiating, and neoplastic cells within the tumor. Recently, CSCs have emerged as a hallmark of cancer and been deemed a promising target for next-generation cancer therapeutics (153). Over the past decade, rapid advances in research have provided increasing evidence of CSCs in solid tumors of the breast, brain, prostate, ovary, colon, liver, lung, and pancreas (154,155). However, because of the heterogeneity of CSCs and their ability to de-differentiate to drive invasion in tumors, targeted therapy remains a challenge. More recently, CSCs have also been associated with tumor resistance, further complicating the targeting of this subpopulation of cells. Interestingly, miRNAs have been reported as novel regulators of stemness in CSCs, where several DEAD-box RNA helicases may be involved (156).

CSCs were first identified in AML, where increased expression of NUP98-DDX10 fusion oncoprotein was reported to dramatically increase proliferation and self-renewal of $CD34^+$ leukemic CSCs (140). Separately, DDX4 was recently found to be a potential stem cell marker for CSCs. Its expression correlated and colocalized with CD133, a stem cell marker in ovarian CSCs. Expression of both CD133 and DDX4 was found to be statistically significantly higher in ovarian cancer cells compared with benign tumors and non-CD133-expressing cells (6). With chemoresistance being a common feature in CSCs, Single-nucleotide polymorphisms (SNPs) in DDX5 have been associated with platinum resistance in ovarian cancer (157). In another study, multiple genomic analyses revealed DDX27 overexpression to be associated with resistance to epirubicin-induced DNA damage and apoptosis in gastric tumors (158). In addition, DDX1 is also linked to testicular cancer through partial transcriptional activation of stem cell genes at chromosome 12p (159). Furthermore, DDX3X and DDX3Y were reported to function as immunogenic antigens in $CD133^+$ CSCs, promoting them as promising immunotherapeutic targets for treatment against CSCs in solid tumors and leukemic stem cells (20,160).

Disruption in miRNA processing has been well associated with cancer and, more recently, reported to be involved in CSC regulation. DICER1 is an important rate-limiting enzyme during miRNA processing. Intriguingly, deletion of *DICER1* led to an increase in CSC-associated stem cell markers CD133, CD166, and CD44 in colon cancer (161). In this regard, aberrant DEAD-box RNA helicase-dependent miRNA processing is involved in various cancers (29). Hence, it is not surprising to find several DEAD-box RNA helicases to be associated with CSCs such as DDX43 (134), which promotes melanoma-initiating tumor cells by inhibiting interferon-α (IFN-α)-induced promyelotic leukemia protein expression (162).

The role of CSCs highlights an exciting area of research in the search for novel potential therapeutics for cancer and regenerative medicine. With increasing knowledge of the roles of noncoding RNAs (eg, miRNA) in CSCs, the importance of DEAD-box RNA helicases and their roles in RNA metabolism in various cancers becomes ever more critical. As we gain a better understanding of CSCs, RNA helicases present themselves as a potential biomarker for cancer therapy and immunotherapy, with the hope of breaking the Achilles heel of tumor-initiating and chemo-resistant CSCs.

Therapeutic Potential of Targeting DEAD/H-Box Helicases in Cancer

The involvement of DEAD/H-box helicases in cancers has led to the discovery of small molecules targeting this family of proteins (163,164). Numerous therapeutic strategies targeting DEAD/H-box helicases have shown to possess antineoplastic

capabilities such as inhibitors against NTPase activity (eg, nucleoside analogues), small molecules affecting RNA binding and helicase activity, and compounds that disrupt specific protein-protein interactions (Table 1) (8,163,165).

Human DEAD/H-box helicases were first identified as antiviral therapeutic targets as many DEAD/H-box helicases' functions have been hijacked at the host-viral interface during viral replication (166). Human immunodeficiency virus (HIV), for example, has been reported to require the involvement of multiple cellular helicases through diverse mechanisms for its replication (167,168). Human DDX1, DDX3, DDX24, and DHX9 DEAD/H-box helicases have all been linked to HIV virus replication (168,169). DDX3 in particular has been found to be an important human host factor for the replication and pathogenesis of multiple viruses, including HIV-1, hepatitis C virus, Dengue virus, and West Nile virus (170). The success of current inhibitors against DDX3 for the treatment of HIV-1 (maraviroc) and hepatitis C infection (alisporivir, phase III trial) demonstrated promising potential for targeting DDX3 in cancers (170–173). In one study, Radi et al. identified small molecule inhibitors targeting the RNA-binding domain of DDX3. Some of these inhibited ATPase and helicase activity and reduced the viral load (174).

The involvement of DDX3 in multiple cancers also makes it a valuable target for the development of DDX3-specific therapeutics for effective cancer treatment (170–173). Apart from targeting viruses, current knowledge of DDX3 inhibitors has been extended to DDX3 targeting in cancer and novel DDX3-specific compounds have been developed (175–180). DDX3 inhibitor RK-33, characterized by Bol et al., has been shown to be effective against several cancers including lung, colorectal, and Ewing's Sarcoma (176,177,179,181). More recently, RK-33 was used for PLGA nanoparticle formulation for improved efficacy (179). Separately, Samal et al. discovered Ketorolac salt as a novel DDX3 inhibitor for oral cancers (178). While targeting DDX3 has shown promise in treating cancers, the contrasting dual roles of DDX3 as an oncogene and tumor suppressor, reported in some cancers, should be considered when treating cancers with DDX3 inhibitors on these cancers (182).

DDX2 or eIF4A has been extensively studied and identified as a potential druggable target for cancers because of its involvement as an important factor in onco-protein synthesis from multiple deregulated signaling pathways through translation. Translational deregulation in cancers has made DDX2 an attractive target for inhibition with the aim of decreasing oncogenic proteins' expression. Several synthetic and natural inhibitors targeting DDX2 are being developed and have shown promise in antitumorigenic effects by inhibiting translational initiation. These include hisppuristanol, silvestrol, and pateamine A. Furthermore, transcriptome-wide analysis of triple-negative breast cancer cells after silvestrol treatment revealed high plasticity in the translational profile of these cells with drastic changes in 284 gene expression, with mainly reduction in cell cycle progression and mitogenic-associated signaling pathways (183). A detailed description of DEAD/H-box helicase inhibitors is summarized in Table 1 (184).

In another study, DDX5, a helicase frequently deregulated and mutated in cancers, has also been identified as a potential candidate for cancer therapeutics (Figure 5) (165,185,186). Post-translational modification of DDX5 by phosphorylation at tyrosine-593 residue was previously identified only in transformed cancer cells, but not in normal cells. Phosphorylated DDX5 (p-DDX5) is found to mediate epithelial-mesenchymal transition by displacing Axin from β-catenin, activating the pathway and increasing transcription of downstream oncogenic Wnt target genes (23,74). p-DDX5 has also been found to regulate EMT epigenetically by facilitating HDAC1 dissociation from the Snail1 promoter (187). In another study, phosphorylation of DDX5 at two tyrosine sites (including Y593) in glioblastoma was

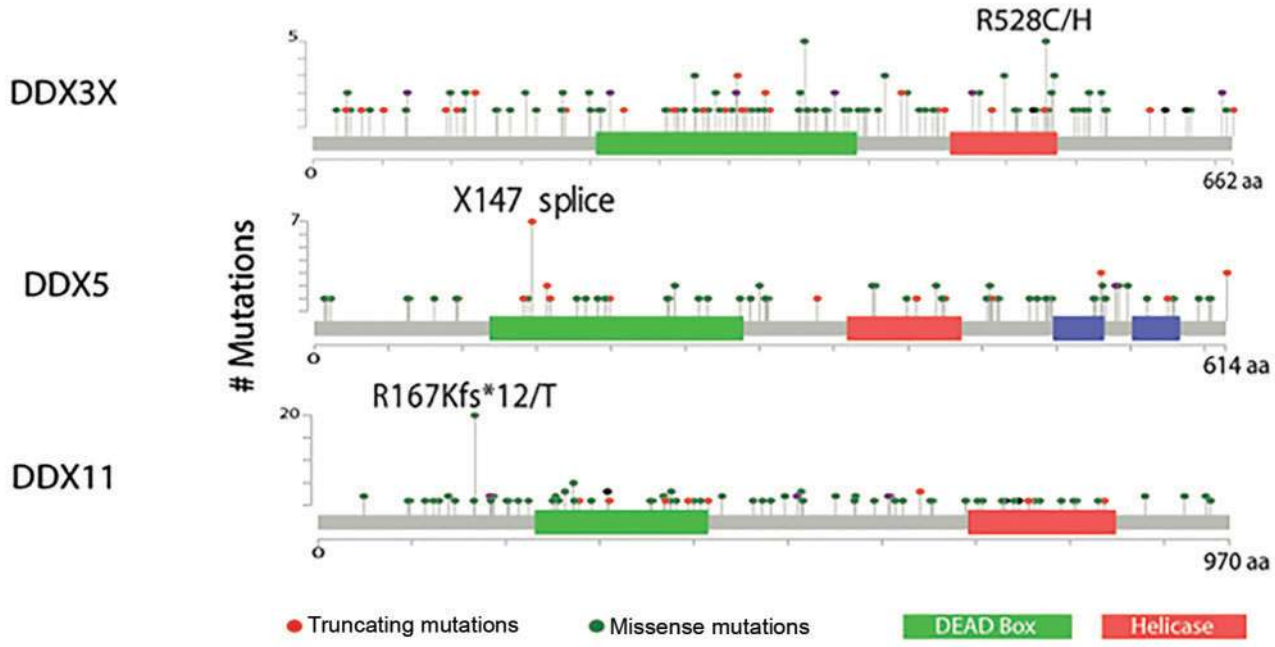

Figure 5. Lollipop plot of selected DEAD/H-box helicases with hotspot mutations. Multiple mutations are observed across DEAD/H helicase genes DDX3X, DDX5, and DDX11. The position(s) of high-frequency hotspot mutation(s) in each gene are indicated.

REVIEW

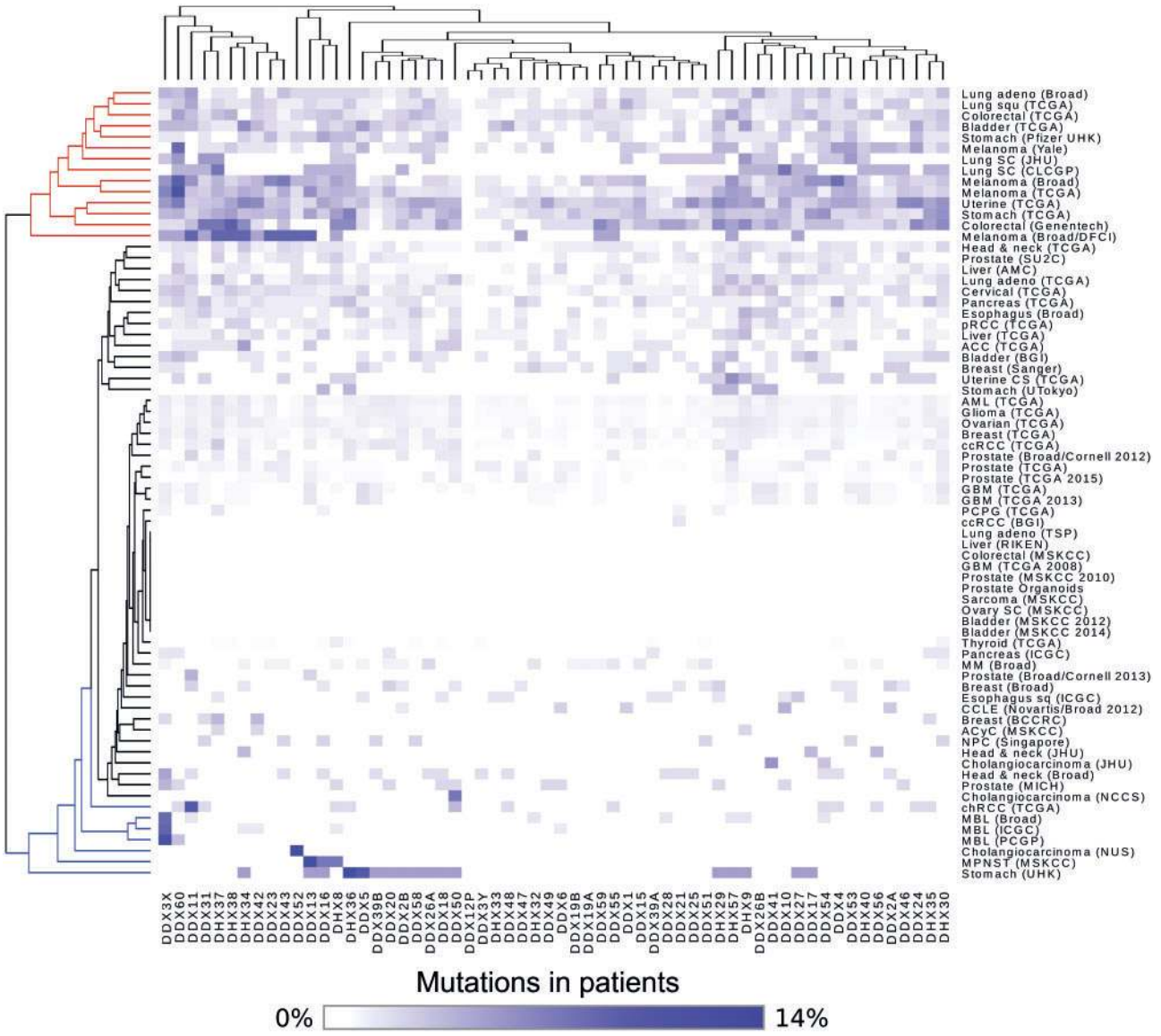

Figure 6. Heatmap shows frequency of mutations for DEAD/H-box helicases in various cancer types. The **right vertical (y-) axis** shows the list of cancer types with DDX/DHX mutations, and the **bottom (x-) axis** shows a list of DEAD/H helicases members from the superfamily. The frequency of mutations is denoted by **shades of purple**, with intensity correlating with frequency. The **red lines** indicate a high frequency of mutations in a particular subgroup of cancers, while the **blue lines** indicate a low frequency of mutations in another subgroup of cancers.

found to confer resistance to TRAIL-induced cell death (188). The increased knowledge of the roles played by p-DDX5 in cancer progression has led to the recent development of a novel, first-in-class, p-DDX5-specific anticancer molecule RX-5902 or Supinoxin (185). RX-5902 binds directly and specifically to p-DDX5 in cancer cells and inhibits β-catenin-dependent ATPase activity (185,189). Since p-DDX5 is only expressed in cancer cells, RX-5902 serves as a useful inhibitor, which could minimize adverse side effects to normal tissues harboring normal DDX5 expression. RX-5902 is currently in phase I clinical trials for numerous cancer types (https://clinicaltrials.gov/ct2/show/NCT02003092) (187,190–192).

Finally, telomerase reverse transcriptase (hTERT) and telomerase RNA component (hTERC) were found reactivated and overexpressed in more than 90% of advanced human cancers (193–195). DEAD/H-box helicases were shown to be associated with telomerase in normal physiology and cancers such as DHX36 (or RHAU), which was reported to interact with a novel motif at the 5'guanosine end of human telomerase RNA, forming a G-quadruplex required for stimulating telomerase-mediated telomere maintenance (196–199). Specifically, DDX39 was identified as interacting with telomeric repeat binding factor 2 (TRF2) and directly affecting telomere length in cancer cells (204). Furthermore, recent developments on the nontelomeric roles of hTERT have demonstrated its involvement in multiple oncogenic signaling pathways. This raises the possibility of helicases being targetable molecules in cancer treatment (197,205).

Conclusions and Future Perspectives

DEAD/H-box helicases have been increasingly associated with multiple cancers in recent years. Numerous studies have demonstrated altered DEAD/H-box helicases expression in cancers,

in some cases impacting the translation of onco-proteins to drive cancer progression (14). Recent advances in high-throughput next-generation sequencing technology such as exome and whole-genome sequencing have opened new avenues in studying gene mutations in an unbiased manner. Indeed, the latest data sets released by worldwide consortia such as The Cancer Genome Atlas (http://cancergenome.nih.gov/) brought to light numerous mutations in DEAD/H-box helicase genes across various cancers such as lung, colorectal, stomach, and melanoma (Figure 6) (36,37,202). In contrast, medulloblastoma harbored only few mutations in DEAD/H-box helicase genes, with accentuated mutations occurring in *DDX3X*, *DDX11*, *DHX36*, and *DDX5* (Figure 6) (118,119,203). Frequent *DDX3X* mutations in medullablastoma were found in a specific Wnt subgroup (Figure 6) (118). Of note are the hotspot mutations identified in several DEAD/H-box genes such as *DDX3X* (R528C/H), *DDX11* (R167Kfs*12/T), and *DDX5* (X147_splice) (Figure 5). The rapid study of the cancer genome of medulloblastoma patients' tumor samples through high-throughput next-generation sequencing techniques has allowed specific molecular classification of medulloblastoma, leading to the discovery and administration of specific targeted treatment options for these patients, who are mostly young pediatric patients (204).

Furthermore, preliminary transcriptome-wide RNA analysis of immortalized cancer cell lines by enhanced UV crosslinking and immunoprecipitation sequencing (eCLIP-seq) from the ENCODE consortium (https://www.encodeproject.org) revealed DEAD/H-box genes *DDX3X* (ENCODE experiment ENCSR930BZL) and *DDX42* (ENCODE experiment ENCSR576SHT) as novel RNA-binding protein (RBP) genes, suggesting a new unexplored layer of gene regulation mechanism by the helicases in regulating cancer progression (https://www.encodeproject.org) (205). Indeed, similar to DEAD/H-box helicases proteins, numerous RBPs are already known to be involved in RNA metabolism in cancers (206–208). In addition, DEAD/H-box helicases RBPs might play a role in RNA regulation, RNA processing, or RNA editing. Therefore, the study of alterations in RNA-DEAD/H-box helicases protein interactions would bring new insights to novel disease prognosis biomarkers and treatment strategies (209,210). The recent discovery of noncoding RNA mechanisms such as RNA editing, miRNA processing, and the existence of large long noncoding RNAs (lncRNAs), have added further insight to the potential involvement of DEAD/H-box helicases in these RNA-related processes that regulate key cancer pathways (29,211–214).

Finally, extensive post-translational modifications (PTMs) undergone by members of the DEAD/H-box helicase family have been important in determining the binding partners of these DEAD/H-box proteins and regulating oncogenic activity in various signaling pathways, distinguishing a new strategy of targeting these post-translationally modified DEAD/H-box proteins in cancer (23,74,185). The success of RX-5902 in specifically binding and inhibiting phosphorylated DDX5 in various cancers provided "proof of concept" evidence for this alternative treatment strategy (215). With the availability of proteomics technologies such as Stable Isotope Labeling with Amino Acids in cell culture (SILAC), large-scale proteomic changes in terms of protein-protein interactions and post-translational modications may be analyzed to complement the drug discovery process (216–218).

Together, these studies highlight the value of analyzing DEAD/H-box helicases. Differential expression, post-translational modification, and the roles of DEAD/H-box helicases in noncoding RNA processes in cancer not only make them attractive therapeutic targets but also potential biomarkers for diagnosis, prognosis, and treatment response when specific mechanisms for molecular targeting are not known. With the development of high-throughput sequencing, transcriptomics, proteomics, and drug screening technologies, the value in studying and understanding the involvement of DEAD/H-box helicases in cancer becomes ever more important as it may be the key to "unwinding" cancers in the near future.

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