

Review Article

Multifunctional DDX3: dual roles in various cancer development and its related signaling pathways

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Received December 29, 2015; Accepted January 12, 2016; Epub January 15, 2016; Published February 1, 2016

Abstract: DEAD-box RNA helicase 3 (DDX3) is a highly conserved family member of DEAD-box protein, which is a cluster of ATP-dependent and the largest family of RNA helicase. DEAD-box family is characterized by the regulation of ATPase and helicase activities, the modulation of RNA metabolism, and the actors of RNA binding proteins or molecular chaperones to interact with other proteins or RNA. For DDX3, it exerts its multifaceted roles in viral manipulation, stress response, hypoxia, radiation response and apoptosis, and is closely related to cancer development and progression. DDX3 has dual roles in different cancer types and can act as either an oncogene or tumor suppressor gene during cancer progression. In the present review, we mainly provide an overview of current knowledge on dual roles of DDX3 in various types of cancer, including breast cancer, lung cancer, colorectal cancer, hepatocellular carcinoma, oral squamous cell carcinoma, Ewing sarcoma, glioblastoma multiforme and gallbladder carcinoma, and illustrate the regulatory mechanisms for leading these two controversial biological effects. Furthermore, we summarize the essential signaling pathways that DDX3 participated, especially the Wnt/ β -catenin signaling and EMT related signaling (TGF- β , Notch, Hedgehog pathways), which are crucial to DDX3 mediated cancer metastasis process. Thoroughly exploring the dual roles of DDX3 in cancer development and the essential signaling pathways it involved, it will help us open new perspectives to develop novel promising targets to elevate therapeutic effects and facilitate the "Personalized medicine" or "Precision medicine" to come into clinic.

Keywords: DDX3, cancer, oncogene, tumor suppressor gene, Wnt/ β -catenin pathway, EMT related pathway

Introduction

DEAD-box protein is the largest family of RNA helicase, which is able to unwind RNA duplexes and is involved in multiple RNA processing procedures, including mRNA splicing, RNA editing, export, RNA decay, ribosome biogenesis, transcriptional and translational regulation and so on [1, 2]. The name of DEAD-box RNA helicase is derived from the conserved amino acid sequence D-E-A-D (Asp-Glu-Ala-Asp) located in the motif II of 12 motifs [3]. The roles of these motifs can be divided into three parts: ATP binding, RNA binding, and link ATP and RNA binding. So DEAD-box family is characterized by the regulation of ATPase and helicase activities, and modulates RNA metabolism in an ATP-dependent manner [4]. Additionally, acting as RNA binding proteins or molecular chaperones,

DEAD-box RNA helicase have interaction with other proteins or different forms of RNA, so as to maintain the integrity of the secondary and tertiary structure of RNA and facilitate the transcriptional activation, translational initiation, post-translational modification or miRNA biogenesis processes [5-7]. DEAD-box protein is a widely dispersed family which can be found in almost all organisms, from yeast to human. The genome of the yeast encodes 25 DEAD-box proteins. Besides the counterparts of each 25 proteins, along with 12 additional DEAD-box genes, are found in the human genome [8].

DEAD-box RNA helicase 3 (DDX3) is a highly conserved family member of DEAD-box proteins. The human genome encodes two types of DDX3 genes and two DDX3 homologs, DDX3X and DDX3Y. Based on their locations in chromo-

Dual roles of DDX3 in cancer development

some, DDX3X is located on the X-chromosome bands p11.3-11.23 region and escapes from X-inactivation [9, 10]. Whereas, DDX3Y is located in the azoospermia factor a (AZFa) region of the Y-chromosome, and is specifically expressed in testis and plays an essential role in spermatogenesis and male fertility [11, 12]. DDX3X and DDX3Y share 92% similarity in protein sequence identity, and encodes for a 662- or 661-amino acid polypeptide depending on mRNA alternative splicing [13]. As the specialized role of DDX3Y in male fertility, usually we focus on our study on DDX3X and refer DDX3 to DDX3X.

Being a key RNA binding protein and transcriptional cofactor, DDX3 exerts its multifaceted roles in viral manipulation (especially for HIV, HCV, and HBV), immunology regulation, cancer progression and so on [14-17]. Moreover, DDX3 is closely related to various biological processes, such as stress response, hypoxia, radiation response, apoptosis, and cell cycle regulation [18, 19]. For the role of DDX3 in cancer development, it is rather complicated and controversial. DDX3 is a “double-edged sword” gene and can act as either an oncogene or tumor suppressor gene during cancer progression, depending on different cancer types. So in this review, we will illustrate the dual roles of DDX3 in multiple cancer development procedures and explore the essential signaling pathways that DDX3 involved to lead these two conflicting biological effects.

Dual roles of DDX3 in cancer development

Breast cancer

Most of the recent studies demonstrated that DDX3 acts as an oncogenic role in breast cancer biogenesis. The report showed that over-expression of DDX3 in immortalized human breast cancer cell line MCF 10A could promote cell growth, proliferation and neoplastic transformation of epithelial cells. Particularly, DDX3 could repress E-cadherin expression, induced an epithelial-mesenchymal like transformation phenotype and increased the motility and invasive properties of breast cancer cells so as to facilitate metastasis process [20]. Further investigation found that hypoxia inducible factor-1 α (HIF-1 α) was a transcriptional activator of DDX3 in breast cancer cells. And it has been verified that there were three putative HIF-1 responsive elements located in the pro-

motor region of DDX3 gene. Thus, the expression level of DDX3 can be elevated during hypoxia with the effect of HIF-1 on its promoter, and help tumor cells to survive in this unfavorable condition [21]. Moreover, in invasive breast cancer, the expression of DDX3 was correlated with over-expression of HIF-1 α and its downstream genes CAIX, GLUT1, and several hypoxia related genes, including EGFR, HER2, ER α , c-Met and FOXO4 [22]. Those genes worked together under hypoxic conditions to promote tumor cell proliferation and transformation (**Figure 1**).

In addition, a latest study explored a novel DDX3 inhibitor, which is a ring-expanded nucleoside analogue (REN), named for NZ51. This inhibitor could be incorporated into the ATP binding pocket of DDX3 and therefore inhibited the ATP dependent helicase activity of DDX3. Using NZ51 treatment on MCF-7 and MDA-MB-231 breast cancer cells, it showed the suppression of cell cycle, the inhibition of cell proliferation, and the dramatic anti-cancer properties on cellular motility. Meanwhile, NZ51 could stabilize DDX3 with inactivation of its function and was not affected by hypoxia. So NZ51 had the equal potency in killing breast cancer cells both under hypoxic and normoxic conditions [23]. Furthermore, in breast cancer cell apoptosis process, the role of DDX3 was a “double-edged sword”. During DNA damage response, DDX3 could associate with p53, promote the retention and accumulation of p53 in the nucleus, and activate its downstream target p21 expression, so as to positively modulate DNA damage induced apoptotic signaling and caspase activation in cells which expressed functional wild-type p53. However, in cells which expressed non-functional or mutant p53, DDX3 otherwise inhibited the activity of apoptotic pathway to reduce caspase activation [24] (**Figure 1**). Overall, these results indicated that DDX3 plays dual roles in regulating apoptosis processes which are closely associated with breast carcinogenesis, radiotherapy and chemotherapy.

Lung cancer

DDX3 acts as diverse roles in lung cancer progression. Some studies showed that DDX3 was over-expressed in lung cancer and associated with lower survival rate and poor prognosis in lung cancer patients. In their studies, Raman et al. designed a novel small molecule inhibitor, RK-33, which could bind to DDX3 and inhibit its

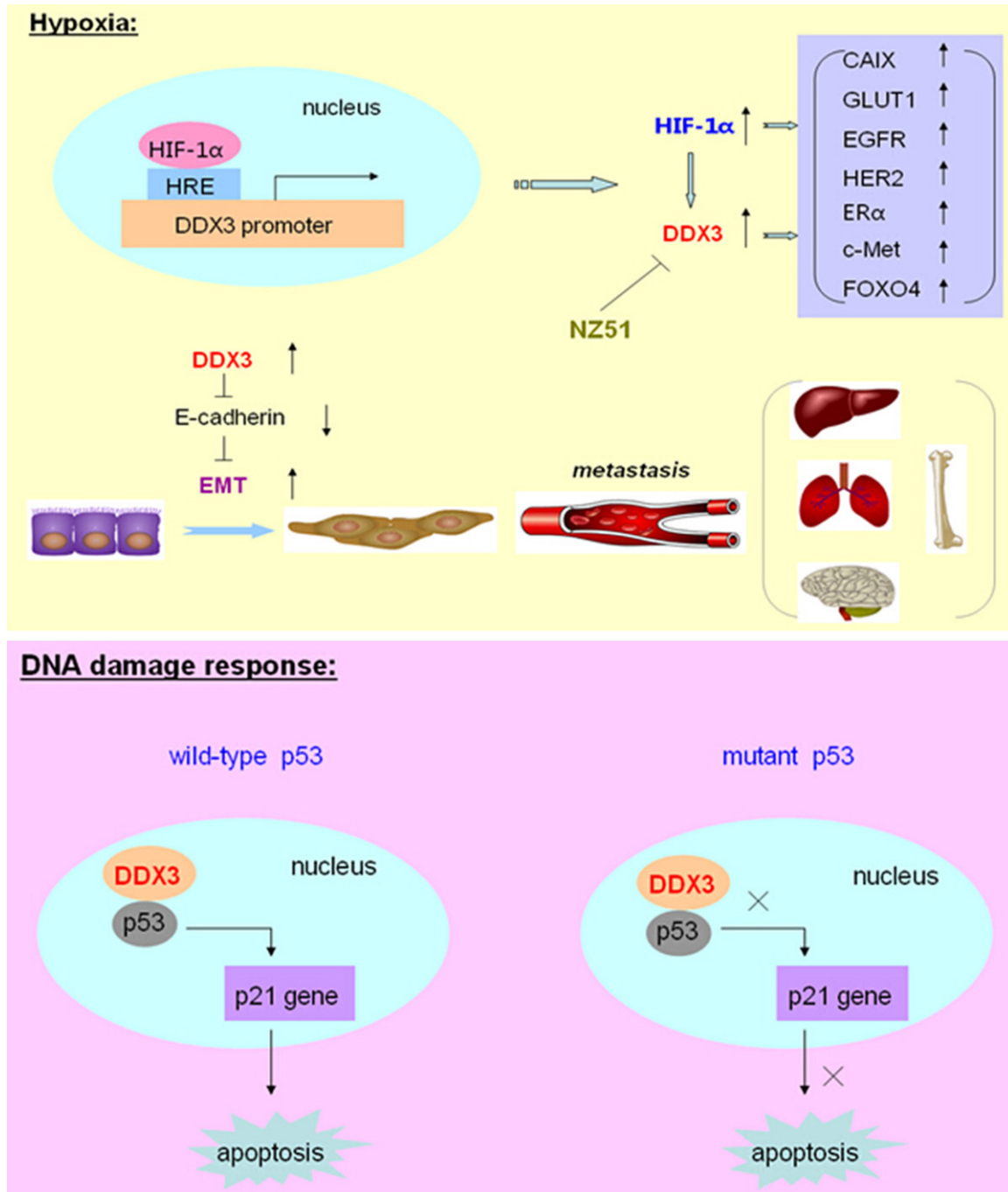


Figure 1. The role of DDX3 in breast cancer. Under hypoxic conditions, hypoxia inducible factor-1 α (HIF-1 α) could bind to the putative HIF-1 responsive elements (HRE) located in the promoter region of DDX3 gene, and transcriptionally activate the expression of DDX3. Meanwhile, the expression of DDX3 was correlated with over-expression of HIF-1 α and its downstream genes CAIX, GLUT1, and several hypoxia related genes, including EGFR, HER2, ER α , c-Met and FOXO4. Moreover, DDX3 could repress E-cadherin expression and induce the Epithelial-mesenchymal transition (EMT) process to facilitate breast cancer metastasis. A novel DDX3 inhibitor, which a ring-expanded nucleoside analogue (REN), named for NZ51, could inhibit the ATP dependent helicase activity of DDX3. During DNA damage response, DDX3 could associate with p53, promote the accumulation of p53 in the nucleus, and activate its downstream target p21 expression, so as to modulate DNA damage induced apoptosis in cells expressed functional wild-type p53. However, in cells expressed non-functional or mutant p53, DDX3 otherwise inhibited the apoptosis process.

Dual roles of DDX3 in cancer development

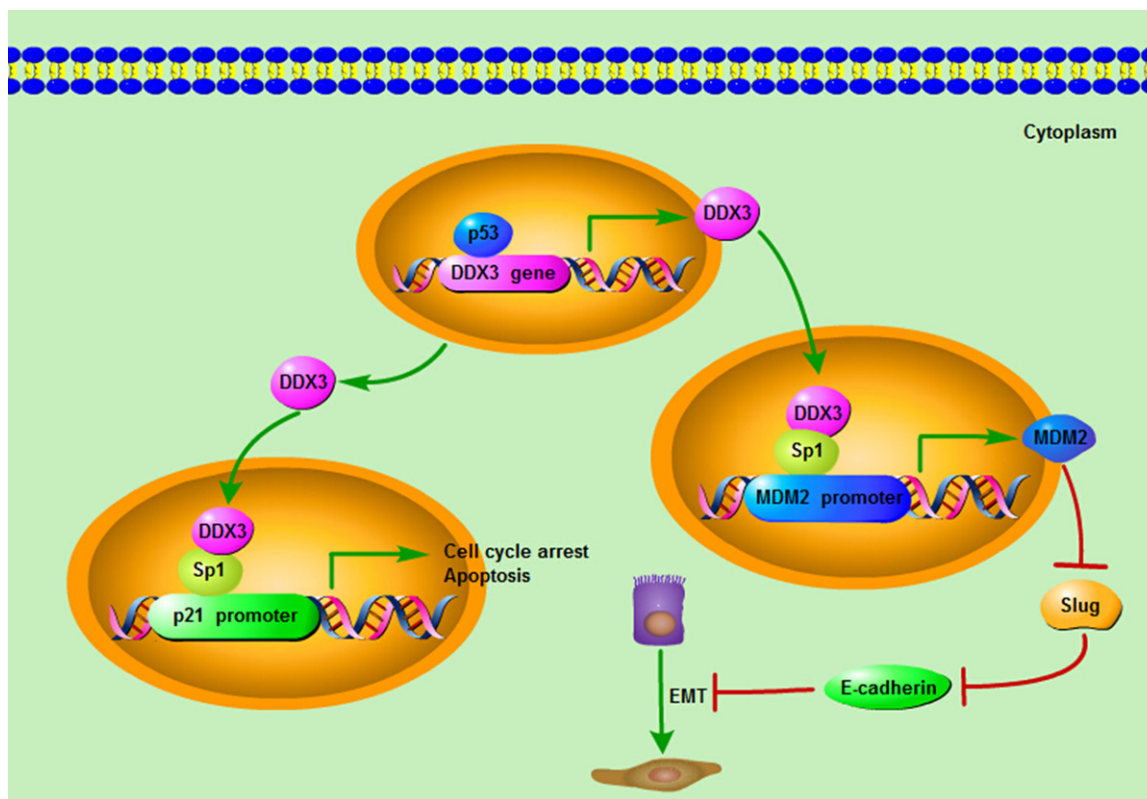


Figure 2. The mechanism of DDX3 acting as a tumor suppressor. The transcription of DDX3 can be regulated by p53, and the expression level of DDX3 is dependent on p53 status. Meanwhile, through interacting and cooperating with Sp1, DDX3 could increase Sp1 binding affinity onto the p21 promoter region and up-regulate the promoter activity of p21 so as to exert its tumor suppressive roles on cell cycle and apoptosis. Moreover, DDX3 could also increase Sp1 binding activity to the MDM2 promoter, and promote the MDM2 transcription process. Consequently, the up-regulation of MDM2 leads to the suppression of Slug, which negatively regulates the expression of E-cadherin. As a result, through increasing MDM2-mediated Slug suppression, DDX3 can act as a tumor suppressor by elevating the E-cadherin expression and impeding the Epithelial-mesenchymal transition (EMT) progression.

helicase activity. Inhibition of DDX3 expression by RK-33 resulted in G1 cell cycle arrest, apoptosis induction, and the promotion of radiotherapy sensitization. Mechanistically, loss of DDX3 functions by RK-33 could disrupt the DDX3- β -catenin regulatory axis and impair key molecules in Wnt signaling pathway. Furthermore, RK-33 could suppress the non homologous end joining (NHEJ) process, which is the major DNA damage repair model in mammalian cells during radiation and DNA damage response [25]. Thus, inhibition of DDX3 by small molecular inhibitors could impede tumor progression and it provided us new insights for developing chemo- or radio-therapy sensitizers.

Whereas, the studies made by another two groups came to quite different conclusions on the role of DDX3 in lung carcinogenesis. In HPV-associated lung cancer, the transcription of

DDX3 was regulated by p53, so the expression level of DDX3 was dependent on p53 status. Meanwhile, through increasing Sp1 binding affinity onto the p21 promoter region, DDX3 synergistically enhanced p53-activated p21 transcription and therefore established the p53-DDX3-p21 regulatory axis (Figure 2). Thus, the DDX3 expression level was negatively associated with HPV oncoprotein E6 and was positively related to p21 expression in lung cancer. So the development of HPV-associated lung cancer, appeared to require E6-mediated inactivation of DDX3, degradation of p53, and synergistic suppression of p21 transcription so as to maintain a malignant phenotype and promote cancer progression [26]. In non-small-cell lung cancer, loss of DDX3 by p53 inactivation or mutation could promote tumor cell colony formation and invasiveness capacities. Mechanically, DDX3 loss could decrease Sp1

Dual roles of DDX3 in cancer development

binding activity to the MDM2 promoter, and the MDM2 transcription process was greatly suppressed. Consequently, Slug expression was elevated due to the suppression of MDM2, and further led to the down-regulation of E-cadherin. Thus, through decreasing MDM2-mediated Slug degradation, loss of DDX3 might result in Slug-suppressed E-cadherin expression [27] (**Figure 2**). Overall, the studies suggested that DDX3 loss by p53 inactivation via the MDM2/Slug/E-cadherin pathway could facilitate tumor malignancy state and lead to poor clinical outcome for lung cancer patients.

Colorectal cancer

The debate on the oncogenic or tumor suppressive role of DDX3 in colorectal cancer is still going on. Some researchers hold the point that DDX3 acted as a tumor suppressive gene and had a significant prognostic predictive power in colorectal cancer. Patients with low DDX3 expression level resulted in poor clinical prognosis and multiple distant metastasis regions. For the molecular mechanisms, down-regulation of DDX3 could lead to the up-regulation of Snail, so as to decrease the expression level of membranous E-cadherin and reduce tumor cell aggregation ability, therefore promoting migration, invasion and metastasis capacities of colon cancer cells [28]. Taken together, these data showed that low DDX3 expression level activated the Snail/E-cadherin pathway, which promoted cancer metastasis process and indicated poor clinical outcome in colorectal cancer patients.

However, other researchers stood at a quite opposite point that DDX3 had an oncogenic role in colorectal cancer. Over-expression of DDX3 was closely correlated with nuclear β -catenin expression, and further activated Wnt signaling pathway. Whereas, down-regulation of DDX3 expression contributed to the reduction of TCF4 reporter activity and the inhibition of mRNA expression levels of TCF4-regulated downstream genes, such as c-MYC, AXIN2, CCND1 and BIRC5A (**Figure 3**). Moreover, using a small molecule inhibitor, RK-33, which binds to the ATP-binding site of DDX3 and inhibits its helicase activity, could effectively suppress tumor cell growth, proliferation, and induce cell cycle G1 phase arrest. Meanwhile, the study found that in APC wild-type tumors harboring an activating CTNNB1 mutation, RK-33 could exert its highest sensitivity in

DDX3 suppression, and therefore had the potential to be a promising treatment strategy for colorectal cancer [29].

Hepatocellular carcinoma

Hepatitis C virus (HCV) is a main leading cause for hepatocellular carcinoma (HCC). HCV infection can lead to different extent of liver damage which includes fibrosis, cirrhosis, and eventually evolving into hepatocellular carcinoma [30-32]. DDX3 has been shown to interact with HCV proteins and regulate HCV replication. HCV core could bind to the C-terminus of DDX3 (amino acids 553-622) and their interaction is mediated by the N-terminus of HCV core (amino acids 1-59). The effect of this interaction between HCV core and DDX3 proposes to be the manipulation of mRNA splicing, transcriptional regulation and translational regulation of HCV, finally affecting HCV replication [33, 34]. In hepatitis virus-associated HCC, including HCV positive patients and HBV positive patients, the DDX3 expression level was different. There was a significant lower DDX3 expression level in HBV-positive HCC patients, but not in the HCV-positive ones. Moreover, the expression level of DDX3 was differentially distinguished by the gender, and the tendency of DDX3 down-regulation in HCC was more frequently found in males rather than in females. Knocking down of DDX3 could up-regulate the expression of cyclinD1 and down-regulate the expression of p21, resulting in an entry to S phase to promote cell cycle progression and facilitate tumor cell growth [35]. Together, these findings suggested that DDX3 was deregulated in hepatitis virus-associated HCC and was involved in cell cycle and cell growth control.

Additionally, another report further verified that DDX3 was a candidate tumor suppressor in HCC. A declined expression of DDX3 could be found in HCC, which was accompanied with the reduction of p21 (waf1/cip1) expression. In detail, through an ATPase-dependent but helicase-independent mechanism, DDX3 could transactivate the functions of p21 (waf1/cip1) promoter and transcriptionally regulate its activity. There were four Sp1 binding sites located in the transcription start site of p21 (waf1/cip1) promoter, which were all essential for DDX3 response. Thus, through interacting and cooperating with Sp1, DDX3 could up-regulate the promoter activity of p21 (waf1/cip1) [36] (**Figure 2**). These findings indicated that DDX3

Dual roles of DDX3 in cancer development

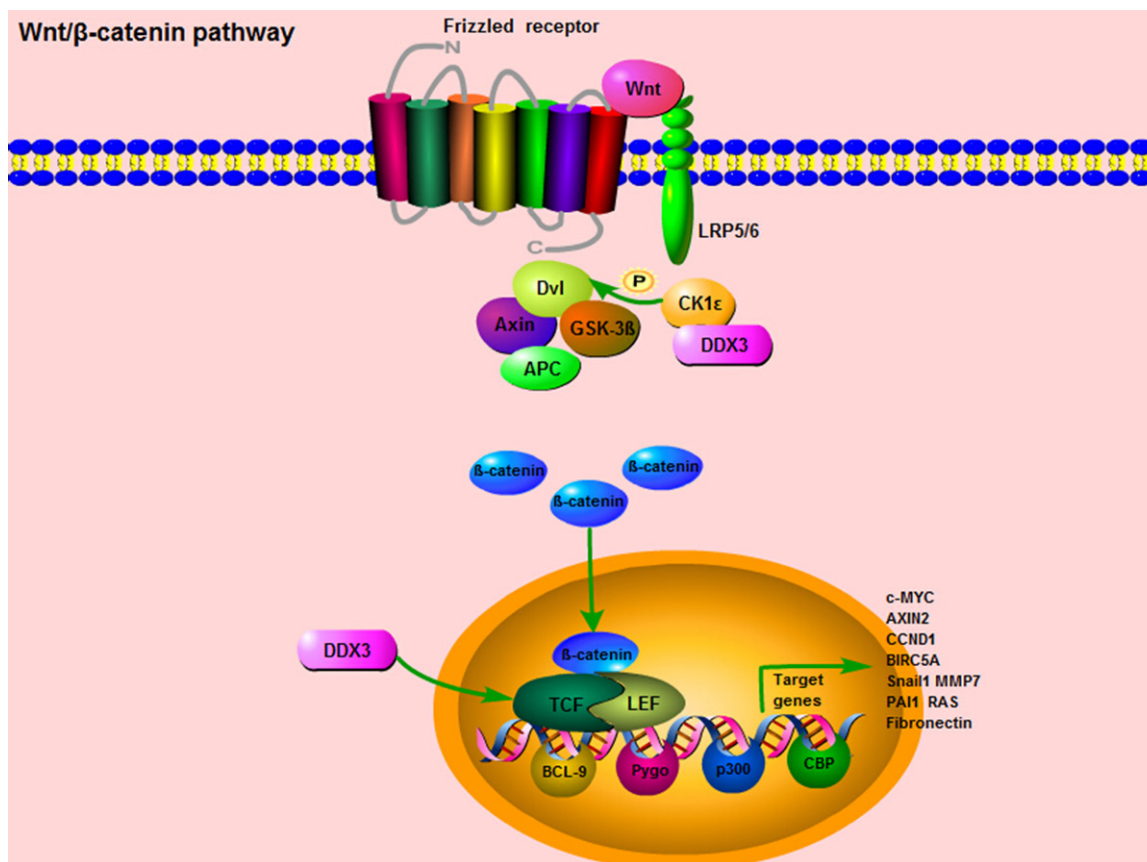


Figure 3. DDX3 is involved in Wnt/ β -catenin pathway. When Wnt/ β -catenin signaling is activated, the Wnt ligands bind to the Frizzled receptor and the co-receptor lipoprotein receptor related protein 5/6 (LRP5/6) in the membrane. Then, the Dishevelled (Dvl) gathers the cytoplasmic Axin and GSK3 β into the membrane, and facilitate the activated β -catenin translocating from cytoplasm to nucleus. The nucleus β -catenin could interact with two major transcription factors, the T-cell factor (TCF) and lymphocyte enhancer factor (LEF), accompanied with several transcription co-activators, BCL9, CBP, p300, Pygo, to regulate multiple genes transcription. These downstream target genes mainly include Snail1, MMP7, PAI1, RAS, and Fibronectin. For the role of DDX3 in Wnt/ β -catenin signaling, DDX3 could directly bind to CK1 ϵ , stimulate its kinase activity and further promote the phosphorylation of the scaffold protein Dvl, so as to facilitate β -catenin translocating into the nucleus. Moreover, up-regulation of DDX3 expression contributes to the induction of TCF reporter activity and the elevation of mRNA expression levels of TCF-regulated downstream genes, such as c-MYC, AXIN2, CCND1 and BIRC5A.

exerted its tumor suppressive properties on cell cycle and tumor growth mainly through transcriptional regulation of p21 (waf1/cip1) promoter activity.

Oral squamous cell carcinoma

The role of DDX3 in oral squamous cell carcinoma (OSCC) is still conflicting. One study led by a Taiwan group found that DDX3 acted as a tumor suppressor and was a protective factor for OSCC patients. Low expression of DDX3 was significantly associated with life behaviors of OSCC patients, such as smoking, alcohol consumption, betel quid chewing, and also with poor clinical outcomes of OSCC patients, including relapse-free survival (RFS) rate and

overall survival (OS) rate. Surprisingly, patients with low/negative DDX3 expression, especially those non-smoker OSCC patients, had relatively worse OS rate compared with smoker patients, and was associated with poor prognosis [37]. All in all, these studies demonstrated that low/negative DDX3 expression was closely correlated with aggressive clinical manifestations and might become a potential survival predictor, particularly for non-smoker OSCC patients.

On the other hand, the research launched by an India group revealed a converse role of DDX3 in OSCC. In their studies, DDX3 was assumed to be an oncogene, which promoted the progression of OSCC. So they developed a new bioactive compound against DDX3, named ketorolac

Dual roles of DDX3 in cancer development

salt. This compound had strong hydrogen bond interactions which were similar to crystallized DDX3 protein, and had less binding free energy than existing synthetic DDX3 inhibitors. Through directly interacting with DDX3, ketorolac salt could inhibit the ATP hydrolysis process of DDX3. Moreover, for in vivo experiment, ketorolac salt could effectively decrease the numbers of neoplastic tongue lesions and reduce the lesion severity in a tongue tumor mouse model [38]. All these data indicated that ketorolac salt might be used as a novel drug candidate to treat DDX3 associated OSCC.

Ewing sarcoma

The latest study showed that DDX3 acted as an oncogenic role in Ewing sarcoma. High expression level of DDX3 could be found in numerous human sarcoma subtypes compared with normal adjacent mesenchymal cells. Knocking down of DDX3 expression by a small molecule inhibitor, RK-33, which was also utilized in lung cancer and colorectal cancer, could efficiently inhibit the oncogenic activities of sarcoma cells and impede Ewing sarcoma progression. Meanwhile, the treatment of RK-33 had preferentially more cyto-toxicity to sarcoma cells, particularly for the chemotherapy-resistant sarcoma stem cells, rather than adjacent non-malignant cells. Moreover, DDX3 inhibition was confirmed to alter the cellular proteome of Ewing sarcoma, especially the proteins which were involved in DNA replication, mRNA translation and proteasome function. For in vivo assay, in human Ewing sarcoma xenograft which expressed high DDX3 level, the tumor growth could be suppressed by RK-33 treatment without overt toxicity [39]. In summary, all these investigations suggested that the development of RK-33 to target DDX3 in Ewing sarcoma could be a promising anti-cancer strategy and more clinical trials should be needed to make it into real clinical use.

Glioblastoma multiforme

In glioblastoma multiforme (GBM), DDX3 was reported to be an oncogene, which facilitated tumor development. In some cases, DDX3 exerted its oncogenic role through impeding the process of death receptor-mediated apoptosis. Meanwhile, there existed another mechanism for DDX3 mediated cancer progression was by elevating the expression levels of tran-

scription factors, such as Snail. Activation of Snail could repress the expressions of several cellular adhesion proteins, and further lead to the malignant phenotypes of cancer cells, including invasion, migration, and metastasis [40-43]. Inhibition of DDX3 expression contributed to the reduced basal level of Snail, which effectively reduced cell proliferation and migration process. In the samples of GBM patients, there was a significantly positive correlation between DDX3 and Snail expression levels [44]. Therefore, these data indicated that DDX3 was required for basal Snail expression, and promoted the expressions of Snail-activated downstream genes, further resulting in tumor progression and development.

Gallbladder carcinoma

DDX3 was found to play an oncogenic role in different pathological subtypes of gallbladder carcinoma, including squamous cell carcinoma, adenocarcinoma, and adenosquamous carcinoma of gallbladder. The study testified that high expression level of DDX3 was significantly correlated with large tumor size, high TNM stage, and lymph node metastasis of gallbladder carcinoma. Using several statistical methods to do univariate Kaplan-Meier analysis and multivariate Cox regression analysis, the results showed that DDX3 expression level, degree of differentiation, tumor size, TNM stage, invasion, lymph node metastasis, and surgical curability were significantly associated with post-operative survival rate in gallbladder patients. And all these risk factors, apart from surgical curability, were essential independent poor-prognostic factors for gallbladder patients [45]. To sum up, this clinical study hinted that high expression of DDX3 was closely related to clinical features, pathological subtypes and biological behaviors of gallbladder carcinoma, thus it provided a promising potential to utilize DDX3 as a novel biomarker for predicting poor prognosis and metastasis in gallbladder carcinoma.

The essential signaling pathways that DDX3 involved

From above studies on the dual roles of DDX3 in multiple cancer development processes, we can summarize several signaling pathways which DDX3 participated in carcinogenesis, including the "HIF-1 α -DDX3-E-cadherin" path-

Dual roles of DDX3 in cancer development

way, the “p53-DDX3-p21” pathway, the “DDX3-Rac1- β -catenin” pathway [46], and the “DDX3-MDM2-Slug-E-cadherin” pathway. Due to the different biological functions that DDX3 exerted, especially for tumor invasion, migration and metastasis, the essential signaling pathways DDX3 involved can be illustrated from the following two aspects.

Wnt/ β -catenin pathway

Wnt/ β -catenin signaling is a conserved indispensable pathway for both embryonic development and adult homeostasis processes [47, 48]. It plays key functions in many types of diseases and various cancers [49-51]. And nearly most of the organs and tissues regeneration need the involvement of Wnt/ β -catenin signaling, such as brain, spinal cord, eyes, heart, liver, kidney, lungs, gut, skin, hair and bone marrow [52-54]. β -catenin is the key factor and the central mediator of Wnt/ β -catenin signaling pathway. Due to its different subcellular locations, in the cytoplasm or in the nucleus, the functions of Wnt/ β -catenin signaling can be activated in varying degrees [55-57]. When the cell is quiescent and the Wnt/ β -catenin signaling is not activated by stimulus, β -catenin is located in cytoplasm and formed a “destruction complex” with several proteins, including Axin, APC, GSK3 β , and CK1 α , and further phosphorylated by CK1 α and GSK3 β at Ser45, 33, 37 and Thr41 [58, 59]. Then, the phosphorylated β -catenin will be marked with ubiquitin and go through proteasome-dependent degradation pathway to degrade β -catenin protein [60, 61].

On the other hand, when the Wnt/ β -catenin signaling is initiated by the stimulus of Wnt ligands, the downstream signal cascades will be activated. Firstly, the Wnt ligands bind to the Frizzled receptor and the co-receptor lipoprotein receptor related protein 5/6 (LRP5/6) in the membrane [62, 63]. Second, the Dishevelled (Dvl) gathers the cytoplasmic Axin and GSK3 β into the membrane, so as to decompose the “destruction complex” and facilitate the unphosphorylated (activated form) β -catenin translocating from cytoplasm to nucleus. Then, nucleus β -catenin could interact with two major transcription factors, the T-cell factor (TCF) and lymphocyte enhancer factor (LEF), and along with several transcription co-activators, BCL9, CBP, p300, Pygo, to regulate

gene transcription and chromatin modification [64-66] (**Figure 3**). Apart from Wnt ligands, integrin-linked kinase (ILK) and MMP-mediated E-cadherin extracellular domain shedding can also release β -catenin in cytoplasm and activate its functions in nucleus [67, 68]. The downstream target genes regulated by Wnt/ β -catenin signaling mainly include Snail1, MMP7, PAI1, RAS, Fsp1, and Fibronectin (**Figure 3**). Most of these genes are involved in the processes of epithelial-mesenchymal transition (EMT), invasion, migration, inflammation, fibrosis and apoptosis [69-73].

For the essential role of DDX3 in the Wnt/ β -catenin signaling pathway, the classical study was published on Science in 2013. The researchers explored that DDX3 acted as a regulator of Wnt/ β -catenin signaling network through modulating a subunit of casein kinase 1 ϵ (CK1 ϵ). This finding was contrary to the previous notions in this field that CK1 members were “rogue” kinases as their enzymatic activities appeared to be unregulated [74, 75]. More specifically, this study verified that DDX3 could directly bind to CK1 ϵ , stimulate its kinase activity and further promote the phosphorylation of the scaffold protein Dishevelled (Dvl), so as to influence Dvl mediated “destruction complex” decomposition, facilitate β -catenin translocating into the nucleus and regulate the activities of Wnt/ β -catenin pathway in a Wnt-dependent manner (**Figure 3**). The regulatory role of DDX3 in Wnt/ β -catenin signaling is universal, not only in mammalian cells, but also in the development processes of *Xenopus* and *Caenorhabditis elegans* as well [76]. In a word, DDX3 is in command of CK1 ϵ activity, and this newly regulatory relationship between DDX3 and CK1 ϵ will open fresh perspectives for searching promising therapeutic targets in Wnt/ β -catenin pathway.

Epithelial-mesenchymal transition (EMT) pathway

Epithelial-mesenchymal transition (EMT) is a key biological process through which an epithelial cell phenotype can be converted into a mesenchymal cell phenotype. During this process, the cells lose epithelial characteristics and acquire mesenchymal features [77-79]. These changes mainly include apical-basolateral polarity losing, cell-cell junctions dissolving, and actin cytoskeleton remodeling [80]. For the

changes of molecular hallmarks in EMT process, the epithelial markers are down-regulated, such as E-cadherin, α -catenin, β -catenin, γ -catenin, CK, ZO-1, while the mesenchymal markers are up-regulated, including N-cadherin, Vimentin, α -SMA, fibronectin and so on [81-84]. Under normal physiological conditions, EMT exerts its important functions in embryogenesis, organ development, tissue remodel and wound repairing [85, 86]. Moreover, under pathological conditions and carcinogenesis procedures, EMT is an essential necessary step, especially for invasion, migration and metastasis of cancer cells [87, 88]. That is because EMT allows cancer cells to leave the primary lesions or environments, cross endothelial barriers, enter blood and lymphatic circulation and migrate to distant locations [89].

Cancer cells can be induced to undergo EMT process by multiple signaling pathways, such as TGF- β signaling, Wnt/ β -catenin signaling, Notch, Hedgehog signaling pathways and so on [90-93]. Additionally, there are several key transcription factors which are closely related to these signaling pathways, including zinc-finger proteins Twist, Snail, Slug, ZEB1, SIP1, E47 and other factors Smads, FOXC2 and so on [94-98]. Apart from these transcription factors that involved in EMT and its related signaling, there are a cluster of growth factors, oncogenes, tumor suppressors and miRNAs which are also participated in the control of EMT [99]. More specifically, the growth factors including TGF- β , EGF, HGF, VEGF and FGF2, could effectively activate various signaling pathways such as TGF- β /Smad, MAPK, NF- κ B, JAK/STAT3, PI3K/AKT/mTOR, ERK pathways so as to facilitate EMT progression [100-102]. The oncogenes, such as HPV16 E6/E7, PIK3CA, BMI1, AKT2, AEG1 [103-105], and the tumor suppressors, such as p53, RASAL2, SFRPs, Klotho [106, 107], along with miR-200 family, miR-155, miR-181, miR-214 and miR-130 [108-110], work together as a complicated network to precisely modulate EMT and the signaling pathways it involved.

Although these signaling pathways may possess different regulatory mechanisms to activate EMT process, they share the common endpoints---E-cadherin, which belongs to adherens junction protein and is a central downstream target being tightly regulated [111,

112]. For the role of DDX3 in EMT, E-cadherin is undoubtedly a key target of DDX3 to mediate EMT process. DDX3 could directly repress E-cadherin expression, or elevate the expression level of transcription factor Snail, and further promote the expressions of Snail-activated downstream genes to induce an epithelial-mesenchymal like transformation phenotype and increase the motility and invasive properties of cancer cells [20, 44]. On the other hand, loss of DDX3 by p53 inactivation via the MDM2/Slug/E-cadherin pathway might result in Slug-suppressed E-cadherin expression, which could promote EMT process and facilitate tumor invasion, migration, and metastasis [27]. Therefore, E-cadherin is the most important mediator in EMT, and the decreased E-cadherin expression level can be a useful predictor to indicate poor survival in cancer patients.

Conclusion

In this review, we mainly discussed about the recent progress of the DDX3 study in cancer and explored the essential signaling pathways that DDX3 involved in for modulating cancer metastasis. Specifically, DDX3 was reported to be an oncogene in breast cancer, Ewing sarcoma, glioblastoma multiforme and gallbladder carcinoma. In hepatocellular carcinoma, DDX3 was found to act as a tumor suppressive role. Meanwhile, DDX3 led dual roles of both oncogene and tumor suppressor in lung cancer, colorectal cancer and oral squamous cell carcinoma (**Table 1**). Moreover, for the two major signaling pathways DDX3 participated, Wnt/ β -catenin signaling and EMT related signaling (TGF- β , Notch, Hedgehog pathways), were crucial to DDX3 mediated biological functions in cancer development.

From the above studies, we can sense that the role of DDX3 in different types of cancer is rather controversial. DDX3 can act as an oncogene in one cancer, but in other type of cancer, it demonstrates a significant tumor suppressor role. Even in same certain type of cancer, the reporters can get to the two totally contradictable conclusions on the role of DDX3. It seems quite confusing about these dual roles of DDX3 in cancer development and progression, and we really want to tell the working mechanisms for this "double-edged sword" gene. Right now, some possible clues might help us to explain these dual roles of DDX3 in cancer. First, as

Dual roles of DDX3 in cancer development

Table 1. Dual roles of DDX3 in cancer development

Cancer type	Roles	Possible pathways and mechanisms	DDX3 inhibitors	Refs
Breast cancer	Oncogene	“HIF-1 α -DDX3-E-cadherin-EMT” pathway, promote metastasis	<u>NZ51</u> (a ring-expanded nucleoside analogue (REN), incorporate into the ATP binding pocket of DDX3)	[20-23]
Lung cancer	Oncogene/TSG	<u>Oncogene</u> : “DDX3-Wnt/ β -catenin” pathway, affect apoptosis process <u>TSG</u> : “p53-DDX3-p21” pathway, affect apoptosis process “p53-DDX3-MDM2-Slug-E-cadherin” pathway, suppress EMT and metastasis	<u>RK-33</u> (a small molecule inhibitor, bind to the ATP-binding site of DDX3)	[25-27]
Colorectal cancer	Oncogene/TSG	<u>Oncogene</u> : “DDX3-Wnt/ β -catenin-TCF4” pathway, affect cell cycle progression <u>TSG</u> : DDX3 inhibits Snail expression, and prevents EMT	<u>RK-33</u> (a small molecule inhibitor, bind to the ATP-binding site of DDX3)	[28, 29]
Hepatocellular carcinoma (HCC)	TSG	DDX3 down-regulates cyclinD1, and up-regulates p21, impede cell cycle progression “DDX3-Sp1-p21” pathway, affect apoptosis process	None	[35, 36]
Oral squamous cell carcinoma (OSCC)	Oncogene/TSG	<u>Oncogene</u> : promote OSCC progression <u>TSG</u> : DDX3 is a protective factor and a good survival predictor	<u>Ketorolac salt</u> (bioactive compound, inhibit the ATP hydrolysis process of DDX3)	[37, 38]
Ewing sarcoma	Oncogene	Promote Ewing sarcoma progression and the resistance to chemotherapy	<u>RK-33</u> (a small molecule inhibitor, bind to the ATP-binding site of DDX3)	[39]
Glioblastoma multiforme (GBM)	Oncogene	Impede the death receptor mediated apoptosis process, elevate Snail expression, and promote metastasis	To be developed	[44]
Gallbladder carcinoma	Oncogene	High DDX3 level is correlated with large tumor size, high TNM stage, lymph node metastasis, and poor prognosis	To be developed	[45]

Abbreviations: TSG: Tumor Suppressor Gene; EMT: Epithelial-Mesenchymal Transition; TCF: T-Cell Factor; TNM: Tumor, Node, Metastasis.

DDX3 has two different phenotypes, unstable and stable fashion, to exert its functions, so in each experiment related to explore cancer cell biological functions, if the experiment system and conditions vary or even change a little bit, the status and activity of DDX3 might be at different degrees, so the conclusions for DDX3 can come to a different direction. Second, DDX3 itself can have mutations in some specific sites and in some types of cancer, so the mutant DDX3 and wild-type DDX3 will exhibit different functions in cancer. Meanwhile, each cancer patients have the heterogeneity for cancer initiation, development and progression, and their cancer samples might have some variations for molecular expressions, which can lead to the dual roles of DDX3 in cancer. Third, DDX3 is closely related to virus infections and replications, particularly for the HIV, HPV, HCV, HBV, so if the cancer cell and cancer patients with different kinds of virus infections, the regulatory mechanisms and roles of DDX3 will not be the same as the cancer patients without virus infections. Virus infections might be a key factor to explain the altered role of DDX3 in different cancers. However, all these possible

explanations will need further experiments to confirm and validate.

In summary, systematically studying the dual roles of DDX3 in cancer development and its related essential signaling pathways, it will provide us new insights to explore novel therapeutic targets or small molecule inhibitors aiming at DDX3 and its signaling pathways. Also, these dual roles of DDX3 in cancer will enlighten us to carry out “Personalized medicine” and “Precision medicine” to each patient and each type of cancer to optimize the maximum therapeutic effects and realize the translational medicine from “bench to bedside”.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (3087-2267, 61572200, 81071290, 81225013, 81-430075), China Postdoctoral Science Foundation, the Postdoctoral Science Foundation of Central South University and the China Scholarship Council (CSC). Dr. Luqing Zhao is right now a Postdoctoral Fellow in Department

of Dermatology, Xiangya Hospital, Central South University, and thanks all members in Dermatology Lab for their critical comments.

Disclosure of conflict of interest

None.

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Dual roles of DDX3 in cancer development

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