[Frontiers in Bioscience, Landmark, 21, 225-250, January 1, 2016]

DEAD box RNA helicases: crucial regulators of gene expression and oncogenesis

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1. ABSTRACT

DEAD box protein family of RNA helicases are vital players of RNA metabolism, and constitute the largest family of RNA helicases. Members of this family share nine conserved motifs including an Asp-Glu-Ala-Asp motif, giving this family its characteristic name as DEAD box RNA helicases. These conserved motifs

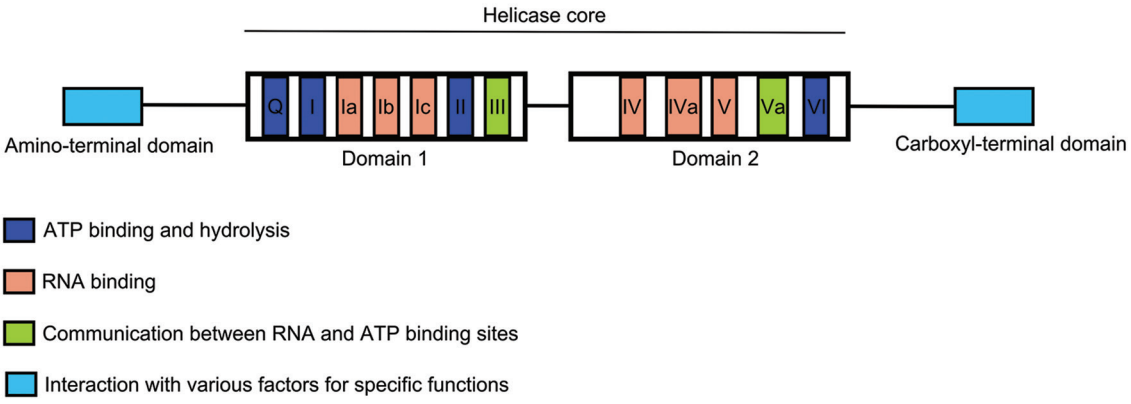

Figure 1. Schematic representation of structure of DEAD box RNA helicases. The helicase core of DEAD box proteins consists of two RecA-like domains, comprising of twelve conserved motifs which functions in ATP binding and hydrolysis, RNA binding, and communication between ATP and RNA binding sites. Additionally, DEAD box proteins also contain ancillary amino-terminal domain and carboxyl-terminal domain which provides specificity of function through interaction with other factors.

confer RNA binding and RNA unwinding properties. Besides functioning in RNA metabolism, emerging evidences suggests several DEAD box RNA helicases to possess potential roles in regulating gene expression by acting as a transcriptional co-activator. Many of them are deregulated in cancers, and are implicated in possessing oncogenic potential. On the contrary, each of them also possesses tumor suppressive property in a context dependent manner. In this review, we discuss the mechanistic insights of gene regulation by DEAD box RNA helicases, and their significance in cancers.

2. INTRODUCTION

The genomic instability is a hallmark of cancer that involves gradual accumulation of genetic alterations leading to aberrations of gene expression programs; it contributes largely to oncogenesis (1). The gene expression programs that maintain cellular homeostasis are controlled by myriads of transcription factors, co-factors, chromatin regulators, and non-coding RNAs such as siRNAs and miRNAs. Defects in these are frequent in cancers. Emerging evidences indicate that overexpressed oncogenic transcription factors can alter the core autoregulatory circuitry of the cell (2). RNA helicases are essential regulator of gene expression, exerting its effect in almost every aspects of RNA metabolism.

RNA helicases are present in almost all organisms. These enzymes unwind double-stranded RNA molecules using energy derived from hydrolysis of NTP. RNA helicases functions in large complexes of ribosome or spliceosome. However, purified RNA helicases demonstrate poor activity compared to when it is present in the complex. This is due to interaction with protein factors present in the complexes that regulate its activity (3). Helicases in general are classified into three superfamilies and two families (SF1 to SF5), based on the presence of characteristics of conserved motifs. The DEAD box along with DEAH, DExH and DExD families are commonly referred to as the DExD/H helicase family, and belongs to SF2 (3-6). The DEAD box family is the largest family of RNA helicases characterized by the presence of about twelve conserved motifs (5-7). They were first identified and reported as a distinct family in 1989 when protein sequence alignments of eight homologues of the yeast eIF4A translation initiation factor showed the presence of several conserved motifs (8). The name was derived from the amino-acid sequence D-E-A-D (Asp–Glu–Ala–Asp) of its motif II (3).

DEAD box RNA helicases are key components of life, and are essential for RNA metabolism. Besides, emanating evidences suggest that many DEAD box RNA helicases possess multiple functions and plays significant roles in transcriptional regulation of gene expression. This is mostly attributed to them as they function as co-activator or co-repressor by their interaction with other factors which are crucial in transcriptional regulation of gene expression. There are numerous studies which links DEAD box proteins to oncogenesis, its deregulations, and ensuing altered gene expressions of crucial oncogenic players. This review presents current evidences of gene regulation by multi-talented DEAD box proteins, in the context of cancer.

3. STRUCTURAL ORAGANIZATION OF DEAD BOX RNA HELICASES

SF2 helicases including DEAD box proteins are comprised of two covalently linked identical globular domains; each contains five beta-strands surrounded by five alpha-helices, resembling bacterial RecA. The two domains (Domain 1 and Domain 2) form the helicase core comprising of at least twelve conserved motifs, serving binding sites for ATP and RNA (Figure 1). Domain

1 and 2 form a cleft between them that harbors the ATP-binding site, and the cleft must be closed to effectively bind and hydrolyse ATP; RNA binds opposite to that of the ATP-binding site. Domain 1 contains the ATP binding and hydrolyzing motifs Q, I and II, the RNA-binding motifs Ia, Ib and Ic, and motif III which coordinates between ATP and RNA binding sites. Motif II includes the D-E-AD sequence (6,7,9). The Q motif and upstream conserved phenylalanine are characteristic and specific features of DEAD box proteins (10). The Q motif serves as adenine recognition motif and regulate ATP binding and hydrolysis. The Q-motif was also reported to affect the helicase activity through regulating the affinity between the proteins with RNA substrates (11,12). Domain 2 contains the RNA-binding motifs IV, IVa and V, motif VI which serves for ATP binding and hydrolysis, and motif Va, which may coordinate ATPase and unwinding activities. An additional beta-strand and two alpha-helices, which are located upstream of motif I form a cap like structure on top of domain 1. In addition to the RecA-like domains, DEAD box proteins have ancillary amino-terminal domain and carboxyl-terminal domains which confers to the diverse functions by allowing interactions with other protein and RNAs (6,7).

4. FUNCTIONS OF DEAD BOX RNA HELICASES

4.1. Basic biochemical properties

4.1.1. ATPase activity

DEAD box RNA helicases possess an ATPase activity; facilitated by RNA. Specific RNA substrate is not required for the stimulation of ATPase activity (6,7,13,14).

4.1.2. RNA binding

DEAD box proteins bind RNA through interactions with the 2′ hydroxyl group. The binding of RNA is stronger in the presence of ATP, but it is drastically reduced upon binding of ADP. The helicase core does not provide substrate specificity. The substrate specificity is likely to be attributed by the ancillary amino-terminal and carboxyl-terminal domains, and extensive interactions with other factors (6,7).

4.1.3. RNA unwinding

RNA unwinding mechanism of DEAD box proteins is unique. Unlike other helicases, they do not employ translocation; rather they deploy local strand separation by a tethering interaction that holds the helicase core in proximity to targeted RNA duplexes. DEAD box helicases binds to the duplex region of RNA, aided by single-stranded or structured nucleic acid regions. These regions needs to be proximal but do not require to be covalently bonded to the helix. Multiple protomers of the protein, or a single helicase possessing accessory domains might be involved while binding. Binding can take place at an end of the RNA, internally, and on either strand. Processivity of DEAD box proteins is low; unwinding becomes ineffective when the length of the helix reaches above 10 to 15 base pairs. Also, efficiency of unwinding is strongly dependent not only on the length but also on the stability of the helix.

Binding of a DEAD box protein requires ATP but not necessarily ATP hydrolysis. Unwinding occurs as long as ATP is bound in the active site, regardless of hydrolysis. However, ATP hydrolysis results in the formation of ADP which promotes the release of the DEAD box protein from the RNA; this step is important for the turnover of multiple RNA substrates. ATPase cycle consists of ATP binding, ATP hydrolysis, strand separation, generation of a segment of single-stranded RNA, and finally release single-stranded RNA from the helicase core. For each unwinding event, a single ATP molecule is involved. Events in the ATPase cycle varies for different DEAD box proteins; unwinding may be coupled to ATP binding in some cases and to ATP hydrolysis in others. As discussed earlier, the two RecA-like domains in the helicase core form a cleft which binds ATP and RNA on opposite sides. But there exists a close energetic flow between them, and each ligand strengthens the binding of the other. This cooperative effect is reversed in the presence of ADP (7,13-15).

4.1.4. RNA clamping

The tethering of DEAD box proteins with the RNA could remain intact through prolonged time period, enabling to unwind the same structure repeatedly or facilitate additional structure disruptions of intermediates (7).

4.1.5. Displacement of proteins from RNA

DEAD box proteins can also remove proteins from RNA in an ATP dependent manner but independent of unwinding. As for example, removal of yeast Mud2 by the DEAD box protein Sub2 during pre-mRNA splicing, and the removal of mRNA export factor Mex67 during mRNA export by DEAD box protein Dbp5 (47–52). Protein displacement is crucial for the physiological function of RNA helicases as RNAs are generally bound to other proteins *in vivo* (7,16).

4.1.6. Strand annealing activity

Several DEAD box proteins also demonstrate strand annealing activity. Ded1 which functions in cytoplasmic translation and Mss116 which is important for mitochondrial RNA metabolism are potent strand annealers. The annealing activity is mostly ATP independent. This trait of DEAD box proteins is crucial in RNA remodelling reactions, and RNA chaperoning (7,13,14).

4.1.7. RNA chaperone activity

DEAD box helicases also acts as RNA chaperones by promoting folding of RNA. For example, Mss116 and CYT19, which act as RNA chaperones

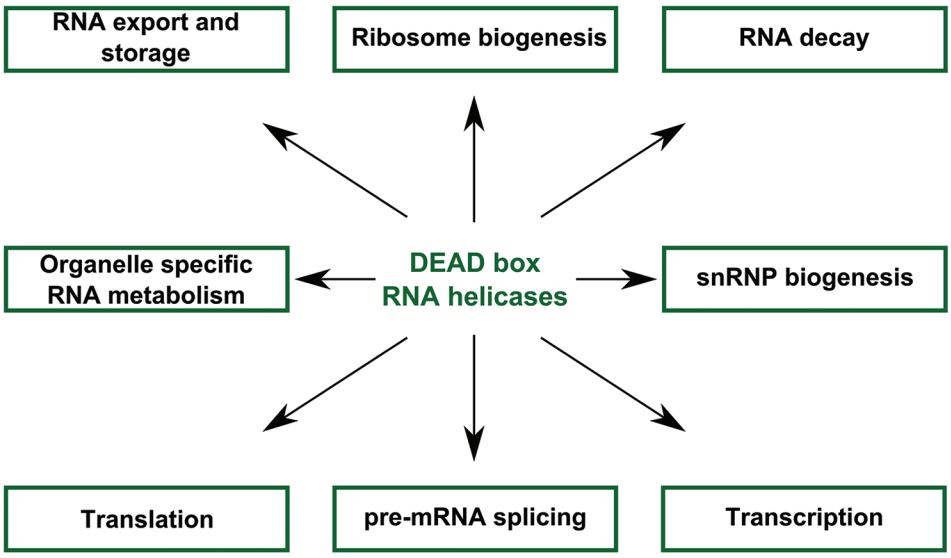

Figure 2. Physiological roles of DEAD box RNA helicases. General functions of DEAD box RNA helicases includes ribosome biogenesis, RNA export, RNA decay, RNA storage, pre-mRNA splicing, snRNP biogenesis, organelle specific RNA metabolism, translation, and in general transcription machinery as well as specific transcriptional regulation of gene expression.

Table 1. DEAD box RNA helicases in various cellular functions (7)

Cellular functions	DEAD box RNA helicases
Ribosome biogenesis	DDX3X, DDX3Y, DDX5, DDX10, DDX18, DDX21, DDX24, DDX27, DDX31, DDX47, DDX48, DDX49, DDX50, DDX51, DDX52, DDX54, DDX55, DDX56
RNA decay	DDX5, DDX6, DDX17, DDX48
Organelle specific RNA metabolism	DDX28
snRNP biogenesis	DDX20
Transcription	DDX5, DDX17, DDX20, DDX21, DDX3
pre-mRNA splicing	DDX3X, DDX3Y, DDX23, UAP56, DDX42, DDX46
Translation	DDX2A, DDX2B, DDX3X, DDX3Y, DDX4, DDX19
RNA storage	DDX6
RNA export	DDX3X, DDX3Y, DDX5, DDX17, DDX19, DDX25, DDX39, UAP56

promotes folding of several mitochondrial RNA introns into their native conformations (7,14,16).

4.1.8. Proofreading activity

DEAD box helicases also act as proofreaders in splicing events. They distinguish between correct and incorrect substrates, and promote processing of correct ones while discarding of incorrect ones (13).

4.2. Cellular functions

In cells, DEAD box RNA helicases plays vital roles in every aspects of RNA metabolism (Figure 2). Most of them perform specialized functions that require them to act on specific RNAs; some participate solely in a single process. Table 1 lists various DEAD box RNA helicases required for specific cellular functions. The specificity for the targets is achieved through interactions either through specific loops or surfaces within the helicase core, or by the ancillary domains adjacent to the helicase core in many DEAD box proteins.

4.2.1. Ribosome biogenesis

A large number of DEAD box proteins are associated with the maturation of rRNA. They are required for the unwinding of short duplexes of snoRNA with rRNAs, or between rRNAs. Also, some of them are required for the dissociation of RNA–protein interactions (6,7,17).

4.2.1. pre-mRNA splicing

Newly transcribed pre-mRNAs are spliced to create mature mRNAs. Splicing requires trans-esterification reactions and structural rearrangements in the stepwise assembly of the large ribonucleoprotein complex known as spliceosome which involves five snRNAs and several proteins including DEAD box helicases. Several DEAD box proteins play vital functions in splicing, majorly at the early events of pre-spliceosome assembly, and the formation of active spliceosome. They are required for the unwinding of short RNA–RNA duplexes that are formed between the different snRNAs or pre-mRNA molecules (6,7,17).

4.2.2. RNA export

Cellular compartmentalization in cells requires regulated transport of RNAs, proteins and complexes

from the nucleus to the cytoplasm, and vice versa. Several DEAD box proteins are involved in this process. Notably, Dbp5 is required for the export of mRNA from the nucleus (6,7,17).

4.2.3. Translation

Yeast DEAD box proteins DDX2A/eIF4A and Ded1, and their homologues in higher eukaryotes are essential for translation initiation. eIF4A rearranges RNA duplexes at the 5′ end of eukaryotic mRNA. It also removes proteins from mRNA, after exiting the nucleus. In higher eukaryotes, DEAD box protein Vasa is required for translational activation of germline-specific mRNAs (6,7,17).

4.2.4. Organelle gene expression

DEAD-box proteins are also required for the expression of mitochondrial genomes in fungi. As for example, *Neurospora crassa* Cyt-19 RNA helicase is required for the splicing of group I introns. Recent reports also suggests that DDX28 resides in RNA granules, and function in assembly of mitochondrial ribosomes (mitoribosomes) (7,17,18).

4.2.5. RNA decay

mRNA turnover is initiated with shortening of the 3′ poly (A) tail followed by decapping complex and 5′–3′ exonucleolytic decay. In mammalian cells, proteins involved in mRNA silencing and degradation localize to specific cytoplasmic foci called processing or P-bodies. DDX6/p54 present in the P-bodies activates decapping by remodelling mRNPs. They are also required for the accumulation of the mRNA degradation factors in P-bodies (19-21). Spliced mRNAs harboring a premature termination codon (PTC) are degraded by nonsense-mediated decay (NMD). In mammals, the termination codon is located at least 50–55 nucleotides upstream of an exon–exon junction. Some mRNAs also possesses uORFs or alternative splicing introducing non-sense codons or frameshifts, are also targeted to NMD as well. Degradation is triggered by the exon junction complex (EJC) through recruitment of upstream frame shifting (Upf) proteins forming Upf complex (22). Several DEAD box proteins are part of NMD. DEAD box protein DDX48/eIF4AIII is an integral part of EJC (22). DDX5/p68 and its homologous protein DDX17/p72 also participates in NMD by binding to the Upf complex (7,23). In *Escherichia coli*, RhlB protein forms a complex with RNase E and PNPase, and unwinds RNA for degradation (17).

4.2.6. RNA storage

In the P-bodies untranslated mRNAs are stored for later release. DDX6/p54 is abundant in P-bodies. After formation of translational repressor complex, DDX6 catalyzes ATP-dependent mRNA unwinding which leads to sequence-independent association with multiple DDX6 molecules. Thereafter, the silenced mRNP is localized to P-bodies and the transcript is either stored and subsequently released for translation, or targeted to decapping and degradation (7,20,21).

4.2.7. snRNP biogenesis

snRNPs combines with pre-mRNAs to form spliceosomes. DDX20/Gemin3 plays essential roles in the biogenesis of snRNPs (7,24).

4.2.8. Transcription

As discussed earlier, the ancillary amino and carboxyl-terminal domains of DEAD box proteins are highly divergent. Some DEAD box RNA helicases, through interaction with components of the transcriptional machinery though their ancillary domains play important roles in transcriptional regulation. Notably, mounting evidences suggests that DDX3, DDX5/p68, DDX17/p72, DP103/DDX20, DDX21 regulates transcription, and are significantly implicated in oncogenesis. Their roles in transcription and significance in cancer are major focus of this review, and discussed in details in the following sections.

5. DEAD BOX RNA HELICASES IN REGULATION OF GENE EXPRESSION AND ONCOGENESIS

Emanating evidences suggests several DEAD box proteins to be critical regulators of gene expression and potent contributors of oncogenesis. This section describes the mechanisms of gene regulation of DEAD box proteins, and their involvement in regulation of other important proteins significantly implicated in cancers.

5.1. DDX1

DDX1 is located on chromosome 2p24 within the distance of 400 kilobases 5’ to the MYCN proto-oncogene (25). DDX1 is expressed in all cell lines and tissues; the highest being in the cells of neuroectodermal origin, and cancer cells (26,27). The possible indications of the involvement of DDX1 in tumorigenesis came from reports which suggested its co-amplification along with *MYCN* gene in retinoblastomas and neuroblastomas (26,28-30). Co-amplification of DDX1 and MYCN was also observed in Wilms tumors and alveolar rhabdosarcomas (31,32). DDX1 was speculated to be tumorigenic as it caused increased cell survival and anchorage-independent growth in non-transformed mice embryonic fibroblasts; these traits being hallmarks of cancer (33). Also, DDX1 and MYCN was frequently co-amplified in higher stages of neuroblastoma, and was associated with reduced disease-free survival as to those having only MYCN amplification, implicating DDX1 in tumorigenesis (28,34). A recent report suggested that in breast cancer, overexpression of the DDX1 RNA and elevated cytoplasmic DDX1 protein are associated with early recurrence, and DDX1 could serve as an independent prognostic marker for early recurrence in breast cancer (35).

There are several contradicting reports regarding the contribution of DDX1 in oncogenesis. One report suggested that co-amplification of MYCN and DDX1 correlated with a better patient survival in neurobastoma (36). A recent study also demonstrated that high DDX1 expression was associated with improved local, metastasis-free and overall survival in early-stage node-negative breast cancer (37). These findings advocate plausible tumor suppressive roles of DDX1. However, there are reports stating that there is no effect of DDX1 amplification on prognosis of patients diagnosed with MYCH-amplified neuroblastomas (38,39). Also, the prognostic effect of DDX1 co-amplification with MYCN is different between subgroups of MYCN-amplified neuroblastomas (40,41). Therefore circumstantial evidences and precise functions of DDX1 in oncogenesis are needed to address the genuine oncogenic role of DDX1.

Functions of DDX1 are well documented in the replication of several viruses, including HIV (27). DDX1 is generally a nuclear protein; present in both nucleus and cytoplasm of cells where it is amplified. It is involved in 3' end processing of pre-mRNAs, RNA transport, cell migration, and a part of several ribonucleoprotein complexes (42). It is known to associate with the hnRNP-K (43). hnRNP-K is a multifunctional protein involved in transcription, translation, nuclear transport, and signal transduction, and implicated in neoplastic transformation and metastasis (44-46). Therefore its association with hnRNP-K might aid in oncogenesis. DDX1 is also reported to interact with 14-3-3 proteins (42) which plays a central role in cell proliferation, survival, and inhibits apoptosis in multiple cancers. It is a potential candidate for cancer therapy (47). This again highlights potential oncogenic role of DDX1.

A direct evidence of DDX1 in tumorigenesis came from a report which stated that DDX1 is critical for testicular tumorigenesis. The study showed that DDX1 is required for the transcriptional activation of stem cell associated genes including cyclin D2; it directly regulated the transcription of cyclin D2 by occupying its promoter. Also, inhibition of DDX1 abrogated tumor formation in mice (48). This study provides a mechanism of oncogenesis by DDX1 through transcriptional regulation of gene expression. In cells exposed to ionizing radiation, DDX1 is phosphorylated by ATM and is involved in RNA clearance at double stranded break sites, suggesting its significance in DNA repair (49). Recently, DDX1 has been identified to promote maturation and expression of miRNAs leading to ovarian cancer suppression. Majority of these miRNAs are induced after DNA damage through ATM mediated phosphorylation of DDX1. This report suggests that inhibition of DDX1 promotes ovarian tumor growth and metastasis in mice. Also, analysis of The Cancer Genome Atlas suggested that low DDX1 levels are associated with poor clinical outcome in patients with serous ovarian cancer (50). Hence, it seems that highly contextual roles of DDX1 in tumorigenesis might be due to tissue specificity. A lot remains to be explored regarding the involvement of DDX1 in other cancers and its mode of actions.

5.2. DDX3

DDX3 has two homologs designated as DDX3X and DDX3Y, located on X and Y chromosomes, respectively. DDX3X is ubiquitously expressed in most tissues, while DDX3Y expression is restricted to the male germline where it functions in male fertility (51). Mutations in DDX3X have been reported in head and neck squamous cell carcinomas, chronic lymphocytic leukemia, Burkitt's lymphomas and medulloblastomas (52). A study reported somatic mutations in DDX3X along with mutations in beta-catenin and stated DDX3X as a part of aberrant beta-catenin signaling in medulloblastoma (53).

DDX3 and its yeast homolog Ded1p function in multiple cellular processes involved in the regulation of gene expression including transcription, pre-mRNA splicing, mRNA export and translation. Ded1p is required for global translation; the role of DDX3 in global translation is controversial (42,51,54). DDX3 is required for the replication of several viruses such as HCV, HBV and HIV. It is considered as a potential therapeutic target for anti-viral drug against HCV and HIV (42,51).

Several evidences suggest that DDX3 regulates transcription of genes which are crucial in oncogenesis. The first connection of DDX3 with oncogenesis was established from a study which showed that DDX3 mRNA is overexpressed in hepatocellular carcinoma tissues. Its ectopic expression in hepatocellular carcinoma cells led to anchorage independent growth, a trait of aggressive cancers. (55). BPDE, present in tobacco smoke is a potent carcinogen. Exposure of BPDE to breast cancer cells increased DDX3 expression (56). EMT is a phenomenon in which epithelial cells lose their cell polarity and gain mesenchymal phenotype which includes increased migration, invasion and evasion of apoptosis. This process is a salient feature of metastasis. Loss of E-cadherin and induction of Vimentin and Snail are critical events of EMT (57). Overexpression of DDX3 in breast cancer cells led to EMT as a consequence of repression of E-cadherin expression. DDX3 was found to occupy E-cadherin promoter and repress its transcription; it also repressed transcription of tumor suppressor p21WAF1/CIP1 (56). Cyclins regulates progression of cell cycle through the activation of cyclin-dependent kinases. p21WAF1/CIP1 is a cyclin-dependent kinase 2 inhibitor, and functions as a crucial regulator of G1/S progression of the cell cycle. It also mediates cellular senescence, and is tightly controlled by tumor suppressor p53 (58). DDX3 also aids in cancer progression by upregulating Snail transcription; Snail represses expression of cellular adhesion proteins for increased cell migration and

metastasis. Expression of Snail and DDX3 exhibited significant correlation in glioblastoma multiforme samples (59). Positive DDX3 expression is also reported to be a biomarker of metastasis and poor prognosis of gall bladder cancers (60).

Other possible oncogenic effect of DDX3 is through the regulation of cell cycle. It induces cell cycle through induction of G1/S transition, thereby promoting cellular growth. It regulates the translation of G1/S specific cyclin E1 mRNA; RNA helicase activity is required for the process (61,62). Moreover, DDX3 interacts with DDX5 in the G2/M phase of the cell cycle, and also regulates cell cycle during mouse embryonic development (63,64). A recent report stated that DDX3 regulated Rac1 mRNA translation through interaction with its 5' UTR, and affected beta-catenin protein stability in a Rac1-dependent manner, thereby modulating beta-catenin target genes. As a consequence, DDX3 was responsible for invasiveness and metastasis through Rac1 dependent signaling (65). Its mechanism of oncogenesis is not only limited to direct regulation of transcription or translation but also in modulation of signaling. DDX3 is also reported to inhibit death receptor mediated apoptosis by associating with TRAIL-R2/DR5; one of the most common death receptor. It blocks death receptor mediated apoptotic signaling by interaction with GSK3 and inhibitor of apoptosis protein-1, forming an antiapoptotic complex (66,67). A recent study also showed that breast cancer cell lines with low expression of DDX3 were more sensitive to antibodies targeting TRAIL-R2 (68). In the presence of Wnt signaling, DDX3 interacts with oncogenic casein kinase CK1 and activates beta-catenin (69). Hypoxia is a characteristic feature of solid tumors. Hypoxia driven gene expression is majorly regulated by hypoxia inducible factors HIFs. In breast cancer, DDX3 is aberrantly expressed and exhibits positive correlation with HIF-1 expression and other related proteins of hypoxia. HIF-1 occupies DDX3 promoter stimulates its transcription (70,71).

Paradoxically, DDX3 also possess tumor suppressive activity. Significant decrease in the expression of DDX3 in hepatocellular carcinoma was observed in case of hepatitis B-positive but not in hepatitis C-positive patients. Moreover, depletion of DDX3 in the non-transformed mouse cell line resulted in enhanced G1/S transition of the cell cycle leading to heightened cell proliferation. This was the consequence of upregulated cyclin D1 and the downregulation of p21WAF1/CIP1 in the DDX3 knockdown cells. Furthermore, reduction in DDX3 expression increased resistance to apoptosis, and enhanced the oncogenic ras-induced anchorage-independent growth (72). Another study revealed that DDX3 inhibited the colony forming ability of various tumor cells, due to upregulation of p21WAF1/CIP1 expression. DDX3 upregulated p21WAF1/CIP1 promoter activity in an ATPase-dependent but helicase-independent manner, and interacted with the Sp1 sites present in the promoter. DDX3 mRNA and protein was found to exhibit diminished expression in 58% to 73% of hepatoma specimens. Additionally, an alteration of subcellular localization from nuclei to cytoplasm was observed in more than 70% of cutaneous squamous cell carcinoma samples (73). Interestingly, DDX3 promoter contains binding sites of tumor suppressor p53 through which it can directly regulate DDX3 expression. In human papilloma virus-associated lung tumorigenesis, altered p53-DDX3 pathway results in reduced p21WAF1/CIP1 expression due to hampered sp1 binding in its promoter, and is associated with poor relapse-free survival (74). DDX3 loss by p53 inactivation also promotes oncogenesis through the MDM2/Slug/E-cadherin pathway and results in poor patient outcome in non-small-cell lung cancer (75). In contrast to the modulation of death receptor mediated extrinsic apoptotic signaling, DDX3 also regulates intrinsic apoptotic signaling following DNA damage. DDX3 associates with p53, increases its accumulation, and positively regulates DNA damage induced intrinsic apoptotic signaling in cells expressing functional wild-type p53. DDX3 inhibits extrinsic apoptotic pathway in cells harboring non-functional p53 (76). Low DDX3 expression is reported to have poor prognostic significance in non-smoker patients with oral squamous cell carcinoma (77).

Taken together DDX3 has both oncogenic and tumor suppressive properties. Further research is required to decipher its exact contribution in cell growth and tumorigenesis, and its dominance as a tumor suppressor or oncogene.

5.3. DDX5 (p68) and DDX17 (p72)

An expansive body of evidences suggests DDX5 and DDX17 to be crucial regulators of gene expression. DDX5 was identified when an antibody against the simian virus 40 large T oncoprotein cross-reacted with a 68 kilo dalton nuclear protein. Sequence analysis revealed homology to the eukaryotic translation initiation factor eIF4A, and it was the first protein to demonstrate RNA helicase activity in an ATP dependent manner. DDX17, the highly homologous partner of DDX5 shared a 90% identical helicase core. DDX17 was shown to encode two isoforms: p72 and p82 RNA helicases. p82 arises due to alternative translation initiation at a non-AUG start codon. These two isoforms are generally expressed at similar levels and possess identical properties. Moreover, p72/p82 and p68 RNA helicases can form both homo- as well as heterodimers, suggesting that these proteins might have overlapping functions (42,52,78-80). Both DDX5 and DDX17 was originally demonstrated to be a nuclear proteins but later DDX5 was established as a nucleo-cytoplasmic shuttling protein (81). mRNA expression of DDX5 and DDX17 is ubiquitous but varies in an organ specific manner (78).

DDX5 and DDX17 regulates various cellular processes including pre-mRNA and rRNA

processing, alternative splicing, RNA export and miRNA biogenesis (78,42,52). Apart from these functions, DDX5 and DDX17 are essential in gene transcription as they act as co-activators of several transcription factors through their ancillary domains. Both DDX5 and DDX17 play essential roles in development. Mutations in yeast ortholog of DDX5 showed lethal phenotype; both DDX5 and DD17 knockout mice exhibited embryonic lethality. DDX5 gene passed three litmus tests for a proto-oncogene: ectopic expression of DDX5 in non-transformed mice embryonic fibroblasts led to formation of colonies in soft agar, implying anchorage-independent growth. Also, these were able to form tumors in nude mice (78). Depletion of DDX5 and DDX17 by RNA interference in colon cancer or cervical carcinoma cells suppressed cell proliferation. Also depletion of DDX17, but not DDX5, in breast cancer cells abrogated estrogen-dependent growth. These evidences suggested DDX5 and DDX17 to have major effects on cell growth and survival, and oncogenesis.

The first link between DDX5 and oncogenesis came from the study which reported it to be overexpressed in colon cancer samples unlike normal tissues, and the expression was more in the higher grade tumour than the lower one (82). In addition, studies showed that DDX5 overexpression protects lung carcinoma cells from the topoisomerase-1 poison, camptothecin, which is often employed against cancer (83). Both DDX5 and DDX17 are overexpressed in tumors of colon and breast (84-86). DDX5 is also overexpressed in prostate cancer, glioma, leukemia, head and neck squamous cell carcinoma, cutaneous squamous cell carcinoma, hepatocellular carcinoma, ovarian carcinoma and multiple myelomas (87-93). Initial reports suggested DDX5 to be phosphorylated in a tyrosine residue (Y593) in several cancer cells unlike normal cells, and this phosphorylation was induced by PDGF and decreased by TNF. Another PDGF induced phosphorylation on Y595 was further reported in glioblastoma cells, which along with Y593 conferred resistance to TRAIL induced apoptosis (94,95).

Ample of evidence suggests that DDX5 and DDX17 is involved in the initiation of gene transcription and are important regulators of gene expression. CBP and p300 are acetyl-transferases that acetylate histones, and PCAF is another acetyltransferase that forms complexes with CBP/p300. Histone acetylation is generally associated with gene transcription, while histone deacetylation induced by HDACs is associated with transcriptional repression (96). These are general transcription co-factors participating in the functioning of myriads of transcription factors (97). Both DDX5 and DDX17 interacts with RNA polymerase II, CBP, p300 and PCAF (98,99). On the other hand, DDX5 and DDX17 also interact with HDACs (100). Therefore DDX5 and DDX17 are vital for transcriptional regulation, and can act as an activator or repressor in a context specific manner. DDX5 and DDX17 were reported to co-activate MyoD; a regulator of muscle differentiation (101). DDX5 was also shown act as a co-activator of Runx2, required for osteoblast development. RNA helicase activity was not required in these cases (102).

A key event during colorectal cancer is the aberrant activation of Wnt/beta-catenin signaling (103). DDX5 and DDX17 acts as a co-activator beta-catenin-dependent transcription of target genes such as c-myc, cyclin D1 and c-jun. Furthermore, depletion of DDX5/DDX17 in colorectal cancer cells induced the expression of p21WAF1/CIP1, reduced cellular proliferation and tumor formation in nude mice (84). Another mode of interplay between DDX5 and beta-catenin is through the phosphorylation of DDX5. In colon cancer cells, PDGF is reported to phosphorylate DDX5 on Y593. This phosphorylation was required for interaction with beta-catenin and the induction of EMT. Also, the RNA helicase activity of DDX5 was required for the upregulation of beta-catenin target genes (104,105). The phosphorylated p68 also activates transcription of the Snail by promoting dissociation of HDAC from the *Snail1* promoter; Snail being a critical player of EMT. PI3K/AKT pathway is largely implicated in cancers; it is a major target for colorectal cancer prevention (106,107). Tumor suppressor FOXO3a, the legitimate target of AKT is reported to be downregulated in colon cancer (108). A recent finding suggested that in colon cancer, DDX5 co-activates beta-catenin and NF-kappaB in upregulating AKT transcription and consequently downregulating tumor suppressor FOXO3a. As a consequence colon cancer cells depleted of DDX5 exhibited reduced cellular proliferation and increased population of cells in G1/S phase of the cell cycle. Moreover, DDX5 overexpression led to increased primary tumor growth and lung metastasis in colorectal mice tumor model (109). Collectively, these evidences highlight DDX5 and DDX17 to be a major contributor of colorectal cancer.

Aberrant estrogen signaling is largely associated with breast cancers and a major chemotherapeutic target; approximately 70% of human breast tumors are positive for ER-alpha (110,111). Both DDX5 and DDX17 co-activate ER-alpha dependent gene transcription, independent of its RNA helicase activity. The steroid receptor co-activator (SRA) gene encodes a non-coding RNA molecule and the SRA protein, SRAP. The co-activation was dependent on interaction with SRA RNA. Moreover, DDX17 was found to be more implicated in estrogen dependent stimulation of endogenous ER-alpha responsive genes (86,112,113). NFAT signaling is gaining importance in oncogenesis, and functions in invasive migration, differentiation and survival of cells in the tumor and its microenvironment. NFAT5 is reported to be involved in the migratory ability of breast cancer cells (114). DDX5 and DDX17 act as co-activators of NFAT5, inducing NFAT5 target genes required in tumor cell migration. DDX5 and DDX17 also regulates splicing

of NFAT5 reducing its protein level thereby exerting fine regulation on NFAT5 signaling (115). DDX5 and DDX17 leads to tumor invasiveness by modulating alternative splicing of several DNA and chromatin-binding factors, including the macroH2A1 histone (116). DDX5 was found to promote G1/S phase progression of the cell cycle by upregulating DNA replication factor expression by promoting the recruitment of RNA polymerase II to its promoters. Additionally, DDX5 locus was observed to be frequently amplified in breast cancer (117). A recent finding suggests that Wnt/beta-catenin signaling upregulates DDX5 transcription in breast cancer. Beta-catenin/TCF4 occupies the promoter of DDX5 in regulating its transcription. Additionally, DDX5 along with beta-catenin upregulates TCF4 expression and maintains a positive feedback loop responsible for EMT in breast cancer (118). DDX5 regulates miRNAs miR-21 and miR-182 in breast cancer cells. Depletion of DDX5 resulted in reorganization of actin cytoskeleton and reduction of cellular proliferation, as a consequence of downregulation of miR-182 (119). Other than transcriptional regulation, DDX5 also interacts with Ca-calmodulin (CaM); a protein triggered by Ca2+ signaling and a major factor in relaying the signaling to cell motility (120). The interaction facilitates cellular migration and promotes metastasis in breast cancer (121). In prostate cancer, DDX5 co-activates AR, and promote tumorigenesis (87). Deregulated androgen dependent signaling mediated though AR is the key cause of prostate cancer (122). DDX5 also potentiate beta-catenin and RNA polymerase II mediated AR dependent gene expression (123). A recent study stated that DDX5 and DDX17 are master regulators of the estrogen and androgen mediated signaling through modulation of transcription, and splicing both upstream and downstream of the ER and AR (124).

NF-kappaB signaling pathway is frequently deregulated in glioma (125). DDX5 acts as a co-activator of NF-kappaB signaling through direct interaction with p50 subunit of NF-kappaB. DDX5 promoted glioma cell proliferation and tumor formation in mice though binding with p50 subunit of NF-kappaB (88). In T-ALL, aberrant activation of Notch1 prevails over 60% of the cases (126). Notch signaling is a cell–cell communication based pathway. Its activation eventually releases the Notch intracellular domain (NICD), which translocates in the nucleus and complexses with RBP-J. This complex is joined by MAML co-activator, activating the transcription of genes containing RBP-J binding sites on their promoters (127). DDX5 has also been shown to interact with RBP-J/NICD and occupy the promoters of Notch target genes thereby functioning as a co-activator in Notch signaling. Again, SRA RNA acts as an additional co-activator in this case (128). A separate study also demonstrated that DDX5 binds MAML1, and is associated with the Notch1 transcription activation complex in human T-ALL leukemic cells thereby acting as a co-activator of oncogenic Notch signaling (126).

Though majority of the evidences proves DDX5 and DDX17 as oncogenic, DDX5 may also possess growth suppressive functions in some specific contexts. p53 is a major tumor suppressor that executes cell cycle arrest, cellular senescence and apoptosis, in response to specific stimulus (129,130). Interestingly, DDX5 acts as a co-activator of p53, and was found to be recruited to p53-responsive promoters in response to DNA damage including p21WAF/CIP1 promoter, facilitating transcription. Hence, p68 helps in maintaining cellular homeostasis in a context specific manner (131,132). Another study showed that p38 MAP kinase activated by drug oxiplatin phosphorylates DDX5 at threonine residues (T564 and/or T446) which cause apoptosis induction (133). Also, DDX5 co-activates the vitamin D receptor, stimulating its response in the presence of vitamin D ligand, suggesting its growth suppressive role (134). This contextual regulation of gene expression by DDX5 and DDX17 is not only exerted through transcriptional regulation but also by miRNA processing. As discussed earlier, DDX5 is essential for miRNA processing, and is a part of the Drosha complex. RNase III endonuclease Drosha is essential for miRNA processing in the nucleus. Interaction with DDX5 leads to the recruitment of the TGF-beta/BMP specific Smad signal transducers, ER-alpha as well as p53 into the Drosha complex; processing of several miRNAs that play key roles in cancer progression are executed. DDX5 and DDX17 have been implicated in promoting the processing of both oncogenic and tumor suppressor miRNAs. DDX5 was found to promote not only the processing of the oncogenic miR-21 through TGF-beta/BMP but also of several growth suppressive miRNAs that are modulated by p53. In a context dependent manner, DDX5 and DDX17 also promote ER-alpha mediated inhibition of tumor suppressive miRNAs (42,80).

Post-translational modifications like ubiquitylation, sumoylation and acetylation are also predominant modes of DDX5/DDX17 modifications. Some reports provide possible causes of overexpression of p68 in cancers. As mentioned earlier, DDX5 mRNA was found to be overexpressed in ovarian carcinoma and multiple myeloma. Moreover Wnt/beta-catenin signaling regulated the transcription of DDX5 in breast cancer. Besides, in colon cancer, poly-ubiquitylated DDX5 was observed implying possible defects in its proteasomal degradation leading to its overexpression. SUMO modifiation stabilizes DDX5 and DDX17 in breast cancer (85). Additionally, sumoylation and acetylation have also been shown to modulate DDX5/DDX17 function in case of ER-alpha and p53 mediated transcription. Sumoylation enhances also repressive function of DDX5 and DDX17 in a contextual manner (85,135,136). SUMO1, the SUMO conjugating enzyme Ubc9 and the SUMO ligase PIAS3 are upregulated in breast cancer cells, whereas the SUMO protease SENP6 is downregulated, suggesting that posttranslational modification by SUMO is generally

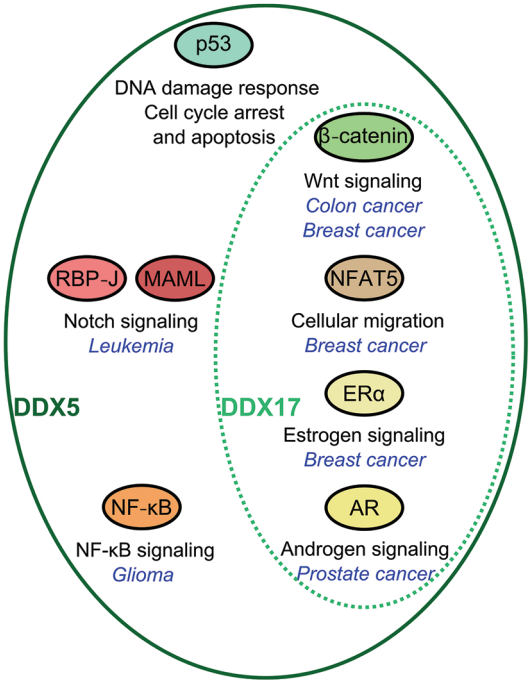

Figure 3. Involvement of DDX5 and DDX17 in oncogenic signaling pathways. DDX5 and DDX17 act as co-activators of several oncogenic transcription factors. Both co-activate beta-catenin and ER-alpha in colon and breast cancers respectively. They also act as a co-activator of NFAT5 in enhancing migration of breast cancer cells. DDX5 also co-activates NF-kappaB in glioma; RBP-J and MAML (mediators of Notch signaling) in leukemia; In DNA damaging stress DDX5 co-activates tumor suppressor p53 to induce cell cycle arrest and apoptosis.

enhanced in breast tumors (78,85). This provides possible explanation of p68 overexpression in breast cancers (78,80). Thus, p68 overexpression may be due to increase in transcript level or stabilization due to post-translational modifications. Altogether, DDX5 and DDX17 are involved with crucial signaling pathways of the cell in regulating gene expression and oncogenesis (Figure 3).

5.4. DDX6/p54/Rck

The first instance of the involvement of DDX6 in cancer came from a study involving its mapping at a chromosomal breakpoint region 11q23 in B-cell lymphoma cell line (137,138). DDX6 was found to be present in majority of human and mouse tissues except undetectable protein levels in lumbar muscle and lung tissues, and very low protein expression in human brain; although it's mRNA was abundant in these tissues. It was overexpressed in neuroblastoma, glioblastoma, rhabdomyosarcoma, lung cancer cell lines, and in hepatocytes of HCV-mediated chronic hepatitis and hepatocellular carcinoma (139,140). Also, it exhibited overexpression in human colorectal tumors, and its abundance strongly correlated with the abundance of proto-oncogene c-myc (141,142). Recently, it has also been shown to be overexpressed in gastric cancer tissues (143).

DDX6 plays significant roles in mRNP assembly, translation regulation, RNA export, RNA degradation, RNA export and viral RNA expression (20,21,42,52). Besides, regulation of expression of genes required for cellular proliferation attributes oncogenic properties to DDX6. In colorectal cancer cells, DDX6 significantly regulates the transcriptional activity of TCF, and expression levels of Wnt target genes. Downregulation of DDX6 by RNA interference significantly diminished the viability of colorectal cancer cells, causing cell cycle arrest in the S phase. It also led to induction of apoptosis, and inhibited tumor growth in mice (144). DDX6 also contributes to the development of human colorectal tumors by stabilization and increased translation of the c-myc (142). Proto-oncogene c-myc is the major downstream effector of Wnt signaling pathway (103). Therefore in colorectal cancer, DDX6 regulates gene expression profile of Wnt signaling by exerting transcriptional as well as translational control. Yeast homologue of DDX6 was also reported to be significant in the recovery of G1/S cell cycle arrest following DNA damage. Its function in the modulation of mRNA metabolism is responsible for the recovery (145). In human cervical cancer cell line Hela, expression of DDX6 was upregulated during cell proliferation and downregulated during differentiation. Depletion of DDX6 hampered cell growth by induction of cell cycle arrest at S phase. Interaction of DDX6 with eIF4E implicated possible modulation of translation initiation of the genes involved in the cell proliferation (146). Expression levels of miRNAs are frequently downregulated in cancers. miR-143 and -145 are downregulated in most cancers. A recent study showed that DDX6 post-transcriptionally downregulated miR-143/145 expression by prompting the degradation of its host gene product, *NCR143*/*145* RNA in human gastric cancer cell line (143).

DDX6 also possess anti-tumorigenic traits. Its overexpression led to inhibition of growth in guinea pig cancer cell line (147). VEGF is critical in angiogenesis; 5'UTR of its mRNA harbors IRES responsible for its sustained translation in hypoxia when cap-dependent mRNA translation is inhibited. DDX6 interacts with the VEGF mRNA 5'-UTR and inhibits IRES-mediated translation in normoxia; its expression declines during hypoxia. Additionally, depletion of DDX6 increases VEGF expression in breast cancer cells (148). Dhh1p, the yeast homologue of DDX6 inhibited growth in yeast through general translation repression acting as an activator of mRNA decapping (149). While several evidences advocate growth promoting roles of DDX6, its specific functions might rely on its tissue specific expression, physiological amount and interaction with other factors.

6. OTHER DEAD BOX RNA HELICASES SIGNIFICANT IN ONCOGENESIS

6.1. DDX2/eIF4A

DDX2 is majorly implicated in eukaryotic translation initiation. DDX2A is a subunit of eIF4F

complex which binds 5' cap structure of mRNA through eIF4E, thereby playing crucial roles in translation initiation. Tumor suppressor PDCD4 controls the availability of eIF4A. Elevated eIF4AI expression has been reported in a several types of tumor cells; physiological significance is poorly understood. DDX2 promotes T-cell acute lymphoblastic leukaemia development *in vivo.* Besides, decreased levels of PDCD4 are found in gliomas, hepatocellular carcinomas, and tumors of lung, colon and breast. Further, depletion of DDX2 resulted in reduced proliferation of melanoma cells. Inhibition of DDX2 displayed reduction in xenograft tumor (52,150). The oncogenic MUC1 C-terminal subunit is overexpressed in most human breast cancer; targeting the DDX2 blocks MUC1-C overexpression in breast cancer cells (151). DDX2 has also been shown to be a potential therapeutic target for modulating tumor cell response to chemotherapy (152). LEF-1, a Wnt mediating transcription factor is important for cell survival and metastasis in cancer, and is produced by IRES-directed translation. LEF-1 mRNA is overexpressed in cancers. Hippuristanol, an inhibitor of DDX2 has been shown to thwart translation of IRES mRNA, and is proposed to be used in combinatorial chemotherapy against chronic myelogenous leukemia (153). Hippuristanol is also reported as a potential therapeutic agent against ATL through inhibition of tumorigenesis (154). An intriguing recent finding reports that DDX2 promotes oncogenic translation through RNA G-quadruplex structures present in the 5' UTR region of its target transcripts which includes several oncogenes (155).

6.2. DDX10

DDX10 maps to a chromosomal location on 11q22–23. It is involved in the leukemia-associated chromosomal translocation Inv11 (p15q22). This leads to fusion of N-terminus of the nucleoporin gene NUP98 to the C-terminal region of DDX10. This has been reported in acute myeloid leukemia, myelodysplastic syndrome, and blast crisis of chronic myelogenous leukemia. NUP98 encodes a member of the nucleoporin complex, which is involved in the active transport of proteins and RNA between the nucleus and cytoplasm (52,150). Ectopic expression of the NUP98–DDX10 fusion increased proliferation and self-renewal property of primary human CD34+ cells, and disrupts their erythroid and myeloid differentiation. The NUP98–DDX10 fusion regulates the transcription of a several oncogenes, including MYCN and COX-2. Mutation of motif VI within DDX10 of the NUP98–DDX10 fusion diminished its transformation potential and transcriptional activation potential, indicating the significance of its helicase activity in oncogenesis (156). Recently, whole genome mate pair sequencing studies revealed fusion between DDX10 and SKA3 in breast cancer. Moreover, RNA interference studies have shown DDX10 to be a as potential cancer gene with significant impact on the growth and proliferation of breast cancer cells (157).

6.3. DDX11

DDX11 is vital for the maintenance of sister chromatid cohesion. DDX11 is overexpressed in melanomas; its depletion by RNA interference halts proliferation, and induces apoptosis and defective chromosomal segregation in melanoma cells (158). DDX11 knockout mice display embryonic lethality (52).

6.4. DDX20/DP103/Gemin3

Apart from its functions in snRNP assembly, murine homologue of DDX20 was shown to interact though its C-terminal domain with the nuclear steroidogenic factor SF-1; crucial in regulation of genes for reproductive and endocrine development. Moreover its C-terminal was shown to possess an intrinsic transcriptional repression activity, and repressed SF-1 function. Moreover, DDX20 was also found to recruit HDAC2/5, suggesting its repressive effect on transcription (159).

It was first implicated in cancer with its association with Epstein–Barr virus proteins EBNA2 and EBNA3C, which are involved in B-cell immortalization. EBNA3C interacts with DDX20 leading to its increased stability. Also, EBNA3C promotes formation of a complex with p53 and DDX20 which blocks the DNA binding affinity of p53, inhibiting its apoptotic functions (160). Recent studies in breast cancer showed that DDX20 increases MMP9 levels, which are associated with metastasis and invasion through activation of NF-kappaB. Also, there exists a positive DDX20/NF-kappaB feedback loop exerting constitutive NF-kappaB activation. DDX20 impeded metastasis in a mice xenograft model. DDX20 has been suggested as a biomarker in human breast cancers (161).

DDX20 also has tumor suppressive functions. It can mediate apoptosis through transcription factor FOXL2, a protein required for proper development and function of the ovaries. DDX20 complexes with mitogenic Ets transcriptional suppressor; blocks transcription of genes required for Ras-dependent proliferation of murine macrophages. DDX20 is a major component of miRNA RNP complexes. Loss of function of miRNAs, and deregulation of miRNA machinery components are involved in hepatocarcinogenesis. Reduced expression of DDX20 is frequent in human hepatocellular carcinomas; DDX20 is a part of miRNP. DDX20 is required for the preferential loading of miRNA-140 into the RNA-induced silencing complex (RISC). Impairment function of miRNA-140 due to reduction of DDX20, leads to hepatocarcinogenesis (162). DDX20 was stated as a tumor suppressor gene in a mouse liver cancer model (52). Inhibition of DDX20 is reported to activate the NF-kappaB pathway. Moreover, DDX20 suppresses NF-kappaB function through miR-140-3p activity; it loads miRNA140-3p into the RISC. Hence, it participates in the loading of specific miRNAs into the RISC complex to control gene expression (163). A study assessing

genetic variations in miRNA biogenesis genes in non-muscle-invasive bladder cancer stated that the most significant single nucleotide polymorphisms is harboured in DDX20 (164).

6.5. DDX21/RHII/Gu

DDX21 is a nucleolar protein, essential in rRNA processing and ribosome biogenesis. It is involved in the processing of 20S rRNA to 18S rRNA (52). Besides it is also a modulator of transcription. A recent report stated that DDX21 can sense the transcriptional status of both RNA polymerase I and II to regulate multiple steps of ribosome biogenesis (165). It is a transcriptional co-activator of c-Jun; its RNA helicase activity was required for the c-Jun mediated transcription. c-Jun play significant roles in G1/S cell cycle progression, cancer cell proliferation and survival. Moreover, it is also involved in rRNA processing (166). The N-terminal transcription activation region of c-Jun interacts with the C-terminal domain of DDX21. It was also shown to be involved in the c-Jun mediated transcription in stress response (167). A recent finding suggests that DDX21 is highly abundant in breast cancer tissues and cell lines. DDX21 in breast cancer cells promotes tumorigenesis by regulating the transcriptional activity of c-Jun, and also through rRNA processing. Depletion of DDX21 results in significant reduction of tumorigenicity *in vitro* and *in vivo* (168). In breast cancer tissues, DDX21 mRNA expression has been correlated with disease-free survival (169); overexpression of accumulation of DDX21 is reported in colon cancers and lymphomas (170,171).

6.6. DDX39

DDX39 is overexpressed in lung squamous cell carcinomas. Its overexpression in HeLa cells enhances cell proliferation and colony formation possibly through elevation of translation (172). DDX39 is stated as a prognostic biomarker of gastrointestinal stromal tumors (173,174). Upregulation of DDX39 was observed in human malignant pleural mesothelioma cell lines (175), and in drug resistant pancreatic cancers cells (176). On the other hand low expression of DDX39was associated with disease progression of bladder cancers. Depletion of DDX39 in bladder cancer cells stimulated their invasion ability, thereby suggesting it as a suppressor of invasion and disease progression in bladder cancers (177). The mechanistic insights of these varied properties of DDX39 remains to be elucidated. One intriguing finding suggests that DDX39 is required for global genome integrity as well as telomere protection, and maintenance by regulating telomere length homeostasis. Overexpression of DDX39 in human cancer cells led to progressive telomere elongation; depletion of DDX39 resulted in telomere shortening. This might be one of the mechanisms of enhanced cell survival and oncogenesis posed by DDX39 (178).

6.7. DDX43/HAGE

DDX43 was identified as an RNA helicase with tumor specific expression in human sarcoma cell line. DDX43 transcript abundance was shown to be 100-fold higher in a number of tumors compared to normal tissues, except testis. DDX43 mRNA was found to be overexpressed in salivary gland neoplasms, CMLs, AMLs, multiple myelomas and melanomas. In protein level, it was shown to be overexpressed in melanomas and cancers of bladder, brain, breast, colon, esophagus, kidney, liver, lung, stomach and small intestine. In normal tissues, the protein level of DDX43 is undetectable with the exception of testis (52,150,179). Recent reports suggest that DDX43 is overexpressed in uveal melanoma cell lines; its mRNA expression is significantly upregulated in MEK inhibitor resistant uveal melanoma, and in liver metastases of patients with uveal melanoma. DDX43 induces RAS protein expression and signaling, mediating MEK inhibitor resistance in uveal melanoma (180). A recent report also states DDX43 to be a biomarker for poor prognosis, and a predictor of chemotherapy response in breast cancer (181). In an approach to assess novel biomarkers and target antigens for immunotherapy in glioma, glioma cell lines derived from high-grade glioma patients were used. DDX43 was identified among the most relevant ones in this regard (182). DDX43 is reported to be over-activated by promoter hypomethylation and this hypomethylation might be a favorable prognostic factor in AML (183). DDX43 is considered a suitable target for immunotherapy due to its expression in broad range of tumours. In deciphering the modes of oncogenesis, recent studies in malignant melanoma-initiating cells (MMIC) which are subpopulation of cells responsible for melanoma growth and progression, DDX43 was found to be required for tumor growth *in vitro* and *in vivo*. DDX43 suppression caused a reduction in N-Ras protein expression, and weakening of its downstream oncogenic signaling pathways. DDX43 colocalizes with N-Ras mRNA and modulates its expression at the posttranscriptional level (184). A separate study showed that DDX43 was responsible for transcriptional repression of tumor suppressor PML in MMICs. DDX43 promoted unwinding of the SOCS1 mRNA, thereby increased its protein expression at the post transcriptional level. SOCS1 is an established inhibitor of JAK-STAT pathway; PML is a downstream target of interferon alpha which acts though JAK-STAT pathway. DDX43 dependent increase in SOCS1 inhibited JAK-STAT pathway mediated gene expression of PML, in response to anti-proliferative effects of interferon alpha (185).

6.8. DDX48

DDX48 is a human nuclear matrix protein (150); it has been identified to be upregulated in a specific type of gastric cancer (186). DDX48 has also been stated to be a potential serum marker for pancreatic cancer (187). It has been also shown to be overexpressed in vaginal carcinoma (188). Its functions in oncogenesis remain to be deciphered.

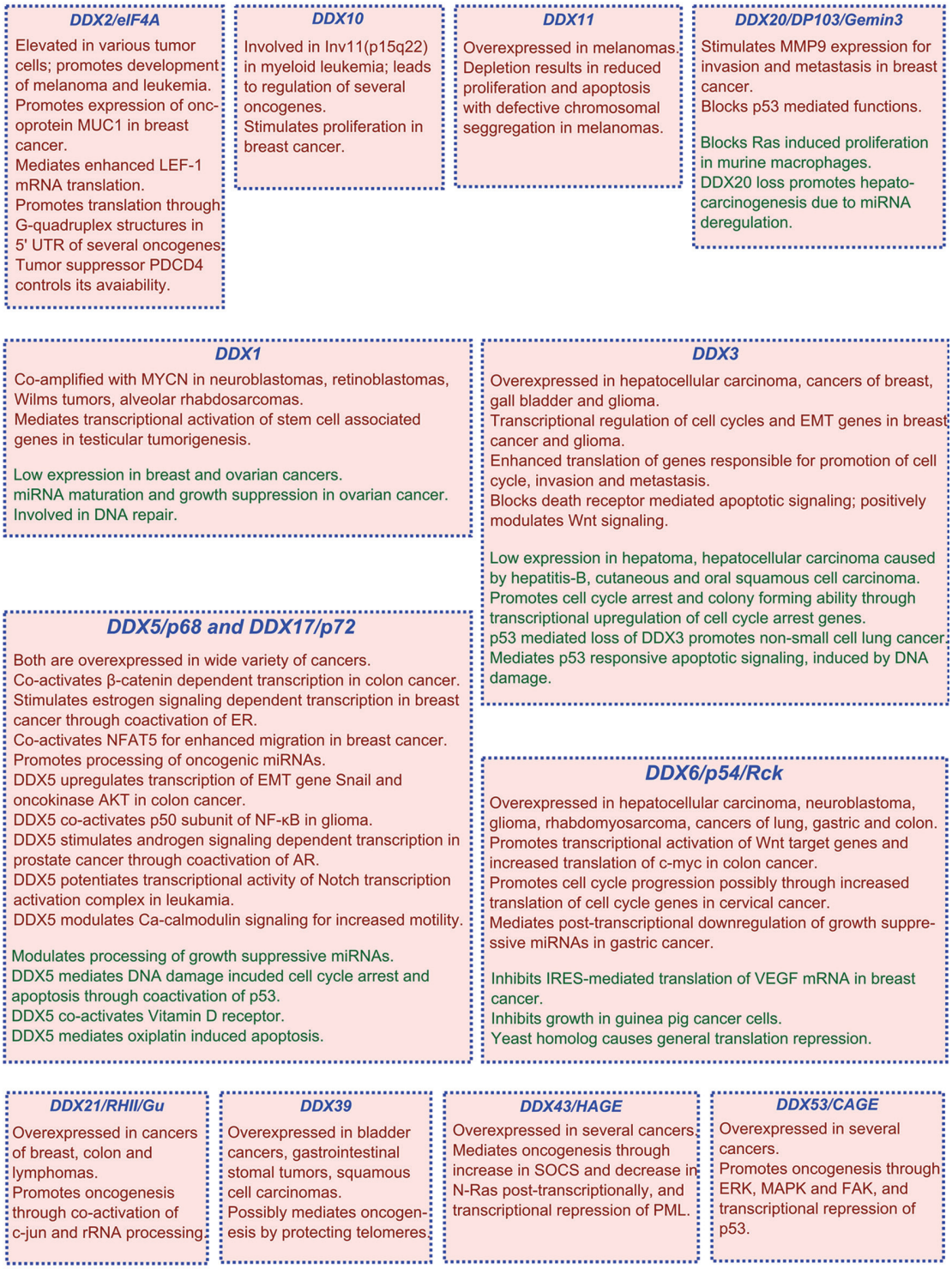

Figure 4. Summary of the involvement of DEAD box RNA helicases in oncogenesis. Substantive evidences implicate role of DDX1, DDX3, DDX5, DDX6 and DDX17 in oncogenesis. Others include DDX2, DDX10, DDX11, DDX20, DDX21, DDX39, DDX43 and DDX53. In the Figure oncogenic properties are depicted in red whereas tumor suppressive traits are presented in green.

6.9. DDX53/CAGE

Like DDX43 whose amino acid sequence closely resembles DDX53, it shows low levels of expression in normal tissues except testis. It is overabundant in various cancer tissues and cell lines. Hypomethylation of the DDX53 promoter was associated

with its overexpression (52,150). DDX53 has been shown to enhance the migration of cervical cancer cells, hepatocellular carcinoma cells, and melanoma cells through ERK and p38 MAPK pathways. It has also been reported to promote motility of hepatocellular carcinoma cells through the activation of focal adhesion kinase, a kinase with recognized tumorigenic potential (52). In cervical cancer cells, DDX53 overexpression stimulated tumor growth *in vitro* and *in vivo* in mice. This was the consequence of DDX53 mediated enhanced cell cycle progression by inducing AP-1 and E2F-dependent expression of cyclins D1 and E (189). Recent evidences suggest DDX53 to be implicated in chemosensitivity. In the drug-resistant human melanoma and the human hepatic cancer cell line showed induction of DDX53 expression. Increased expression of DDX53 resulted from displacement of DNMT1 from its promoter sequence**.** DDX53 conferred resistance to drugs through negative regulation of p53. DDX53 suppression led to induction of p53 expression, and activation of apoptosis. Furthermore, reduced expression of DDX53 decreased their invasion potential. DDX53 induced the interaction between HDAC2 and Snail, exerting a negative effect on p53 expression (190). In a recent finding miRNA miR-200b was shown to occupy the 3'-UTR of DDX3, and negatively regulate the transcription of DDX3 in the abovementioned cell lines. miR-200b enhanced the sensitivities to chemotherapeutic drugs and had negative effects on the tumorigenic and metastatic potential *in vitro* and *in vivo*. Additionally, miR-200b and CAGE constitutes a feedback loop, and regulate the chemosensitivity, invasion, tumorigenic potential, and angiogenic potential (191).

7. CONCLUSIONS

This review addresses the physiological functions of DEAD box RNA helicases having significant impact on gene regulation and oncogenesis (summarised in Figure 4). From the findings, it is evident that many DEAD box proteins play crucial roles in oncogenesis. Though DEAD box proteins present themselves as attractive targets of combinatorial chemotherapy yet it poses challenges in this regard. They cannot be made non-functional as they are vital players in normal physiology. In several cases, the knockout mice exhibit embryonic lethality. Moreover, most of them possess context-dependent tumor suppressive functions. As discussed before, specific functions of individual DEAD box proteins depends on the interaction with other factors. Therefore inhibiting specific interactions or targeting the interaction partner might be of translational use against cancer. However, several aspects remain to be understood regarding the mechanistic insights of gene regulation and oncogenesis with respect to DEAD box proteins. Therefore further research would shed light into the specific mechanisms of oncogenesis which would aid in designing of better therapeutic agents to combat their oncogenic functions.

8. ACKNOWLEDGEMENTS

The laboratory is supported with grants provided by Council of Scientific and Industrial Research (CSIR) [EMPOWER (OLP-002); MEDCHEM (BSC-0108)] and CSIR-MAYO Clinic (MLP-0017), Govt. of India.

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Abbreviations: siRNAs: small interfering RNAs; miRNAs: microRNAs; NTP: nucleoside triphosphate; RecA: recombinase A; pre-mRNA: precursor messenger RNA; Mex67: mRNA export factor 67; Dbp5: DEAD box protein 5; rRNA: ribosomal RNA; snoRNAs: small nucleolar RNAs; snRNAs: small nuclear RNAs; eIF4A: eukaryotic initiation factor-4A; mRNPs: messenger ribonucleoprotein particles; PTC: premature termination codon; uORFs: upstream open reading frames; EJC: exon junction complex; Upf: upstream frame shifting; PNPase: polynucleotidephosphorylase; snRNPs: small nuclear ribonucleoproteins; MYCN: v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog; HIV: human immunodeficiency virus; hnRNP-K: heterogeneous nuclear ribonucleoprotein protein K; ATM: ataxia telangiectasia mutated; HCV: hepatitis C virus; HBV: hepatitis B virus; BPDE: Benzo(a)pyrene diol epoxide; EMT: epithelial-mesenchymal transition; 5'UTR: 5' untranslated region; TRAIL-R2/DR5: tumor necrosis factor-related apoptosis-inducing ligand receptor-2/death receptor 5; GSK3: glycogen synthase kinase-3; PDGF: platelet derived growth factor; TNF: tumour necrosis factor; TRAIL: tumor necrosis factor related apoptosis inducing ligand; CBP: CREB-binding protein; PCAF: p300/CBP-associated factor; HDACs: histone deacetylases; PI3K: phosphoinositide 3-kinase; ER-alpha: Estrogen receptor alpha; NFAT: nuclear factor of activated T cells; CaM: Ca-calmodulin; AR: androgen receptor; T-ALL: T-cell acute lymphoblastic leukemia/lymphoma; NICD: Notch intracellular domain; RBP-J: recombination signal binding protein J kappa; MAML: mastermind-like; TGF-beta: transforming growth factor beta; BMP: bone morphogenic protein; SUMO: small ubiquitin-related modifier; TCF: transcription factor; VEGF: vascular endothelial growth factor; IRES: internal ribosome entry sites; PDCD4: programmed cell death protein 4; LEF-1: lymphoid enhancer factor-1; ATL: acute T-cell leukemia; MMP9: matrix metallopeptidase 9; miRNP: miRNA-containing ribonucleoprotein; RISC: RNA-induced silencing complex; CMLs: chronic myeloid leukaemias; AMLs: acute myeloid leukaemias; MMIC: malignant melanoma-initiating cells; PML: promyelocytic leukaemia protein; SOCS1: suppressor of cytokine signaling 1; JAK-STAT: janus kinase–signal transducers; ERK: extracellular signal-regulated kinase; MAPK: mitogen-activated protein kinase; DNMT1: DNA methyltransferase 1

Key Words: RNA helicase, DEAD box proteins, Gene expression, Oncogenesis, Review

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