Genes & Diseases (2023) 10, 1220–1241

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REVIEW ARTICLE

DEAD-box RNA helicases with special reference to p68: Unwinding their biology, versatility, and therapeutic opportunity in cancer

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Received 9 August 2021; received in revised form 13 January 2022; accepted 6 February 2022
Available online 21 March 2022

KEYWORDS
Cancer;
DDX5;
DEAD-box RNA helicases;
Gene expression;
Oncogene;
Signaling;
Therapy;
Transcription factor

Abstract In the era of advancement, the entire world continues to remain baffled by the increased rate of progression of cancer. There has been an unending search for novel therapeutic targets and prognostic markers to curb the oncogenic scenario. The DEAD-box RNA helicases are a large family of proteins characterized by their evolutionary conserved D-E-A-D (Asp-Glu-Ala-Asp) domain and merit consideration in the oncogenic platform. They perform multidimensional functions in RNA metabolism and also in the pathology of cancers. Their biological role ranges from ribosome biogenesis, RNA unwinding, splicing, modification of secondary and tertiary RNA structures to acting as transcriptional coactivators/repressors of various important oncogenic genes. They also play a crucial role in accelerating oncogenesis by promoting cell proliferation and metastasis. DDX5 (p68) is one of the archetypal members of this family of proteins and has gained a lot of attention due to its oncogenic attribute. It is found to be overexpressed in major cancer types such as colon, brain, breast, and prostate cancer. It exhibits its multifaceted nature by not only coactivating genes implicated in cancers but also mediating crosstalk across major signaling pathways in cancer. Therefore, in this review, we aim to illustrate a comprehensive overview of DEAD-box RNA helicases especially p68 by focusing on their multifaceted roles in different cancers and the various signaling pathways affected by them. Further, we have also briefly discoursed the therapeutic interventional

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Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2022.02.008

approaches with the DEAD-box RNA helicases as the pharmacological targets for designing inhibitors to pave way for cancer therapy.

Introduction

Cancers are a large family of diseases characterized by abnormal cell growth forming neoplasms. The tumor cells are discerned by six cardinal hallmark features such as gaining replicative immortality, sustained cell proliferation, evading apoptosis, evading immune surveillance, promoting angiogenesis, undergoing invasion, and metastasis.[1] It has led to immense mortality of patients all around the world. To combat this fatal disease, scientists have been putting relentless efforts to materialize the basic scientific research into effective translational strategies for effective cancer therapy. Extensive deregulation of gene expression profiles due to genomic alterations, and aberrant proteins and RNA production disrupts the homeostatic machinery of cells. The identification of the molecular players involved in the abnormality of biochemical pathways of cells may help in the prognostic approach for therapy. This is where the RNA helicases come into play.

In the 1970s, DNA helicases were the first eukaryotic helicases discovered from the lily plant before human RNA helicases came one decade later.[2] RNA helicases have ascertained importance due to their biological roles in RNA metabolism and implications in cancer. DEAD-box family of RNA helicases form the largest helicase family (37 members in humans and 26 in *Saccharomyces cerevisiae*). They are categorized by the presence of an evolutionary conserved Asp-Glu-Ala-Asp (DEAD) motif. These proteins play diversified roles concerning RNA metabolism and cancer progression. They are involved in the modulation of complex RNA structures, pre-mRNA processing, RNA export, ribosome biogenesis, and transcriptional regulation. Moreover, they also act as molecular drivers in steering oncogenesis and their roles in various cancers have been elaborated over the years.[3,4]

p68 (DDX5) is a nuclear representative member of the DEAD-box family discovered through an antibody cross-reaction with simian virus SV-40 large T antigen.[5] It was the first identified RNA helicase and bears extensive amino-acid sequence homology to eukaryotic translation initiation factor eIF-4A. p68 is a multifunctional protein with varied roles in transcription, RNA processing, and miRNA processing.[6] Supporting pieces of evidence demonstrate that aberrant expression of p68 exists in several types of cancers due to their potential roles in cell proliferation and transformation. It has gathered immense attention due to its transcriptional coactivator roles leading to its oncogenic property. It acts as an important co-activator of countless transcription factors, namely, estrogen receptor α (ERα), myogenic regulatory factor Myo-D, Runx2, androgen receptor (AR), and p53, all of which are significantly important in cancer.[7]

Thus, the DEAD-box RNA helicases play critical roles in the development and progression of cancer, and it is necessary to have a thorough understanding of these aspects to shed light upon the therapeutic routes of research. Our review aims to summarize the importance of DEAD-box proteins especially p68 against the cancer backdrop as well as gain insights into their multidimensional roles to envisage their roles as suitable therapeutic markers of cancer.

Structure of DEAD-box RNA helicases

Looking at DEAD-box proteins from the structural perspective, it is known that they consist of two identical globular domains that are covalently linked. They are in turn made up of five β-strands surrounded by five α-strands resembling bacterial RecA. The helicase core is made up of these two domains, serving as binding sites for RNA and ATP. DEAD-box helicase family of proteins bears an evolutionally conserved general architecture. The helicases are composed of two RecA-like domains connected via a short flexible linker. This facilitates the enzymatic function with respect to changing orientation and flexibility. The C- and N-terminals contain a few to several hundred amino acids, which allow interaction with other proteins or RNA. RecA-like domains have nine conservative motifs involved in ATP and RNA binding, ATP hydrolysis, and RNA strands unwinding. In DEAD-box helicases, the conserved amino acid sequence 'DEAD' is found in motif II as well as an additional upstream Q motif.

Briefing about the p68 (DDX5) structure, we can summarize that its core is divided into two RecA-like domains, Domain 1 (D1) and Domain 2 (D2). The D1, consisting of Q-motif, motifs I, II, and III, serves for ATP-binding. The D2 (motifs IV, V, and VI) exhibits an RNA-duplex recognition domain. Q-motif is present at the N-terminus of the catalytic core and is preceded by an upstream conserved phenylalanine (17 amino acids) sequence. This aromatic group and the Q-motif are identified as adenine recognition motifs and can regulate ATP binding and hydrolysis. Further, the Q-motif was reported to affect the helicase activity. Motifs I and II (Walker A and B) are capable of binding ATP. ATP hydrolysis is coupled to RNA unwinding by motif III and cooperates with other motifs to create a high-affinity RNA binding site. Motif IV, motif Ia, Ib, and V, are engaged in ATP-dependent binding with RNA substrates.[7,8]

Functions of DEAD-box RNA helicases

DEAD-box RNA helicases perform diversified functions ranging from key cellular roles such as modulation of RNA structures, splicing, RNA processing, ribosome biogenesis, and transcription to specialized functions such as acting as coactivators/repressors, regulating cell cycle, and promoting oncogenesis (Fig. 1). A brief description of some of the functions has been described in the paragraphs ahead.

Modulating RNA structures

DEAD-box RNA helicases play an important role in RNA metabolism and modifying the secondary and tertiary structures of RNA. RNA helicases are capable of binding and re-modeling RNA and RNP complexes in an ATP- (or NTP-) dependent manner. The conserved helicase core sequences and other additional domains confer specificity to interact with RNA and target RNPs. The ATP-dependent RNA unwinding activity stimulates several biochemical processes such as unwinding of the RNA duplex,[9,10] RNA-protein complex (RNP) re-modeling, and "clamping" of multiprotein complexes onto RNA.[11] Moreover, DEAD-box helicases also act as chaperones to promote RNA folding.[12,13] Human DEAD-box helicases, including DDX5 (p68) and DDX17 (p72), are involved in secondary RNA structure metabolism and regulation of alternative splicing.[14,15] Also, it was recently reported that DDX1 acts as a novel cofactor in the Drosha microprocessor, which governs the post-transcriptional maturation of a subset of miRNAs.[16]

Ribosome biogenesis

DEAD-box RNA helicases also play a role in ribosome biogenesis in bacteria, yeast, and humans. Ribosomes are protein synthesizers of cells that sediment at 70S (smaller 30S and larger 50S) in bacteria and 80S (smaller 40S and larger 60S) in eukaryotes. They further consist of different ribosomal proteins (RPs) and ribosomal RNA (rRNA). Since ribosomes mediate the translation process, the biogenesis of ribosomes is a key interventional step in the central dogma.[17,18] There are innumerable reports of DEAD-box RNA helicases and their intricate association with ribosomal biogenesis but we will discuss only a few of them as the rest are beyond the scope of this review. Proteomic analysis has identified more than 30 putative RNA helicases in the human nucleus. The further screening revealed certain key DEAD-box proteins (DDX5, DDX10, DDX18, DHX15, DHX37, DDX24, DDX56, etc.) which are found to be involved in ribosome biogenesis. DDX10 was found together with nucleolin, RRP5, and the U3 snoRNP in a 50S subunit, which accrued in response to inhibition of Pol-I transcription by actinomycin D treatment or upon depletion of tUTP proteins. DDX56 was testified to associate with pre-60S particles. Its involvement in ribosome biogenesis is additionally reinforced by nuclear accumulation of RPL29-GFP upon knockdown of DDX56.[19] It was also demonstrated that DDX5 (p68) localized to the nuclei and nucleoli and then gets associated with the rDNA promoter in a p19Arf-regulated manner. ARF inhibits the interaction between DDX5 and nucleophosmin, thereby affecting the nucleolar DDX5 recruitment. Co-silencing of DDX5 and its paralog DDX17 disturbed nucleolar structure and 32S rRNA precursor processing. Reduced levels of mature 5.8S rRNA is found in mutant mice lacking DDX5 and DDX17.[20]

Transcriptional cofactors

The DEAD-box RNA helicases mediate various diseases through their roles as important transcriptional co-factors. They cannot directly bind to their target sites, instead, they coactivate/co-repress their target genes through

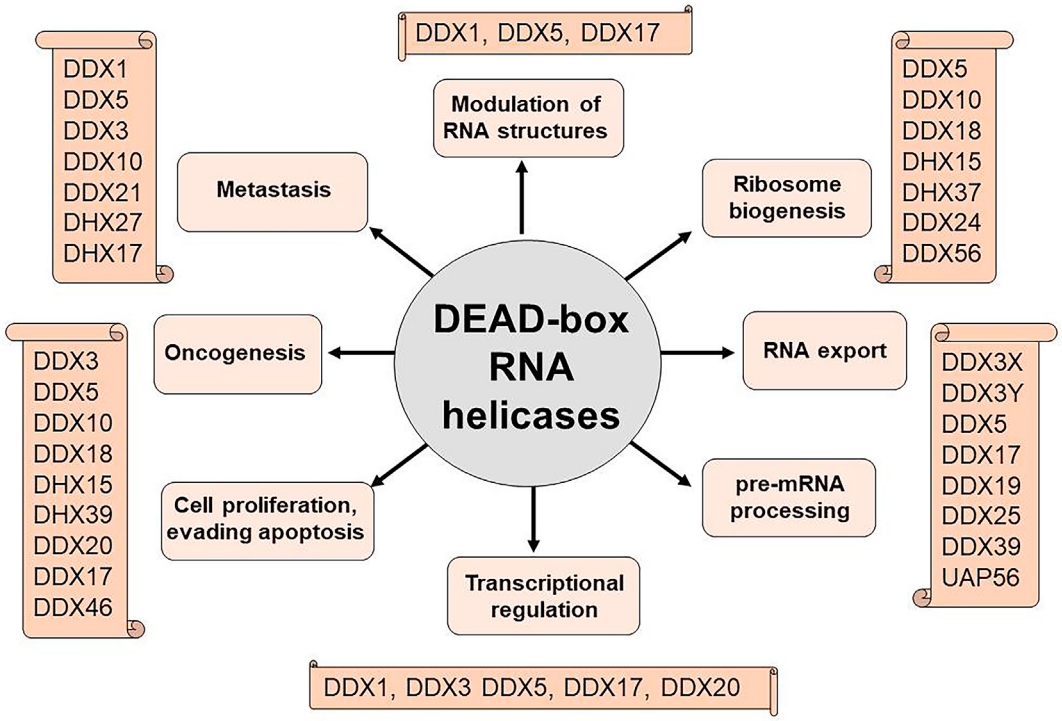

Figure 1 Versatile functions of DEAD-box RNA helicases. A summary of the myriad roles played by the DEAD-box RNA helicases. They help in modulating the secondary RNA structures, RNA export, ribosome biogenesis as well as play an important role in oncogenesis by acting as transcriptional regulators, helping in cell proliferation and evading apoptosis.

effector molecules which directly bind upon the promoter of the genes and modulate their expression levels. DDX3 has been shown to coactivate p21-waf1 promoter,[21] be involved in the activation of Snail1 and IFNβ, and represses expression from the E-cadherin promoter.[22] DDX20 represses the activity of both the Egr2/Krox20 and the steroidogenic factor 1 (SF-1) transcription factors.[23] DDX5 (p68) and DDX17 (p72) proteins interact with, and function as coactivators of, several transcription factors such as estrogen receptor alpha (ERα),[24] androgen receptor,[25] p53 tumor suppressor (for p53-dependent DNA damage response),[26] myogenic regulatory factor MyoD, and Runx2.[27,28]

Cell proliferation and survival

DEAD-box RNA helicases are multifaceted in their functional diaspora. They are also involved with cell cycle regulation, subsequent cell proliferation, and survival. DDX3 maintains cell survival and augments cell proliferation in hepatocellular carcinoma, colorectal cancer, prostate cancer, glioma, lung cancer, and breast cancer. Similarly, DDX5 and DDX17 also increase cell proliferation and survival capability thereby potentiating their tumorigenic nature. Silencing of p68 is linked with diminished cell progression.[29] It also suppresses the JAK/STAT pathway thereby decreasing the proliferation and migration of basal cell carcinoma cells.[29] It also promotes cell proliferation and evasion of apoptosis by regulating Akt/mTOR and Wnt signaling pathways. Human DEAD-box/RNA helicase 6 or RCK/p54 helps in maintaining cell growth by affecting the cell cycle in cultured cells. Downregulation of RCK/p54 expression inhibits proliferation of human colorectal cancer cells.[30,31] Silencing of DDX10 inhibited proliferation, invasion, and migration of osteosarcoma cells *in vitro*. Contrarily epigenetic downregulation of DDX10 promotes ovarian cancer cell proliferation through the nuclear factor (NF)-κB pathway.[32,33] Numerous other examples exist which justify the role played with DEAD-box proteins in promoting cell proliferation and tumorigenesis. This knowledge further helps in selecting proper prognostic markers for further therapeutic intervention.

Metastasis

The property of the cancer cell to invade other distant sites from its primary site of occurrence through the circulatory system is referred to as metastasis. It is one of the major hallmarks of cancer. The DEAD-box RNA helicases have been overtly related to this phenomenon and their roles in cancer are being delineated more and more exhaustively with each passing day. The metastasis-inducing property of DDX3 encompasses a wide variety of cancer types. Cytoplasmic DDX3 is highly expressed in metastatic prostate cancer and knockdown of DDX3 diminishes metastatic growth by decreasing proliferation and motility.[34] High DDX3 levels are also associated with poor prognosis in distant breast cancer metastasis events. DDX3 is also a strong metastatic prognostic marker in colorectal cancer.[35,36] Similarly, DDX5 has been widely associated with increased metastasis and helping in the epithelial to mesenchymal transition (EMT) through regulating key molecular players. Inhibition of DDX5 attenuated metastasis and proliferation of lung cancer with miR-431 playing the suppressor role.[37] Post-translational modification in terms of tyrosine phosphorylation also mediates its role in EMT and invasion. Platelet-derived growth factor (PDGF)-induced EMT is dependent on the phosphorylation of DDX5 at Y593. The interaction of DDX5 and Ca-calmodulin provides evidence supporting the role of DDX5 in metastasis.[28,38,39]

DEAD-box RNA helicases in cancers

DEAD-box proteins generally play critical roles in cellular maintenance and in doing so they have often been found to be closely associated with cellular proliferation and/or neoplastic transformation.[28] Various reports exist that delineate the involvement of DEAD-box proteins in cancer. Amidst all these proteins, the oncogenic capabilities of DDX5 (p68) stand out and it proves itself as a major factor involved in most cancers such as colon cancer, brain cancer, breast cancer, prostate cancer, lung and ovarian cancers to name a few (Fig. 2). Its expression levels have been concomitant with the severity of tumorigenesis. Therefore, we sought to review the roles of DEAD-box proteins in cancer and also implicate the importance of DDX5 as a crucial player in causing different cancers.

Colon cancer

Colon cancer is one of the most common malignancies in terms of morbidity and mortality in the world and there is a lack of efficient therapeutic avenues after surgery. The DEAD-box family of RNA helicases are involved in a multitude of way in the progression of colon carcinoma. Being typical oncogenes, they play various roles in establishing their importance in the carcinogenic scenario which can be later studied further for the therapeutic research arena.

The homologous proteins p68 (DDX5) and p72 (DDX17) are members of the DEAD-box family of RNA helicases. It had been reported long back that their expression strongly increases during the polyp to adenocarcinoma transition in the colon. They form complexes with β-catenin and regulate the downstream signaling scenario by regulating β-catenin-regulated genes, *c-Myc*, *cyclin D1*, *c-jun*, and *fra-1* (proto-oncogenes). p68/p72 contributes to colon cancer formation by directly up-regulating proto-oncogenes and indirectly by down-regulating $p21^{WAF1/CIP1}$. Accordingly, knockdown of p68 and p72 in colon cancer cells inhibits proliferation and diminishes tumor growth *in vivo*.[40] The most popular DEAD-box RNA helicase is p68 or DDX5. It is highly implicated in colon carcinogenesis through its multiple signaling empowerment façades. Our lab has extensively worked upon its signaling aspect and crucial roles in oncogenesis. We have previously reported that DDX5 interacts with oncogenic transcription factor STAT3 and leading to an increase in its downstream target genes leading to oncogenesis. Furthermore, our lab has also shown that it acts as a coactivator of AKT gene expression and further attenuates FoxO3a downstream in colon cancer cell lines. We have also discerned the molecular mechanism

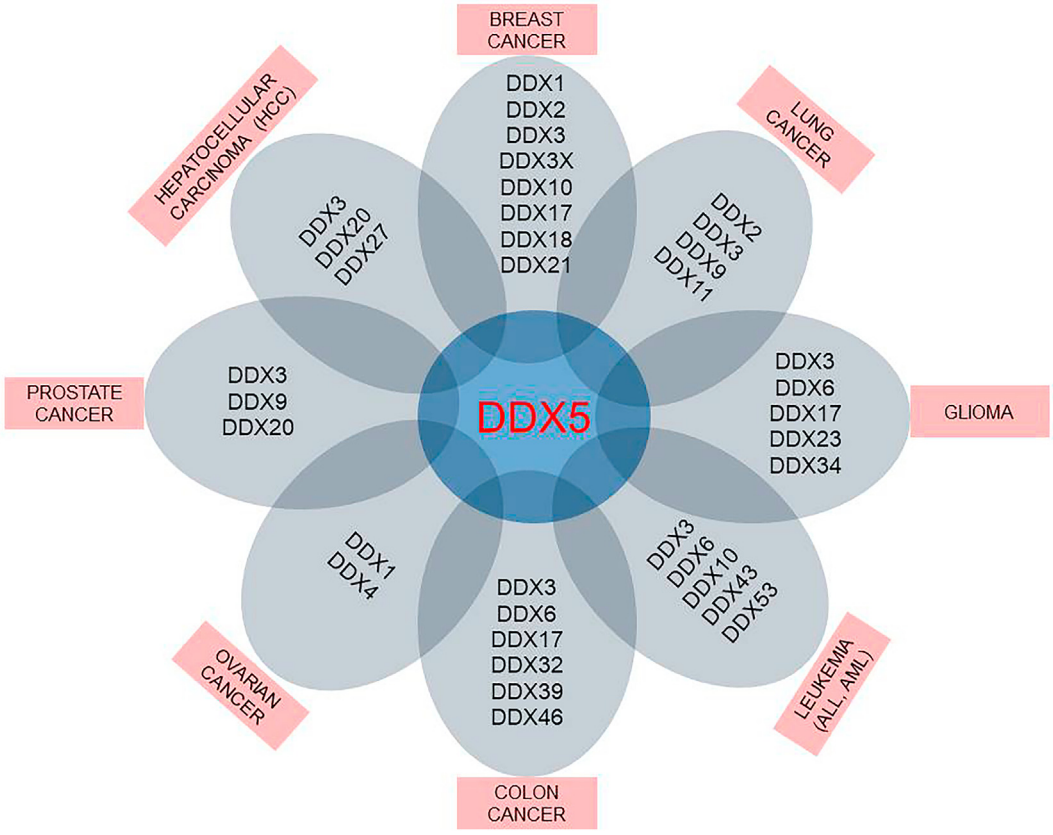

Figure 2 DEAD-box RNA helicases in various cancers. DEAD-box RNA helicases are implicated in different types of cancers namely breast cancer, colon cancer, glioma, prostate cancer, lung cancer, ovarian cancer, hepatocellular carcinoma and leukemia. In all these types of cancer, DDX5 (p68) stands out as the common factor that is highly implicated in each of these cancers, thereby triggering its role as a major player in oncogenesis.

of p68, Wnt/β-catenin to regulate the expression of RelA towards driving colon carcinogenesis.[41–43]

Meanwhile, pondering over other DEAD-box RNA helicases, we can elaborate as follows. On analyzing the relationship between DDX39 expression levels with the prognosis of colorectal cancer, it was found that DDX39 expression was differential expressed in epithelial and stromal regions and served as a new prognostic marker. Among the 824 patient samples, there were 541 cases of high and 283 cases of low DDX39 expression in the epithelium; there were 424 cases of high and 400 cases of low DDX39 expression in the stroma. The expression profiles in epithelial and stromal regions of colorectal tumors were related with tumor location, degree of tumor differentiation, tumor recurrence, and metastasis.[44] There are numerous reports on the multifunctionality of DDX3 in cancer scenarios. In large cohort survival analysis, DDX3 has a significant prognostic power in colorectal cancer (CRC) at both RNA and protein levels. Patients with low DDX3 expression had a poor prognosis and frequent distant metastasis.[36] DDX3 has been identified as a regulator of the β-catenin/TCF signaling that acts as a regulatory subunit of CK1ϵ to promote phosphorylation of disheveled segment polarity protein 2 (Dvl2). It was found that β-catenin expression was decreased by DDX3 knockdown and increased by its overexpression in CRC cells upon checking by Western blot analysis. The authors also reported that the invasion capability in CRC cells and xenograft lung tumor nodules induced by DDX3-overexpression were suppressed by inhibitors of CK1ϵ and β-catenin/TCF signaling. Thus, DDX3 might promote tumor invasion via the CK1ϵ/Dvl2 axis due to β-catenin/TCF activation.[45] In another group of studies, it was found that DDX3 plays an oncogenic role in CRC *via* the β-catenin/ZEB1 axis by increasing KRAS transcription. DDX3 enhances oncogenic KRAS transcription *via* an increase in SP1 binding to its promoter whereas the β-catenin/ZEB1 axis is responsible for DDX3-induced cell invasiveness and xenograft lung tumor nodule formation.[46] Upon studying the importance of DDX46 concerning colon cancer, 87.04% of the columnar adenocarcinoma cases displayed high levels of focal nuclear DDX46 staining, and DDX46 expression was strongly increased in CRC tissues compared to adjacent tissues. Cells treated with DDX46-RNAi-LV exhibited reduced cell proliferation, apoptotic induction *via* increased expressions of cleaved caspase-3 and PARP thereby pointing towards its importance in CRC progression and as a tumor marker.[47] The RCK/p54 protein belongs to the DEAD-box protein/RNA helicase family and in colorectal adenocarcinoma tissues, it was found to be overexpressed and indicated to play an important role in CRC.[48] The therapeutic efficacy of RCK/p54 was tested in human colorectal carcinoma. RNAi-mediated knockdown of

p54 drastically affected the proliferative propensity of colon cancer cells *in vitro* as well as the tumor-forming ability *in vivo*. This indicates that p54 can serve as a prognostic marker in colon cancer[31] and further research are underway to tap its potential. Proteomic analysis conducted in CRC patients by LC-MS revealed that DDX54 was highly expressed in tumor tissues. Also, tissue microarray data unveiled that CRC patients' survivability is inversely correlated with DDX54 levels. Mechanistically, DDX54 activated NF-κB and AKT-mTOR signaling pathway and thereby promoted tumor cell proliferation and metastasis. Upon blocking these signaling pathways with inhibitors like BTZ (Bortezomib) and MK2206, the proliferative role of DDX4 on CRC cells was repressed thus indicating the cumulative action of NF-κB and AKT on DDX4 in accelerating colon cancer.[49] Recently, the effect of aberrant fucosylation associated with DEAD-box proteins was found to play a role in CRC progression. RNA-sequencing (RNA-seq) and RNA-binding protein immunoprecipitation-sequencing (RIP-seq) showed direct binding of DDX39B with FUT3 pre-mRNA thereby upregulating FUT3 expression. Upregulation of FUT3 accelerates the fucosylation of TGFβR-I and activates the TGFβ signaling pathway which ultimately causes the EMT and cancer progression.[50]

Brain cancer

The heterogeneous malignancy of the central nervous system caused by glial cells neoplasm leads to gliomas. They constitute the most common type of primary tumors of the brain and are further classified based on the aggressiveness and invasiveness of tumor mass. Glioblastoma multiforme (GBM) exemplifies the rapacious astrocytoma grade IV and is associated with poor patient survival. There is an imperative need to focus on the signaling dysregulation associated with this disease and search for novel molecular markers for a plausible future therapy.

For this aspect, DEAD-box RNA helicases have been getting researched for quite some time. Wang et al reported that p68 (DDX5) was overexpressed in glioma cells and tissues and plays a chemotactic role in oncogenesis. They found using microarray that DUSP5 is a novel target of p68. Both of them colocalized in glioma cells. Moreover, in high-grade glioma tissues, DUSP5 expression was lower, indicating its role as a tumor suppressor. p68 induced negative regulation of DUSP5 and subsequently mediated ERK signaling pathway activation. Additionally, upon downregulating DUSP5 and p68 singly and in combination, the capacity of the cells to grow and invade was stronger in co-transfected cells than in cells treated with p68 knockdown alone. Hence, p68/DUSP5 promotes proliferation, invasion, and migration in glioma cells.[51] The same group of researchers also reported that p68 interacts with the N-terminal of NF-κB p50 through the release of inhibitory activity of IκBα and consequently increased NF-κB p50 target luciferase transcription activity in glioma cell lines. The regulatory activity of p68 in NF-κB signaling and its regulatory activity led to glioma progression.[52] Post-translational modification in terms of tyrosine double phosphorylation of p68 at Y593 and Y595 in TRAIL-resistant T98G glioblastoma cells conferred apoptosis resistance and protected the cells from programmed cell death. An autocrine loop of PDGF induced the post-translational modification. Thus, p68 plays a role in overcoming TRAIL resistance from glioma perspective.[53] Another DEAD-box protein namely p72 when upregulated led to enhanced migration and invasion of glioma cells by decreasing Beclin1 expression and increasing the expression of miR-34-5p and miR-5195-3p in A172 and T98G cells. Apoptosis and colony formation ability was found to be significantly decreased with p72 inhibition. Furthermore, Beclin1 contributes to A172 cell autophagy, invasion, and apoptosis through its upregulation.[54]

Similarly, DDX23 controls glioma malignancy by upregulating miR-21 biogenesis. Also, the inhibitor ivermectin inhibited DDX23 activity which decreased miR-21 levels and blocked invasion and cell proliferation. It also diminished glioma growth in mouse xenografts.[55] Genome-wide association studies (GWAS) identified a risk locus proximal to the *PHLDB1* gene on 11q23.3. Through bioinformatic studies to identify the associated SNPs, a subset of 10 functional SNPs in the promoters of PHLDB1 and DDX6 were identified. Chromatin conformation capture (3C) recognized a physical interaction between the enhancer element containing a functional SNP (rs73001406) and the *DDX6* gene promoter. This may help in assessing the glioma-risk studies in the future.[56] Meanwhile, for radio- and chemoresistance in GBM, DDX6 was found to play a key role. CCRT (concomitant chemotherapy and radiation therapy) leads to poor patient prognosis in GBM therapy. Through genome-wide screening of infected shRNA pools in cells derived from patients, DDX6 appeared as a promising candidate for treatment. Decreased DDX6 leads to increased clonogenic ability and resistant response against radiation treatment *in vivo* and *in vitro*.[57] These studies regarding DEAD-box proteins envisage their role in glioma progression and opens up the prospect of therapeutic research by identifying suitable players of this family.

Breast cancer

Globally, amongst women, the most common cancer is breast cancer. Molecular markers namely estrogen receptor (ER), progesterone receptor (PR), and ErbB2 (HER2/Neu) have been related with the five major subtypes of breast cancer: basal-like, luminal A, luminal B, ErbB2^{+}/ER^{-}, and basal breast cancer.[58] A lot has been studied concerning the progression of breast cancer especially triple-negative breast cancer (TNBC, having the worst prognosis). Molecular players and their roles have been discovered throughout these years and therein comes the mediative role of DEAD-box proteins. They have been closely linked with the oncogenicity of breast tumors. In 2019, Kim et al demonstrated that PARP-1-directed RNA immunoprecipitation (IP) coupled with deep sequencing (RIP-seq) in MCF7 cell line, led to preferential binding of PARP-1 with snoRNAs. In turn, snoRNAs interacted with the PARP-1 DNA-binding domain and stimulated the catalytic activity of PARP-1. snoRNA-activated PARP-1 ADPRylates DDX21 to promote its nucleolar localization. Thereafter, DDX21 drives ribosome biogenesis and cancer cell growth. This study reveals the mechanistic rationale of PARP signaling in breast cancer

cells.[59] Reports have also suggested that an antagonistic feedback loop between DDX21 and Snail transcription, as well as, the crucial role of miR-218-5p in decreasing the ratio of DDX21/Snail leads to epithelial—mesenchymal transition (EMT) and metastasis in breast cancer.[60] In a report published this year, a novel interaction between DEAD-box helicase 5 (DDX5) and the BRCA2 DNA repair associated protein (BRCA2) at regions of DNA breaks revealed unwinding of DNA-RNA hybrids within transcribed chromatin and facilitates repair process. In breast cancer patients expressing the BRCA2T207A missense variant, BRCA2—DDX5 interaction gets hampered.[61]

The oncogenic role of DDX3 in breast cancer had gained popularity long back (Farabaugh et al). It is upregulated in breast cancer metastases cases.[35] DDX21[62] and DDX3X (homolog of DDX3) have been reported to be overexpressed in breast cancers. *In vitro*, over-expression of DDX3X in non-tumorigenic breast epithelial cells (MCF10A) results in increased invasive cell properties whereas knockdown of DDX3X decreases cell proliferation in breast cancer cell lines (MCF-7 and MDA-MB-231). DDX3X promotes MCF7 cell proliferation, by inhibiting the expression of the key negative cell cycle regulator, Kruppel-like factor 4 (KLF4). This highlighted the oncogenic role of DDX3X.[63] Coming to the enormous reports on the role of DDX5 in breast cancer, we can summarize as follows: Concerning post-translational modification of p68 in breast cancers, reports have suggested that tyrosyl phosphorylation affects its ATPase and helicase activity. Tyrosine 593 (Y593) phosphorylation promotes PDGF-induced EMT.[64] Besides, PDGF and TNF-α enhance the tyrosine phosphorylation of p68.[65] Phosphorylated p68 upregulates c-Myc and Cyclin D1 and thus aids in PDGF mediated cell proliferation and apoptosis resistance (induced by TNF-related apoptosis-inducing ligand or TRAIL).[53] Phosphorylated p68 can also stimulate Snail1 transcription through dissociation of histone deacetylase 1 (HDAC1).[66] A strong correlation between p68 expression and the oncogenic PLK1 protein kinase expression exists in human breast cancers. For transcriptional activity, reports can be enumerated as follows; Downregulation of PLK1 and p68 expression by p53 in human breast cancers depend on high levels of PLK1 and poor clinical outcomes.[67] Jacob et al showed that interaction of p68 with lemur tyrosine kinase-3 (LMTK3) and modulation of its target genes expression, especially of the miRNAs, occurs in breast cancer. LMTK3 inhibits the expression of miR-34a, miR-196-a2, and miR-182 in breast cancer cells and curbed cancer cell proliferation, invasion, and migration.[68] p68 is also involved in cell cycle progression. Δ133p53 (p53-spliced variants) negatively regulates p53-mediated apoptosis (Bourdon et al). It has been shown that siRNA-mediated p68 knockdown upregulates Δ133p53 in a p53-independent manner. Also, it could adversely affect the ability of p68 to coactivate p53-mediated induction of p21.[69] Wnt signaling is crucial in the development of breast cancer. Guturi et al reported the direct inductional effect of β-catenin on p68 promoter or indirect effect through regulation of c-Myc in both human and mouse breast cancer cells. Additionally, a positive feedback regulation exists for the expression of TCF4 by p68.[70] Altogether, these phenomena demonstrate an important role of p68 in EMT and breast cancer progression. In breast cancers, p68 and p53 interplay stimulates expression of oncogenic protein kinase, Polo-like kinase-1 (PLK1), and is associated with poor prognosis.[67] Another study claimed that p72 plays a crucial role in ER-α co-activation and estrogen-dependent cell growth and is associated with improved survival in ERα-positive breast cancer.[71] Additionally, the DDX5 locus is amplified in breast cancer. Thus, it contributes towards G1-S progression, DNA replication, gene expression, and recruitment of RNA polymerase II to promoters of DNA replication genes, all concomitantly linked with breast cancer progression.[72] Further studies on the activity of p68 might allow the progress of improved therapeutic interventions in breast cancer.

Prostate cancer

Malignancy of benign prostatic epithelial cells leads to prostate cancer (PCa) through neoplastic conversion. It is the most common and major type of cancer in men, especially in western countries. There is distinct progression involved in the formation of high-grade prostatic intraepithelial neoplasia, invasive adenocarcinoma, distant metastatic disease, and finally lethal castration-resistant metastatic disease. There is a search for better biomarkers of this disease and regarding this context, the DEAD-box proteins come into play. DDX3 expression was quantified in 320 human prostate samples, revealing an increase in epithelial whole cell, cytoplasmic, and nuclear DDX3 in primary prostate cancer compared with benign prostate or hyperplasia. Inhibition of DDX3 affects metastatic growth indicating a novel role of DDX3 in prostate cancer progression.[34] Apart from cancer progression, there is also a need to look out for novel therapeutic strategies to overcome the hurdles. Bol et al reported the overexpression of DDX3 in prostate cancer tissue samples. Upon knocking down DDX3 in three prostate cancer cell lines (PC3, DU145, and 22Rv1), cell proliferation and clonogenicity decreases in DU145 and 22Rv1, but not in PC3 (because it already has a low level of DDX3).[73] Another key protein that needs to be mentioned is DDX20 (an oncogenic marker). Oncomine database revealed the significantly high occurrence of DDX20 in prostate cancer tissue which was validated by patient sample studies and quantitative RNA analysis both *in vivo* and *in vitro*. It contributed to increased cell migration, proliferation, and invasiveness in PCa cell lines and upon further analysis, Chen et al did luciferase assays to further report the promigratory effects of DDX20 upon NF-κB signaling in PCa cells.[74]

Interestingly, DDX5 has shown equal paramountcy in prostate cancer progression. Clark et al had already reported in 2008 that the RNA helicase p68 is a novel androgen receptor coactivator and found to be overexpressed in prostate cancer. Building upon this finding, they further reported in 2013 that p68 mediates β-catenin and AR transcriptional co-activity in PCa cells. p68 is not only vital in AR regulated transcription at the promoter level, but it also helps in elongation and transcriptional progression through direct interaction with RNAP II and influencing AR regulated genes thereafter.[75] In the quest of therapeutic-based research, a group of scientists reported that resveratrol via inhibition of the mTORC1 pathway led

to directly targeting DDX5.[76] Resveratrol, a dietary phytochemical is synonymous with the prevention of cancer, neurodegenerative disorders, and obesity-related diseases. Thus, there was a search for its interacting partners. In PCa cell line, using resveratrol immobilized beads, DDX5 was reported to be a novel target. Treatment with resveratrol induced degradation of DDX5 in prostate cancer cells. On the other hand, knockdown of DDX5 attenuated the inhibitory activities of resveratrol against mTORC1 signaling, leading to the role of resveratrol & DDX5 in therapeutic ways. So, this was an interesting discovery along the clinical lines of prostate cancer therapy. RK-33 (small molecule inhibitor of DDX3) inhibits DDX3 dependent proliferation of prostate cancer cell lines and cell-cycle arrest. Combinatorial therapy using RK-33 and radiation had synergistic effects in the xenograft model of prostate cancer.[77] There is still a long way to go before we can arrive at something breakthrough for proper clinical treatment of prostate cancer.

Other cancers

DEAD-box RNA helicases are also involved in myriad types of cancers. DEAD box helicases are implicated in lung cancer. Survival and differential expression analysis reveal that DDX11, DDX55, and DDX56 act as negative prognostic factors, whereas DDX5 acts as a positive prognostic factor. Pathway enrichment analysis indicates that MYC signaling and expression profile of the *DDX5* gene is negatively correlated whereas that with *DDX11*, *DDX55*, and *DDX56* is positively correlated. Low mutation levels of TP53 and MUC16 correlated with high expression levels of the *DDX5* gene lung cancer.[78] On the other hand, inhibition of DDX3 using specific inhibitory peptide RK-33 caused G1 cell cycle arrest, apoptosis, and tumor regression in lung cancer mouse models.[79] Moreover, DDX3 was also identified as a molecular marker in ovarian cancer. An association exists between DDX3 and miR-196a in ovarian cancer, and miR-196a can promote tumor proliferation and attenuate apoptosis by downregulating DDX3 through the PTEN/PI3K/AKT pathway.[80] Liu et al demonstrated that DDX10 is significantly overexpressed in lung cancer and is connected with the proliferation, apoptosis, and cell cycle of tumor cells. It interacts with U3 small nucleolar ribonucleoprotein IMP4 and a positive correlation exists between them such that overexpression of IMP4 reverses the effect of knockdown of DDX10 in lung cancer cells (A549).[81] Recently, the tumor-suppressive role of DEAD-box protein 1 (DDX1) had been reported in ovarian cancer progression. Similarly, DDX10 is significantly downregulated in ovarian cancer tissues. Knockdown of DDX10 encourages ovarian cancer cell proliferation through Akt/NF-κB pathway.[32] DEAD-box protein DDX46 plays important role in various cancers such as squamous cell carcinoma and osteosarcoma. High levels of DDX46 were observed in human osteosarcoma tissues and cell lines. Its downregulation inhibited osteosarcoma cell proliferation, migration, and invasion *in vitro* and tumor growth *in vivo*.[82] In esophageal squamous cell carcinoma (ESCC), DDX46 is significantly upregulated. DDX46 knockdown inhibited cell growth, and induced apoptosis, thereby signifying its role in ESCC proliferation.[83]

Meanwhile, DDX5 (p68) is clinically significant in numerous cancers such as ovarian cancer, lung cancer,[79] esophageal cancer, gastric cancer[84] and, liver cancer.[85] In hepatocellular carcinoma (HCC) tissues, it is found to be overexpressed. Mediating the Akt pathway, p68 promoted tumorigenesis *in vitro*. Knockdown of p68 adversely affected the cell migration, invasion, and EMT process.[86] In esophageal cancer (EC), p68 knockdown inhibited the proliferation of EC cells *in vitro* and the growth of EC xenografts *in vivo*.[87] In another study, it was demonstrated that when DDX5 gets down-regulated, CDK2, Cyclin D1, and Vimentin were also downregulated but E-cadherin was upregulated thereby inhibiting esophageal squamous cell carcinoma (ESCC) cell proliferation and metastasis. Also, DDX5 and the expression of phospho-eIF2α, phospho-PERK, and P62 are positively correlated. This highlights that decreased levels of DDX5 are related to inhibition of endoplasmic reticulum (ER) stress and the recovery of autophagy flux and subsequent inhibition of ESCC.[88] In renal cell carcinoma (RCC), it was reported that Simvastatin inhibited RCC cell viability, migration, invasion, and induced apoptosis. Mechanistically, simvastatin significantly suppresses DDX5 and promotes DUSP5 expression thereby implicating the role of DDX5 in RCC.[89] Also, in gastric cancers, it was reported that HSP90 directly interacts with DDX5 which inhibits DDX5 protein degradation via the AMPK/ULK1-regulated autophagy pathway. Thereafter, gradual accumulation of DDX5 and subsequent activation of β-catenin signaling pathway triggers the malignant HCC phenotype. Thus DDX5 and HSP90 can be potential therapeutic targets in HCC.[90] In gastric cancer, LINC01207 and DDX5 are up-regulated, whereas miR-671-5p is down-regulated. It was found that LINC01207 interacts with miR-671-5p and negatively regulates it. On the other hand, DDX5 is a downstream target of miR-671-5p and is positively correlated with LINC01207.[91] Thus, long noncoding RNA LINC01207 promotes gastric cancer cell proliferation and metastasis by modulating the miR-671-5p/DDX5 axis.

Role of DEAD-box protein p68: a double-edged sword in oncogenesis

Amidst all the DEAD-box RNA helicases, p68 is the most abundantly researched protein. It has been implicated as an oncogenic factor and is gradually gaining recognition as a molecular prognostic marker of oncogenesis. Therefore, we sought to summarize its role as a double-headed agent in the process of cancer progression by not only coactivating major transcription factors but also being intricately involved in the signaling switch of various signaling pathways (Fig. 3). A deeper understanding of more such mechanisms is required for a holistic knowledge regarding p68. It is an important co-activator of several oncogenic transcription factors including β-catenin, Stat3, p53, ER, AR amongst others that have been discussed in detail below:

β-catenin

β-catenin is encoded by the *CTNNB1* gene in humans. It is a multi-functional protein involved in the regulation and

coordination of cell–cell adhesion as well as in gene transcription and regulating developmental processes. It is ubiquitously present in many tissues. Its aberration plays a major role in hampering developmental stages, cardiac diseases, and driving oncogenesis. Both p68 and β-catenin are overexpressed in cancer. In 2013, it was reported that both of them co-localize and functionally interact with each other as well as with AR in prostate cancer cell lines. Overexpression of p68 had an additive effect on the coactivation of β-catenin and thereby mediated transcription of AR responsive genes whereas the knockdown showed opposite effects. Also, p68 immunoprecipitated with RNA polymerase II (RNAP II) and recruited to elongating regions of AR-mediated PSA gene thereby implicating its role in facilitating β-catenin and AR transcriptional activity in prostate cancer cells.[75] DDX5 also played a role in promoting non-small-cell lung cancer (NSCLC) through activating the β-catenin pathway. The co-immunoprecipitation assay showed a direct physical interaction between DDX5 and β-catenin. Nuclear accumulation of β-catenin was significantly increased by DDX5 overexpression (and transcriptional and translational levels of β-catenin target genes *cyclin D1* and *c-Myc* were also upregulated by DDX5 overexpression.[92] In our lab, we have shown that p68 deploys β-catenin to drive RelA/p65 gene expression in colon cancer. p68 along with β-catenin occupied RelA promoter at the TCF4/LEF (TBE) sites and hence potentiates RelA transcription.[43] Recent reports have suggested that long noncoding RNAs (lncRNAs) are involved in pro-tumorigenesis activity. One such lncRNA NHEG1 (neuroblastoma highly expressed 1) bound to DEAD-box helicase 5 (DDX5) protein through repressing proteasome-mediated degradation, causing β-catenin transactivation and neuroblastoma progression. This indicates an interplay between the trio of 'lncRNA/p68/β-catenin' resulting in a cascading turn of oncogenic events.[93] Liu et al have demonstrated that lentiviral particles carrying human hepatoma-derived growth factor (HDGF) short hairpin RNA and plasmids for HDGF, DDX5, and β-catenin expression, were introduced into endometrial cancer (EC) cells and were reported to affect proliferation, migration, invasion, and metastasis. Thus, HDGF and DDX5 activate β-catenin in promoting carcinogenesis in EC.[94] Another study focused that in lung adenocarcinoma, HDGF interacted with DDX5 to cause nuclear translocation of β-catenin and stimulated TCF4 and its downstream EMT signal. In subsequent investigations, it was revealed that overexpressed HDGF reversed miR-1254-mediated suppression by activating DDX5/β-catenin/TCF4-stimulated EMT signal. Later, a novel correlation between DDX5 and β-catenin was shown in nasopharyngeal carcinoma (NPC). The authors demonstrated that down-regulating miR-1254 promotes NPC pathogenesis. It gets induced by nasopharyngeal epithelium-specific protein 1 (NESG1) and targeted HDGF to diminish DDX5/β-catenin/TCF4 and its downstream EMT signal.[95] Therefore, we can conclude that the combinatorial effect of p68 and β-catenin interplay can lead to cancer.

Stat3

The signal transducer and activator of the transcription (Stat) family of transcription factors consists of seven highly conserved proteins, including Stat3. It is associated with numerous processes such as embryonic development, adult homeostasis, immune response, cell survival, proliferation, and oncogenesis. p68 and Stat3 are overexpressed in a multitude of cancers and we hereby aim to list a few examples of their inter-relation against the cancer backdrop. In our lab, we recently reported for the first time that p68

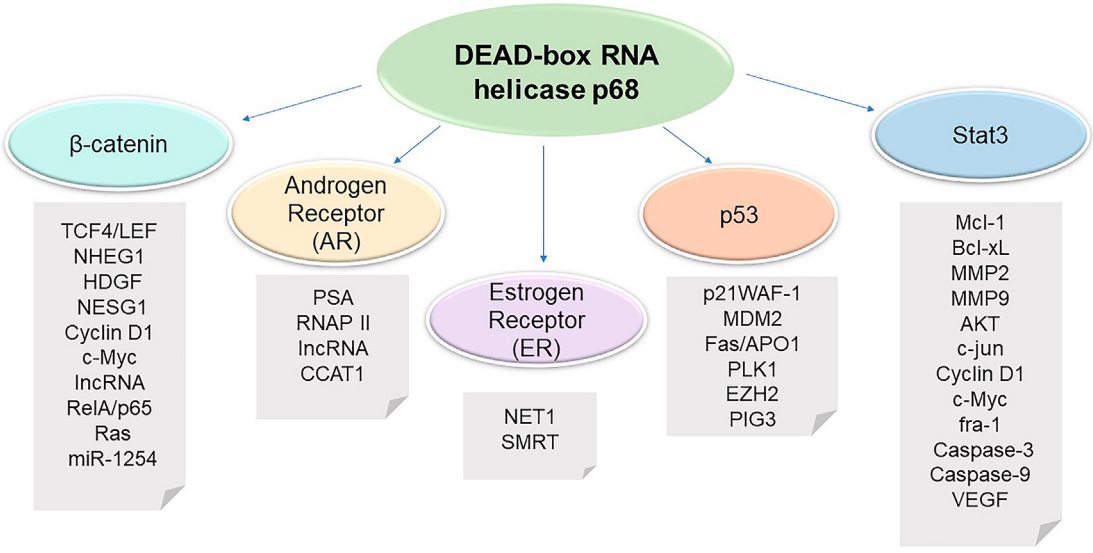

Figure 3 p68: an important co-activator of several transcriptional machineries. DEAD-box RNA helicase p68 (DDX5) acts as a transcriptional coactivator of various major oncogenic transcription factors such as β-catenin, androgen receptor, estrogen receptor, p53 and Stat3. Further these factors trigger several important downstream effector genes as mentioned briefly in the diagram.

physically interacts with Stat3 (proved through IP, reverse IP, Co-IP, and GST-pull down assays) and thereafter stimulates its target genes namely *Cyclin D1*, *Mcl-1*, *Bcl-xL*, *MMP2*, *MMP9*, and *VEGF* by enhancing the Stat3 transcriptional activity. Overexpression and knockdown of p68 affected the target genes directly and the "p68 and Stat3 alliance" led to coactivation of Stat3 transcriptional activity, thereby potentiating oncogenic effect (proved by Western blot, qPCR assay, ChIP assay, and luciferase reporter assay).[41] p68 enhances the expression of Cyclin D1 in a STAT3-and β-catenin-dependent manner.[29] It increases the expression of VEGF, c-Jun, c-Myc, fra-1, AKT, matrix metalloproteinase-2 (MMP2), and MMP9, wherein all of these are regulated by β-catenin and STAT3.[96] Additionally, DDX5 interferes with JAK2/STAT3 signaling pathway in BCC (basal cell carcinoma). Activating the STAT3 pathway in BCC contributes to tumor growth and tumor progression. STAT3 expression was decreased by JAK2 inhibitor which abolished the regulatory effects of DDX5 knockdown on apoptosis, migration, and invasion of BCC cells. STAT3 inhibitor also abolished DDX5 knockdown-regulated caspase-3, caspase-9, Bcl-2, and Bcl-xl gene and protein expression in BCC cells, indicating a mediative mechanism between these two players.[97]

p53

p53 referred to as the "guardian of the genome", is a critical tumor suppressor. It is induced and activated in response to several stresses, including DNA damage. Once it gets activated, p53 induces transcription of its downstream target genes, found to be involved in growth arrest, apoptosis, and DNA repair. In addition, p53 also induces expression of its negative regulatory partner Mdm2.[98,99] The role of p68 as a direct co-activator of p53 is interesting given the importance of these two major proteins against the cancer backdrop. p68 interacted with p53 both *in vitro* and *in vivo* and endogenous p68 and p53 co-immunoprecipitated together from nuclear extracts. Furthermore, silencing of p68 expression, in wild-type (WT) p53 expressing cells showed that p68 is specifically required for upregulating expression of p53 target genes $p21^{WAF-1}$, *MDM2*, *Fas/APO1*, and *PIG3* in response to treatment with the DNA-damaging agent etoposide, but it showed no effect on non-p53-responsive genes. p68 is recruited to the p21 promoter in a p53-dependent manner thereby promoting transcriptional initiation.[26] But p68 is a selective regulator of the p53-mediated DNA damage response. The decision between cell cycle arrest and apoptosis is intricately balanced in a tissue- and context-dependent manner. It was shown that p68 is critical for p53-mediated transactivation of the pro-survival cell cycle arrest gene *CDKN1* (*p21*) and the G1/S cell cycle checkpoint. Also, siRNA depletion of p68 inhibited the recruitment of p53 and RNA Pol II to the *p21* promoter but not to the *Bax* or *PUMA* promoters. Finally, using inducible *p68* knockout mouse model, it was discovered that p68 is required for p53-dependent induction of p21 in many tissues in response to γ-irradiation and is not required for induction of pro-apoptotic genes highlighting the novel function of p68 as a modulator of the decision between p53-mediated growth arrest and apoptosis.[100] In breast cancer, the p68/p53 interplay regulates the PLK1 transcriptional levels. It was found that repression of PLK1 expression in response to etoposide treatment (activation of p53) is proportionately reduced upon silencing of p68 expression. The p68 and PLK1 collaboration in cancer cells might hint at a future plausible role of novel PLK1 inhibitors to target the cancers in which the *TP53* (*p53*) gene is mutated.[67] Apart from all these, it is also known that p68 (DDX5) is a p53-independent target of ARF and mediates ribosome biogenesis.[101] Conversely, mutant p53 by disrupting p68-Drosha complex assembly and attenuating miR-26a processing induces EZH2 expression and promotes EMT.[102] Certain p53 isoforms can modulate the action of full-length p53 (p53α). The Δ133p53α isoform inhibits p53-dependent apoptosis in mammalian cell lines. p68, p53, and Δ133p53α form a complex to regulate the expression of Δ133p53 and its consequent modulation of p53-mediated transcription. In breast cancers, p68 expression is inversely associated with Δ133p53 expression.[69] Thus, all these reports emphasize the major role played by p68 in the coactivation of p53 as well as the feedback interaction between them in cancer.

Androgen receptor (AR)

The androgen receptor (AR), also known as NR3C4 (nuclear receptor subfamily 3, group C, member 4) is a nuclear receptor that is activated by binding any of the androgenic hormones in the cytoplasm and then translocating into the nucleus. AR is as a DNA-binding transcription factor that regulates gene expression. It is involved in the occurrence of prostate cancer in men. The role of p68 in coactivating AR was discovered in 2009. It was found that p68 is a novel nuclear androgen receptor-interacting protein in prostate cancer cells. ChIP assays proved that p68 and AR co-occupied androgen-responsive elements (ARE) within the promoter and enhancer regions of the androgen-responsive prostate-specific antigen (*PSA*) gene and luciferase assays showed the transcriptional activation of PSA by AR in presence of p68. AR-regulated repression of CD44 splicing and post-translational modification of p68 in terms of tyrosine phosphorylation led to enhanced co-activation of ligand-dependent transcription of AR-regulated luciferase reporters independent of ATP-binding. Therefore, p68 may serve as a common link between transcription and splicing.[25] Moreover, p68 is not only vital for AR-regulated transcription at the promoter level but also equally important during elongation and transcriptional progression. p68 is required for transcriptional regulation of AR-mediated genes and also for the recruitment of AR co-factors to the AR transcriptional complex. This was proved by the finding that p68/DDX5 supports β-catenin and RNAPII (RNA polymerase II) during AR-mediated transcription in prostate cancer.[75] Recruitment of p68 to endogenous AR responsive genes may facilitate spliceosome assembly and increase the elongation rate of RNAPII. p68 has been shown to interact with the C-terminal domain of RNAPII[25]; AR enhances elongation by interacting with TFIIH (transcription factor IIH) and P-TEFb (positive transcription elongation factor b), which phosphorylates the C-terminal domain of RNAPII, switching it from a non-processive to a

processive form.[103] Further studies need to be done to properly unveil the therapeutic potential of the p68/AR reciprocity.

Estrogen receptor (ER)

Estrogen receptor-α (ERα) belongs to the nuclear hormone receptor family of ligand-activated transcription factors that are activated by estrogen and regulate the expression of a plethora of target genes. Approximately, 50% of breast tumors are ER-positive (ER^+) and can be growth inhibited by pharmacologic blockade of estrogen. Furthermore, the ER status is one of the most widely used markers of breast cancer prognosis. The first breakthrough report of the involvement of p68 in transcriptional regulation of ERα came from the identification of p68 as a protein that interacts with and coactivates estrogen receptor alpha (ERα) in response to estrogen.[15] They reported that p68 binds to transcriptional coactivator CREB-binding protein (CBP) and helicase activity was superfluous for the ability of p68 to coactivate ERα in promoter-reporter assays. Furthermore, p72 was also shown to interact with ERα and to potentiate its activity. Pan genomic exon arrays are conducted to investigate the transcriptional regulation from alternative promoters of ERα-regulated genes in MCF7 cells. Recently, several alternative promoters that are differentially regulated by estrogen were identified.[104] Surprisingly, simultaneous si-RNA mediated knockdown of both p68 and p72 may have the ability to switch expression between promoters of certain target genes in an estrogen-dependent manner.[15] NET1 (a RhoA specific guanyl nucleotide exchange factor) that is differentially regulated by estrogen was found to be associated with shorter metastasis-free survival in ERα-positive breast cancers, as was high p68/p72 mRNA expression, which is unswerving with the knowledge that p68 and p72 arbitrate the estrogen-controlled promoter switching. There have also been reports of DDX5 and DDX17 acting upstream of the estrogen and androgen signaling pathways. At the splicing level, the expression of several key regulators of the hormone-signaling pathways is controlled by them. The authors reported that DDX5 and DDX17 controlled the expression level of the SMRT transcriptional coregulator. DDX17 depletion led to the production of a SMRT splicing variant that is degraded by the NMD pathway, leading to a concomitant decrease of SMRT protein level. Also, they regulated alternative splicing of GSK3β, thereby controlling AR and ERα protein levels.[24] Thus, p68 and other DEAD box proteins such as Ddx17 play important role in mediating breast cancer through the ER pathway regulation.

Role of p68 and other DEAD-box RNA helicases in mediating the major cancer signaling pathways

The 11 most important signaling pathways that are involved with the pathology of human cancers are EGFR, RAS, PI3K, NF-κB, Wnt, STAT, MAPK, p53, TGF-β, NOTCH, and Hedgehog pathways. Out of these pathways, our review focuses on 4 key pathways namely EGFR, NF-κB, Wnt, and Notch pathway for their immense importance in cancer and also to delineate the role of p68 as a crucial signaling switch in the coordination of these signaling network. The role of other DEAD-box proteins in regulating these pathways have also been discussed ahead (Fig. 4).

EGFR pathway

EGFR pathway plays pleiotropic roles in developmental and physiological functions. Mutations of the *EGFR* gene lead to EGFR overexpression. Dysregulation of EGFR or its members and associated proteins have been implicated in different cancers. There is a need to properly investigate the mechanistic routes of aberrations for proper cancer management and target-specific drug development. The epidermal growth factor receptor (EGFR) belongs to the ErbB family which is a prototypical receptor tyrosine kinase (RTK) family of receptors. They consist of an extracellular ligand-binding domain (13 conserved EGF-domain containing polypeptide ligands), a transmembrane hydrophobic domain, and a tyrosine kinase cytoplasmic domain. The ErbB family comprises four receptors *viz.*, EGFR (ErbB-1/HER1), ErbB-2 (Neu, HER2), ErbB-3 (HER3), and ErbB-4 (HER4). The receptor dimerizes (homo or heterodimers) upon ligand binding, leading to autophosphorylation and/or cross-phosphorylation of the cytosolic tail. Finally, there is an activation of numerous downstream kinases and transcription factors affecting various signaling pathways.[105] Consequently, they regulate the expression of genes involved in survival, proliferation, differentiation, and migration. EGFR pathway affects many signaling cascades *viz.*, JAK/STAT, RAS/RAF/MAPK, PI3K-Akt, etc. It is one of the major pathways being perturbed in cancers and its multifaceted nature is being vastly studied for cancer therapy.[106] The earliest report of a plausible link between the EGFR pathway and p68 dates back to 1999. Immunoaffinity and chromatography analysis had identified five novel EGFR putative substrates such as p97, p68, p61, p56, and p23. These proteins were found to be phosphorylated on tyrosine residue after EGF treatment assessed by phospho-amino acid analysis.[107] Such experimental results paved way for further research upon the EGFR-mediated mitogenic signaling. PI3K/AKT/mTOR pathway plays a major role in the regulation of cell growth, cell cycle, and apoptosis. Sarkar et al from our laboratory reported a direct link between the p68 and STAT3 pathway as part of the EGFR cascade. p68 was reported to be a novel coactivator of Stat3 and through their direct interaction, p68 mediated the effects of Stat3 upon its downstream target genes and consequently triggered colon carcinoma.[41] The same group of authors had also reported earlier that p68 transcriptionally regulates the expression levels of oncogenic AKT which in turn affects the nuclear exclusion and degradation of tumor suppressor FoxO3a.[42] The mammalian target of rapamycin (mTOR) is an important downstream effector molecule of numerous signaling pathways, such as PI3K and AMPK. It is intricately linked with cancer progression. The mTOR is a key downstream effecter of several signaling pathways that are involved in cancer progression, including PI3K/Akt and AMPK pathways. The upregulation of p68 was

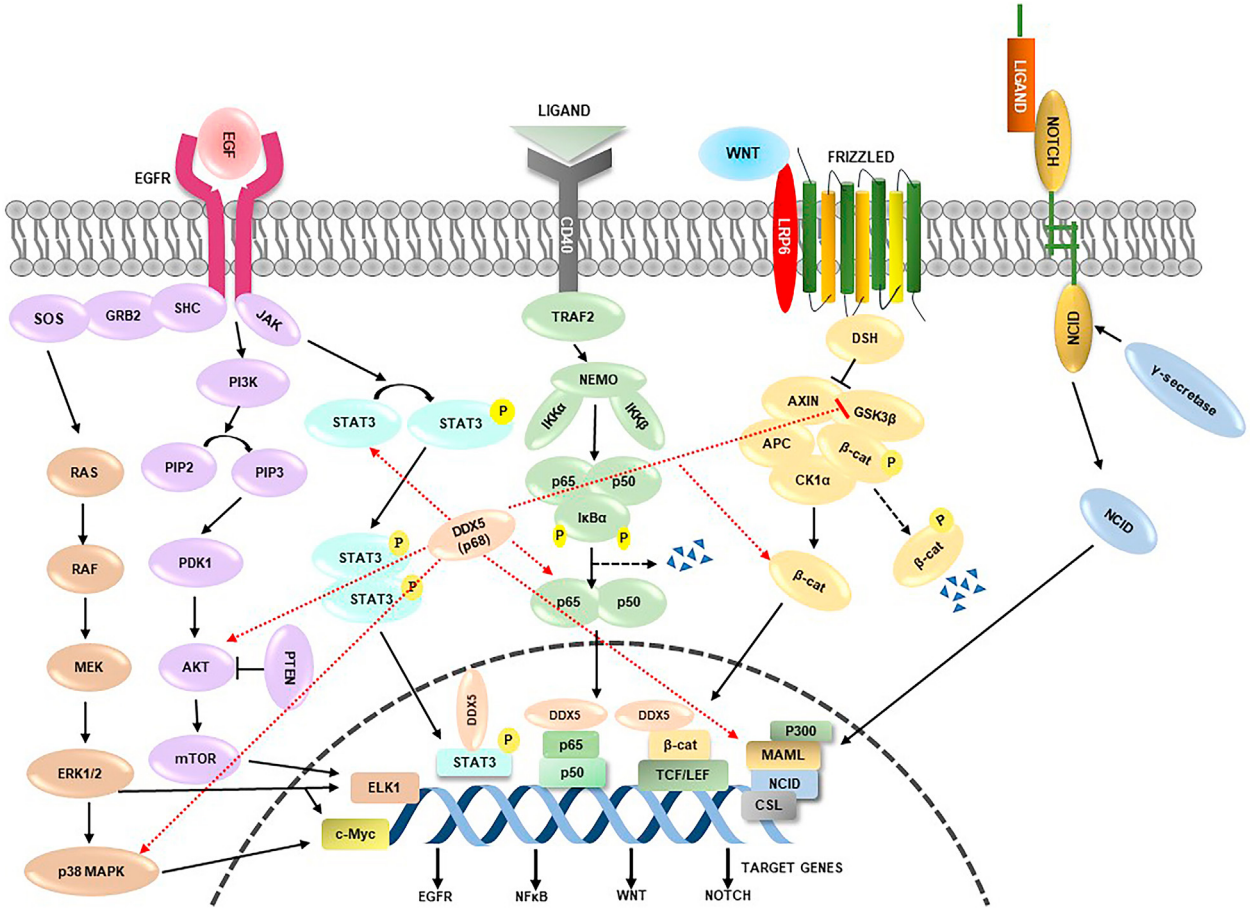

Figure 4 p68: a crucial switch for the major signaling pathways and crosstalks. In the signaling crosstalk between the four major pathways namely EGFR pathway, Wnt pathway, NF-κB pathway and Notch pathway, p68 plays a crucial role as a connecting link between these pathways and helping in the switch from one pathway to another. Due to this, the downstream target genes get activated and thereafter mediate the progression of cancer through their physiological functions. This highlights the effective role played by p68 in being the unique common player that helps in regulating the factors like p38 MAP kinase, Stat3, Akt, β-catenin and MAML1 involved in the major signaling axis.

reported to activate mTOR/S6K1 leading to increased cell proliferation in gastric cancer cells. These findings were in similar lines with previous reports that the mTOR pathway mediated DDX5-dependent cell growth in prostate cancer.[84] Therefore, modulating the DDX5/mTOR/S6K1 axis may help in targeting gastric cancer. There have also been reports of the involvement of p68 in directly regulating the mTOR pathway. Inhibiting p68 activates the mTOR/MDM2 survival mechanism which finally represses p53.[108] The mitogen-activated protein kinase (MAPK) pathway plays an important role in tumorigenesis, cell growth, differentiation, proliferation, apoptosis, and migration. Alteration of the RAS-RAF-MEK-ERK-MAPK (RAS-MAPK) pathway is linked with the progression of several types of cancer. MK2 kinase lies downstream of the p38 MAPK signaling pathway, and it affects p38 MAPK-mediated post-transcriptional gene regulation. p68 was found to directly interact with MK2. It then led to phosphorylation of p68 necessary for pre-miRNA processing. Also, inhibition of p38 MAPK enhanced c-Myc expression levels by suppressing the biogenesis of miR-145. Subsequently, the growth of wild-type MEFs and breast cancer MCF7 cells was promoted via p38 MAPK—MK2 signaling axis.[109] Thus, ample indications are hinting towards the role of p68 in mediating the EGFR signaling network and affecting the ensuing oncogenic loop.

Involvement of other DEAD-box RNA helicases in EGFR pathway

DDX31, a nucleolar protein is overexpressed in human renal cell carcinomas (RCC). Upon investigation, it was revealed that its nuclear-cytoplasmic shuttling mediates its oncogenic properties. On one hand, the overexpressed nuclear DDX31 forms a transcriptional complex with mutant p53 (mutp53). SP1 upregulates its transcriptional activity and target gene expression leading to increased bladder cancer invasion and migration, whereas on the other hand cytoplasmic DDX31 complexes with EGFR by interacting with phospho-NCL (nucleolin), which causes EGFR stabilization and constitutive activation of EGFR/Akt signaling. Using inhibitory peptides to hamper DDX31-NCL interaction led to anti-tumor effects, thereby highlighting the importance of DDX31 in oncogenesis.[110] The RAS/RAF/MEK/ERK pathway is altered in cutaneous melanoma and

uveal melanoma. The therapeutic intervention involves the use of MEK inhibitors, but resistance posed by cells to such inhibitors provides a hindrance in the treatment stage. It was reported that in uveal melanoma cell lines, the continual inhibition of MEK increased DDX43 expression. DDX43 was found to induce RAS and downstream pathways and thus mediated the resistance of cells to MEK inhibitor.[111] Furthermore, the use of epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors (TKIs) also helps in targeting cancers that harbor specific EGFR mutations. Recently, the role of DDX3X in conferring EGFR-TKI resistance was studied. The researchers found that overexpression of DDX3X induced CSC-like phenotypes, EMT, and increased EGFR-TKI resistance in lung cancer cell lines, whereas its knockdown sensitized the cells. Moreover, there was a lack of phosphorylation of Tyr residues in EGFR of the lung cancer cells overexpressing DDX3X. Therefore, inhibition of DDX3X may serve as a promising therapy for lung cancer patients harboring EGFR-activating mutations.[112] Along similar lines, it has been reported that upregulation of DDX17 augmented the gefitinib (EGFR inhibitor) resistance in non-small cell lung cancer cells (NSCLC). The mechanism of gefitinib resistance involves a unique exportin/importin-dependent cytoplasmic shuttling, which consequently causes DDX17 to disassociate the E-cadherin/β-catenin complex, triggering increased β-catenin nuclear translocation and expression of its target genes. So, DDX17 inhibition may be utilized to overcome gefitinib resistance in NSCLC patients.[113] Osteosarcoma (OS) is an invasive skeletal system cancer. DDX10 was over-expressed in OS patients and linked with poor prognosis. Down-regulation of DDX10 inhibited MAPK signaling pathway and decreased the expression of p-MEK and p-ERK. It further inhibited proliferation, invasion, and migration of MG63 osteoblast-like cells *in vitro* due to suppression of MAPK pathway.[33] Therefore, these reports strengthen our understanding of the interplay between EGFR signaling and DEAD-box proteins, making them promising therapeutic targets.

NF-κB pathway

The NF-κB transcription factor family consists of NF-κB1 (p50 and its precursor p105), NF-κB2 (p52 and its precursor p100), RelA (p65), RelB, and c-Rel. Classical (or canonical) and Alternative (non-canonical) pathways exist for NF-κB signaling. In the classical pathway different stimuli, such as B cell receptors (BCR) and some tumor necrosis factor receptors (TNFR) activates the cascade. Following stimulation through TNFR-associated factors (TRAF5, TRAF2), RIP, TAK1, and IKKβ (which is part of an IKKα-IKKβ-IKKγ complex) are activated. NF-κB/Rel proteins which are bound and inhibited by IκB proteins (IκBα, IκBβ, and IκBϵ), get released due to phosphorylation and ubiquitination mediated degradation of the inhibitory subunits by IKKβ. As a result, NF-κB homodimers and heterodimers accumulate in the nucleus and induce target gene expression of genes bearing consensus κB sequences in the promoter or enhancer regions. The p50:p65 and p52:RelB heterodimers are the most common NF-κB complexes in cells. The canonical pathway plays a major role in the control of the immune response, inflammation, cell survival, and proliferation.[114] The alternative pathway is activated by a more restricted set of cytokines, including CD40L, LTαβ, BAFF, RANKL (receptor activator of NFκB ligand), and TWEAK (TNF-related weak inducer of apoptosis). After activation, NIK (NFκB-inducing kinase) activates IKKα, resulting in the phosphorylation of NFKB2. An inhibitory C-terminal IκBδ domain is degraded by the proteasomal pathway and thereby generating the p52 subunit, which leads to the accumulation of p52/RelB heterodimers in the nucleus. This pathway is important in lymphoid development and B-cell maturation.[115] Additionally, it was reported that IFNs also mediate the NF-κB signaling pathway apart from their usual role in JAK/STAT pathway. STAT3, TYK2, PI3K, AKT, IKK, TRAF, and NIK have been recognized in the IFN-activated NF-κB signaling pathway.[116]

DEAD-box RNA helicase p68 is associated with the NF-κB pathway and cooperatively regulates the oncogenic loop. A study showed that p68 enhances glioma cell proliferation *in vitro* and *in vivo*. It positively correlated with increased pathological grade of gliomas and inversely correlated with overall survival in patients which highlights the clinical significance of p68 in the glioma progression and therapy. It was found that the N-terminal of p68 binds directly with the p50 subunit of NF-κB to stimulate glioma cell proliferation. Overexpression of p68 induced the localization of NF-κB p50 into the nucleus. Also, it can induce NF-κB p50 release from IκBα. NF-κB p50 prevents glioma cell proliferation induced by p68. This was shown by pretreatment of p68-overexpressing glioma U-251 cells with pyrrolidine dithiocarbamate (inhibitor of NF-κB p50) which led to decreased cell proliferation. Knockdown of endogenous NF-κB p50 in p68-overexpressing U-251 cells inhibited glioma cell proliferation *in vitro* as well as drastically inhibited *in vivo* tumor growth. These results demonstrated that NF-κB p50 activation is necessary for p68 induced glioma cell proliferation.[116] A noteworthy finding was reported from our lab in the year 2019 which emphasized the cooperative role of p68 and β-catenin in mediating the regulation of RelA (*p65*) gene expression and thereby showed crosstalk between Wnt signaling and NF-κB pathway. We reported that a strong positive correlation exists between p68 and RelA in both normal and colon carcinoma tissue samples. Overexpression and knockdown of p68 and β-catenin resulted in a concomitant increase and decrease of RelA at the protein and mRNA levels respectively. Moreover, there was physical occupancy of p68, TCF4, and β-catenin on the RelA promoter *in vitro*, as demonstrated by ChIP assays. p68, Wnt3a, and β-catenin overexpression significantly increased RelA promoter activity. It diminished upon mutation of TCF4/LEF (TBE) sites in the RelA promoter. p68 and β-catenin alliance also controlled the expression of signature NF-κB target genes. Thus, our studies reveal a novel mode of p68-mediated colon carcinogenesis against the backdrop of NF-κB signaling.[43] Concerning post-translational modifications, Tanaka et al have found that DDX5 knockdown attenuates serine-311 phosphorylation of NF-κB p65 subunit and selectively downregulates anti-apoptotic downstream target of NF-κB, Bcl2, via TNF-α stimulation. Diacylglycerol kinase ζ (DGKζ), is an enzyme that phosphorylates lipid secondary messenger diacylglycerol to phosphatidic acid. DGKζ depletion facilitates

IκBα degradation, as a result of which nuclear translocation of NF-κB p65 subunit increases upon TNF-α stimulation. DDX5 was found to be a novel interacting partner of DGKζ and thereafter together they negatively modulate the NF-κB pathway.[117] Further studies will strengthen our understanding of the role of p68 in mediating the oncogenic switch in NF-κB pathways and help in assessing the therapeutic wing.

Involvement of other DEAD-box RNA helicases in NF-κB pathway

In ovarian cancer, DDX10 is found to be downregulated. It shows low expression profiles in the ovarian tissue samples. Yet it plays a major role in promoting ovarian cancer proliferation *in vitro* and the subcutaneous xenograft tumor formation *in vivo*. miR-155-5p silences it epigenetically in ovarian cancer and DDX10 regulates the Akt/NF-κB pathway in ovarian cancer cells to promote cell proliferation and tumorigenesis. This depicts the crosstalk between these two pathways in ovarian cancer and a DEAD-box protein at the crossroads mediating this interaction.[32] NF-κB necessitates multiple coactivator proteins like CREB-binding protein (CBP)/p300,[118] CBP-associated factor, and steroid receptor coactivator 1 for the transcription machinery functioning. These proteins modify chromatin through their histone acetyltransferase activity. Various shreds of evidence indicate the transcriptional co-activator role of RNA helicase A (RHA). It also interacts with CREB-binding protein (CBP).[119] This triggered a study where it was discovered that the NF-κB p65 subunit interacts with RHA *in vitro* and *in vivo*. Downregulation of RHA reduces NF-κB-dependent gene expression of E-selectin, ICAM-1, and IFN-β. The mutant form of RHA lacking ATP-binding activity inhibited NF-κB dependent reporter gene expression thus implying that the enzymatic mode of action of RHA necessitates NF-κB transactivation.[119]

NF-κB reporter gene assay in HEK293T cells led to the discovery of DDX1 as a co-activator to enhance NF-κB transcription activation. The carboxy-terminal transactivation domain of RelA interacts with the amino-terminal ATPase/helicase domain of DDX1. The negative mutant form of DDX1 failed to induce transcriptional activity. Depletion of endogenous DDX1 reduced NF-κB-dependent transcription. Taken together, it suggests that DDX1 may help in NF-κB-mediated transactivation. On the other hand, DDX3 modulates the NF-κB pathway by monitoring the phosphorylation of PP2A-C subunit.[120,121] Additionally, DDX3 also plays a role in antiviral immune signaling pathways by directly interacting with IKKϵ (IκB kinase ϵ) and IKKα, activating them and further facilitating downstream NF-κB signaling.[122] Both DDX3 and DDX1 are oncogenic. Therefore, the opportunity to study their interaction with NF-κB components against a cancer situation needs to be further evaluated in the future.

Canonical Wnt pathway

The Wnt signaling is an evolutionarily conserved developmental pathway that regulates cell fate determination, cell migration, cell polarity, neural patterning, and organogenesis during embryonic development.[123] In canonical Wnt signaling, the absence of Wnt ligands leads to phosphorylation of β-catenin by the destruction complex (comprises of Axin, APC, and the kinases GSK3β and casein kinase CK1α). Finally, it undergoes proteasomal degradation. The canonical pathway is activated upon binding of Wnt ligands (Wnt3a and Wnt1) to Frizzled receptors and LRP co-receptors. Phosphorylation of LRP recruits and activates Dishevelled (Dvl) proteins which inactivate the destruction complex. Thus, β-catenin gets stabilized and translocate into the nucleus. There, it forms an active complex with LEF (lymphoid enhancer factor) and TCF (T-cell factor) proteins and activates downstream signaling.[124] Dysregulation of the canonical Wnt/β-catenin signaling pathway is synonymous with cancer progression. We have already discussed in the earlier sections, the role of p68 as a coactivator of β-catenin and thereby its affiliated association with the regulation of Wnt/β-catenin signaling. Apart from it, a direct link between p68 and Wnt pathway was demonstrated by Yang et al. Treatment with PDGF in HT29 cells (colon cancer cell lines) activated the pool of c-Abl kinase. In turn, it phosphorylated p68 at Y593. By blocking GSK3β and displacing Axin, phosphorylated p68 facilitates β-catenin nuclear translocation. It affects the downstream TCF/LEF effectors and in turn augmented EMT.[125] Besides, Wnt/β-catenin pathway also directly regulates p68. Guturi et al reported that the β-catenin/TCF4 complex along with c-Myc upregulated p68 in breast cancer. A positive feedback loop existed between the p68 and Wnt/β-catenin pathway leading to breast carcinoma.[70] p68 also affects the Wnt/β-catenin pathway in a unique way to drive colon carcinogenesis via its interaction with a long non-coding RNA NEAT1. The direct interaction promotes β-catenin nuclear translocation and activation of downstream genes leading to oncogenesis.[126] Recent therapeutic intervention in the p68 and Wnt interplay can be accredited to the study related to RX-5902, a first-in-class anticancer agent targeting phosphorylated-p68 and affecting the nuclear shuttling of β-catenin in TNBC cells. The anti-tumor efficacy of this drug is now well established, and its anti-tumorigenic property was characterized in a large panel of TNBC cell lines *in vitro* and PDX models *in vivo*. It inhibits the Wnt pathway by attenuating the nuclear translocation of β-catenin and delineates the role of p68 as a therapeutic target.[127] RX-5902 phase I dose-escalation studies have yielded positive results and recommended phase 2 trials, which are currently on the way.

Involvement of other DEAD-box RNA helicases in Wnt pathway

There have been few reports regarding the involvement of other DEAD-box proteins with the Wnt pathway. In HCC, an interactive loop exists between DDX39 and Wnt/β-catenin pathway. DDX39 overexpression increased β-catenin expression levels and its downstream target gene levels whereas knockdown had the opposite effect. This regulative aspect of the Wnt/β-catenin pathway promoted HCC migration, invasion, growth, and metastasis.[128] DDX3 is also intricately associated with the canonical Wnt/β-catenin pathway. In colon cancer cells, there exists a correlation between cytoplasmic DDX3 expression and nuclear β-catenin expression. In-vitro inhibition of DDX3 reduced TCF4-reporter activity and decreased mRNA levels of the

downstream TCF4-regulated genes. It also reduced proliferation and caused cell cycle arrest.[129] Overexpression of Disheveled segment polarity protein 2 (Dvl2) can potently activate the β-catenin/TCF signaling. DDX3 acts as a regulatory subunit of CK1ϵ to encourage phosphorylation of Dvl2. DDX3 modulates the CK1ϵ—Dvl2 axis and thus activates β-catenin/TCF signaling, promoting cell invasiveness and tumor formation in lung cancer xenograft models.[45] To develop proper targeted therapeutic modalities, it is extremely imperative to have proper knowledge regarding the mechanistic pathways of regulation that drive the oncogenic axis. Thus, the research upon the DEAD-box proteins may help in the further translational aspect of studies in cancer therapy.

Notch pathway

Notch signaling is a developmental signaling pathway, regulating cell proliferation, differentiation, and survival. In mammals, four different notch receptors are found *viz.*, NOTCH1, NOTCH2, NOTCH3, and NOTCH4. The Notch receptors are single-pass transmembrane receptor proteins. It is composed of a large extracellular portion, a single transmembrane-pass, and a small intracellular region. Perturbed regulation of Notch signaling may lead to many epithelial cancers and hematological malignancies. The Notch pathway begins with the ligand-binding step. Ligands may be either transmembrane ligands such as Delta (termed Delta-like in humans) or Serrate (termed Jagged in humans) present on neighboring cells. Binding of ligand leads to cleavage and release of the Notch intracellular domain (NICD), which then travels to the nucleus to regulate transcriptional complexes containing the DNA-binding protein CBF1/RBP-J/Su(H)/Lag1 (CSL). CSL is a DNA-binding adaptor that interacts with proteins resulting in the formation of either repressor complexes (e.g., histone deacetylases or HDACs) or activating complexes (e.g., NICD along with other proteins like histone acetyltransferases or HATs). In canonical Notch signaling, NICD translocates to the nucleus, where it binds to CSL, and thereafter helps in recruitment of adaptor protein Mastermind-like (MAML). MAML in turn recruits the HAT (p300) and other transcriptional machinery components. Biochemical details of the non-canonical Notch pathway are not solidified yet.[130—132]

Reports have suggested that DDX5 is highly expressed in human T cell acute lymphoblastic leukemia (T-ALL) patient samples. Notch signaling is a major oncogenic pathway in the pathogenesis of T-ALLs. To delve further the authors discovered that proteomic studies identified DDX5 as a component of the MAML1 protein complex. Both of them interact with each other *in vitro* and *in vivo*, leading to endogenous NOTCH1 transcription activation complex. Upregulation and downregulation of DDX5 led to an increase and decrease of the expression levels of Notch signature genes (*HES1*, *HEY1*, *MYC*, and *DTX1*). Also, it gets recruited to the Notch transcription activation complex as well as gets physically localized upon the Notch responsive *HES1* promoter. DDX5 knockdown inhibited leukemic cell growth and sponsored cell apoptosis. Furthermore, reduced leukemic xenograft growth was also associated with the DDX5 knockdown state. Thus, the above findings enumerate the functions of DDX5 as a novel regulator of oncogenic Notch signaling in leukemic cells and reveal a close relation between DDX5 and NOTCH pathway, wherein it acts as a crucial molecular switch for the cascading interplay.[133] In line with these results, another group of researchers headed by Jung et al showed that p68 directly interacts with the transcription factor RBP-J. p68 localizes at RBP-J binding sites within the Notch target genes *preTCRα*, *Hes1*, and *CD25* in a Notch-dependent manner. RNA coactivator SRA which is a cofactor of p68 downregulates Hes1 and preTCRα. Hence their data demonstrated that Ddx5 and SRA function as coactivators of Notch signaling.[134] The notch pathway also mediates the regulation of β-catenin stability. As we have already discussed that p68 and Wnt signaling have a major role in most of the cancers and are linked intricately with other pathways too, it would be interesting to delve further into the signaling crosstalk between Wnt and Notch pathways with p68 at the crossroads.[135]

Involvement of other DEAD-box RNA helicases in Notch pathway

There has been a spurt of research in discovering molecules governing the pathway and modifying it. Amidst all this, the DEAD-box RNA helicases are also being researched upon for exhibiting their roles in modulating the Notch pathway. A long non-coding RNA Linc00630 is closely associated with the development of non-small-cell lung cancers (NSCLCs). RNA pulldown assay and RNA-seq assays revealed that Linc00630 can physically interact with HDAC1 and DDX23. It also stabilizes the protein level of HDAC1. Overexpression of DDX23 increases the luciferase activity of Linc00630 and knockdown shows a reverse effect. Thus, DDX23 transcriptionally regulates Linc00630 in A549 cells. RNA-seq data showed that the Notch signaling pathway was found to be affected by these mechanistic interplays. DDX23-Linc00630-HDAC1 further activates Notch signaling pathway to exert its oncogenic regulation.[136] The other DEAD-box helicases haven't shown any conclusive effect for the regulation of Notch pathways. Thus, there are ample opportunities to focus on this path and look for novel modes of regulation of the Notch signaling pathway and interaction of Notch members with DEAD-box proteins.

Therapeutic opportunities

Despite improved knowledge and research in the field of cancer, the therapeutic road faces several challenges. The biological circuit is extremely complex and unpredictable. The homeostasis-evading properties of cancer cells provide hindrance in suitable target-specific therapies. Therefore, it is important to have holistic information about the various signaling pathways involved as well as the different molecular players that intervene, to design novel targeted therapeutic strategies. Target-specific interventional methods involve the use of inhibitory peptides, CRISPR-mediated knockout of specific genes, and miRNA or lncRNA-based approaches that target specific DEAD-box RNA helicases or suitable target molecules of the signaling pathway

in which the DEAD-box proteins are involved (Fig. 5). The revolutionary role played by small molecule inhibitors in the context of targeting DEAD-box RNA helicases has been of utmost importance. An interesting class of DEAD-box helicase inhibitors is ring-expanded nucleosides containing imidazo [4,5-e][1,3]diazepine ring or imidazo [4,5-e] 1,2,4 triazepine ring systems (RENs). They strongly inhibit human DDX3 helicase. NZ51, a synthesized REN analog, suppresses ATPase/helicase of DDX3 at low micromole concentration and shows the antiproliferative effect, and blocks cell replication in aggressive breast cancer cell cultures. Unfortunately, at the primary tumor growth level, NZ51 failed to elicit a significant response even though DDX3 knockdown causes reduced tumor volume and metastasis.[39] Among ring-expanded derivatives, the compound RK33 is very interesting and perspective for medicine. RK-33 displays antiproliferative activity against Ewing sarcoma, breast cancer, colorectal, prostate, and lung cancer through G1 arrest. RK-33, an inhibitor of DDX3, impedes non-homologous end-joining repair of DNA breaks and consequently, it has been used for *in vitro* cancer therapy studies.[137] Similarly, a novel anti-cancer drug, 1-(3,5-dimethoxyphenyl)-4-[(6-fluoro-2-methoxyquinoxalin-3-yl) aminocarbonyl] piperazine (RX-5902) was demonstrated to act as phospho-p68(DDX5) inhibitor. It interferes with the nuclear translocation of β-catenin and thereby extends its anti-cancer role.[138] Phase I clinical trials of RX-5902 are underway for numerous cancer types. Another drug, resveratrol directly targets DDX5(p68) and suppresses the mTOR pathway in prostate cancer. Recently the inhibitory role of resveratrol (3,4′,5 tri-hydroxystilbene), a naturally occurring polyphenolic compound, against p68 is being studied. Earlier reports had revealed its anti-cancer properties and pro-apoptotic effects. In human colon carcinoma cells, resveratrol treatment induces signature ER stress markers and up-regulation of glucose-regulated protein (GRP)-78, signifying the induction of ER stress in mediating the resveratrol-induced apoptosis.[139] Thus, resveratrol as a drug possesses the immense capability to be used in translational medicine research. Unfortunately, the DEAD-box inhibitors (Table 1) that are being developed have failed to reach the stage of clinical assessment and human phase I trial. Preclinical studies of eIF4A inhibitors *viz.*, silvestrol, hippurastinol, and the DDX3 inhibitor (RK-33) have been successful in mice models but the road ahead is still complex.[140] miRNA and lncRNA (microRNA and long non-coding RNA) based approaches are gradually gaining approval. For example, miR-431 inhibits DDX5 and therefore suppresses lung cancer proliferation.[37] Similarly, miR-5590-3p intervenes the DDX5/AKT/mTOR pathway and inhibits gastric cancer propensity.[141] miR-141 targets DDX5 directly and this interaction is further regulated by lncRNA MIAT to promote gastric cancer progression.[142] siRNA-based methods to downregulate such lncRNA may help in controlling cancer progression. Apart from these conventional forms of RNA, recently circular RNAs (circRNAs) are being hailed as a new class of RNAs that mediate different types of tumor development. Mao et al worked with circRNAs in osteosarcoma (OS) progression. Using bioinformatics tools, a particular upregulated circRNA (Circ-XR1) was chosen, and RT-PCR confirmed its abundant expression in OS tissue. Gain- and loss-of-function studies revealed the correlation of Circ-XPR1 with sponging miR-214-5p to regulate DDX5 expression. Thus, Circ-XPR1/miR-214-5p/DDX5 axis is a novel and potentially useful axis for therapeutic targeting in the future.[143] Apart from proper drug design, the mode

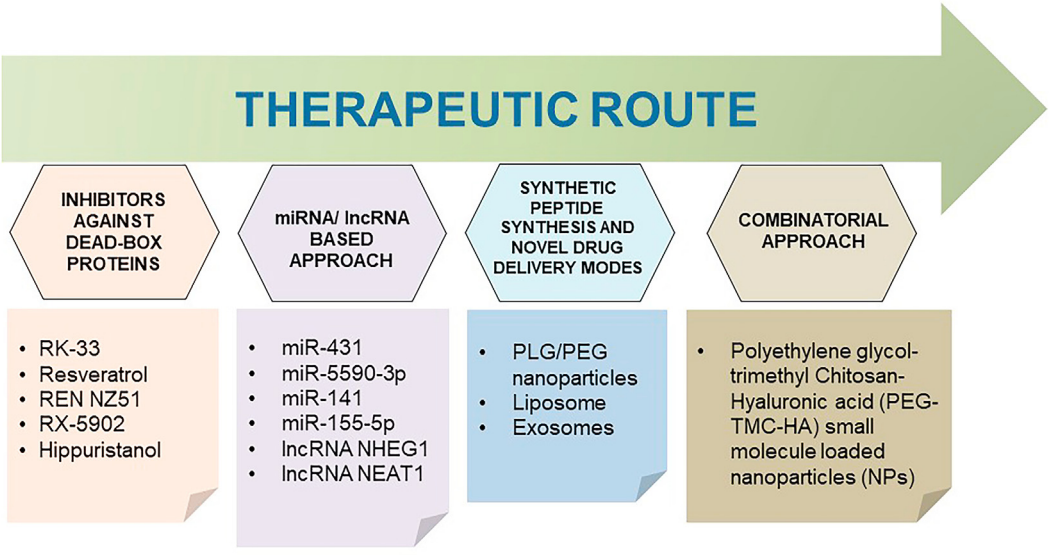

Figure 5 Therapeutic opportunities. The route towards therapeutic alleviation of cancer consists of the four mentioned therapeutic strategies. Below them, various factors are listed that are involved in completion of that strategy so that the DEAD-box RNA helicases can successfully curb oncogenesis in the future. Use of inhibitors against these proteins, microRNA or long non-coding RNA based approaches, synthetic peptide design, novel modes of drug delivery and combinatorial approach together constitute the route towards therapeutic opportunities for targeting DEAD-box proteins.

Table 1 Summary of the different inhibitors against DEAD box proteins as well as their modes of action for therapeutic opportunities.

DEAD-box RNA helicase	Molecules/ Inhibitors	Status/Stage of development	Mode of Action	References
DDX5	RX-5902	Phase I/II	Inhibits phosphorylated-p68 and thus attenuates nuclear β-catenin signaling	[138]
	Resveratrol	Pre-clinical studies	Inhibit the mTORC1 pathway by targeting DDX5	[76]
DDX3	Ketorolac salt	Pre-clinical studies	Downregulates DDX3 and inhibits ATP hydrolysis by directly binding with it	[146]
	Ring-expanded nucleoside analog (REN) NZ51	Tissue culture-based studies	Inhibit the ATP-dependent helicase activity of DDX3	[147]
	RK33	Pre-clinical studies	Docks into the ATP-binding domain of DDX3 and inhibits its activity	[77]
	Rhodamine and Triazine derivatives	Tissue culture-based studies	Targets ATP-binding domain of DDX3	[148]
eIF4AI/II/III (DDX2)	Hippuristanol	Pre-clinical studies	Allosteric inhibitor of eIF4A RNA binding	[149]
eIF4AI/II/III	Rocaglamide (silvestrol)	Pre-clinical studies	Inhibits translation initiation through RNA-mediated sequestration of eIF4A	[150]
eIF4AI/II/III	Episilvestrol	Pre-clinical studies	eIF4A-targeting translation inhibitor, antitumor activity through inducing apoptosis	[151]
eIF4AI/II	Hypericin	Pre-clinical studies	Inhibitor of ATPase activity or helicase activity	[152]
eIF4AI/II/III	Pateamin-A (DMDA)	Pre-clinical studies	Target eIF4A by interfering with the helicase's RNA-binding activity	[153]
ES-DEAD box proteins	AMP-acrylate	Pre-clinical studies	It specifically binds to electrophile sensitive (ES) DEAD box proteins and inhibits duplex unwinding by electrophile-sensitive DEAD-box proteins	[154]

of drug delivery and its specificity is equally important so that it affects the target cells effectively. Novel drug delivery approaches include nano-vehicular modes such as liposomes, exosomes, and nanoparticles-based routes of delivery. Recently, RK-33 was transported using PLGA nanoparticle formulation for improved efficacy.[144,145]

Combinatorial approach in cancer therapy

DDX3 inhibition by RK-33 in combination with PARP inhibitor was used to synergize breast cancer treatment.[137] PARP inhibitor resistance in BRCA1-deficient cancers is common and sensitivity is limited in BRCA1-proficient breast cancers. Combination treatment using DDX3 and PARP inhibitors causes lethality in BRCA1-proficient breast cancer, giving way to a therapeutic wing. The conjugative role of p68 and STAT3 in oncogenesis marks their role as efficient targets for translational studies. Recently, a group of researchers suppressed the p68/STAT3 axis in different cancer cells by using polyethylene glycol-trimethyl chitosan-hyaluronic acid (PEG-TMC-HA) nanoparticles (NPs) loaded with siRNA (p68 and Stat3 siRNA respectively) molecules.[29] Such combination therapy reduced apoptosis, proliferation, and tumor growth, both *in vitro* and *in vivo*. Such target-specific therapeutic routes hold great prospects and may even pave the way ahead for personalized medicines. Using high-throughput sequencing, transcriptomics, proteomics, innovative drug delivery mechanisms, and drug screening technologies, the prognostic and diagnostic value of DEAD-box RNA helicases in cancer can be judged to overcome the therapeutic glitches and positively impact cancer therapy.

Conclusion

Stephen Hawking had quoted long back that, "Scientists have become the bearers of the torch of discovery in our quest for knowledge". In the quest for finding therapeutic routes to alleviate cancer, the knowledge database contributed by scientists all around the world is impeccable. Every little contribution marks its imprint across the pages of time. Our review too aims at steering the ship of

knowledge ahead and help in the path of cancer research. We have put forward various key research findings related to DEAD-box RNA helicases especially DDX5 (p68) in the field of cancer and highlight their promising roles as suitable targets for translational research. The DEAD-box RNA helicases perform diverse functions in enhancing oncogenesis or suppressing tumors and depending on their context of action, the various signaling pathways they affect as well as the necessary cofactors they interact with; we can look out for a novel interventional approach to overcome their tumorigenic propensity or augment their tumor-suppressive roles. p68 has shown immense caliber as a prognostic marker of cancer. A systematic and detailed characterization of the structure of p68 and proper development of specific inhibitors will help in the therapeutic road ahead.

Author contributions

MKG and ST conceived the idea of the manuscript and wrote it. All authors read and approved the final manuscript.

Conflict of interests

Authors declare no conflict of interests.

Funding

This work was jointly supported by the Department of Science and Technology (Nano Mission: DST/NM/NT/2018/105(G) and SERB: EMR/2017/000992/HS & EMR/2017/001183) and CSIR (FBR Project #31-2(274)2020-21), Govt. of India.

Acknowledgements

We acknowledge Department of Science and Technology (DST) and Council of Scientific and Industrial Research-Indian Institute of Cancer Biology (CSIR-IICB). Authors are happy to acknowledge Ms. Pratyasha Ghosh, Department of Economics, Bethune College, University of Calcutta, WB, India and Dr. Malini Basu, Assistant Professor, Department of Microbiology, Dhruba Chand Halder College, Dakshin Barasat, India for editing the manuscript.

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