The emerging role of RUNX3 in cancer metastasis (Review)

FEIFEI CHEN[1*], XIN LIU[1*], JIN BAI[1], DONGSHENG PEI[1] and JUNNIAN ZHENG[1-3]

[1]Cancer Institute and [2]Jiangsu Center for the Collaboration and Innovation of Cancer Biotherapy, Cancer Institute, Xuzhou Medical College; [3]Cancer Center, Affiliated Hospital of Xuzhou Medical College, Xuzhou, Jiangsu 221002, P.R. China

Abstract. Metastasis remains the major driver of mortality in patients with cancer. The multistep metastatic process starts with the dissemination of tumor cells from a primary site and leading to secondary tumor development in an anatomically distant location. Although significant progress has been made in understanding the molecular characteristics of metastasis, many questions remain regarding the intracellular mechanisms governing transition through the various metastatic stages. The runt-related transcription factor 3 (RUNX3) is a downstream effector of the transforming growth factor-β (TGF-β) signaling pathway, and has critical roles in the regulation of cell death by apoptosis, and in angiogenesis, epithelial-to-mesenchymal transition (EMT), cell migration and invasion. RUNX3 functions as a bona fide initiator of carcinogenesis by linking the Wnt oncogenic and TGF-β tumor suppressive pathways. RUNX3 is frequently inactivated in human cancer cell lines and cancer samples by hemizygous deletion of the Runx3 gene, hypermethylation of the Runx3 promoter, or cytoplasmic sequestration of RUNX3 protein. Inactivation of RUNX3 makes it a putative tumor suppressor in human neoplasia. In the present review, we summarize the proposed roles of RUNX3 in metastasis and, when applicable, highlight the mechanism by which they function.

Contents

1. Introduction
2. RUNX3 inactivation in human cancers
3. Overview of RUNX3 signaling in cancer metastasis
4. RUNX3 in cancer metastasis
5. Conclusion

Correspondence to: Dr Junnian Zheng, Jiangsu Key Laboratory of Biological Cancer Therapy, Xuzhou Medical College, 84 West Huai-hai Road, Xuzhou, Jiangsu 221002, P.R. China
E-mail: cff@xzmc.edu.cn

*Contributed equally

1. Introduction

Whereas surgical resection and adjuvant therapy can cure well-confined primary tumors, metastatic disease is largely incurable due to its systemic nature and the resistance of disseminated tumor cells to existing therapeutic agents. This explains why above 90% of mortality from cancer is attributable to metastases, but not the primary tumors from which these malignant lesions arise (1,2). Thus, our ability to effectively treat cancer is largely dependent on our capacity to interdict, and perhaps even reverse, the process of metastasis.

Metastasis is commonly viewed as a linear chain of events resulting in the re-localization of tumorigenic cells from the primary tumor site to a distant location. The stages of metastasis may be divided into the following categories: i) invasion locally through surrounding extracellular matrix (ECM) and stromal cell layers; ii) intravasation into the lumina of blood vessels; iii) survival of the transport through the vasculature; iv) arrest at distant organ sites; v) extravasation into the parenchyma of distant tissues; vi) initial survival in these foreign microenvironments in order to form micrometastases; and vii) re-initiation of their proliferative programs at metastatic sites, thereby generating macroscopic, clinically detectable neoplastic growths (the step often referred to as 'metastatic colonization') (3). Molecules involved in the metastasis process are potential therapeutic target for cancer metastasis (4).

The RUNX factors describe a family of evolutionarily conserved metazoan proteins [RUNX1, RUNX2 and runt-related transcription factor 3 (RUNX3)], each of which is capable of forming heterodimers with the common β-subunit CBFβ. RUNX families share the highly conserved homologous N-terminal runt domain, a DNA-binding and heterodimerization region of ~120 amino acids. Although their strong homology indicates a certain degree of redundancy as observed during mouse embryogenesis (5) and transcription regulation of various genes (for example Sgpp1, Ncam1 and p21WAF1) (6), the RUNX proteins have been shown to perform distinct functions that are dependent on tissue type (7).

Among the RUNX families, RUNX3 was demonstrated to be the oldest of the three genes by sequence analysis, and is found in cnidarians, the most primitive animals, where it regulates the growth of their primitive gastrointestinal system. RUNX3 is involved in a variety of biological activities, including the development of the gastrointestinal tract (8), neurogenesis of dorsal root ganglia (9) and T-cell differentiation (10). The RUNX3 gene is located on 1p.13-p36.11, where

loss of heterozygosity (LOH) is often reported in many types of cancers, indicating its potential role of tumor suppressor in cancers (11). RUNX3 was first claimed to be a tumor suppressor in gastric cancer more than a decade ago. This assertion was based on the following observations: i) high expression of Runx3 in normal mouse gastrointestinal tract (GIT) epithelium; ii) gastric hyperplasia in Runx3$^{-/-}$ newborn mice; iii) RUNX3 LOH in gastric cancer patients; iv) DNA hypermethylation of RUNX3 promoter in gastric cancer; and v) reduced RUNX3 mRNA in gastric cancer vs. normal gastric tissue in 60% of gastric cancer patients assayed by RNA *in situ* hybridization (RISH). Since then, RUNX3 has emerged as a candidate tumor suppressor that is epigenetically inactivated in a wide spectrum of invasive and pre-invasive epithelial and mesenchymal neoplasms (12). Hypermethylation of RUNX3 promoter is the leading cause which results in RUNX3 inactivation in cancer tissues (13-15). Besides hypermethylation, inactivation of RUNX3 also occurs by mislocalization of RUNX3 protein from the nucleus to the cytoplasm (16). RUNX3 can function as a tumor suppressor and is involved in tumor development and metastasis at different levels, such as EMT (17), adhesion (18), invasion (18,19), apoptosis (20) and angiogenesis (21). However, it is not clear how and where RUNX3 interacts with pathways that regulate tumor development and metastasis. Investigations of the factors controlling RUNX3 function and its downstream effectors have begun to unravel this biological mystery. The present review will focus on the existing knowledge of the role of RUNX3 gene in regulating cancer metastasis, and provide some considerations for future investigations.

2. RUNX3 inactivation in human cancers

The RUNX3 gene is located on 1p.13-p36.11, a deletion hotspot in diverse cancers of epithelial, hematopoietic and neural origins, indicating its potential role of tumor suppressor in cancers (11). RUNX3 was first found lost in gastric cancer, since then, more and more studies have reported that the loss or reduction of the RUNX3 protein can be detected in various cancers, such as colorectal cancer, glioma and melanoma (15,22,23). In our previous studies, we showed the reduction of RUNX3 was correlated with tumor stage and grade in prostate cancer and renal cell carcinoma (18,24). Analysis of clinical tissue samples from peritoneal metastases arising from gastric cancers provides evidence that RUNX3 expression significantly decreased in the metastatic tissue, compared to normal gastric mucosa or primary main tumors (19). Conversely, RUNX3 overexpression correlates with reduced invasive potential of breast cancer cells (25). Similarly, RUNX3 expression in the breast stroma is associated with low rate of recurrence, and thus, a good clinical outcome in breast cancer (26). Furthermore, the presence of nuclear RUNX3 protein in esophageal squamous cell carcinomas correlates with increased sensitivity to radiotherapy, whereas RUNX3 inactivation is linked to radioresistance and poor prognosis (27). The above suggests that RUNX3 inactivation is a significant risk factor for tumorigenesis and metastasis.

Aberrant methylation is the main reason that results in RUNX3 inactivation in human cancers. Although it is unclear what causes aberrant methylation of RUNX3, hypermethylation

of the CpG islands at the RUNX3 promoter is one of the most common aberrant methylation events in cancer. Diverse tumor tissues and cancer cell lines from human patients have RUNX3 promoter hypermethylation and inactivation, as identified by methylation-specific PCR or bisulfate genomic sequencing analysis. These tissues and cells include those derived from gastric, bladder, colorectal, breast, lung, pancreatic and brain cancers, and hepatocellular carcinoma (12). RUNX3 hypermethylation in these cancer tissues is strongly correlated with poor prognosis and a low patient survival rate (28). Recently, RUNX3 hypermethylation status has been used not only as a helpful and valuable biomarker for diagnosis of breast cancer, particularly in ER-negative breast cancer (29), but also a factor in predicting or monitoring postoperative recurrence of papillary thyroid cancer patients (30). These reports suggest that RUNX3 methylation occurs early in tumorigenesis and may increase with age. To date, only three contributors to the increase in RUNX3 promoter hypermethylation have been identified. First, *Helicobacter pylori*, a well-known factor involved in gastric cancer, induces nitric oxide production, liposaccharide production and inflammation, leading to hypermethylation and silencing of RUNX3 (31). Second, inflammatory insults such as that caused by lipopolysaccharide (LPS) enhance RUNX3 methylation at the promoter through increased nitric oxide production (31). Third, estrogen induces hypermethylation and silencing of RUNX3 in mammosphere-derived cells (32).

Enhancer of zeste homologue 2 (EZH2)-mediated histone methylation is another epigenetic mechanism that is involved in RUNX3 inactivation. Overexpression of EZH2 caused H3K27 trimethylation of the RUNX3 promoter, which led to repression of RUNX3 expression in the absence of DNA methylation. EZH2 binds to the RUNX3 promoter, resulting in upregulation of H3K27 methylation and concomitant downregulation of RUNX3 expression (33). Lee *et al* extended this finding by showing that hypoxia-induced upregulation of G9a histone methyltransferase and histone deacetylase 1 (HDAC1) expression also cause epigenetic RUNX3 silencing through H3K9 methylation and decreased H3 acetylation at RUNX3 promoter. This proposed that hypoxia silences RUNX3 by epigenetic histone regulation during the progression of gastric cancer (34).

MicroRNAs are the third epigenetic mechanism responsible for silencing of RUNX3. MicroRNAs have been recently highlighted as regulators of gene expression at the post-transcriptional level. A recent study found that frequent overexpression of EZH2 in aggressive solid tumors is largely due to the loss of its regulator miR-101. Specifically, reintroduction of miR-101 or knockdown of EZH2 expression was sufficient to reduce H3K27 trimethylation and increase RUNX3 mRNA levels (35). Recently, miR130b was proposed as a candidate for silencing RUNX3 in gastric cancer cells and tissues. Overexpression of miR-130b increases cell viability and decreases TGF-β-induced apoptosis, resulting in RUNX3 protein expression (36). In melanoma tissues, RUNX3 silencing is linked to miR-532-5p (37). The role of these miRNAs in silencing RUNX3 should be explored further to evaluate the restoration of RUNX3 expression for therapeutic approaches.

Mislocalization of RUNX3 protein from the nucleus to the cytoplasm also contributes to inactivation of RUNX3. RUNX3 was reported primarily confined to the cytoplasm of cells in
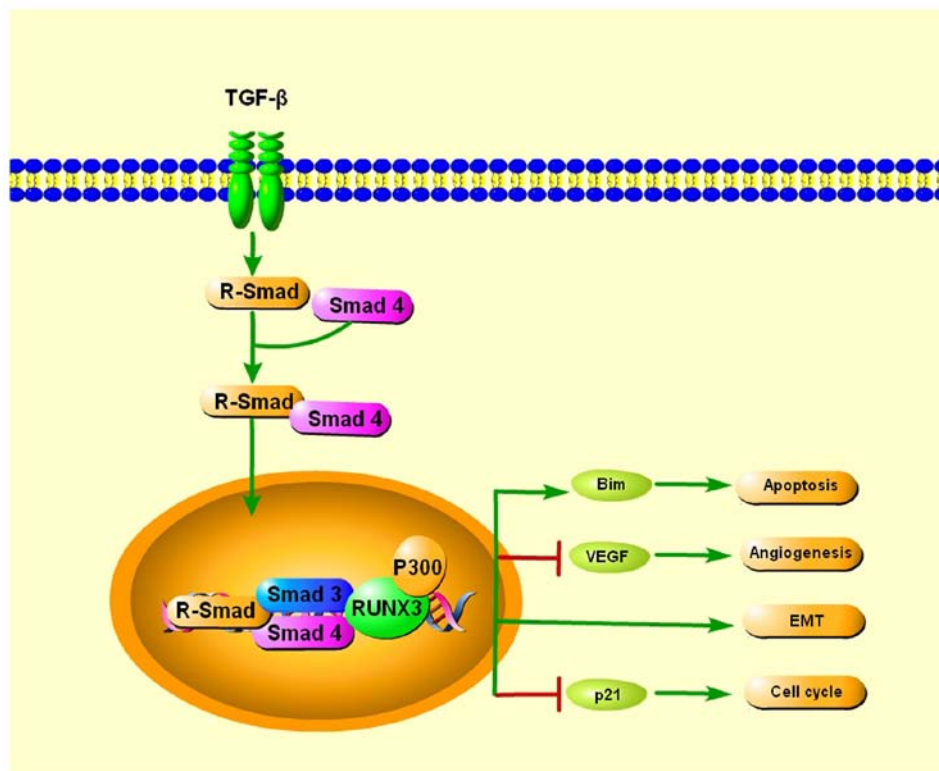
Figure 1. The pleiotropic effects of RUNX3 during tumor metastasis suppression in TGF-β signaling pathway. RUNX3 cooperates with SMAD3/SMAD4 to activate TGF-β-dependent growth inhibition and apoptosis by induction of p21 and Bim, respectively. Lack of RUNX3 may induce EMT. RUNX3 represses VEGF expression, which may prevent angiogenesis.

RUNX3 induced tumors, whereas a substantial fraction of RUNX3 was present in the nucleus of cells in control tumors. Cytoplasmic RUNX3 protein does not elicit tumor suppressive activity, and is therefore a novel mechanism of RUNX3 inactivation (16). Several mechanisms for excluding RUNX3 from the nucleus have been suggested. Src kinase phosphorylates tyrosine residues in RUNX3, and overexpression of activated Src correlates with RUNX3 cytoplasmic localization (38). In contrast, Pim-1 phosphorylates four Ser/Thr residues within the runt domain and thereby stabilizes the RUNX3 protein. It alters the cellular localization of RUNX3 from the nucleus to the cytoplasm (39). Phosphorylation of the RUNX3 by Jun-activation domain-binding protein 1 (Jab1/CSN5) is also a major cause of cytoplasmic sequestration (40).

In summary, RUNX3 inactivation can be regulated by several epigenetic mechanisms, including DNA and histone methylation, and some types of miRNAs. Another mode of RUNX3 inactivation is the mislocalization of RUNX3.

3. Overview of RUNX3 signaling in cancer metastasis

TGF-β is a ubiquitously expressed cytokine that plays very important roles in many cellular functions. When TGF-β binds to the respective cognate receptors, the serine kinases are activated, which then phosphorylate signal transducers called R-Smad. TGF-β activates R-Smad, which associate with a common Smad4 and enter the nucleus, where the R-Smad/Smad4/Smad3 complexes bind to transcription factors in order to regulate the transcription of target genes (41). Runx3$^{-/-}$ gastric epithelial cells were insensitive to the growth inhibitory effects

of TGF-β, indicating a potential role of RUNX3 as a downstream effector of TGF-β (42), providing a possible explanation for its broad involvement in human tumorigenesis and metastasis. Chi et al showed that RUNX3 inhibits gastric epithelial cell growth by inducing p21 gene expression in response to TGF-β (43). Yamamura et al reported that RUNX3 works together with FoxO3a/FKHRL1 in the induction of apoptosis by activating Bim when exposed to TGF-β and may play an important role in tumor suppression of cancer (44). Peng et al found three RUNX3 binding sites in the vascular endothelial growth factor (VEGF) promoter. Restoration of RUNX3 expression inhibited the angiogenic potential of gastric cancer cells, and correlated with downregulated VEGF expression via promoter repression (21). Furthermore, Runx3-null gastric epithelial lines are unexpectedly sensitized to TGF-β-induced EMT, indicating a potential role of RUNX3 in inhibiting EMT in TGF-β signaling (17) (Fig. 1).

The Wnt/β-catenin signaling pathway has a well-established role in the regulation of cell invasion, EMT, cell growth and proliferation, as well as in cancer stem cell differentiation, and its constitutive activation is commonly found in human cancers (45). Notably, RUNX3 was demonstrated to inhibit EMT, which promotes metastasis, through the regulation of Wnt signaling pathways (17,46). The detection of Runx3 in the epithelial cells of small and large intestines in mice hinted that RUNX3 may function as a tumor suppressor in the intestinal epithelium. Indeed, the intestinal epithelia of Runx3$^{-/-}$ mice exhibit hyperplasia that is associated with robust proliferation. Further examination revealed that this phenomenon is the likely consequence of enhanced Wnt signaling activity and

transcriptional upregulation of Wnt target genes such as *c-Myc* and *cyclin D1*. Further discovery indicated that RUNX3 forms a ternary complex with β-catenin/TCF4, and attenuates Wnt signaling activity and reduced ability to bind DNA, and activate transcription of Wnt target genes. RUNX3, therefore, antagonizes aberrant Wnt signaling, which substantial evidence has shown, provides an oncogenic impetus to sustain colorectal cancer growth (47). However, RUNX3 stabilizes the TCF4/β-catenin complex on the Wnt target gene promoter in the gastric cancer cells Kato III, leading to activation of Wnt signaling, and its expression resulted in suppression of tumorigenesis of Kato III cells, indicating that RUNX3 plays a tumor-suppressing role in Kato III cells through a Wnt-independent mechanism, suggesting RUNX3 can either suppress or activate the Wnt signaling pathway through its binding to the TCF4/β-catenin complex by cell context-dependent mechanisms (48) (Fig. 2).

Oncogenic Ras induces premature senescence in primary cells, except when its expression is accompanied by coincident loss of tumor-suppressor activity, suggesting the existence of a cellular defense mechanism against oncogenic insults (49). RUNX3 is preferentially inactivated in K-RAS-induced lung adenocarcinoma, indicating its potential role of tumor suppressor in lung adenocarcinoma (50). ARF and p21 play essential roles in the p53-mediated cellular defense against oncogenic insult (51), and both genes are targets of Runx proteins (43,52). Transcriptional activation of ARF is essential for oncogenic RAS-induced stabilization of p53, which plays major roles in tumor suppression (53). In addition, p21, which is induced in both p53-dependent and -independent manner, also plays a key role in defense against oncogenic RAS (51). Lee *et al* found that both p21 and ARF were transiently induced by serum stimulation, and the induction of both genes was dependent on both K-RAS activity and RUNX3 (54). BRD2 is a member of the bromodomain extra terminal (BET) protein family, which consists of four chromatin-interacting proteins that regulate gene expression (55). They further found that serum stimulation induces K-RAS activation, which triggered the formation of RUNX3-BRD2 complex which is responsible for oncogenic K-RAS-induced expression of p21 and ARF. When K-RAS activity was reduced and cyclin D1 was induced, and subsequently bound to RUNX3. At the same time, RUNX3 interacted with histone deacetylase 4 (HDAC4), resulting in deacetylation of RUNX3 (56) and RUNX3 was released from both p300 and BRD2, converting the RUNX3-BRD2 complex to a RUNX3-HDAC4 complex. As a result, the expression of ARF and p21 is switched off. When K-Ras is constitutively activated, the oncogenic Ras-activated MEK1 pathway inhibits conversion of the RUNX3-BRD2 complex into the RUNX3-HDAC4 complex, which switches off ARF and p21 expression in lung adenocarcinoma (54) (Fig. 3).

4. RUNX3 in cancer metastasis

RUNX3 in EMT. EMT is a process whereby epithelial cells undergo a shift in plasticity and acquire the ability to disseminate, invade and cause metastasis. Established as a central process during the early stages of development, it is now clear that EMT has implications on cancer metastasis by triggering the loss of cell-cell adhesion to facilitate tumor cell invasion

and remodeling of the extracellular matrix. While epithelial cells express high levels of E-cadherin and are closely connected to each other by tight junctions, mesenchymal cells express N-cadherin, fibronectin and vimentin, have a spindle-shaped morphology and less tight junctions (57).

Many of the pathways known to be involved in metastasis also have close connections with EMT. Prominent among these extracellular signals are the Wnt and TGF-β/BMP signaling pathways, which contribute to the EMT activation network via β-catenin-mediated activation of EMT inducing factors and SMAD protein interactions, respectively (58). The Wnt signaling pathway participates in EMT by inhibiting glycogen synthase kinase-3β (GSK3β) to stabilize β-catenin, promoting a gene expression program that favors EMT (59). During the later stages of carcinogenesis, TGF-β signaling plays a paradoxical role in the promotion of the invasion and metastasis of cancer through its induction of EMT (57). However, the convergence of signaling pathways is necessary for EMT. While RUNX3 acts concurrently as a mediator of TGF-β signaling and an antagonist of Wnt, RUNX3 was demonstrated to inhibit EMT, which promotes metastasis, through the regulation of these signaling pathways (17,46). Gastric epithelial cells undergo spontaneous EMT in the absence of Runx3 and p53, which induces the formation of a tumorigenic subpopulation in immortalized Runx3$^{-/-}$p53$^{-/-}$ gastric epithelial (GIF) cells. Remarkably, the mesenchymal-like subpopulation also possesses stem-like properties, as reflected in the enrichment of the gastrointestinal stem cell marker Lgr5 and greater sphere-initiating and colony-forming abilities. Contrary to their resistance to TGF-β-mediated apoptosis, Runx3-null GIF lines are unexpectedly sensitized to TGF-β-induced EMT, indicating a rerouting of the TGF-β signal. In addition, a greatly increased Wnt-responsiveness was observed in Runx3-null GIF cells, which acts synergistically with TGF-β to induce Lgr5 expression. These findings point to the possibility that RUNX3 acts as a tumor suppressor by safeguarding gastric epithelial differentiation and phenotypes; in its absence, gastric epithelial cells are prone to spontaneous EMT and aberrant TGF-β and Wnt signaling, giving rise to a tumorigenic stem cell-like subpopulation (17). Lee *et al* used Runx3-null mouse to explore the role of RUNX3 in lung tumorigenesis. During lung development, various cellular and molecular events occur such as cell proliferation, cell death, differentiation and EMT. Excessive EMT was observed in lungs in Runx3-null mice and PN18 in Runx3 heterozygous mice. Pharmacologic inhibition of EMT resulted in increased life spans of newborn mice were and lung hyperplasia was partially rescued by downregulated EMT (60). In hepatocellular carcinoma, ectopic RUNX3 expression had an anti-EMT effect in low-EMT HCC cell lines characterized by increased E-cadherin expression and decreased N-cadherin and vimentin expression (46).

Numerous studies have suggested that miRNAs contribute to the invasion and metastasis of various types of human cancers by regulating the EMT (61,62). Vimentin is one of the targets that can be regulated by miRNAs. Yamasaki *et al* showed that miR-138 targeted vimentin and inhibited cell migration and invasion in renal cell carcinoma (63). Cheng *et al* showed that miR-30a could directly bind to the 3'UTR of vimentin and inhibited its translation, then reduced the protein level of vimentin in breast cancer (64). In addition, overexpression of
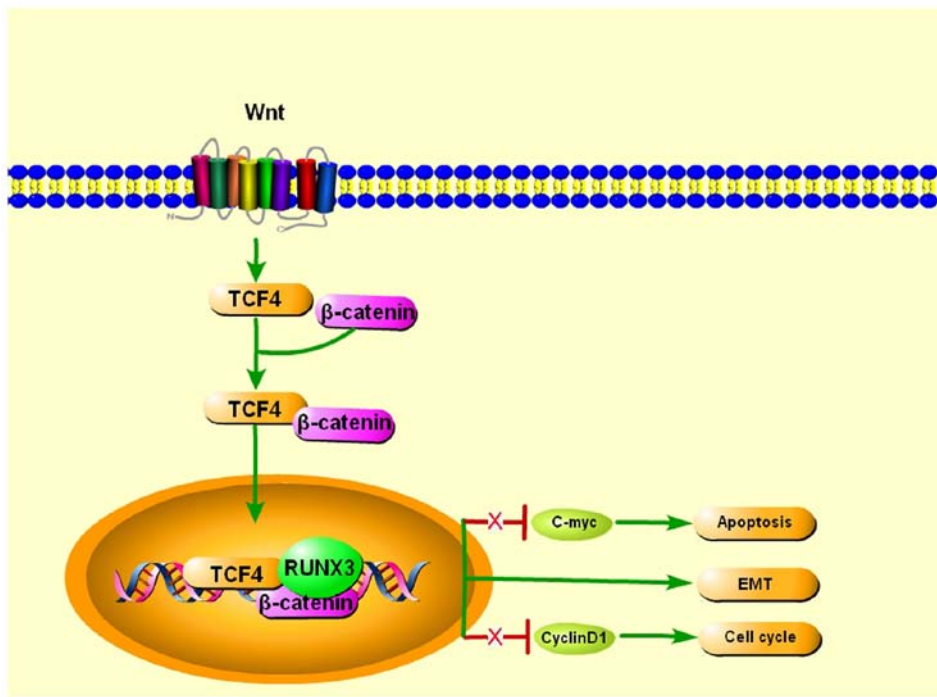
Figure 2. The effect of RUNX3 during suppression of tumor metastasis in Wnt signaling pathway. RUNX3 inhibits the oncogenic Wnt signaling pathway by forming a complex with the TCF4-β-catenin complex and preventing it from binding to target promoters such as c-Myc and cyclin D1, regulating apoptosis and cell cycle, respectively. RUNX3 suppresses EMT via inhibiting Wnt signaling.
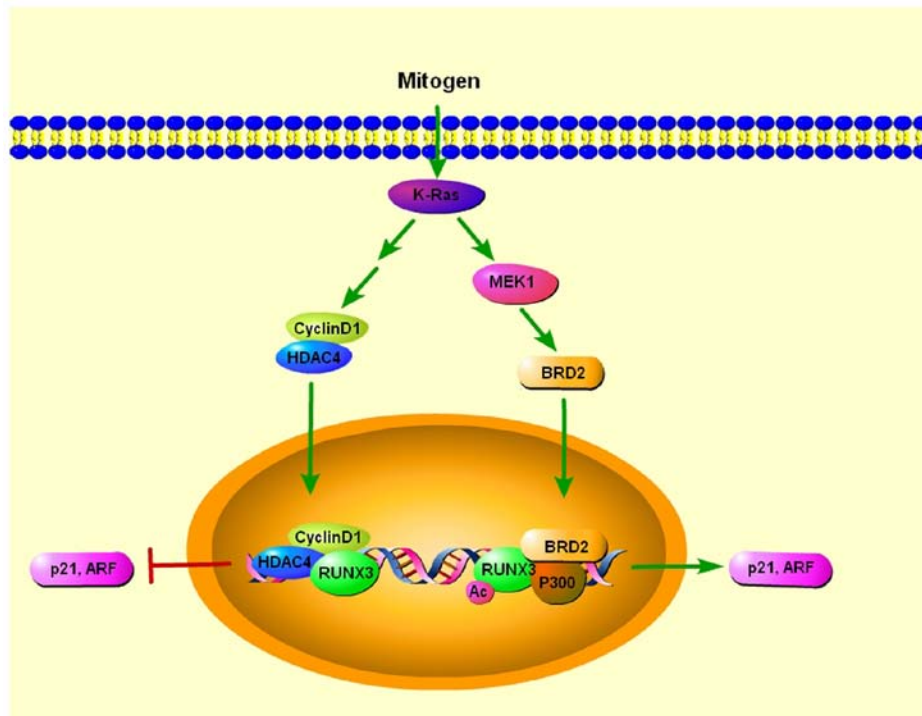

Figure 3. The role of RUNX3 in Mitogenic-K-Ras signaling pathway. Mitogenic stimulation induces RUNX3-BRD2 complex formation and expression of p21 and ARF. A decrease in K-Ras pathway activity and an increase in cyclin D1 convert the RUNX3-BRD2 complex to a RUNX3-HDAC4 complex. As a result, the expression of ARF and p21 is switched off. When K-Ras is constitutively activated, the oncogenic Ras-activated MEK1 pathway inhibits conversion of the RUNX3-BRD2 complex into the RUNX3-HDAC4 complex. As a result, expression of ARF and p21 remains switched on.

RUNX3 increased the expression of miR-30a, which directly targeted the 3'UTR of vimentin and decreased its protein level. The miR-30a inhibitor abrogated RUNX3-mediated inhibition of cell invasion and downregulated vimentin. In gastric cancer patients, levels of RUNX3 were positively correlated with miR-30a and negatively associated with vimentin. Thus,

RUNX3 plays a significant role in regulating the EMT by activating miR-30a (65).

RUNX3 in angiogenesis. Folkman, proposed the hypothesis that tumor growth is dependent on the formation of new blood vessels (66). Tumor vessels eventually formed are different when compared with normal vasculature: they are disorganized with irregular structure and with altered interaction between endothelial cells (67). Once cancer cells generate their own blood supply, they are capable of further invasion and have the capacity to metastasize. Consequently, angiogenesis is essential for the development and evolution of neoplastic disease, as both tumor growth and metastasis require persistent new blood vessels and ongoing angiogenesis is necessary for rapid expansion of a tumor mass (68). Targeting of tumor-related vessels has been regarded as an effective strategy to treat cancer. The vast majority of anti-angiogenic agents being tested in the clinic are based on the strategies that either interfere with pro-angiogenic ligands or block the signaling of pro-angiogenic receptor tyrosine kinases (69).

The most well-described angiogenic factor is VEGF. The VEGF pathway holds the key in regulating angiogenesis, vasopermeability as well as the proliferation and migration of endothelial cells. Targeted therapies against this pathway have been established in the clinic. In several types of cancer (colon carcinoma, soft tissue sarcomas and gastric cancer), serum VEGF level is a marker for disease stage and an indicator of metastasis. VEGF level is significantly elevated in uveal melanoma patients with metastatic disease compared to patients without metastases (70). In our previous studies, we injected nude mice with RUNX3 stably expressed prostate cancer cells and our results showed reduced angiogenesis and metastasis *in vivo*. The VEGF secretion was inhibited by RUNX3 *in vitro* (24). Lee *et al* used Runx3 knock-out mice to detect angiogenesis and liver differentiation. The staining and real-time quantitative polymerase chain reaction (RT-qPCR) displayed that VEGF were markedly upregulated by the loss of RUNX3. Clarifying the mechanisms of angiogenesis and liver differentiation may aid in the design of efficient and safe anti-angiogenic and gene therapies for liver disorders (71). Similarly, Runx3 knockout mice displayed vasculogenesis and angiogenesis by increased CD31, VEGF and vWF (72). Furthermore, Peng *et al* found three RUNX3 binding sites in the VEGF promoter. Restoration of RUNX3 expression inhibited the angiogenic potential of gastric cancer cells, and correlated with downregulated VEGF expression via promoter repression *in vitro*, and attenuation of tumorigenicity and abrogation of metastasis in animal models (21).

In solid tumors >1 mm^3, tumor cells removed from blood vessels experience hypoxia (34,73). Hypoxia stabilizes hypoxia-inducible factor (HIF)-1α, which dimerizes with aryl hydrocarbon receptor nuclear translocator or HIF-1β, a constitutively expressed binding partner of several bHLH-PAS domain proteins, to increase the HIF-1-mediated gene transcriptions involved in tumor angiogenesis (74). Hypoxic conditions also contribute to RUNX3 silencing by the induction of histone methylation and the deacetylation of RUNX3 promoter by G9a histone methyl transferase and HDAC1. Thus, this decreased expression of RUNX3 under hypoxia may potentiate tumor progression and metastasis in gastric

cancer by aggravating the angiogenic microenvironment (34). Lee *et al* further investigated cross-talk between RUNX3 and HIF-1α under hypoxia. They demonstrated that RUNX3 overexpression significantly inhibited hypoxia-induced angiogenesis *in vitro* and *in vivo*. RUNX3 directly interacted with the C-terminal activation domain of HIF-1α and prolyl hydroxylase (PHD) 2 and enhanced the interaction between HIF-1α and PHD2, which potentiated proline hydroxylation and promoted the degradation of HIF-1α. RUNX3 appears to be a novel suppressor of HIF-1α and of hypoxia-mediated angiogenesis in gastric cancer cells (75).

miRNAs are key regulators of diverse cellular processes, including angiogenesis. In has been reported that downregulated miR-130a in patients with type 2 diabetes mellitus (DM) results in endothelial progenitor cells (EPC) dysfunction, via its target RUNX3 (76). However, the relationship between miRNAs and RUNX3 in regulating tumor angiogenesis has not been reported. Therefore, further investigations need to focus on miRNAs that may possess potential ability to regulate angiogenesis through target RUNX3 in human cancers.

RUNX3 in programmed cell death. During the process of metastasis, any mistakes made by a metastatic cell during cellular events may lead to cell death. Therefore, the regulation of cell death is critical for cancer cells to survive during metastasis. Programmed cell death is defined as regulated cell death mediated by an intracellular program. Stresses include the loss of cell-cell contacts, the recognition and destruction of the cancer cells by the immune system, and the lack of necessary growth factors, all of which may trigger programmed cell death (77).

RUNX3 and apoptosis. Apoptosis is a type of programmed cell death that characterized by cell membrane blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation (78,79). Apoptosis may block metastatic dissemination by killing misplaced cells. Thus, apoptosis serves as an important process for inhibiting metastasis. It has been reported that lack of endogenous RUNX3 helps HCC to escape apoptosis (80). The combination of demethylation drugs 5-aza-CdR and TSA induced the translocation of RUNX3 from the cytoplasm into the nucleus, induced caspase-3-dependent apoptosis in leukaemia cell lines (81). Apoptosis is notably reduced in Runx3$^{-/-}$ gastric epithelial cells. Further study showed that in Runx3$^{-/-}$ mouse gastric epithelium, pro-apoptotic gene Bim was downregulated, and apoptosis was reduced to the same extent as that in Bim-/- gastric epithelium. RUNX3 is responsible for transcriptional upregulation of Bim via binding to Bim promoter in TGF-β-induced apoptosis (20). Notably, both RUNX3 and FoxO3a/FKHRL1 were required for induction of Bim expression. RUNX3 cooperates with FoxO3a/FKHRL1 to participate in the induction of apoptosis by activating Bim and play an important role in tumor suppression in gastric cancer (44). Survivin is a member of the inhibitor of apoptosis (IAP) family and has been shown to inhibit cell apoptosis and promote cell proliferation. RUNX3 binds to survivin promoter, leads to significantly inhibition of survivin expression (82). Therefore, as a transcriptional factor, RUNX3 is involved in regulating apoptosis via its target gene.

p53 acts as a tumor suppressor by initiating cell cycle arrest, apoptosis, and senescence in response to cellular stress

to maintain the integrity of the genome (83). In response to the anticancer drug adriamycin, RUNX3 was induced to accumulate in the cell nucleus and co-localized with p53. COOH-terminal portion of p53 is required for the interaction with RUNX3. RUNX3 induced the phosphorylation of p53 at Ser-15, thereby promoting p53-dependent apoptosis (84). Murine double minute 2 (MDM2) is a well known E3 ubiquitin ligase that binds p53 at its transactivation domain blocking p53-mediated transcriptional regulation, while simultaneously promoting its polyubiquitination and proteasome-dependent degradation (83). Intriguingly, RUNX3 directly binds MDM2 through its runt-related DNA-binding domain. MDM2 blocks RUNX3 transcriptional activity by interacting with RUNX3 through an acidic domain adjacent to the p53-binding domain of MDM2 and ubiquitinates RUNX3 on key lysine residues to mediate nuclear export and proteasomal degradation (52). Consequently, as a cofactor of p53, RUNX3 and the ubiquitous p53 protein are both principal responders of the p14 (ARF)-MDM2 cell surveillance pathway that prevents pathologic consequences of abnormal oncogene activation.

In addition, metastatic cells must develop a mechanism to evade cell death resulting from recognition and destruction by cytotoxic lymphocytes such as natural killer (NK) cells. Natural cytotoxicity receptor 1 (NCR1) is an NK lymphocyte-activating receptor. The key *cis*-regulatory element in the immediate vicinity upstream of the gene contains a RUNX recognition motif that preferentially binds RUNX3. Higher level of RUNX3 expression in NK cells may result in preferred binding of RUNX3 at the NCR1 promoter and lead to NCR1 expression. Thus, the role of RUNX3 in the control of an important NK-activating receptor may promote cell death resulting from recognition NK cells (85). Moreover, RUNX3 impaired interleukin-15 (IL-15)-dependent accumulation of mature NK cells. RUNX3 cooperates with ETS and T-box transcription factors to drive the IL-15-mediated transcription program during activation of these cells (86).

Resistance to chemotherapy is a significant barrier for effective cancer treatment. miRNAs promote chemoresistance of cancer cells, and suppress drug-induced apoptosis by target RUNX3 in human cancers. MiR-106a in highly expressed in gastric cancer and promotes chemoresistance of gastric cancer cells, accelerates ADR efflux, and suppresses drug-induced apoptosis. miR-106a may modulate drug resistance of gastric cancer cells partially by targeting RUNX3 (87). In additional, Li *et al* identified miR-185 to be a contributor to chemosensitivity in gastric cancer. Knockdown of endogenous miR-185 prevented high-dose chemotherapy-induced apoptosis via a direct target apoptosis repressor with caspase recruitment domain is of miR-185. RUNX3 was involved in the activation of miR-185 at the transcriptional level (88).

RUNX3 and autophagy. Autophagy is an evolutionarily conserved catabolic process in which intracellular membrane structures package protein complexes and organelles to degrade and renew these cytoplasmic components (89). The role of autophagy in cancer metastasis is complex, as reports have indicated both pro-metastatic and antimetastatic roles of autophagy. Stage-specificity may affect the cellular response to autophagy during cancer metastasis (90). During the early stage of cancer metastasis, autophagy may act as an inhibitor of metastasis by restricting tumor necrosis and inflammatory

cell infiltration and by alleviating oncogene-induced senescence. These processes may help to suppress the invasion and dissemination of cancer cells from the primary site. During the advanced stages of metastasis, autophagy tends to act as a promoter of metastasis by promoting ECM detached metastatic cell survival and colonization in a distant site and by inducing metastatic cells that fail to establish contact with the ECM in the new environment to enter dormancy.

RUNX3 has been reported to be involved in regulating autophagy mediated by microRNA in dysfunction of endothelial progenitor cells (EPC). miR-130a is important for maintaining normal autophagy levels and promoting the survival of EPC. Overexpression of miR-130a decreased the number of autophagosomes, cell death and Beclin 1 expression, but promoted Bcl2 expression; these effects were mediated by RUNX3 (91). RUNX3 may be an autophagy regulator linking apoptosis and the autophagy of EPC. However, the role of RUNX3 in regulating autophagy in human cancers has not been explored. Thus, further investigations needs to be carried out to focus on the role of RUNX3 in autophagy in human cancers.

RUNX3 in migration and invasion. Tumor cell migration and invasion are essential steps in the process of metastasis. The deregulation of cell migration during cancer progression determines the capacity of tumor cells to escape from the primary tumors. Then, tumor cells invade adjacent tissues to finally form metastases. As cell migration and invasion a prerequisite for metastasis, strategies targeting cell migration and invasion are a major focus of current efforts to develop cancer treatments.

Cancer cell migration and invasion are regulated by numerous, interconnected molecular networks. In fact, there are many intracellular molecules belonging to the Wnt, Notch, sonic Hedgehog, NF-κB, Ras/Raf/MEK/MAPK, as well as the AKT/ERK signaling pathways, which control every aspect of each of the stages of cancer cell migration and invasion (4,5,10). In contrast, extracellular molecules also contribute in a critical way to the progress of cancer cell invasion. These molecules can be: i) part of the ECM; ii) secreted in the ECM; iii) secreted but also attached on the cell surface; and iv) cell membrane proteins such as receptors. The role of RUNX3 in regulating these molecules that related cancer cell migration and invasion may elucidate how RUNX3 function in cancer metastasis from another points.

OPN. Osteopontin (OPN) is a secreted multifunctional glycophosphoprotein that is expressed in various tissues and plays important roles in a wide range of biological processes, such as inflammation, angiogenesis, bone remodeling, cell adhesion and migration (92). OPN binds to various receptors, including integrins and CD44, which potentially allow it to stimulate different signaling pathways and ultimately leading to tumor progression (93). Studies have indicated that OPN induces the PI3K-Akt signaling pathway through the αvβ3 integrin-mediated pathway and promotes cell migration (94,95). The constitutive expression of OPN is involved in the invasion, progression and metastasis in different cancers (96,97). Elevated OPN and its receptor CD44v9, are correlated with the degree of lymphatic vessel invasion and lymph node metastasis in gastric cancer (98). Cheng *et al* identified one RUNX3 binding site in the OPN promoter.

The binding of RUNX3 to the OPN promoter significantly decreased OPN promoter activity. The knockdown of OPN or overexpression of RUNX3 inhibited cell migration in gastric cancer cells; however, the coexpression of RUNX3 and OPN reversed the RUNX3-reduced migration ability in these cells. Obviously, RUNX3 is important to control cell migration through OPN, which promotes migration in gastric cancer cells (99).

MMPs. Invasion of basement membrane (BM) and extracellular matrix (ECM) is an essential event in tumor invasion and metastasis. ECM provides both structural support and extracellular cues that regulate invasive tumor growth, and tumor-associated changes in ECM contribute to cancer progression. MMPs are a family of zinc-binding endopeptidases that participate in the ECM degradation molecular machinery during tumor invasion (100,101). In order for tumor neo-vascularization and cell invasion processes to occur, degradation of the basement membrane as well as matrix remodeling is essential. Amongst the many known MMPs, MMP-2 and MMP-9 degrade gelatin as well as type IV collagen, the central component of the basement membrane. These MMPs are secreted in an inactive form and acquire their active form extracellularly (102,103). MMP-2 and MMP-9 are enriched in the invadopodia where they contribute to ECM degradation *in vitro* and *in vivo* (104). In our previous studies, we provided evidence that RUNX3 can suppress MMP-2 activities in prostate and breast cancers, but MMP-9 in renal cell carcinoma (18,24,105). We suppose that RUNX3 regulate cell invasion through different MMPs depending on the cancer tissue.

MMPs activity is controlled by specific, endogenous tissue inhibitors of metalloproteinases (TIMPs). TIMPs bind non-covalently with 1:1 stoichiometry to the active form of MMPs to inhibit their proteolytic activity (106). We found that the suppression of RUNX3 on MMP-2 may be due to upregulation of TIMP-2 in prostate cancer. However, in gastric cancer, the inhibition of MMP-9 induced by RUNX3 overexpression attributed to TIMP-1. Two RUNX3 binding sites were identified in the TIMP-1 promoter and direct interaction of RUNX3 with the TIMP-1 promoter was confirmed *in vitro* and *in vivo*.

5. Conclusion

In the present review, we highlighted important findings that shape our current understanding of RUNX3 as a tumor suppressor in the process of cancer metastasis. RUNX3 is deeply involved in multiple cancer metastasis processes, such as apoptosis, angiogenesis, EMT, migration and invasion in various types of tissues. Identification of key tissue- and stage-specific target genes of RUNX3 functioning as a tumor suppressor or an oncogene is an important step forward, and combination of these genes would improve the potential of usage of RUNX3 as a biomarker for cancer diagnosis. Aberrant methylation is the leading reason that results in RUNX3 inactivation in human cancers. Reversal of DNA methylation by demethylating agents and inhibitors of DNA-methyltransferases restores RUNX3 activation. Therefore, development of drugs targeting DNA methylation of RUNX3 may have vital clinical implications for the treatment or prevention of cancers. An understanding of the upstream and downstream signaling pathways of RUNX3

in the context of physiological and pathological processes is necessary if we are to harness our growing knowledge of RUNX3 for successful applications in cancer therapeutics.

References

1. Gupta GP and Massagué J: Cancer metastasis: Building a framework. Cell 127: 679-695, 2006.
2. Steeg PS: Tumor metastasis: Mechanistic insights and clinical challenges. Nat Med 12: 895-904, 2006.
3. Al-Mehdi AB, Tozawa K, Fisher AB, Shientag L, Lee A and Muschel RJ: Intravascular origin of metastasis from the proliferation of endothelium-attached tumor cells: A new model for metastasis. Nat Med 6: 100-102, 2000.
4. Fidler IJ: The pathogenesis of cancer metastasis: The 'seed and soil' hypothesis revisited. Nat Rev Cancer 3: 453-458, 2003.
5. Fukushima-Nakase Y, Naoe Y, Taniuchi I, Hosoi H, Sugimoto T and Okuda T: Shared and distinct roles mediated through C-terminal subdomains of acute myeloid leukemia/Runt-related transcription factor molecules in murine development. Blood 105: 4298-4307, 2005.
6. Wotton S, Terry A, Kilbey A, Jenkins A, Herzyk P, Cameron E and Neil JC: Gene array analysis reveals a common *Runx* transcriptional programme controlling cell adhesion and survival. Oncogene 27: 5856-5866, 2008.
7. Brady G and Farrell PJ: RUNX3-mediated repression of RUNX1 in B cells. J Cell Physiol 221: 283-287, 2009.
8. Fukamachi H and Ito K: Growth regulation of gastric epithelial cells by Runx3. Oncogene 23: 4330-4335, 2004.
9. Levanon D, Bettoun D, Harris-Cerruti C, Woolf E, Negreanu V, Eilam R, Bernstein Y, Goldenberg D, Xiao C, Fliegauf M, *et al*: The Runx3 transcription factor regulates development and survival of TrkC dorsal root ganglia neurons. EMBO J 21: 3454-3463, 2002.
10. Woolf E, Brenner O, Goldenberg D, Levanon D and Groner Y: Runx3 regulates dendritic epidermal T cell development. Dev Biol 303: 703-714, 2007.
11. Bagchi A and Mills AA: The quest for the *1p36* tumor suppressor. Cancer Res 68: 2551-2556, 2008.
12. Subramaniam MM, Chan JY, Yeoh KG, Quek T, Ito K and Salto-Tellez M: Molecular pathology of RUNX3 in human carcinogenesis. Biochim Biophys Acta 1796: 315-331, 2009.
13. Oshimo Y, Oue N, Mitani Y, Nakayama H, Kitadai Y, Yoshida K, Ito Y, Chayama K and Yasui W: Frequent loss of *RUNX3* expression by promoter hypermethylation in gastric carcinoma. Pathobiology 71: 137-143, 2004.
14. Lau QC, Raja E, Salto-Tellez M, Liu Q, Ito K, Inoue M, Putti TC, Loh M, Ko TK, Huang C, *et al*: RUNX3 is frequently inactivated by dual mechanisms of protein mislocalization and promoter hypermethylation in breast cancer. Cancer Res 66: 6512-6520, 2006.
15. Ahlquist T, Lind GE, Costa VL, Meling GI, Vatn M, Hoff GS, Rognum TO, Skotheim RI, Thiis-Evensen E and Lothe RA: Gene methylation profiles of normal mucosa, and benign and malignant colorectal tumors identify early onset markers. Mol Cancer 7: 94, 2008.
16. Ito K, Liu Q, Salto-Tellez M, Yano T, Tada K, Ida H, Huang C, Shah N, Inoue M, Rajnakova A, *et al*: RUNX3, a novel tumor suppressor, is frequently inactivated in gastric cancer by protein mislocalization. Cancer Res 65: 7743-7750, 2005.
17. Voon DC, Wang H, Koo JK, Nguyen TA, Hor YT, Chu YS, Ito K, Fukamachi H, Chan SL, Thiery JP, *et al*: Runx3 protects gastric epithelial cells against epithelial-mesenchymal transition-induced cellular plasticity and tumorigenicity. Stem Cells 30: 2088-2099, 2012.
18. Chen F, Bai J, Li W, Mei P, Liu H, Li L, Pan Z, Wu Y and Zheng J: RUNX3 suppresses migration, invasion and angiogenesis of human renal cell carcinoma. PLoS One 8: e56241, 2013.
19. Sakakura C, Hasegawa K, Miyagawa K, Nakashima S, Yoshikawa T, Kin S, Nakase Y, Yazumi S, Yamagishi H, Okanoue T, *et al*: Possible involvement of RUNX3 silencing in the peritoneal metastases of gastric cancers. Clin Cancer Res 11: 6479-6488, 2005.

20. Yano T, Ito K, Fukamachi H, Chi XZ, Wee HJ, Inoue K, Ida H, Bouillet P, Strasser A, Bae SC, et al: The RUNX3 tumor suppressor upregulates Bim in gastric epithelial cells undergoing transforming growth factor beta-induced apoptosis. Mol Cell Biol 26: 4474-4488, 2006.

21. Peng Z, Wei D, Wang L, Tang H, Zhang J, Le X, Jia Z, Li Q and Xie K: RUNX3 inhibits the expression of vascular endothelial growth factor and reduces the angiogenesis, growth, and metastasis of human gastric cancer. Clin Cancer Res 12: 6386-6394, 2006.

22. Mei PJ, Bai J, Liu H, Li C, Wu YP, Yu ZQ and Zheng JN: RUNX3 expression is lost in glioma and its restoration causes drastic suppression of tumor invasion and migration. J Cancer Res Clin Oncol 137: 1823-1830, 2011.

23. Zhang Z, Chen G, Cheng Y, Martinka M and Li G: Prognostic significance of RUNX3 expression in human melanoma. Cancer 117: 2719-2727, 2011.

24. Chen F, Wang M, Bai J, Liu Q, Xi Y, Li W and Zheng J: Role of RUNX3 in suppressing metastasis and angiogenesis of human prostate cancer. PLoS One 9: e86917, 2014.

25. Chen W, Salto-Tellez M, Palanisamy N, Ganesan K, Hou Q, Tan LK, Sii LH, Ito K, Tan B, Wu J, et al: Targets of genome copy number reduction in primary breast cancers identified by integrative genomics. Genes Chromosomes Cancer 46: 288-301, 2007.

26. Finak G, Bertos N, Pepin F, Sadekova S, Souleimanova M, Zhao H, Chen H, Omeroglu G, Meterissian S, Omeroglu A, et al: Stromal gene expression predicts clinical outcome in breast cancer. Nat Med 14: 518-527, 2008.

27. Sakakura C, Miyagawa K, Fukuda KI, Nakashima S, Yoshikawa T, Kin S, Nakase Y, Ida H, Yazumi S, Yamagishi H, et al: Frequent silencing of RUNX3 in esophageal squamous cell carcinomas is associated with radioresistance and poor prognosis. Oncogene 26: 5927-5938, 2007.

28. Chuang LS and Ito Y: RUNX3 is multifunctional in carcinogenesis of multiple solid tumors. Oncogene 29: 2605-2615, 2010.

29. Yu YY, Chen C, Kong FF and Zhang W: Clinicopathological significance and potential drug target of RUNX3 in breast cancer. Drug Des Devel Ther 8: 2423-2430, 2014.

30. Wang D, Cui W, Wu X, Qu Y, Wang N, Shi B and Hou P: RUNX3 site-specific hypermethylation predicts papillary thyroid cancer recurrence. Am J Cancer Res 4: 725-737, 2014.

31. Katayama Y, Takahashi M and Kuwayama H: Helicobacter pylori causes runx3 gene methylation and its loss of expression in gastric epithelial cells, which is mediated by nitric oxide produced by macrophages. Biochem Biophys Res Commun 388: 496-500, 2009.

32. Cheng AS, Culhane AC, Chan MW, Venkataramu CR, Ehrich M, Nasir A, Rodriguez BA, Liu J, Yan PS, Quackenbush J, et al: Epithelial progeny of estrogen-exposed breast progenitor cells display a cancer-like methylome. Cancer Res 68: 1786-1796, 2008.

33. Fujii S, Ito K, Ito Y and Ochiai A: Enhancer of zeste homologue 2 (EZH2) down-regulates RUNX3 by increasing histone H3 methylation. J Biol Chem 283: 17324-17332, 2008.

34. Lee SH, Kim J, Kim WH and Lee YM: Hypoxic silencing of tumor suppressor RUNX3 by histone modification in gastric cancer cells. Oncogene 28: 184-194, 2009.

35. Varambally S, Cao Q, Mani RS, Shankar S, Wang X, Ateeq B, Laxman B, Cao X, Jing X, Ramnarayanan K, et al: Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. Science 322: 1695-1699, 2008.

36. Lai KW, Koh KX, Loh M, Tada K, Subramaniam MM, Lim XY, Vaithilingam A, Salto-Tellez M, Iacopetta B, Ito Y, et al; Singapore Gastric Cancer Consortium: MicroRNA-130b regulates the tumour suppressor RUNX3 in gastric cancer. Eur J Cancer 46: 1456-1463, 2010.

37. Kitago M, Martinez SR, Nakamura T, Sim MS and Hoon DS: Regulation of RUNX3 tumor suppressor gene expression in cutaneous melanoma. Clin Cancer Res 15: 2988-2994, 2009.

38. Goh YM, Cinghu S, Hong ET, Lee YS, Kim JH, Jang JW, Li YH, Chi XZ, Lee KS, Wee H, et al: Src kinase phosphorylates RUNX3 at tyrosine residues and localizes the protein in the cytoplasm. J Biol Chem 285: 10122-10129, 2010.

39. Kim HR, Oh BC, Choi JK and Bae SC: Pim-1 kinase phosphorylates and stabilizes RUNX3 and alters its subcellular localization. J Cell Biochem 105: 1048-1058, 2008.

40. Kim JH, Choi JK, Cinghu S, Jang JW, Lee YS, Li YH, Goh YM, Chi XZ, Lee KS, Wee H, et al: Jab1/CSN5 induces the cytoplasmic localization and degradation of RUNX3. J Cell Biochem 107: 557-565, 2009.

41. Sheen YY, Kim MJ, Park SA, Park SY and Nam JS: Targeting the transforming growth factor-β signaling in cancer therapy. Biomol Ther 21: 323-331, 2013.

42. Ito Y and Miyazono K: RUNX transcription factors as key targets of TGF-beta superfamily signaling. Curr Opin Genet Dev 13: 43-47, 2003.

43. Chi XZ, Yang JO, Lee KY, Ito K, Sakakura C, Li QL, Kim HR, Cha EJ, Lee YH, Kaneda A, et al: RUNX3 suppresses gastric epithelial cell growth by inducing p21$^{WAF1/Cip1}$ expression in cooperation with transforming growth factor β-activated SMAD. Mol Cell Biol 25: 8097-8107, 2005.

44. Yamamura Y, Lee WL, Inoue K, Ida H and Ito Y: RUNX3 cooperates with FoxO3a to induce apoptosis in gastric cancer cells. J Biol Chem 281: 5267-5276, 2006.

45. Yao H, Ashihara E and Maekawa T: Targeting the Wnt/β-catenin signaling pathway in human cancers. Expert Opin Ther Targets 15: 873-887, 2011.

46. Tanaka S, Shiraha H, Nakanishi Y, Nishina S, Matsubara M, Horiguchi S, Takaoka N, Iwamuro M, Kataoka J, Kuwaki K, et al: Runt-related transcription factor 3 reverses epithelial-mesenchymal transition in hepatocellular carcinoma. Int J Cancer 131: 2537-2546, 2012.

47. Ito K, Lim AC, Salto-Tellez M, Motoda L, Osato M, Chuang LS, Lee CW, Voon DC, Koo JK, Wang H, et al: RUNX3 attenuates beta-catenin/T cell factors in intestinal tumorigenesis. Cancer Cell 14: 226-237, 2008.

48. Ju X, Ishikawa TO, Naka K, Ito K, Ito Y and Oshima M: Context-dependent activation of Wnt signaling by tumor suppressor RUNX3 in gastric cancer cells. Cancer Sci 105: 418-424, 2014.

49. Palmero I, Pantoja C and Serrano M: p19^{ARF} links the tumour suppressor p53 to Ras. Nature 395: 125-126, 1998.

50. Lee KS, Lee YS, Lee JM, Ito K, Cinghu S, Kim JH, Jang JW, Li YH, Goh YM, Chi XZ, et al: Runx3 is required for the differentiation of lung epithelial cells and suppression of lung cancer. Oncogene 29: 3349-3361, 2010.

51. Kruse JP and Gu W: Modes of p53 regulation. Cell 137: 609-622, 2009.

52. Chi XZ, Kim J, Lee YH, Lee JW, Lee KS, Wee H, Kim WJ, Park WY, Oh BC, Stein GS, et al: Runt-related transcription factor RUNX3 is a target of MDM2-mediated ubiquitination. Cancer Res 69: 8111-8119, 2009.

53. Efeyan A and Serrano M: p53: Guardian of the genome and policeman of the oncogenes. Cell Cycle 6: 1006-1010, 2007.

54. Lee YS, Lee JW, Jang JW, Chi XZ, Kim JH, Li YH, Kim MK, Kim DM, Choi BS, Kim EG, et al: Runx3 inactivation is a crucial early event in the development of lung adenocarcinoma. Cancer Cell 24: 603-616, 2013.

55. Hnilicová J, Hozeifi S, Stejskalová E, Dušková E, Poser I, Humpolíčková J, Hof M and Staněk D: The C-terminal domain of Brd2 is important for chromatin interaction and regulation of transcription and alternative splicing. Mol Biol Cell 24: 3557-3568, 2013.

56. Jin YH, Jeon EJ, Li QL, Lee YH, Choi JK, Kim WJ, Lee KY and Bae SC: Transforming growth factor-beta stimulates p300-dependent RUNX3 acetylation, which inhibits ubiquitination-mediated degradation. J Biol Chem 279: 29409-29417, 2004.

57. Thiery JP, Acloque H, Huang RY and Nieto MA: Epithelial-mesenchymal transitions in development and disease. Cell 139: 871-890, 2009.

58. Padua D and Massagué J: Roles of TGFbeta in metastasis. Cell Res 19: 89-102, 2009.

59. Lamouille S, Xu J and Derynck R: Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol 15: 178-196, 2014.

60. Lee JM, Shin JO, Cho KW, Hosoya A, Cho SW, Lee YS, Ryoo HM, Bae SC and Jung HS: Runx3 is a crucial regulator of alveolar differentiation and lung tumorigenesis in mice. Differentiation 81: 261-268, 2011.

61. Chang CJ, Chao CH, Xia W, Yang JY, Xiong Y, Li CW, Yu WH, Rehman SK, Hsu JL, Lee HH, et al: p53 regulates epithelial-mesenchymal transition and stem cell properties through modulating miRNAs. Nat Cell Biol 13: 317-323, 2011.

62. Kumarswamy R, Mudduluru G, Ceppi P, Muppala S, Kozlowski M, Niklinski J, Papotti M and Allgayer H: MicroRNA-30a inhibits epithelial-to-mesenchymal transition by targeting Snai1 and is downregulated in non-small cell lung cancer. Int J Cancer 130: 2044-2053, 2012.

63. Yamasaki T, Seki N, Yamada Y, Yoshino H, Hidaka H, Chiyomaru T, Nohata N, Kinoshita T, Nakagawa M and Enokida H: Tumor suppressive microRNA-138 contributes to cell migration and invasion through its targeting of vimentin in renal cell carcinoma. Int J Oncol 41: 805-817, 2012.

64. Cheng CW, Wang HW, Chang CW, Chu HW, Chen CY, Yu JC, Chao JI, Liu HF, Ding SL and Shen CY: MicroRNA-30a inhibits cell migration and invasion by downregulating vimentin expression and is a potential prognostic marker in breast cancer. Breast Cancer Res Treat 134: 1081-1093, 2012.

65. Liu Z, Chen L, Zhang X, Xu X, Xing H, Zhang Y, Li W, Yu H, Zeng J and Jia J: RUNX3 regulates vimentin expression via miR-30a during epithelial-mesenchymal transition in gastric cancer cells. J Cell Mol Med 18: 610-623, 2014.

66. Folkman J: Tumor angiogenesis: Therapeutic implications. N Engl J Med 285: 1182-1186, 1971.

67. Nishida N, Yano H, Nishida T, Kamura T and Kojiro M: Angiogenesis in cancer. Vasc Health Risk Manag 2: 213-219, 2006.

68. Hanahan D and Folkman J: Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 86: 353-364, 1996.

69. Jain RK: Antiangiogenesis strategies revisited: From starving tumors to alleviating hypoxia. Cancer Cell 26: 605-622, 2014.

70. Yang M, Kuang X, Pan Y, Tan M, Lu B, Lu J, Cheng Q and Li J: Clinicopathological characteristics of vascular endothelial growth factor expression in uveal melanoma: A meta-analysis. Mol Clin Oncol 2: 363-368, 2014.

71. Lee JM, Lee DJ, Bae SC and Jung HS: Abnormal liver differentiation and excessive angiogenesis in mice lacking Runx3. Histochem Cell Biol 139: 751-758, 2013.

72. Lee JM, Kwon HJ, Lai WF and Jung HS: Requirement of Runx3 in pulmonary vasculogenesis. Cell Tissue Res 356: 445-449, 2014.

73. Semenza GL: Angiogenesis in ischemic and neoplastic disorders. Annu Rev Med 54: 17-28, 2003.

74. Harris AL: Hypoxia - a key regulatory factor in tumour growth. Nat Rev Cancer 2: 38-47, 2002.

75. Lee SH, Bae SC, Kim KW and Lee YM: RUNX3 inhibits hypoxia-inducible factor-1α protein stability by interacting with prolyl hydroxylases in gastric cancer cells. Oncogene 33: 1458-1467, 2014.

76. Meng S, Cao J, Zhang X, Fan Y, Fang L, Wang C, Lv Z, Fu D and Li Y: Downregulation of microRNA-130a contributes to endothelial progenitor cell dysfunction in diabetic patients via its target Runx3. PLoS One 8: e68611, 2013.

77. Luzzi KJ, MacDonald IC, Schmidt EE, Kerkvliet N, Morris VL, Chambers AF and Groom AC: Multistep nature of metastatic inefficiency: Dormancy of solitary cells after successful extravasation and limited survival of early micrometastases. Am J Pathol 153: 865-873, 1998.

78. Nagasaka A, Kawane K, Yoshida H and Nagata S: Apaf-1-independent programmed cell death in mouse development. Cell Death Differ 17: 931-941, 2010.

79. Burgess DJ: Apoptosis: Refined and lethal. Nat Rev Cancer 13: 79, 2013.

80. Nakanishi Y, Shiraha H, Nishina S, Tanaka S, Matsubara M, Horiguchi S, Iwamuro M, Takaoka N, Uemura M, Kuwaki K, *et al*: Loss of runt-related transcription factor 3 expression leads hepatocellular carcinoma cells to escape apoptosis. BMC Cancer 11: 3, 2011.

81. Zhai FX, Liu XF, Fan RF, Long ZJ, Fang ZG, Lu Y, Zheng YJ and Lin DJ: RUNX3 is involved in caspase-3-dependent apoptosis induced by a combination of 5-aza-CdR and TSA in leukaemia cell lines. J Cancer Res Clin Oncol 138: 439-449, 2012.

82. Liu Z, Zhang X, Xu X, Chen L, Li W, Yu H, Sun Y, Zeng J and Jia J: RUNX3 inhibits survivin expression and induces cell apoptosis in gastric cancer. Eur J Cell Biol 93: 118-126, 2014.

83. Meng X, Franklin DA, Dong J and Zhang Y: MDM2-p53 pathway in hepatocellular carcinoma. Cancer Res 74: 7161-7167, 2014.

84. Yamada C, Ozaki T, Ando K, Suenaga Y, Inoue K, Ito Y, Okoshi R, Kageyama H, Kimura H, Miyazaki M, *et al*: RUNX3 modulates DNA damage-mediated phosphorylation of tumor suppressor p53 at Ser-15 and acts as a co-activator for p53. J Biol Chem 285: 16693-16703, 2010.

85. Lai CB and Mager DL: Role of runt-related transcription factor 3 (*RUNX3*) in transcription regulation of natural cytotoxicity receptor 1 (*NCR1/NKp46*), an activating natural killer (NK) cell receptor. J Biol Chem 287: 7324-7334, 2012.

86. Levanon D, Negreanu V, Lotem J, Bone KR, Brenner O, Leshkowitz D and Groner Y: Transcription factor Runx3 regulates interleukin-15-dependent natural killer cell activation. Mol Cell Biol 34: 1158-1169, 2014.

87. Zhang Y, Lu Q and Cai X: MicroRNA-106a induces multidrug resistance in gastric cancer by targeting RUNX3. FEBS Lett 587: 3069-3075, 2013.

88. Li Q, Wang JX, He YQ, Feng C, Zhang XJ, Sheng JQ and Li PF: MicroRNA-185 regulates chemotherapeutic sensitivity in gastric cancer by targeting apoptosis repressor with caspase recruitment domain. Cell Death Dis 5: e1197, 2014.

89. Boya P, Reggiori F and Codogno P: Emerging regulation and functions of autophagy. Nat Cell Biol 15: 713-720, 2013.

90. Kenific CM, Thorburn A and Debnath J: Autophagy and metastasis: Another double-edged sword. Curr Opin Cell Biol 22: 241-245, 2010.

91. Xu Q, Meng S, Liu B, Li MQ, Li Y, Fang L and Li YG: *MicroRNA-130a* regulates autophagy of endothelial progenitor cells through Runx3. Clin Exp Pharmacol Physiol 41: 351-357, 2014.

92. Denhardt DT, Lopez CA, Rollo EE, Hwang SM, An XR and Walther SE: Osteopontin-induced modifications of cellular functions. Ann NY Acad Sci 760: 127-142, 1995.

93. Anborgh PH, Mutrie JC, Tuck AB and Chambers AF: Role of the metastasis-promoting protein osteopontin in the tumour microenvironment. J Cell Mol Med 14: 2037-2044, 2010.

94. Fong YC, Liu SC, Huang CY, Li TM, Hsu SF, Kao ST, Tsai FJ, Chen WC, Chen CY and Tang CH: Osteopontin increases lung cancer cells migration via activation of the alphavbeta3 integrin/FAK/Akt and NF-kappaB-dependent pathway. Lung Cancer 64: 263-270, 2009.

95. Wang Y, Yan W, Lu X, Qian C, Zhang J, Li P, Shi L, Zhao P, Fu Z, Pu P, *et al*: Overexpression of osteopontin induces angiogenesis of endothelial progenitor cells via the avβ3/PI3K/AKT/eNOS/NO signaling pathway in glioma cells. Eur J Cell Biol 90: 642-648, 2011.

96. Singhal H, Bautista DS, Tonkin KS, O'Malley FP, Tuck AB, Chambers AF and Harris JF: Elevated plasma osteopontin in metastatic breast cancer associated with increased tumor burden and decreased survival. Clin Cancer Res 3: 605-611, 1997.

97. Schneider S, Yochim J, Brabender J, Uchida K, Danenberg KD, Metzger R, Schneider PM, Salonga D, Hölscher AH and Danenberg PV: Osteopontin but not osteonectin messenger RNA expression is a prognostic marker in curatively resected non-small cell lung cancer. Clin Cancer Res 10: 1588-1596, 2004.

98. Ue T, Yokozaki H, Kitadai Y, Yamamoto S, Yasui W, Ishikawa T and Tahara E: Co-expression of osteopontin and CD44v9 in gastric cancer. Int J Cancer 79: 127-132, 1998.

99. Cheng HC, Liu YP, Shan YS, Huang CY, Lin FC, Lin LC, Lee L, Tsai CH, Hsiao M and Lu PJ: Loss of RUNX3 increases osteopontin expression and promotes cell migration in gastric cancer. Carcinogenesis 34: 2452-2459, 2013.

100. Min KW, Kim DH, Do SI, Kim K, Lee HJ, Chae SW, Sohn JH, Pyo JS, Oh YH, Kim WS, *et al*: Expression patterns of stromal MMP-2 and tumoural MMP-2 and -9 are significant prognostic factors in invasive ductal carcinoma of the breast. APMIS 122: 1196-1206, 2014.

101. Stellas D and Patsavoudi E: Inhibiting matrix metalloproteinases, an old story with new potentials for cancer treatment. Anticancer Agents Med Chem 12: 707-717, 2012.

102. Galis ZS and Khatri JJ: Matrix metalloproteinases in vascular remodeling and atherogenesis: The good, the bad, and the ugly. Circ Res 90: 251-262, 2002.

103. Zuo JH, Zhu W, Li MY, Li XH, Yi H, Zeng GQ, Wan XX, He QY, Li JH, Qu JQ, *et al*: Activation of EGFR promotes squamous carcinoma SCC10A cell migration and invasion via inducing EMT-like phenotype change and MMP-9-mediated degradation of E-cadherin. J Cell Biochem 112: 2508-2517, 2011.

104. Murphy DA and Courtneidge SA: The 'ins' and 'outs' of podosomes and invadopodia: Characteristics, formation and function. Nat Rev Mol Cell Biol 12: 413-426, 2011.

105. Bai ZK, Guo B, Tian XC, Li DD, Wang ST, Cao H, Wang QY and Yue ZP: Expression and regulation of Runx3 in mouse uterus during the peri-implantation period. J Mol Histol 44: 519-526, 2013.

106. Jinga DC, Blidaru A, Condrea I, Ardeleanu C, Dragomir C, Szegli G, Stefanescu M and Matache C: MMP-9 and MMP-2 gelatinases and TIMP-1 and TIMP-2 inhibitors in breast cancer: Correlations with prognostic factors. J Cell Mol Med 10: 499-510, 2006.