Mini Review

BIO Integration

RUNX3: A Location-oriented Genome Coordinator

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Abstract

Transcription factors are key components in gene expression and are associated with various diseases. Transcription factors maintain the stability of gene transcription and cell function. Among the transcription factors, the Runt-related transcription factor (RUNX) family regulates growth and development in a tissue-specific manner and is involved in tumorigenesis. The function of an important member of the RUNX family, RUNX3, was shown to be closely related to its subcellular localization. Normally, RUNX3 promotes or represses gene transcription in the nucleus; however, when RUNX3 is restricted in the cytoplasm, RUNX3 fails to function and only has a minor effect o gene expression. Hence, the risk of tumorigenesis cannot simply be equated with the level of RUNX3 expression, which makes the diagnosis and treatment of cancer more complicated. The cytoplasmic localization of RUNX3 has been shown to be associated with a variety of tumors. Herein we have summarized the current information on RUNX3 mis-localization and RUNX3 promotion of tumorigenesis, thus providing new insight for future investigations to elucidate the mechanisms by which RUNX3 regulates tumorigenesis.

Keywords

Cytoplasmic localization, mis-localization, RUNX3, tumorigenesis.

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Introduction

Transcription factors are a group of proteins that bind to a specific sequence upstream of the 5' end of a gene, thereby enabling the transcriptional regulation of downstream genes [1]. Transcription factors are key components in the nucleus that control gene expression, determine cell function, and the response to the environment.

As a main member of the Runt-related transcription factor (RUNX) family of transcription factors, RUNX3 is essential for regulating growth and development, and regulates the development, differentiation, and maintenance of the gastric epithelium [2, 3], nervous system [4], and immune system [5–7]. The RUNX3 N-terminus is mainly composed of the highly-conserved Runt homology domain (RHD) [8], which is the characteristic structure of the RUNX family and participates in the direct transcriptional regulation of DNA. The C-terminus is composed of similar transactivation domains (TADs), inhibitory domains (IDs), and proline-tyrosine (PY) and VWRPY motifs [9], but RUNX3 exhibits different protein interactions and post-translational modifications than RUNX1 and RUNX2. Moreover, the sequence structure of RUNX3 is more compact and conserved [10], thus RUNX3 is considered a unique gene in this family and has received much attention.

In recent years, an unusual relationship between RUNX3 cytoplasmic localization and a tumor-promoting phenotype have been reported [11]. Therefore, we focused on the impact of unusual localizations of the RUNX3 transcription factor in cancer by comparing localization in the nucleus and cytoplasm, and combining that information with literature reports to determine the possible causes of mis-localization and impact on cell fate.

RUNX3 in the nucleus, an important regulator of gene homeostasis

RUNX3 was first identified in the nucleus [10]. As a transcription factor, RUNX3 has long been considered a tumor suppressor gene that directly or indirectly suppresses the expression of cancer-related genes by binding to DNA promoters [12] or protein–protein interactions [13, 14]. Under different stimulation signals, RUNX3 can

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independently or cooperatively maintain homeostasis at the genomic and cellular levels.

RUNX3 balances TGF-β signaling

The TGF-β signaling pathway is a characteristic signaling pathway involved in cancer progression that promotes cell proliferation and malignant phenotype transformation in tumor cells, and also activates RUNX3 [2, 15]. Under the stimulation of TGF-β signaling, reactive-suppressor of mothers against decapentaplegic (R-SMAD) of the Smad protein family, which is anchored to the plasma membrane, forms heterodimeric SMAD complexes with cooperative SMAD (Co-Smad). The complexes bind to RUNX3 and transport to the nucleus together [16]. After reaching the nucleus, under promotion of CBF-β, the runt homology domain (RHD) of RUNX3 directly binds to the DNA promoter [17, 18], upregulates the expression of p21, Bim, and Claudin1 [12, 19, 20], silences Trkb, promotes apoptosis and inhibits tumor formation and progression [21–25] (**Figure 1A**). Moreover, RUNX3 also inhibits the transcriptional activity of TGF-β [26], thus forming a negative feedback pathway to balance the tumor-promoting effect brought about by the upregulation of TGF-β [27].

RUNX3 competitively inhibits oncogene transcription

In addition to direct effects, RUNX3 also indirectly inhibits the transcriptional activity of cancer-promoting genes by competing for the DNA-binding sites of cancer-promoting factors, i.e., protein–protein interactions (PPIs). In fact, RUNX3 mostly affects the activity of cancer-promoting genes in an indirect way, blocking multiple cancer signaling pathways. RUNX3 inhibits the formation of the β-catenin/TCF4 complex in the Wnt signaling pathway [13, 28], thereby blocking the transcription of Cdx2, Axin2, Cyclin D1, and c-Myc, and inhibiting proliferation and invasion [14, 29, 30] (**Figure 2A, C**). RUNX3 also directly binds to the Akt1 promoter and inhibits the Akt1/β-catenin/cyclin D1 signaling axis [30], blocking the Wnt signaling pathway in an alternative way. In addition, BMP proteins cooperate with RUNX3 to bind to the c-Myc promoter, thus inhibiting transcriptional activity [31] (**Figure 2D**). Similarly, RUNX3 binds to the TEAD-YAP complex in the nucleus to form a YAP-TEAD-RUNX3 ternary complex in the Hippo pathway [32, 33], which accelerates the dissociation of TEAD-YAP; the RAC signal can even promote this process [34]. RUNX3 also blocks binding of TEAD-YAP to CTGF and CYR61, inhibiting tumor proliferation activity [33, 35–37] (**Figure 3A**).

Moreover, RUNX3 acts on the downstream target, STAT4, inhibits STAT5 [38] and JAK3/STAT3 signaling [39], and downregulates c-Myc [40–42], Bcl [43–45], and cyclin D [46, 47] (**Figure 3B**). With respect to DNA damage repair, RUNX3 recruits FANCD2-FANCI through the Fanconi anemia (FA) pathway to repair the DNA fork of interstrand crosslinks (ICLs) [48–50] and activates transcription of redox regulator heme oxygenase 1 (HO-1 or HMOX1), ameliorating the DNA damage caused by oxidative stress and thereby maintaining cellular homeostasis [47].

RUNX3 synergizes with P53 to exert antitumor effects

In addition to independent RUNX3-independent tumor suppressor regulation, DNA damage and activation of oncogenes (Myc and K-Ras) cause RUNX3 and p53 to exhibit a synergistic role [51–56]. In this context, RUNX3-p53 forms a genome surveillance system to cooperatively regulate gene transcription activity [57]. RUNX3 simultaneously recruits phosphorylated ATM and p300 to activate p53 downstream target genes [58, 59], execute DNA damage repair or promote cell cycle arrest [60], and apoptosis

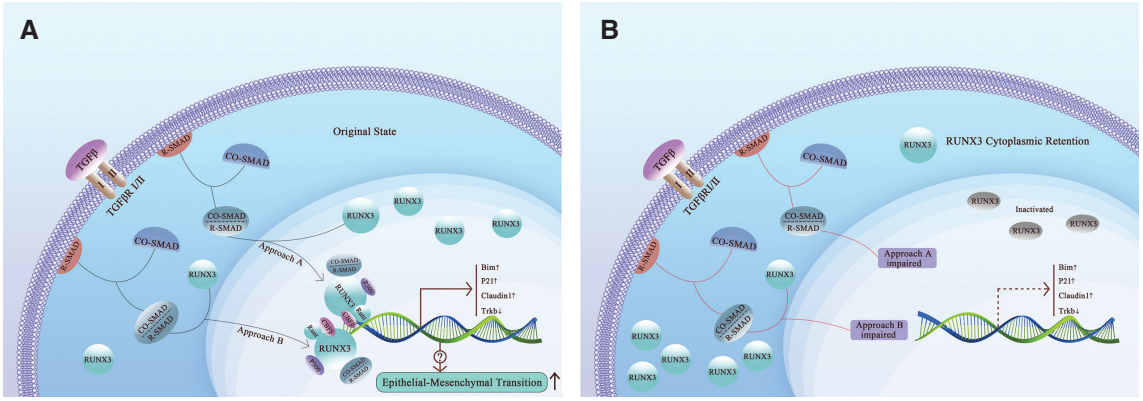

Figure 1 TGFβ pathway and RUNX3 cytoplasmic retention. A. RUNX3 enters the nucleus under TGFβ signaling. RUNX3 exerts DNA binding ability with the assistance of CBFβ. After stimulation by TGFβ, R-Smad combined with Co-Smad form the Smads complex, which binds to RUNX3 and promotes RUNX3 nuclear translocation and transactivation. In the original state, Smads complex and P300 activate RUNX3, the Runt domain binds to DNA, promotes the upregulation of Bim, P21, and Claudin 1, and down-regulation of TrkB. B. Impaired TGFβ pathway leads to cytoplasmic retention of RUNX3. When the TGFβ signaling pathway is injured, malfunctioning of TGFβ signaling or SMAD protein family occurs. RUNX3 fails to bind with SMADs and is retained in the cytoplasm in an inactive state. The aforementioned dysregulation result in downregulation of Bim, P21, and Claudin 1, and upregulation of TrkB.

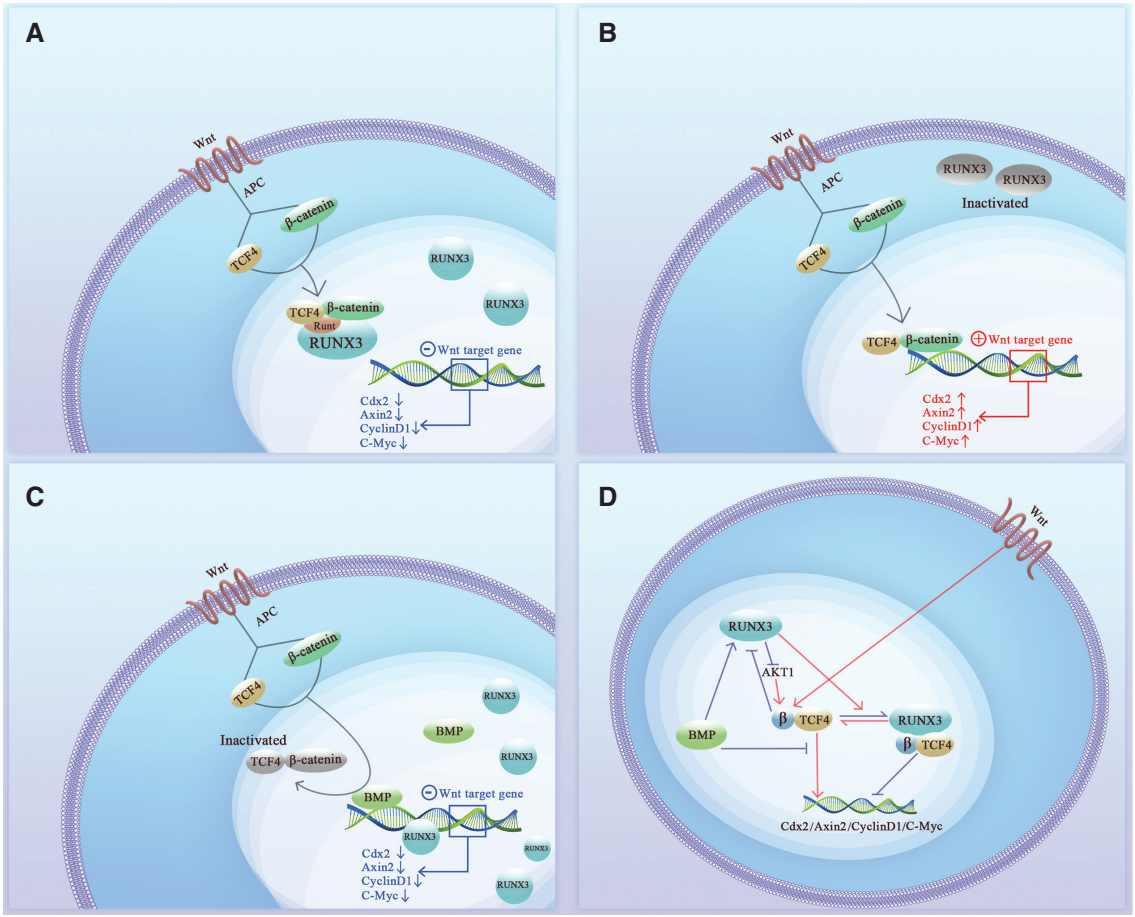

Figure 2 The relationship between RUNX3 and the Wnt signaling pathway. A. Nuclear RUNX3 blocks the Wnt signaling pathway through "protein-protein interactions." In the original state, TCF4 binds to β-catenin and translocates into the nucleus after Wnt signaling stimulation. The Runt domain of RUNX3 binds to the DNA-binding region of TCF4 in nucleus, thus preventing the TCF4-β-catenin complex from binding to DNA and inhibiting the target genes (Cdx2, Axin2, CyclinD1, and c-Myc) of the Wnt signaling pathway; B. Cytoplasmic RUNX3 fails to inhibit the Wnt signaling pathway. RUNX3 is localized in the cytoplasm in an inactive state. RUNX3 in the nucleus is absent or insufficient to counteract the TCF4-β-catenin complex, which subsequently binds to DNA and promotes the transcription of Cdx2, Axin2, CyclinD1, and c-Myc oncogenes. C. RUNX3 cooperates with the BMP family to suppress oncogenes. BMP occupies the DNA binding site of TCF and RUNX3 binds to the DNA binding site to jointly exert a tumor suppressor effect. D. The relationship between RUNX3 and Wnt signaling pathway. The Runt domain of nuclear RUNX3 combines with the DNA binding region of TCF4 to form a RUNX3-TCF4-β-catenin trimer, which blocks binding of the TCF4-β-catenin complex to the target gene promoter and inhibits the target genes (Cdx2, Axin2, CyclinD1, and c-Myc). When RUNX3 is localized in the cytoplasm and the nucleus RUNX3 is absent or deficient, the TCF4-β-catenin complex smoothly binds to the promoters of target genes to promote the transcription of Cdx2, Axin2, CyclinD1, and c-Myc oncogenes. RUNX3 can also inhibit the effect of AKT1 on CTNNB1 (β-catenin encoding gene) and indirectly inhibits the formation of TCF4-β-catenin complex. BMP interacts with the TCF4-β-catenin complex and upregulates the expression of RUNX3, directly or indirectly regulating Cdx2, Axin2, CyclinD1, and c-Myc transcription.

[51, 61]. In addition, p53 inhibits the overexpression of RUNX3, thereby forming a stable RUNX3-p53 negative feedback loop [62]. Therefore, the combination of RUNX3 and p53 is thought to be the gatekeeper and guardian of the genome, ensuring genomic stability. The synergistic effect of RUNX3 on p53, however, is destabilized by the p53 high mutation rate in many cancer types [63]. Mutant p53 (p53R175H-human/p53R172H-mouse) binds RUNX3 and promotes Myc transcription in osteosarcomas [64]. In summary, the RUNX3-p53 negative feedback loop monitors the genome, but the stability of p53 cannot be overlooked.

In general, under the action of various post-translational modifications, RUNX3 has an important role in the regulatory balance between oncogenes and tumor suppressor genes.

Cytoplasmic localization: a constraint of RUNX3

RUNX3 is normally localized and functions in the nucleus, but recent studies have shown that RUNX3 has an unusual cytoplasmic localization in many tumor cell lines, which is also called "cytoplasmic sequestration" or "mis-localization" of RUNX3 [11]. The cytoplasmic localization of RUNX3 is associated with the gastric epithelium in gastric cancer, suggesting a role in carcinogenesis [11, 65, 66]. Subsequent reports on RUNX3 mis-localization confirmed that cytoplasmic localization of RUNX3 is not a rare event [11], and cytoplasmic localization occurs in 80% of breast cancer patients [67]. Cytoplasmic localization of RUNX3 protein also exists in 46.0% and 30% of ovarian cancer and oral squamous cell

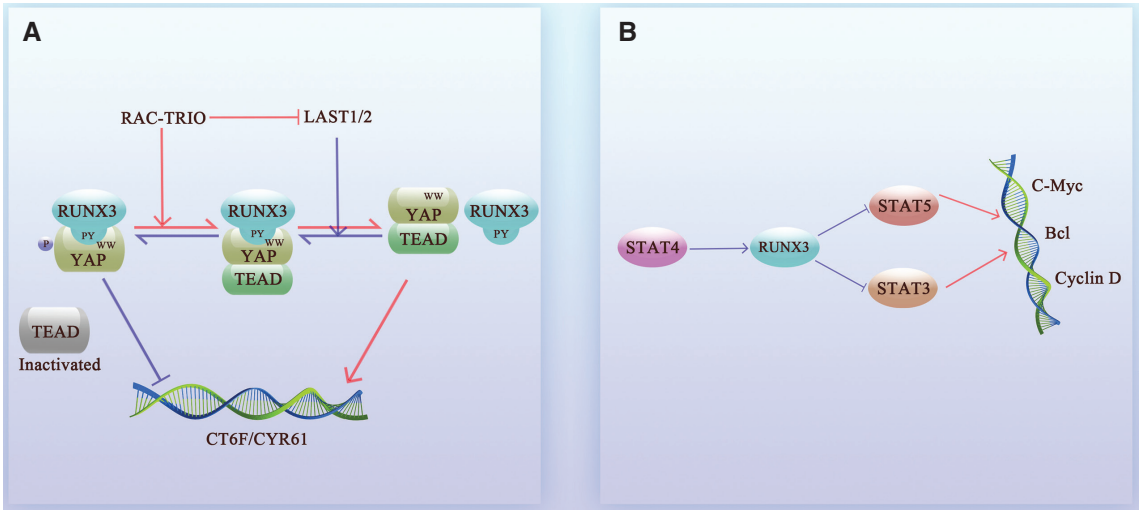

Figure 3 RUNX3 competitively inhibits oncogene transcription. A. RUNX3 inhibits the Hippo pathway. The TEAD-YAP complex of the Hippo signaling pathway promotes the transcription of the oncogenes, CTGF and CYR61, and promotes cancer progression. Nuclear NRUNX3 directly binds to YAP, thus preventing the formation of the TEAD-YAP complex, and forming a triple complex with TEAD-YAP for degradation. When RAC-TRIO is downregulated, the downstream LAST1/2 renders YAP more likely to interact with RUNX3 by phosphorylating YAP and prevents the formation of TEAD-YAP. B. Relationship between RUNX3 and the STAT family. STAT4 upregulates the expression of RUNX3. RUNX3 inhibits the promoting effect of STAT3 and STAT5 on c-Myc, Bcl, and Cyclin D.

carcinoma patients [68, 69], respectively. Cytoplasmic localization of RUNX3 has also been observed in colon cancer and is associated with tumorigenesis and metastasis [70]. The cytoplasmic localization of RUNX3 in lung small cell carcinoma is thought to lead to a higher probability of postoperative distant metastasis and is significantly associated with lymph node-positive involvement and margins indicating lymphatic invasion [71]. Cytoplasmic localization of RUNX3 greatly limits RUNX3 function, resulting in protein conversion to a tumor-promoting phenotype. Interestingly, although the three members of the RUNX family share a high degree of homology, only RUNX3 exhibits cytoplasmic localization that leads to functional differences.

Current studies suggest that the cytoplasmic sequestration of RUNX3 originates via two mechanisms: ① blockade of the RUNX3 nuclear import pathway (cytoplasmic retention); and ② abnormal activation of the nuclear RUNX3 nuclear export signal (nuclear exclusion). We will discuss the impact on tumor formation from the perspective of these two mechanisms.

Cytoplasmic retention of RUNX3: RUNX3 nuclear import pathway dysfunction

The nuclear import of RUNX3 can be mediated in two ways: ① TGFβ-SMAD signaling [11]; and ② DNA damage or proto-oncogene activation signaling [51]. TGFβ signaling is the initiation signal by which RUNX3 enters the nucleus to regulate transcription. Dysregulation of one or more molecules in the TGFβ pathway upstream of RUNX3, such as downregulated expression of Smad4 and TGF-βI/II receptors and mutation of TGF-βII receptors, may lead to plasma retention of RUNX3. Occupation of SMAD-binding sites can also lead to retention of RUNX3. This process leads to the continuous activation of intracellular TGF-β-SMAD signaling without negative feedback inhibition, stimulates cell proliferation, and induces tumorigenesis [11, 67] (**Figure 1B**). In addition, RUNX3 can also rapidly enter the nucleus and co-localize with p53 under the stimulation of DNA damage or proto-oncogene activation [51]. Given that RUNX proteins rely on nuclear localization signals (NLSs) [72] and nuclear matrix targeting signal (NMTS) sequences for nuclear import [73–75], cytoplasmic retention could be related to inactivation of these two nuclear import-related signals. The absence of effective repair methods after DNA damage makes it possible for the aggravation of abnormal DNA mutations and the occurrence of tumor signals [53, 64, 76, 77].

Nuclear exclusion of RUNX3: aberrant activation of nuclear export signaling

RUNX3, which is localized in the nucleus, can also be exported to the cytoplasm. After being exported, RUNX3 loses tumor suppressor regulatory function and DNA damage monitoring, and indirectly promotes tumorigenesis.

This nuclear export program is activated by phosphorylation- or ubiquitination-modifying enzymes or other types of molecules. Specifically, abnormal phosphorylation of RUNX3 is one of the main triggers of nuclear exportation, and this process can be mediated by phosphokinases, such as the Src kinase family [65, 78], Pak1 [79, 80], and Pim-1 [81, 82]. Histone deacetylase (HDAC) produced by oxidative stress or the Cag. oncoprotein produced by *Helicobacter pylori* (H.p.), can increase the level of Src in cells [70]. Src phosphorylates multiple tyrosine residues on the surface of the RHD of RUNX3, initiating the RUNX3 nuclear export program [65]. Subsequently, chromosome

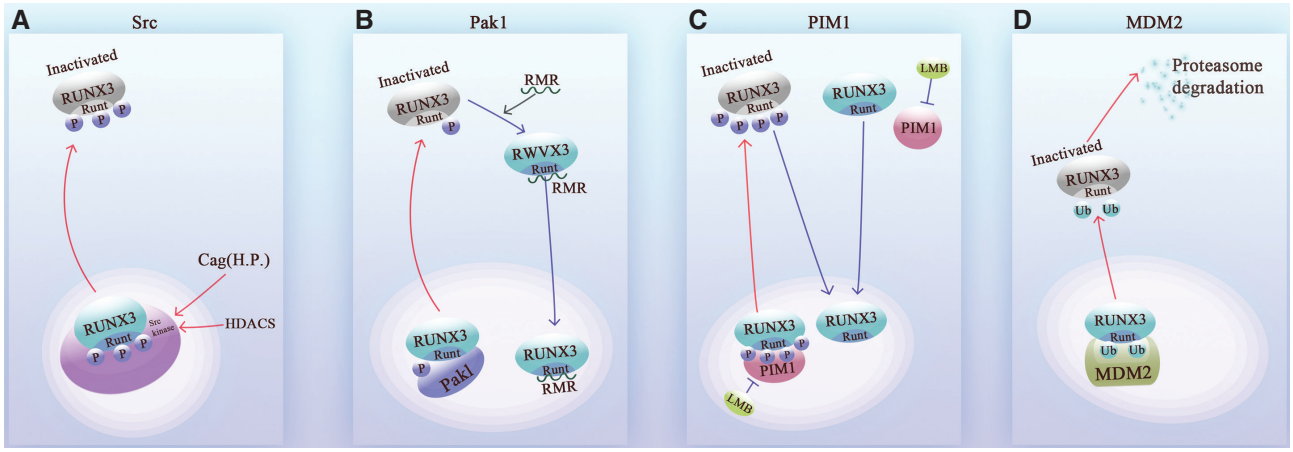

Figure 4 The mechanisms underlying RUNX3 nuclear exportation. A. Src kinase phosphorylates the tyrosine residues of the Runt domain of RUNX 3, promoting RUNX3 nuclear exportation. Scr kinase can be induced by *Helicobacter pylori* (H.p.) and HDACs. B. Pak1 phosphorylates the serine and threonine sites of the Runt domain to promote inactivation of RUNX3 after nuclear exportation. The discovered RMR peptide competitively inhibits Pak1 and restores RUNX3 activity and nuclear localization. C. PIM-1 shuttles between the cytoplasmic and nucleus. PIM-1 phosphorylates and inactivates of RUNX3 after nuclear exportation. This process can be reversed by LMB, the Pim-1 inhibitor. D. The acidic domain of MDM2 interacts directly with Runt, and the RING finger domain of MDM2 is the active region for ubiquitination. After ubiquitination on the surface of the Runt domain, RUNX3 is exported from the nucleus and degraded by the proteasome.

region maintenance protein 1 (CRM1) binds the RUNX3-containing nuclear export signal (NES) to the nuclear pore complex and mediates nuclear export [83, 84] (**Figure 4**).

Thus, tyrosine-phosphorylated RUNX3 is mainly present in the cytoplasm, while non-tyrosine-phosphorylated RUNX3 is present in the nucleus [85]. In addition, the Src kinase family members, Fyn and Lck, are also able to phosphorylate RUNX3, which shows that this family has multiple roles in RUNX3 nuclear export [65]. Moreover, Pak1 and PIM-1 kinases also promote the nuclear export of RUNX3 in a similar manner. The three phosphorylation sites are located on the surface of the RHD [80, 81]. Moreover, the nuclear export of ubiquitinated RUNX3 is mediated by MDM2. The acidic domain of MDM2 directly ubiquitinates lys94 and lys148 of the RHD of RUNX3 [52] (**Figure 4**).

Furthermore, the Jab1/CSN protein complex is also responsible for the nuclear export and degradation of RUNX3. Jun activation domain-binding protein 1 (Jab1) binds to RUNX3 through the MPN domain and initiates complex nuclear export via the NES of Jab1 [86]. The Mpr1p Pad1p N-terminal (MPN) domain of Jab1 has a role in the physical interaction between RUNX3 and Jab1, while the NES is the receptor of the CRM1 export substrate. Moreover, the COP9 signalosome complex (CSN complex) is regulated by CSN-associated kinases that degrade RUNX3 via the proteasomal pathway [86, 87].

Prospects

As a tumor suppressor in the RUNX family, RUNX3 attenuates multiple oncogenic signals. Restoring RUNX3 activity in nuclear RUNX3-negative cells significantly reverses the tumor phenotype [79, 88], suggesting that remobilization of cytoplasmic RUNX3 into the nucleus or restoring the level of nuclear RUNX3 expression exogenously may be a possible therapeutic strategy for the treatment of cancer.

It is worth mentioning that among the many mechanisms of protein dysregulation, only cytoplasmic localization causes a functional restriction through a change in the spatial physical position, rather than the common mechanism underlying changes in protein levels. Simply overexpressing RUNX3 to reverse the tumor phenotype will not completely overcome this challenge. This RUNX3 mislocalization undoubtedly increases the complexity of tumor progression and the difficulty of diagnosis and treatment. Overall, cytoplasmic localization of RUNX3 promotes oncogenesis. Thus, it is necessary to pay more attention to this unconventional phenomenon.

Abbreviations

RUNX3, Runt-related transcription factor 3; RHD, Runt homology domain; TADs, transactivation domains; IDs, Inhibitory domains; PY motifs, proline-tyrosine motifs; R-SMAD, reactive SMAD; Co-SMAD, cooperative SMAD; P21, cyclin-dependent kinase inhibitor 1A; Claudin1, senescence associated epithelial membrane protein 1; Trkb, tyrosine kinase receptor B; TGF-β, transforming growth factor-β; PPIs, protein–protein interactions; TCF4, transcription factor 4; Cdx2, caudal-type homeobox protein 2; Axin2, axis inhibition protein 2; cyclin D1, G1/S-specific cyclin-D1; c-Myc, Myc proto-oncogene protein; Akt1, v-akt murine thymoma viral oncogene homolog 1; BMP, bone morphogenetic protein; TEAD, TEA domain family member; YAP, Yes-associated protein; RAC protein, Ras-related C3 botulinum toxin substrate protein; CTGF, connective tissue growth factor; CYR61, 61 cysteine-rich, angiogenic inducer; STAT, signal transducer and activator of transcription; JAK3, Janus kinase 3; FA pathway, Fanconi anemia pathway; ICLs, interstrand crosslinks; HO-1 or HMOX1, heme oxygenase 1; K-Ras, Kirsten rat sarcoma viral oncogene homolog; ATM protein, ataxia telangiectasia-mutated

protein; NLS, nuclear localization signal; NMTS, nuclear matrix targeting signal; Pak1, p21 activated kinase 1; Pim-1, provirus integration site for Moloney murine leukemia virus kinase-1; HDAC, histone deacetylase; CRM1, chromosome region maintenance protein 1; NES, nuclear export signal; MDM2, mouse double minute 2 homolog; CSN, constitutive photomorphogenic signalosome; Jab1, Jun activation domain-binding protein 1; MPN domain, Mpr1p Pad1p N-terminal domain.

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Conflict of Interest

This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.Tianshu Xu, Yancan Liang, Zhiquan Huang and Zixian Huang declare that they have no conflicts of interest.

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