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REVIEW

RUNX2 as a promising therapeutic target for malignant tumors

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Abstract: The transcription factor runt-related protein 2 (RUNX2) has an important impact on the transformation of bone marrow mesenchymal stem cells to osteoblasts. Further studies have shown that RUNX2 plays a key role in the invasion and metastasis of cancers. RUNX2 is a "key" molecule in the regulatory network comprised of multiple signaling pathways upstream and its target downstream molecules. Due to the complex regulatory mechanisms of RUNX2, the specific mechanism underlying the occurrence, development and prognosis of malignant tumors has not been fully understood. Currently, RUNX2 as a promising therapeutic target for cancers has become a research hotspot. Herein, we reviewed the current literature on the modulatory functions and mechanisms of RUNX2 in the development of malignant tumors, aiming to explore its potential clinical application in the diagnosis, prognosis and treatment of tumors.

Keywords: RUNX2, malignant tumor;, research progress

Introduction

In mammals, RUNX represents a family of three transcription factors, which share a common DNA binding domain-Runt domain, homologous to the Drosophila Runt gene. RUNX family members (including RUNX1, RUNX2 and RUNX3) combine with core-binding factor β to form heterodimers that enhance their ability to bind to DNA, and participate in subsequent transcriptional regulation.[1,2] The transcription factor RUNX2 is closely related to the differentiation of human osteoblasts and the maturation of chondrocytes by regulating multiple signaling pathways and transcriptional activation of a series of downstream molecules.[3,4] (figure 1, figure 2, figure 3, table 1Current researches have confirmed that RUNX2 is closely related to the proliferation, invasion and bone metastasis of multiple cancers such as osteosarcoma, breast cancer (BC), prostate cancer, gastric cancer and colorectal cancer. This article summarizes the research progress of RUNX2 in malignant tumors, which focuses on the involvement of signaling pathways miRNAs regulations, histone modification and so on.

The Transcription Factor RUNX2 and Malignant Tumors

RUNX2 and Breast Cancer

BC is one of the common malignant tumors in women. RUNX2 plays a vital role in BC development. RUNX2 and estradiol have opposite effects on BC.[5] Previous studies have found that estrogen can trigger BC progression in situ, while presents

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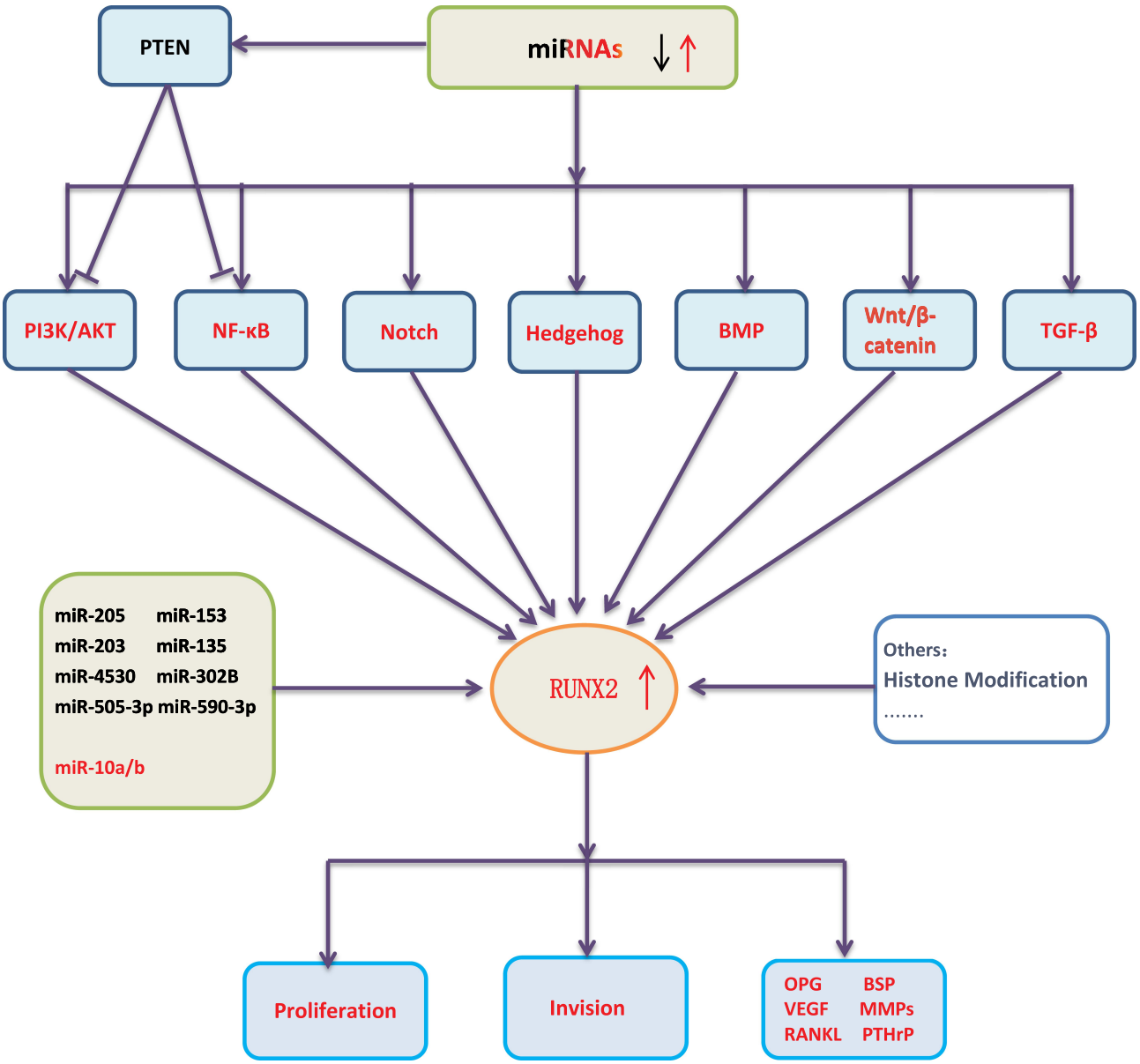

Figure 1 Direct or indirect regulation of RUNX2 by miRNAs via the targeting of signaling pathways in BC. In BC, the miRNAs marked in red color play an oncogenic role, their expression levels are down-regulated; the miRNAs marked in black color act as tumor suppressor genes, their expression levels are up-regulated.

anti-metastatic properties. Its mode of action is opposite to RUNX2. Overexpression of RUNX2 in BC cell line MCF7 induces epithelial-mesenchymal transition (EMT), which relies on signaling pathways of TGF β and Wnt.[6] The high nuclear expression level of RUNX2 is related to the state of human epidermal growth factor receptor type 2 (HER2) in BC cells, and the poor prognosis is correlated with high RUNX2 expression level and negative HER2 expression in BC patients.[7]

Micro ribonucleic acid (miRNAs) can control gene expressions at the post-transcriptional level, and act as oncogene or tumor suppressor in BC. miRNAs directly[8] or indirectly affect the expression of RUNX2 in BC cells by controlling different signaling pathways, such as PI3K/AKT, NF-κB, Wnt/β-catenin, TGF-β, BMP, Notch and Hedgehog (Figure 1).[9–17] miRNAs can regulate these signaling pathways by targeting their molecular signaling components. Reduced expression levels of tumor-suppressive miRNAs and increased expression levels of oncogenic miRNAs can activate the signal transduction pathways, subsequently up-regulating the expression level of RUNX2. The enhanced expression level of RUNX2 stimulates the expressions of metastatic marker genes such as vascular endothelial growth factor (VEGF),

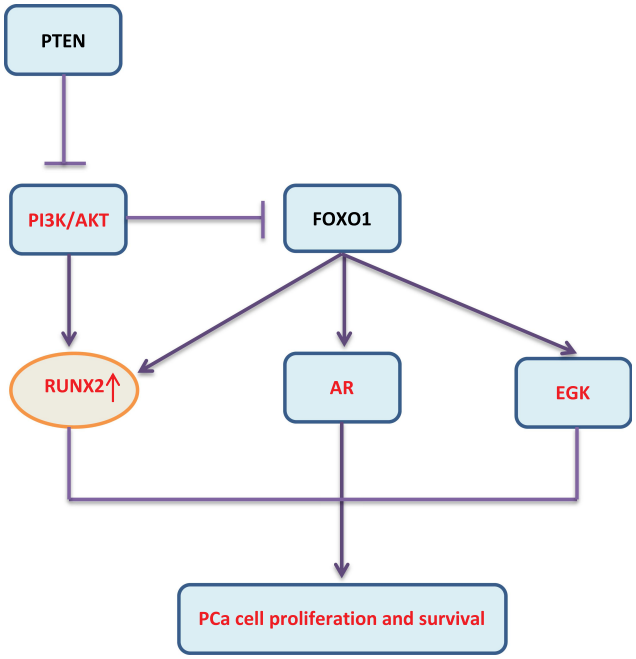

Figure 2 PI3K/AKT-dominated signaling pathway regulates RUNX2 in PCa.

metalloproteinase 2 (MMP-2), osteopontin (OPN), bisulfite sequencing PCR (BSP), parathyroid hormone-related peptide (PTHrP) and receptor activator of nuclear factor κB ligand (RANKL), thus promoting bone metastasis, increasing BC cell proliferation, and ultimately leading to poor prognosis of BC.[18]

With the increase of whole-genome acetylation level, histone deacetylase inhibitor (HDACi) promotes delocalization of bromodomain-containing protein 4 (BRD4) from active Enhancers (ENHs) to other sites and reduces the expression of highly expressed genes; JQ1 inhibits RUNX2 by blocking the recruitment of BRD4 to the RUNX2 promoter and ENHs; It is also speculated that HDACi and bromodomain and extraterminal inhibitors (BETi) synergistically inhibit RUNX2 and other cancer driver genes, supporting the rational combined use of these drugs in cancer treatment.[19,20]

RUNX2 has an important impact on osteogenesis and BC-mediated bone metastasis. It also plays a critical role in osteolytic lesions. BC cells with a lack of RUNX2 expression suppress osteoblasts differentiation and increase osteoclast differentiation.[21] RANKL expression is positively correlated with connective tissue growth factor (CTGF) in BC tissues and the expression levels of RANKL and CTGF are higher in bone metastasis tissues than in other sites. CTGF also promotes the recruitment of RUNX2 to the RANKL promoter, thereby increasing the production of RANKL in tumor cells, and subsequently stimulating osteoclastogenesis.[22] In BC cells, RUNX2 is implicated in an adhesion-dependent mechanism of bone tropism and bone colonization, which is a potential target for predicting and treating bone metastasis of BC.[23] In the process of BC metastasis, cancer cells promote survival

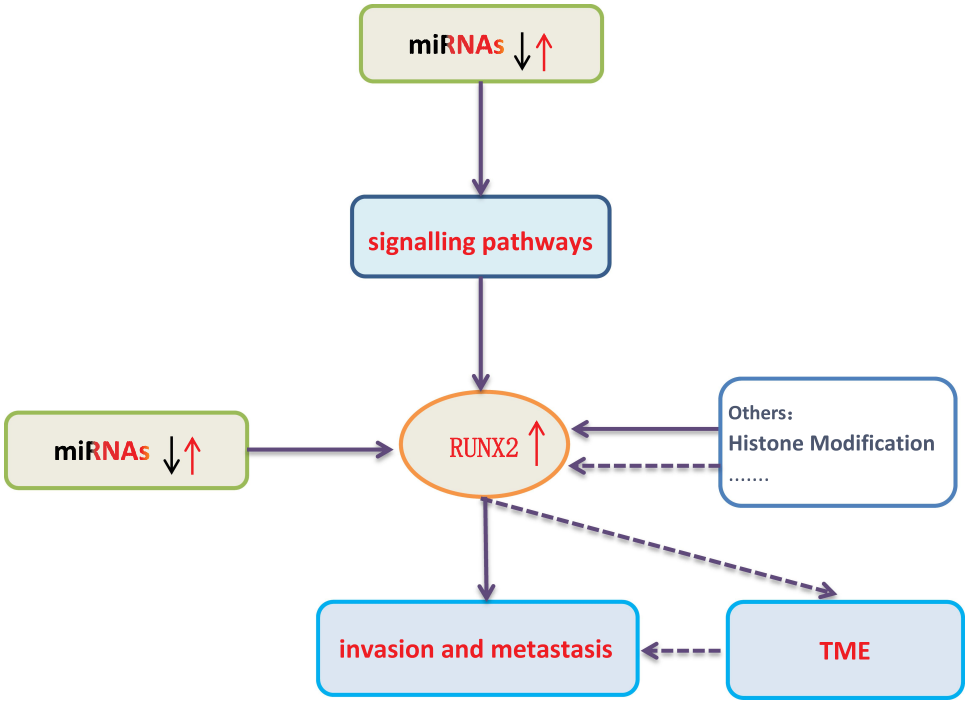

Figure 3 Simulation diagram of RUNX2′ s regulation mechanism in malignant tumors. The dotted line indicates that further research is required.

Table 1 Regulation of RUNX2 Expression by miRNAs and lncRNAs in Osteosarcoma

No.	miRNA (s) or lncRNAs	Interaction Site	Role	Signaling Pathway	References
1	miR-150	Directly	Tumor Suppressor	Apoptosis proteins	[7]
2	LncRNA TUG1	Directly	Oncogene	-	[8]
3	miR-338-3p	Indirectly	Tumor Suppressor	MAPK	[9]
4	LncRNA SNHG20	Directly	Tumor Suppressor	Mitochondrial apoptosis	[10]
5	miR-340	Indirectly	Tumor Suppressor	Notch	[11]
6	miR-302B miR-203 miR-205	Directly	Tumor Suppressor	-	[12–14]

under nutrient starvation by autophagy. In addition, RUNX2 promotes autophagy through α-tubulin acetylation and autophagic vesicle transport. Therefore, the levels of LC3B and RUNX2 can be used to predict metastasis of Bethesda categories (BCs).[24]

The mechanism of RUNX2 underlying BC is relatively thorough, which provides reference for the study of other malignant tumors.

RUNX2 and Osteosarcoma

Osteosarcoma is a malignant bone tumor, which occurs more commonly in adolescents or children under the age of 20. RUNX2 has the potential to regulate osteoblast differentiation and carcinogenesis, and it exerts an important impact on the development and progression of osteosarcoma. RUNX2 is overexpressed in human osteosarcoma tissues, especially in tumors that respond poorly to chemotherapy.[25] Increasing number of studies have confirmed that miRNAs and long non-coding RNAs (lncRNAs) are aberrantly expressed in osteosarcoma and can directly or indirectly participate in the regulation of RUNX2 expression. The potential diagnostic and therapeutic values of the differentially expressed miRNAs and lncRNAs by targeting RUNX2 are extremely important in the clinic (Table 1).[26–34] For example, miR-150 is suggested as the therapeutic target of osteosarcoma due to its anti-tumor function in promoting chemotherapy sensitivity and inhibiting tumor cell proliferation via RUNX2. In details, the results of luciferase reporter assay demonstrated that RUNX2 is a direct target of miR-150 and miR-150-RUNX2 axis affects the chemical sensitivity of osteosarcoma cells by regulating the expression levels of apoptosis proteins such as increasing the expression levels of cleaved caspase-3 and cleaved caspase-8 while reducing the expression levels of cleaved caspase-3 and cleaved caspase-8.[27] With the deepening of research, the regulatory signaling pathways, potential targets and corresponding drugs related to RUNX2 are emerging research hotspot against osteosarcoma.[35–37]

RUNX2 and Prostate Cancer

Prostate cancer (PCa) is a heterogeneous disease at both the genetic and clinical levels. At present, it is divided into different genotypes and treatment methods were applied based on a large amount of clinical data. The molecular mechanism remains to be clarified.[38] Through RUNX2 immunohistochemical staining analysis on PCa tissues, it was found that patients with the positive RUNX2 staining had higher PSA levels, higher Gleason grades, and stronger metastatic ability than the negative staining ones.[39] Overexpression of RUNX2 is related to the up-regulation of matrix metalloproteinases, bone resorption factors and the enhanced metastasis of PCa cells to bone.[40] The increased RUNX2 protein level is related to the decrease of phosphatase and tensin homolog (PTEN) protein expression. The expression of RUNX2 is down-regulated in PCa cells by interacting with Forkhead Box O1 (FOXO1). Correspondingly, FOXO1/PTEN expression is decreased in patients with bone metastasis, while RUNX2 expression is increased, suggesting the potential value of FOXO1 in the metastasis of PCa to bone.[41] Further studies have confirmed that PTEN deletion promotes the activation of the AKT-RUNX2-OCN-GPRC6A-CREB signal axis and further induces the expressions of cytochrome P450 family 1 subfamily A1 (CYP1A1) and CYP17A1 in PCa cells and intratumoral androgen synthesis (IAS).[42] An important target of the PI3K/AKT signaling pathway is FOXO1 protein that can be phosphorylated directly by

AKT leading to translocation of FOXO1 from the cytoplasm to the nucleus. This not only impairs FOXO1 activities on transactivation of downstream target genes, but also abolishes its transcriptional activity-independent inhibitory effect on other targets such as androgen receptor (AR), extracellular regulated protein kinases (ERK) and RUNX2 (Figure 2). Additionally, miR-466 inhibits tumor growth and bone metastasis in PCa by direct regulation of RUNX2 expression.[43]

Furthermore, RUNX2 S319 phosphorylation plays an important role in the development of PCa; P-S319-Runx2 is a marker for more aggressive metastatic disease in a patient population, which has an exclusively nuclear localization and is regulated by both RAS/MAPK and PI3K/AKT signaling pathways.[44] Therefore, based on the conventional signaling pathways research, the research of histone modification function of RUNX2 should be paid more attention, and it may be used as a potential target for the diagnosis and treatment of prostate cancer.

RUNX2 and Colorectal Cancer

Colorectal cancer (CRC) is a common malignant tumor in the digestive system. Most CRC-related deaths are attributed to liver metastases. Researches have shown that RUNX2 is closely related to Duke staging, liver metastasis and ERβ, and is an independent factor in the prognosis of colon cancer patients.[45] OPN in CT26 CRC cells is regulated by RUNX2 and ETS-1. Therefore, inhibiting these transcription factors could result in a significant down-regulation of the osteopontin transfer proteins.[46] RUNX2, OPN and MMP-7 are highly expressed in CC531 colon cancer cells metastases explanted from the liver, while the expressions are reduced and/or disappeared in cell culture in vitro. The opposite expression profiles of Hoxc8, OPN and RUNX2 indicate that these genes may be regulated in a feedback loop way. Transforming Growth Factor β-1 (TGFβ-1) induces the overexpression of OPN and RUNX2 in hepatocytes, but does not show the same effect on hepatocytes co-cultured with CC531 cells.[47] Small nucleolar RNA host gene 3 (SNHG3) binding to miR-539 up-regulates RUNX2 expression, which promotes the growth and metastasis of CRC. SNHG3 may be a potential target for CRC treatment.[48] RNA-metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) binding to miR-15 family members affects the expression of LRP6, thereby enhancing β-catenin signaling and leading to an increase in the transcription level of the downstream target gene RUNX2. Moreover, MALAT1 can also bind to the splicing factor proline/glutamine-rich (SFPQ) protein, contributing to dissociation of the SFPQ/polypyrimidine tract binding protein-2 (PTBP2) dimer, the release of PTBP2 and the increase of RUNX2 expression by interplaying with the IRES domain of the corresponding mRNA 5′UTR region.[49]

Our previous studies indicate that miR-455 inhibits the progression of CRC via RAF. Plasmacytoma variant translocation 1 (PVT1) silencing can inhibit the progression of CRC by miR-455 in vivo. RUNX2 increases the expression level of PVT1 in CRC, while miR-455 inhibits RUNX2 expression, which forms a feedback loop between RUNX2/PVT1/miR-455. In conclusion, the RUNX2/PVT1/miR-455/RAF-1 axis has been considered a potential target for CRC treatment.[50,51]

RUNX2 and Gastric Cancer

Gastric cancer (GC) remains to be one of the world's leading malignant tumors of the digestive system. Half of the new affected cases occurred in East Asia, including China, Japan and South Korea. RUNX2 in GC tissue is related to the degree of differentiation, depth of invasion, and lymph node metastasis. RUNX2 is one of the independent prognostic factors for patients with GC. RUNX2 enhances transcription and promotes the progression of GC by directly binding to CXCR4.[52] In GC patients, RUNX2 plays a regulatory role by interacting with lncRNAs under certain conditions. The expression levels of lncRNA EPEL and RUNX2 are up-regulated in GC. Overexpression of lncRNA EPEL leads to the up-regulation of RUNX2 expression, while overexpression of RUNX2 does not affect EPEL expression. Thence, lncRNA EPEL may be regulated by the interaction with RUNX2.[53] MiR-539 as a tumor suppressor inhibits GC progression by targeting RUNX2.[54] JQ1 can inhibit the progression of GC by down-regulating chromatin accessibility and inactivating the RUNX2/Nidogen 1 (NID1) signaling pathway. In addition, NID1 may be a new therapeutic target for GC.[55]

RUNX2 and Lung Cancer

Non-small cell lung cancer (NSCLC) accounts for nearly 80% of lung cancers. The expression level of RUNX2 in NSCLC is significantly correlated with tumor size, stage and lymph node metastasis. RUNX2 is an independent risk factor in NSCLC.[56] Besides, the co-expression network of RUNX2 in lung squamous cell carcinoma (LUSC) reveals that the complex interactions between RUNX2 and 45 co-

expressed genes, which are involved in extracellular matrix-receptor interactions, local adhesion, protein digestion and absorption, human papillomavirus infection and PI3K/AKT signaling pathway, etc.[57] In lung adenocarcinoma, RUNX2 transcriptionally regulated its potential target genes are involved in cell cycle-related proteins and MAPK signaling pathways, providing a new perspective for targeted drug development and drug resistance research.[58] WW domain-containing oxidoreductase (WWOX) is a tumor suppressor gene.[59] Overexpression of WWOX in highly aggressive H1299 lung cancer cells suppresses cell motility and invasiveness, and inhibits the expressions of RUNX2 and its target gene MMP-9.[60] BMP-2 induces lung cancer migration through increasing the activation of ERK and p38 and up-regulating Runx2 and Snail expressions. ERK-RUNX2-Snail and p38-RUNX2-Snail can induce lung cancer cell migration and EMT. It is also shown that RUNX2 can increase the expression of Snail to regulate the early metastatic events of lung cancer.[61] In addition, miR-218 and lncRNA H19 directly or indirectly regulate RUNX2 in lung cancer.[62,63]

RUNX2 and Other Cancers

Malignant melanoma is highly aggressive and resistant to chemotherapy. RUNX2 is overexpressed in melanoma cells, and its expression is related to the process of proliferation and migration.[64] The RUNT domain in RUNX2 is responsible for the proliferation and migration of melanoma.[65] The RUNT domain increases the angiogenic properties of melanoma cells. Proteomic analysis allows us to point out that the RUNT domain is involved in the process of neovascularization.[66] RUNT domain promotes bone metastasis of melanoma cells through the complex interactions with genes involved in bone remodeling through the ERK/p-ERK and AKT/p-AKT pathways.[67] All these findings indicate that the RUNT domain is involved in melanoma metastasis and cell migration. RUNX2 can reactivate the MAPK and PI3K/AKT pathways, thereby endowing melanoma cells with high metastatic potential.[68]

In patients with thyroid cancer, the expression level of RUNX2 was higher in patients with microcalcification, compared with those without microcalcification.[69] RUNX2 promotes bone homing and bone metastasis by interacting with its target genes such as Stromal Sell-Derived Factor 1 (SDF1), CXCR7 and BSP.[70] Thyroid hormone receptor β, (TRβ) modulating RUNX2 expression is a signal axis shared by thyroid cancer and BC. TRβ directly interacts with the proximal promoter of RUNX2 through the thyroid hormone response element, resulting in the reduced RUNX2 promoter activity.[71] As a cis-regulatory element, RAIN promotes carcinogenic characteristics in thyroid and BC cells, promoting the expression of RUNX2 through two patterns, including binding to WD repeat domain 5 (WDR5) and facilitating its positioning on RUNX2 promoter; and changing the transcription status of the RUNX2 locus and promoting transcription initiation. RAIN acts as a bait for the negative elongation factor (NELF) complex to inhibit its inhibitory effect on transcription elongation.[72] Ectopic expression of miR-218 inhibits the development of papillary thyroid cancer by targeting RUNX2 to inactivate the PTEN/PI3K/AKT pathway.[73]

Transcription Factor RUNX2 and Cancer Stem Cells in Some Cancers

The theory of cancer stem cells (CSCs) suggests that there is a group of "stem" cell populations with self-renewal, multidirectional differentiation, multi-drug resistance to radiotherapy and chemotherapy, and invasion and metastasis.[74] CSCs were first found in hematological malignant tumors, and then many CSCs or tumor stem cell-like cells have been identified and isolated from solid tumors, such as BC, PCa, colon cancer, etc.[75–77] CSCs are usually hidden in the cancer nest, in a static state, in which DNA replication is not active, cells can escape DNA damage induced by chemotherapeutic drugs, enhance the ability to repair, and maintain the stable inheritance of genes. CD44 is one of the most commonly used markers for identifying CSCs. Essentially, cell-cell adhesion proteins have an important impact on tumor invasion and metastasis.[78] These biological characteristics of CSCs are controlled by complex intracellular and extracellular regulatory networks. RUNX2 is closely related to the biological behavior of malignant tumors, and tumor stem cells are the root causes of the malignant behavior of tumors. There are a few related studies. BMP-2 can induce bone formation and restrain CSCs in the human osteosarcoma OS99-1 cell line, accompanied by the accumulation of RUNX2 and Osx.[79] The function of RUNX2 begins with breast stem cells that differentiated into progenitor cells which develop into luminal and basal breast lineages. RUNX2 can promote the activity of $CD44^{+}/CD24^{-/low}$ BC stem cells and regulate the malignant phenotype of BC.[80] The prostate cancer stem cells

are characterized by high expression of CD49f and RUNX2, low expression of CD44, CD133 and Androgen Receptor.[81]

Outlook

In recent years, RUNX2 as a specific transcription factor in malignant tumors has attracted much attention, and its related molecular pathways have been put forward as research hotspots. Current research mainly focuses on its upstream signaling pathways and the regulation of miRNAs, and the researches of its downstream mechanisms are relatively few (Figure 3). Its research on and histone modifications has also begun to take shape and has excellent research value. At the same time, combined with the regulatory characteristics of the RUNX family in malignant tumors, both RUNX1 and RUNX3 can regulate the differentiation and activity of immune cells. Combined with rare reports in previous studies, we believe that RUNX2 has a unique mechanism in regulating the tumor microenvironment (TME), which can not only reshape the microenvironment also regulate the activity of immune cells, leading to tumor immune escape. With the in-depth study of RUNX2-related regulatory mechanisms, RUNX2 is expected to become a new therapeutic target and contribute to the development of new drugs and the improvement of clinical efficacy.

Abbreviations

RUNX2, transcription factor runt-related protein 2; BC, Breast cancer; HER2, human epidermal growth factor receptor type 2; EMT, epithelial-mesenchymal transition; VEGF, metastatic marker genes vascular endothelial growth factor; MMP-2, metalloproteinase 2; OPN, osteopontin; BSP, bisulfite sequencing PCR,; PTHrP, parathyroid hormone-related peptide; RANKL, receptor activator of nuclear factor κB ligand; miRNAs, micro ribonucleic acid; HDACi, histone deacetylase inhibitor; BRD4, bromodomain-containing protein 4; ENHs, Enhancers; BETi, bromodomain and extraterminal inhibitors; CTGF, connective tissue growth factor; BCs, Bethesda categories; lncRNAs, long non-coding RNAs; Pca, Prostate cancer; PTEN, phosphatase and tensin homolog; FOXO1, Forkhead Box O1; CYP1A1, cytochrome P450 family 1 subfamily A1; IAS, intratumoral androgen synthesis; AR, androgen receptor; ERK, extracellular regulated protein kinases; CRC, Colorectal cancer; TGFβ-1, Transforming Growth Factor β-1; SNHG3, small nucleolar RNA host gene 3, MALAT1, RNA-metastasis-associated lung adenocarcinoma transcript 1; SFPQ, splicing factor proline/glutamine rich; PTBP2, polypyrimidine tract binding protein-2; PVT1, Plasmacytoma variant translocation 1; NID1, Nidogen 1; EMT, epithelial-mesenchymal transition; WWOX, WW domain-containing oxidoreductase; NSCLC, Non-small cell lung cancer; LUSC, lung squamous cell carcinoma; SDF1, Stromal Sell-Derived Factor 1; TRβ, Thyroid hormone receptor β; WDR5, WD repeat domain 5; NELF, negative elongation factor; CSCs, cancer stem cells; TME, tumor microenvironment.

Consent for Publication

All authors have agreed to the publication of this manuscript.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

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