DDX3-mediated translational activation drives β-catenin signaling and cancer cell motility

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DDX3 is a versatile protein involved in transcription, RNA export, translation, and signal transduction [1]. DDX3 modulates several cellular processes, such as cell growth, apoptosis, innate immunity, and viral infection [1] and may function as an oncogene [1, 2]. Examination of cancerous cells and clinical samples shows that DDX3 expression is positively correlated with malignancy and the stemness of different types of cancers [3-6]. A gene dysregulation analysis of healthy human tissues and cancer genomes from The Cancer Genome Atlas project indicated that DDX3mRNA is significantly upregulated in seven of nine cancers analyzed [7]. Ectopic expression of DDX3 in hepatocellular carcinoma and immortalized breast cells induces anchorage-independent growth [3, 4], which is consistent with attenuation of cell proliferation in DDX3-knockdown cells [3, 4]. Moreover, DDX3 has been proposed to have an anti-apoptotic role through inhibiting TRAIL receptor activation and/or promoting mutant p53 accumulation [8, 9]. Moreover, DDX3 knockdown in two-cell murine embryos results in cell cycle arrest and apoptosis with p53 accumulation, indicating that DDX3 plays similar roles in cell cycle progression and anti-apoptosis in both cancer and stem cells [10].

Compared with cell proliferation and apoptosis, less is known about the mechanism(s) for DDX3 in the regulation of cell adhesion, migration, and metastasis, which are crucial for cancer malignancy. Based on a variety of cell motility assays, DDX3 can activate the migration of immortalized and cancer cells [3, 11]. Nevertheless, mitogen and/or serum present in those experiments may possibly affect the results due to cell proliferation. We have recently reported that knockdown of DDX3 increases cell-cell adhesion and decreases cell-extracellular matrix adhesion, and also suppresses cell migration under the conditions without interfering cell proliferation [12]. However, DDX3 depletion inhibited cell migration to different extents among cell lines examined. Such a differential effect might be due to various intrinsic motility factors in different cell types. Moreover, we
noted that although the migration speed of DDX3 knockdown HeLa cells was partially maintained (~40% decrease), the cell invasion ability was largely abolished (~80% decrease). This observation suggests that DDX3 has an additional role in the control of cell motility in three-dimensional environments.

Using time-lapse recording, we observed that DDX3 knockdown in HeLa cells disrupts lamellipodia formation and migration directionality toward the scratch space in a wound-healing assay, indicating that DDX3 is required for lamellipodia-dependent migration and cell polarity. We further provided evidence that DDX3 is essential for cancer cell metastasis. Tail vein injection of HeLa and Neuro2A (N2A) cells causes lung and multiple-organ metastases, respectively. Knockdown of DDX3 generally decreases the ability of those cells to colonize and form tumors [12], whereas ectopic expression of DDX3 enhanced the ability of N2A cells to form tumors in mice (unpublished data). Our results underscore the oncogenic role of DDX3 and suggest a possibility for inhibition of oncogenic DDX3 as an adjuvant strategy for cancer therapy.

DDX3 is essential for cell cycle progression via its role in promoting the translation of cyclin E1 mRNA [13]. We speculated that the function of DDX3 in cell adhesion and motility control at least partially involves DDX3-mediated translation. DDX3-activated translational targets were identified by a comparative microarray analysis of mRNAs that cofractionated with the translational pre-initiation complex of control and DDX3-knockdown cells [13]. Among these, Rac1 holds great potential because of its oncogenic activity and role in cytoskeletal remodeling. We confirmed that DDX3 is required for Rac1 expression at the translational level [12]. The 5' untranslated region (UTR) of Rac1 mRNA has a high GC content and may fold into a stable secondary structure; this feature conforms to previously determined DDX3 targets [13-15]. Knockdown of DDX3 reduces the translation of a reporter containing the Rac1 5' UTR. Accordingly, the RNA helicase activity of DDX3 is essential for reporter translation, suggesting that DDX3 activates Rac1 translation through unwinding and/or remodeling of its 5' UTR, as observed in DDX3-assisted cyclin E1 translation [13]. Furthermore, functional analyses have confirmed that Rac1 is a major downstream factor in DDX3-modulated cell adhesion, invasion, and metastasis [12].

While we identified downstream targets of DDX3 signaling, we found that the mRNA levels of several Wnt-β-catenin targets were reduced in DDX3-knockdown cells, suggesting that DDX3 deficiency specifically affects β-catenin signaling [12]. We then confirmed that DDX3 deficiency indeed impedes β-catenin-activated transcription. However, the extent of DDX3 deficiency-suppressed β-catenin signaling varied somewhat between cell lines, suggesting that cell-specific environments or epigenetic factors may influence the transcriptional networks involved in β-catenin signaling. We further determined that diminished β-catenin signaling activity occurs because of β-catenin destabilization in DDX3-depleted cells. Consistent with a previous finding that Rac1 can prevent proteasome-dependent degradation of β-catenin [16], ectopic expression of Rac1 in DDX3-knockdown cells restored β-catenin protein expression. Our finding establishes a new Rac1-mediated DDX3-β-catenin pathway, which is distinct from the known pathway involving a casein kinase [17] (see below for the details).

A high frequency of somatic DDX3 mutations has recently been identified in various cancers [1]. In Wnt-type medulloblastoma, DDX3 mutations often occur concurrently with a β-catenin mutation [18-20]. Some DDX3 mutants can potentiate the activity of the mutated β-catenin in TCF/LEF-responsive reporter assays, suggesting the oncogenic nature of DDX3 mutations in medulloblastoma [19]. Cruciat et al. first reported the mechanism underlying the role of DDX3 in Wnt-β-catenin signaling [17]. DDX3 acts as a CKI activator to enhance disheveled phosphorylation and hence potentiate the activity of Wnt-β-catenin signaling. Notably, the RNA helicase activity of DDX3 is dispensable for this pathway [17]. Because all of the oncogenic mutations of DDX3 identified so far are located in either the RNA-binding or the ATP-binding region, we deduced that the mutations may tilt the balance between helicase-dependent and helicase-independent activation of canonical Wnt signaling. Furthermore, Rac1 has been suggested to be a component of a no canonical Wnt pathway involved in cell polarity and migration [18]. Further biochemical characterization and functional analysis of the oncogenic DDX3 mutants will help to distinguish the contribution of DDX3 to different Wnt pathways.

The versatile nature of DDX3 may lead to diversification of its downstream effects on cell cycle, apoptosis, and cell migration in different genetic backgrounds and/or cellular environments [3, 8, 11]. DDX3 has also been proposed to promote anapathelial-to-mesenchymal–like transition and suppress E-cadherin in mesenchymal–like transition and suppress E-cadherin in metastatic cancer cells via direct inactivation of the E-cadherin promoter or stabilization of Snail protein [3, 11]. In contrast, a clinical study revealed a positive correlation between DDX3 and E-cadherin expression in invasive breast cancers [21]. Our study showed a considerable decrease in E-cadherin mRNA and an increase in E-cadherin protein on the plasma membrane in DDX3-knockdown HeLa and HEK293 cells. We thus
assume that DDX3 knockdown impedes recycling of E-cadherin–bound vesicles and hence increases cell–cell adhesion. Moreover, because E-cadherin mRNA was not detected in N2A cells, DDX3-induced N2A cell aggregation may involve other, not yet determined, adhesion molecules.

Rac1 has an important regulatory role in cell growth and in motility control and has long been implicated in cancer progression and malignancy. As a signaling transducer, the activity of Rac1 is principally regulated by the switch between the GTP- and GDP-bound states. Control of Rac1 at the level of translation was recently revealed by genome-wide ribosome profiling in HeLa cells [22]. Perhaps DDX3 is involved in cell cycle–dependent Rac1 translation and has a special role in cell cycle regulation. Moreover, it has been reported that the cyclin-dependent kinases cyclin B/Cdc2 and cyclin E/Cdk2 can phosphorylate DDX3 [23]. Future studies may show how cell cycle-induced phosphorylation of DDX3 modulates its biochemical activity and cellular function and further influences the Rac1-related signaling networks.

Conflicting interests

The authors have declared that no competing interests exist.

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References