Wrong place, wrong time: Runt-related transcription factor 2/SATB2 pathway in bone development and carcinogenesis

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Abstract:
Upregulation or aberrant expression of genes such as special AT-rich sequence-binding protein 2 (SATB2) is necessary for normal cell differentiation and tissue development and is often associated with carcinogenesis and metastatic progression. SATB2 is a critical transcription factor for biological development of various specialized cell lineages, such as osteoblasts and neurons. The dysregulation of SATB2 expression has recently been associated with various types of cancer, while the mechanisms and pathways by which it mediates tumorigenesis are not well elucidated. Runt-related transcription factor 2 (RUNX2) is a master regulator for osteogenesis, and it shares common pathways with SATB2 to regulate bone development. Interestingly, these two transcription factors co-occur in several epithelial and mesenchymal cancers and are linked by multiple cancer-related proteins and microRNAs. This review examines the interactions between RUNX2 and SATB2 in a network necessary for normal bone development and the circumstances in which the expression of RUNX2 and SATB2 in the wrong place and time leads to carcinogenesis.

Keywords:
Carcinogenesis, microRNA, osteogenesis, Runt-related transcription factor 2, SATB2

Introduction
Special AT-rich sequence-binding protein 2 (SATB2) is a nuclear matrix-associated protein that regulates gene transcription by binding to the nuclear matrix-attachment regions and altering organization of eukaryotic chromosomes. It is an important regulator of the osteoblastic, craniofacial, and nervous system development. SATB2 was identified at locus 2q32, in which the chromosomal changes contributed to the pathogenesis of isolated cleft palate. SATB2-knockout mice presented a similar phenotype as observed in humans, such as delayed bone formation and mineralization, resulting in death shortly after birth. Further examination of SATB2-knockout mice during embryonic development showed that the observed cleft palate malformation was caused by a defect in skeletal patterning, which suggested failure to form areas of condensed mesenchymal progenitor cells or improper differentiation of chondrocytes or osteoblasts.

While SATB2 has been identified mainly as a gene contributing to embryonic development, it was recently found expressed in adenocarcinomas, as well as in many other tumors, such as breast, ovarian, lung, and sinonasal carcinomas. Recent studies have revealed an important role of SATB2 in mediating heavy metal-induced cell transformation. SATB2 plays an important role in the cell development and differentiation, while its pathway and mechanisms in cancers are not well understood. Thus, this review aims to...
explore a potential signaling pathway in which SATB2 and Runx-related transcription factor 2 (RUNX2) cooperate to promote carcinogenesis.

**Runx-related Transcription Factor 2 and SATB2 in Bone Development**

RUNX2 is another important regulator in the bone development. RUNX2 expression establishes commitment to the osteoblast lineage and is necessary for proliferation of osteoprogenitors and immature osteoblasts, as well as upregulation of the bone matrix molecules needed for differentiation into mature osteoblasts.[7] Like SATB2-knockout mice, mice lacking RUNX2 exhibited an absence of mature osteoblasts and ossification and neonatal mortality.[8,9] If RUNX2 is a master switch for inducing osteoblast differentiation, then SATB2 is believed to be a molecular node in the transcriptional network regulating osteoblast differentiation.[10] SATB2 is not considered as a direct interacting transcription factor with RUNX2, but the connection between RUNX2 and SATB2 through their participation in the bone development and formation has been described.[11] The interaction between RUNX2 and SATB2 is supported by pathway analysis.[12] As shown in the Gene Oncology Study of String (https://string-db.org), a potential SATB2/RUNX2 pathway is mainly involved in osteoblast differentiation and development, and bone morphogenetic protein (BMP) signaling pathway, as well as many other cancer-associated pathways, such as extracellular matrix organization, epithelial–mesenchymal transition (EMT), cell proliferation, and cell death signaling pathways [Table 1].

**Common Upstream Regulators**

RUNX2 and SATB2 share common upstream regulators during osteoblast differentiation, such as BMP-2 and SMAD pathways. While BMP-2 is not required for

| Biological process                                      | \( P \)     |
|--------------------------------------------------------|-------------|
| Osteoblast differentiation                             | 6.32E-09    |
| Osteoblast development                                 | 4.82E-08    |
| BMP signaling pathway                                  | 4.80E-06    |
| Positive regulation of extracellular matrix organization| 5.32E-06    |
| Positive regulation of epithelial to mesenchymal transition | 3.46E-05    |
| Regulation of epithelial cell proliferation             | 0.0002      |
| Apoptotic process                                       | 0.0054      |
| Stress-activated MAPK cascade                           | 0.0059      |
| Negative regulation of cell death                       | 0.0061      |
| Notch signaling pathway                                 | 0.0067      |

BMP: Bone morphogenetic protein, MAPK: Mitogen-activated protein kinase

RUNX2 to induce the expression of osteoblast genes, the formation of RUNX2–SMAD complex is necessary for BMP-2 to transduce osteoblastogenic signals and to complete osteogenic processes.[13] A study also found that BMP-2 activated RUNX2 in the early osteogenesis mediated by two homeodomain proteins Dlx3 and Dlx5, which are essential mechanisms in the commitment to osteogenic lineage, and in later stages, BMP-2 facilitated RUNX2 in cell differentiation.[14] BMP-2 also stimulated SATB2 expression in a time- and concentration-dependent manner in C2C12 cells, a mouse myoblast cell line used to study the differentiation of myoblasts and osteoblasts under expression of various target proteins, which was mediated through SMAD1/5 by directly binding and activating SATB2 promoter to mediate myoblast/osteoblast transdifferentiation.[15]

Mitogen-activated protein kinases/extracellular signal-regulated kinases (MAPK/ERK) is the upstream of RUNX2 and phosphorylates and activates RUNX2, promoting osteoblast differentiation.[16-19] MAPK/ERK was reported to participate in SATB2-mediated osteogenesis,[20] and many downstream microRNAs (miRNAs) targeted by SATB2 in mouse bone mesenchymal stem cells (BMSCs) were also involved in MAPK/ERK signaling as determined by pathway analysis.[21] In contrast, continuous activation of ERK by extracellular stimuli had a negative effect in BMP-2 induction of osteoblast genes and an inhibitory effect on osteogenesis.[22,23] It was pointed out that ERK phosphorylated BMP-associated SMAD1/5/8 and mediated degradation of SMAD proteins in a cell-specific manner.[22] ERK inhibited SATB2 activity,[23] supporting the role of MAPK/ERK signaling in osteoblast differentiation showing that SATB2 was downstream of MAPK/ERK pathway.

**Common Downstream Targets**

Genes regulated by RUNX2 and SATB2 individually have been identified, and there are also genes controlled by both of these transcription factors.[11] RUNX2 was found to directly target many osteoblast-specific genes. The osteoblast-specific cis-acting element 2 (OSE2, ACCACA) is a binding site for RUNX2, and it has been characterized in the promoters of many osteo-specific genes: osteocalcin (OCN), bone sialoprotein (BSP), COL1A1, osterix (OSX), and osteopontin (OPN).[24] SATB2 is also found to target a few genes regulated by RUNX2, such as BSP, COL1A1, and OCN.[1,25] BSP is an essential component of bone extracellular matrix and an important protein for bone development.[26] Several studies have demonstrated that SATB2 regulated BSP by binding to its promoter.[1,10,27] Microarray analysis indicated COL1A1 as a downstream of SATB2.[1] COL1A1 is another osteomarker gene
induced by RUNX2 during osteoblast development that encodes fibrillar collagen in connective tissues.\textsuperscript{[44,28]} One study found that lower RUNX2 level failed to decrease COL1A1 expression in mature osteoblasts,\textsuperscript{[29]} indicating that the expression of COL1A1 can be maintained through other pathways independent of RUNX2 and may be a compensatory pathway aided by SATB2.

SATB2 can act as a protein scaffold to enhance the activity of other DNA-binding proteins. Although SATB2 was shown to directly bind to the promoter of OCN, OCN was activated by SATB2 binding and promoting the activity of RUNX2.\textsuperscript{[1]} It was suggested that the interaction of SATB2 with RUNX2 occurs at the 108 N-terminal residues of RUNX2, where a QA domain is present to mediate this activation.\textsuperscript{[1]} QA domain of RUNX2 was believed to be responsible for preventing the heterodimerization with core-binding factor (CBF)-β, a co-factor for RUNX2 transactivity.\textsuperscript{[30,31]} SATB2 may have compromised the function of the QA domain of RUNX2, promoting RUNX2 binding with CBF-β, thereby stimulating the transcription of genes controlled by the RUNX2/CBF-β complex, e.g., OCN, and other targets necessary for bone development.

**Intermediate Genes between Runx-related Transcription Factor 2 and SATB2**

RUNX2 and SATB2 are also connected by osteomarkers. OSX is a critical transcription factor in osteoblast differentiation and is downstream of RUNX2 regulated by an OSE2 element that binds RUNX2.\textsuperscript{[32-34]} OSX was reported to regulate SATB2 expression in C2C12 cells by promoter activation.\textsuperscript{[27]} Another study reported that SATB2 increased RUNX2 transactivity on OSX promoter and SATB2 also increased OSX expression independent of RUNX2.\textsuperscript{[10]} This indicates a positive feedback signal in the RUNX2/OSX/SATB2 pathway and suggests SATB2 also functions independently of RUNX2 to regulate OSX expression.

Although many studies placed SATB2 downstream of RUNX2 during osteogenesis, SATB2 can also regulate upstream. HOXA2 is a direct target of SATB2 and a negative regulator of RUNX2.\textsuperscript{[1,35]} HOXA2 was upregulated in SATB2−/− embryos and osteoblasts, and SATB2 was able to bind to the EII enhancer of HOXA2 \textit{in vivo} and decrease H3K4me at the endogenous HOXA2 promoter.\textsuperscript{[1]} HOXA2 inhibits skeletal formation,\textsuperscript{[38,39]} and inactivation of the HOXA2 gene in SATB2−/− embryos rescues the delay in calvarial bone formation.\textsuperscript{[1]} RUNX2 expression was found increased in the HOXA2-mutated embryos,\textsuperscript{[35]} indicating that HOXA2 might modulate SATB2 and RUNX2 signaling by SATB2-repressing HOXA2, activating RUNX2 expression. In fact, studies reported that overexpression of SATB2 in the BMSCs rapidly induced osteogenic differentiation with increased expression of RUNX2 as well as other osteo-specific markers such as BSP, OSX, and ALP.\textsuperscript{[37]} Another study suggested that SATB2 interacted and upregulated RUNX2 in the early stages of osteoblast differentiation.\textsuperscript{[38]} During craniofacial development, SATB2 was activated early, allowing the induction of RUNX2 by inhibiting HOXA2.\textsuperscript{[38]} SATB2-induced RUNX2 was reported by Mi et al., although it was not clear whether the induction was a direct interaction between SATB2 and RUNX2 or mediated through other factors. The authors suggested that SATB2 might be involved in the BMP-2/RUNX2 pathway with BMP-2–activating SATB2, which then induces RUNX2 to mediate osteoblast differentiation.\textsuperscript{[39]}

miRNAs are short noncoding RNA molecules that target mRNA degradation, inhibiting protein translation and altering chromatin structure to repress gene expression.\textsuperscript{[40-43]} A group of miRNAs associated with osteogenic lineage commitment in the mesenchymal stem cells (MSCs) has been characterized and termed osteo-miRNA.\textsuperscript{[44]} many of which are involved in RUNX2/SATB2 network during osteogenesis. miR-31 and miR-23a–27a–24-2 are negatively regulated by RUNX2 to suppress SATB2 expression in osteogenesis.\textsuperscript{[40-47]} SATB2 was found to control the expression of many miRNAs that negatively correlated with the expression of SATB2, including miR-125b, miR-132, miR-128, miR-127, miR-143, and miR-22,\textsuperscript{[48]} which many of which also target RUNX2 as confirmed by the TargetScan (http://www.targetscan.org). Some miRNA downstream of RUNX2, such as miR-690 and miR-185, was found to target SATB2.\textsuperscript{[49,50]} A list of miRNAs involved with SATB2 and RUNX2 is shown in Table 2 along with their role in osteogenesis.

An illustration of SATB2 and RUNX2 network in the bone development is shown in Figure 1. In total, SATB2 and RUNX2 function in the same network but have independent roles in osteoblast differentiation. In addition, SATB2 induces expression of factors that limit osteoblast differentiation, such as ATF3 and AP2-β,\textsuperscript{[10,27]} indicating that RUNX2 and SATB2 might act to balance differentiation at certain stages. Considering this intricate interaction between RUNX2 and osteoblast differentiation during normal bone development, it is important to consider the conditions in which they are aberrantly expressed and the targets they induce to initiate cancer and promote disease progression.

**Runx-related Transcription Factor 2 and SATB2 in Carcinogenesis**

Transfoming from a normal cell to a cancer cell occurs through germline mutations or epigenetic alterations caused by exposure to carcinogens. These
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Table 2: The microRNAs cooperating with Runx-related transcription factor 2 and SATB2 in osteogenesis

| Genes | Target gene | Regulator | Study model | Function |
|-------|-------------|-----------|-------------|----------|
| miR-31 | SATB2* | RUNX2* | Rat BMSC; Human primary dental follicle cell | Inhibit osteogenic transcription factors; Regulate matrix remodeling and osteoclast activity |
| miR-23a–27a–24–2 | SATB2* | RUNX2* | ROB, MC3T3-E1 | Suppress osteogenic differentiation |
| miR-690 | SATB2 | RUNX2* | C2C12 | Suppress osteogenic differentiation |
| miR-185 | SATB2 | RUNX2* | MC3T3-E1 | Suppress amelogenesis and osteogenesis |
| miR-205 | RUNX2*, SATB2* | | Rat BMSC | Suppress osteogenic differentiation |
| miR-127* | RUNX2* | SATB2 | | Promote chondrogenic and cartilage differentiation |
| miR-128* | RUNX2* | SATB2 | Human MSCs | Promote osteogenic differentiation |
| miR-132* | RUNX2* | SATB2 | Human osteoblast | Suppress osteoblast differentiation |
| miR-22* | RUNX2* | SATB2 | Mouse pre-osteoblast | Stimulate osteoblast differentiation; Suppress adipogenic differentiation |
| miR-218 | RUNX2* | SATB2 | MEC3T3-E1 | Suppress osteoblast differentiation |
| miR-103a | RUNX2* | SATB2 | hFOB 1.19 | Inhibit bone formation |
| miR-125b | RUNX2* | SATB2 | Human BMSC | Suppress osteogenic differentiation |

*Experimentally confirmed. BMSC: Bone mesenchymal stem cell, ROB: Rat primary osteoblasts, MC3T3-E1: Mouse pre-osteoblast cell line, C2C12: Myogenic progenitor cell, MSCs: Mesenchymal stem cells, hFOB 1.19: Human osteoblast.

Figure 1: An illustration of SATB2 and Runx-related transcription factor 2 network in bone development. Runx-related transcription factor 2 and SATB2 share common upstream regulators such as MAPK/ERK pathway and bone morphogenetic protein/SMAD pathway, and they have overlapping downstream genes such as osteocalcin, bone sialoprotein, and COL1A1 in osteogenesis. They are connected by HOX2, osterix, and various microRNAs in either direction, allowing Runx-related transcription factor 2 and SATB2 to act as an upstream regulator for one another. Among the microRNAs connecting SATB2 and Runx-related transcription factor 2, miR-31 and miR-23-27a–24–2 have solid evidence supporting the pathway axis in osteoblast differentiation. In addition, SATB2 also displays physical interaction with Runx-related transcription factor 2 to regulate its transcription activity and induce the expression of osteocalcin.

Runt-related transcription factor 2 and SATB2 as tumor suppressor

The role of SATB2 in cancers has been reviewed by Chen et al., suggesting that SATB2 can function either as an oncogene or tumor suppressor. For example, SATB2 is recognized as a biomarker for colorectal cancer (CRC). However, in some studies, CRC cells exhibit low SATB2 mRNA expression; however, when SATB2 expression was turned on, cancer cell proliferation was reduced. RUNX2 also exerts paradoxical effects in carcinogenesis, acting as a tumor suppressor or oncogene. RUNX2 serves as a regulator for cell cycle and inhibits cell proliferation during osteogenesis. RUNX2 can arrest the cell cycle in the G1 phase, allowing cells to exit the cell cycle and avoid abnormal cell proliferation. A loss of RUNX2 function was related to enhanced in vitro growth potential in RUNX2-deficient osteoblasts.

Runt-related transcription factor 2 and SATB2 as oncogene

On the other hand, RUNX2 and SATB2 are well-known oncogenes. While SATB2 expression is associated with normal embryonic development, its expression in cancers, however, determines overall progression and patient prognosis. SATB2 was first reported to mediate head and neck squamous cell carcinoma (HNSCC) as a novel binding partner for p63 and p73 promoting chemoresistance, and its expression was associated with advanced-stage HNSCC. SATB2 was also a diagnostic marker for CRC. Increased expression of SATB2 has been observed in many other cancers such as osteosarcoma, pancreatic cancer, breast cancer, endometrial cancer, and cancer stem cells (CSCs). In addition, many downstream targets of SATB2 such as B-cell lymphoma 2 (BCL-2), BSR, c-MYC, KLF-4, HOXA2, and NANOG are key regulators of cell pluripotency and survival.

Germline mutations or epigenetic changes may result in haploinsufficiency of tumor suppressors or increased expression of oncogenes. Tumor suppressors activate pathways that limit cell proliferation, promote contact inhibition, prevent cell migration and invasion, and support normal autophagy and apoptosis. Oncogenes, however, activate pathways that increase cell proliferation, migration, and invasion while preventing contact inhibition and programmed cell death. It is important to understand the roles of RUNX2 and SATB2 as either tumor suppressors or oncogenes as they cooperate or antagonize one another in promoting cancer progression.
RUNX2 is expressed only in a limited number of nonosseous tissues, including testis, mammary epithelium, thymus, endothelial cells, and transformed cells. Aberrant expression of RUNX2 has been described in the progression and metastasis of several human cancers, including pancreatic cancer, thyroid cancer, bone cancer, breast cancer, and prostate cancer. RUNX2 promoted cell proliferation and inhibited cell death by repressing expression of p21/CDKN1A in fibroblasts and osteosarcoma cells, which act to arrest in the G1 phase. RUNX2 activated Gpr30 expression and reduced Rgs2 level to increase mitogenic signaling through cAMP and G-protein-coupled receptors. Moreover, downstream genes of RUNX2, such as MMPs, vascular endothelial growth factor (VEGF), BSP, and c-MYC, are involved in promoting cancer cell metastasis. RUNX2-regulated genes such as SOX9, SNAI2, SNAI3, TWIST1, and SMAD3 are potential inducers of cell invasion.

Runt-related transcription factor 2/SATB2 in osteosarcoma

Osteosarcoma is an aggressive bone cancer with poor clinical outcome. It was conventionally believed that osteosarcoma originated from osteoblasts due to the presence of the bone matrix osteoid, whereas it was later found that it can originate from the differentiation of MSCs to a mature osteoblasts. RUNX2 and SATB2 are both key transcription factors in osteoblast lineage commitment and development; thus, cell transformation and metastasis would be supported by aberrant expression of these proteins during osteogenesis.

Improper expression of the development-related genes, at the wrong time, contributed to carcinogenesis. The expression level of RUNX2 was dynamic from pre-osteoblast to immature osteoblasts, and finally to the maturation of osteoblast. Early onset of RUNX2 expression in MSCs may direct the cells to osteoblast progenitors, which is required for their expansion. The expression of RUNX2 increases in immature osteoblast, regulating the expression of many bone matrix proteins. However, in the late stage of osteogenesis, RUNX2 was largely decreased to allow maturation of osteoblasts. RUNX2 was not required for the expression of major bone matrix genes in mature osteoblast, and continuous expression of RUNX2 at high levels was found to inhibit and impair bone formation in immature mouse osteoblasts. RUNX2 overexpression was confirmed in osteosarcomas and associated with poor tumor response to chemotherapy. A frequent amplification and rearrangement where the RUNX2 gene is located (6p12–p21) was found in osteosarcoma. Deregulation of cell cycle control might be a factor promoting osteosarcoma mediated by RUNX2, since studies had reported that high levels of RUNX2 in SAOS-2, a human osteogenic sarcoma cell line, lost cell cycle control. Further studies indicated that the function of RUNX2 in osteosarcoma was related to pRb and p53. Activation signaling pathways for RUNX2, such as PI3K/Akt, Wnt, BMP/ TGF-β, MAPK/ERK, and Notch, were all found turned on in osteosarcoma, indicating that increased expression of RUNX2 at the wrong time, e.g., final stage of osteogenesis, caused formation of too many immature osteoblast cells, resulting in osteosarcoma.

SATB2 was also found commonly expressed in osteosarcoma and has been suggested as a sensitive marker for this cancer. However, SATB2 expression was not specific for osteosarcoma since it was also expressed in other types of bone sarcomas, such as undifferentiated pleomorphic sarcoma and bone fibrosarcoma, and high-grade chondrosarcomas. It is a marker for osteoblast differentiation in both benign and malignant bone tumors. One study reported that SATB2 enhanced osteosarcoma stem cell-like characteristics through the induction of N-cadherin and NF-κB signaling, and knockdown of SATB2 repressed these genes, leading to impaired osteosarcoma sphere formation and tumor cell proliferation. In normal cells, NF-κB is activated by host defense mechanisms in response to inflammation, but this pathway activates osteosarcoma tumorigenesis. However, NF-κB is an inhibitor for RUNX2 in osteoblasts since induced expression of NF-κB was found to repress the binding of RUNX2 with β-catenin and with the downstream target genes such as BSP and OCN, leading to decreased expression of matrix protein and bone formation. Although NF-κB failed to affect RUNX2 expression, considering the transactivity of RUNX2 can be mediated by co-factors, a compensation of RUNX2 expression would be possible by prolonged inflammation. In addition, SATB2 is an activator of β-catenin, allowing a counteracting effect with NF-κB on RUNX2, which contributes to SATB2-driven osteosarcoma.

c-MYC is a transcription factor that plays an important role in cell proliferation, apoptosis, and metabolism. It is involved in cancers such as ovarian, breast, pancreatic, gastric, and uterine cancer and CRC. It was also found overexpressed in osteosarcoma and this was related with metastasis. MYC promoted OS cell invasion by MEK/ERK pathway. c-MYC was a target for RUNX2, and it was overexpressed in human and mouse OS cells compared to MSCs. RUNX2 promoted c-MYC expression by recruiting the COMPASS-like complex to the promoter of c-MYC, and RUNX2/c-MYC pathway contributed to survival of OS cells. Although RUNX2 is required to maintain the expression of c-MYC, in OS cells, c-MYC can be induced through other factors. In fact, c-MYC is also a downstream
target for SATB2. It was found that SATB2 can directly target c-MYC promoter and regulate its transcription in CSCs.[70] Ectopic expression of c-MYC in mature fibroblasts inhibited cell differentiation and promoted formation of multifunctional progenitor cells. SATB2 alone increased expression of c-MYC as well as other pluripotency factors such as Oct-4, SOX-2, and KLF-4, to induce de-differentiation and transformation of human mammary epithelial cells (HMEGs) into progenitor cells.[70]

### Runt-related Transcription Factor 2 and SATB2 in Other Cancers

A search of the cBio genomic cancer portal (http://www.cbioportal.org) for RUNX2 and SATB2 in the Cancer Genome Atlas database found expression of these two transcription factors in cancers, including testicular, bladder, and lung squamous carcinoma, HNSCC, melanoma, CRC, and prostate, breast, papillary thyroid, and cervical cancer [Table 3].

Lung squamous cell carcinoma is a major subtype of nonsmall cell lung cancer (NSCLC) and accounts for about 25%–30% of all lung cancers. RUNX2 was suggested as a novel prognostic marker in NSCLC since its expression correlated with tumor size, tumor stage, and lymph node metastasis.[112] Although SATB2 has been characterized as a tumor suppressor for NSCLC,[113] SATB2 mediated Beas2B cell transformation by metal carcinogens.[8] Inhibition of SATB2 in transformed Beas2B cells reduced cell proliferation and anchorage-independent growth.[6] SATB2 was induced by arsenic exposure in Beas2B by the downregulation of miR-31, following induction of RUNX2 during cellular transformation,[114] miR-31 was implicated as an important negative regulator in osteogenesis with RUNX2 and SATB2 in an osteoblast differentiation pathway.[45,46] MiRNA-31 is a pleiotropically acting miRNA involved in CRC,[115-117] squamous cell carcinoma,[118] and breast, gastric,[120] and pancreatic cancer.[121] Two binding sites for miR-31 were characterized in the 3'UTR of SATB2 and identified as a direct target for miR-31 in cancer-associated fibroblasts.[122] The miR-31/SATB2 axis was also active in CRCs in which upregulated miR-31 reduced SATB2 level promoting cell proliferation, invasion, and metastasis.[117] Wnt/β-catenin pathway plays an important role in SATB2-induced CRC,[123,124] while RUNX2 is known to be downstream of Wnt/β-catenin pathway in osteogenesis.[125] An increased level of RUNX2 was found in CRC cells, confirming its function in CRC metastasis, TMN stages, and prognosis.[126-128] SATB2 was suggested to be a diagnostic marker for CRCs since SATB2 stained positively in 71%–97% of primary and metastatic CRCs biopsies with immunohistochemistry. Reduced expression of SATB2 in colon cancer was associated with poor prognosis, while increased expression enhanced therapeutic sensitivity.[66,129-132] One study found that SATB2 induction and subsequent transformation of colon epithelial cells (CRL-1831) were mediated through Wnt/β-catenin/TCF/LEF pathway.[109] Many downstream genes of the pathway have been associated with cell transformation, including c-MYC, cyclin, and VEGF.[109] Many studies have indicated the role of VEGF in colon cancer.[133] VEGF is an important factor during osteogenesis in directing the invasion of blood vessels into cartilage, and it is also a shared downstream target by SATB2 and RUNX2.[10,134] However, VEGF was not directly targeted by SATB2. As a target of Wnt/β-catenin signaling, RUNX2 is very likely involved in the SATB2-induced VEGF via Wnt/β-catenin pathway in CRC carcinogenesis. The HDAC4/RUNX2/VEGF pathway was investigated in chondrosarcoma, suggesting that reduced HDAC4 induced expression of RUNX2 and thereby increased VEGF to enhance angiogenesis, tumor growth, and metastasis.[86] RUNX2 level was found aberrantly increased in pancreatic cancer, and its expression was associated with poor prognosis.[27] SATB2 was also found highly expressed in pancreatic CSCs causing cell proliferation and EMT by inducing the expression of many pluripotency and stem cell markers such as NANOG, OCT-4, CD24, CD44, as well as BCL-2.[68] BCL-2 regulates cell apoptosis and is a pluripotency-maintaining factor directly targeted by SATB2.[68] However, in osteoblasts, deficient in BCL-2 was associated with increased expression of RUNX2 and other osteogenic markers such as OSX, COL1A1, OCN, and OPN, indicating a negative relationship between BCL-2 and osteoblast differentiation.[135,136] In MG-62, nitric oxide induced expression of BCL-2 by

### Table 3: SATB2 and Runt-related transcription factor 2 co-occurred in cancers as in the cBio genomic cancer portal queried Cancer Genome Atlas Database

| Cancer type                      | Log2 OR | P     | Q     | Tendency         |
|----------------------------------|---------|-------|-------|------------------|
| Testis cancer                    | >3      | 0.002 | 0.002 | Co-occurrence    |
| Head and neck squamous cancer    | >3      | 0.002 | 0.002 | Co-occurrence    |
| Bladder cancer                   | >3      | <0.001| 0.005 | Co-occurrence    |
| Melanoma                         | 2.47    | 0.006 | 0.14  | Co-occurrence    |
| Lung squamous cell carcinoma     | 1.846   | 0.026 | 0.219 | Co-occurrence    |
| Colorectal adenocarcinoma        | 2.409   | 0.087 | 0.087 | Co-occurrence    |
| Prostate cancer                  | 0.129   | 0.618 | 0.658 | Co-occurrence    |
| Breast cancer                    | 0.879   | 0.249 | 0.358 | Co-occurrence    |
| Papillary thyroid cancer         | 2.338   | 0.085 | 0.312 | Co-occurrence    |
| Cervical cancer                  | 2.249   | 0.247 | 0.829 | Co-occurrence    |

OR: Odds ratio
inducing RUNX2 and promoted cancer cell survival under oxidative stress conditions. Hypoxia-induced expression of RUNX2 increased cell viability by inducing the cell apoptosis-associated factor, BCL-2, resulting in apoptosis resistance in prostate cancer cells.

RUNX2 was upregulated in the HNSCC with lymph node metastasis and was identified as a key transcription factor in promoting HNSCC progression and metastasis. RUNX2 level was found negatively regulated by miR-376c, which mediated cell metastasis via the RUNX2/inhibitin subunit beta A axis. SATB2 was highly expressed in advanced HNSCC enhancing resistance to chemotherapy and radiation-induced cell death by promoting the dominant-negative p63a level and subsequently inhibiting proapoptotic Tap73b and p53 signaling. SATB2 is also a direct target of miR-376c (http://www.targetscan.org), indicating that RUNX2 and SATB2 could be co-regulated in HNSCCs.

RUNX2 expression is increased in bladder cancers (compared to noncancerous bladder tissue/cells), and it has been suggested that RUNX2 level in bladder cancer cells was regulated by miRNAs such as miR-217 and miR-154. Calcification is one of the symptoms of papillary thyroid carcinoma (PTC), and RUNX2 when induced by HOXA9 was associated with PTC calcification and invasiveness. An increased expression of RUNX2 was observed in melanoma cells, and RUNX2 regulated expression of matrix metalloproteases implicated in tumor invasion and metastasis, which further the conclusion that regulated RUNX2 expression is important to maintain specialized mesenchymal lineages. A Runt domain of RUNX2 was necessary for melanoma cell proliferation and cell migration, and RUNX2 may probably mediate cell malignancy by regulating downstream genes such as BSP, MMPs, and COL3. SATB2 was also found increased by an unknown mechanism in PTC, breast cancer (BC), and melanoma cancer cells as shown in the Human Protein Atlas Database. A summary of RUNX2 and SATB2 in various cancers is listed in Table 4.

**MicroRNAs in Runt-related transcription factor 2/SATB2 network in carcinogenesis**

Both SATB2 and RUNX2 have been implicated in the regulation of miRNA in cancers. MiRNAs connecting them with osteogenesis might also be important in RUNX2/SATB2-mediated carcinogenesis. miR-31 not only links RUNX2 and SATB2 with osteogenic differentiation but also negatively regulates SATB2 in mediating lung and colon cancer. miR-23a connects RUNX2 and SATB2 in osteoblasts, and it is also targeted by RUNX2 in mouse liver cancer cells to increase metastatic potential. A summary of miRNAs involved in RUNX2/SATB2 pathway and carcinogenesis is listed in Table 5.

miR-127 is an upstream regulator of RUNX2 in rat BMSCs (bone mesenchymal stromal cells). Osteosarcoma cells had reduced miR-127 level, while the restoration of miR-127 inhibited cell migration, invasion, and increased apoptosis. An inverse expression level between miR-153 and RUNX2 was observed in breast cancers and RUNX2 overexpression reversed cell proliferation, migration, and invasion of breast cancer cells which was suppressed by miR-153, miR-103 inhibited bone formation by acting as a mechanosensitive miRNA and directly targeting RUNX2 at its 3’UTR. It also prolonged the Wnt signaling and promoted CRC stemness along with miR-107 pathway. miR-34c downregulated RUNX2 in OS cells and was negatively regulated by p53, creating a novel network p53-miR-34c-RUNX2 in osseous cells and osteosarcoma (OS) cells. SATB2 was found upregulated when miR-34c was silenced by DNA methylation increasing cell metastasis and EMT in the CRC cells. miR-211 significantly reduced SATB2 level in hepatocellular carcinoma, resulting in the reduction of cell proliferation and migration. miR-211 with its homologous miR-204 negatively regulated RUNX2 to inhibit osteogenesis and promoted adipogenesis in mesenchymal progenitor cells and BMSCs. miR-205 was found to target RUNX2 in inhibiting pancreatic cancer progression. Together, this suggests that SATB2-driven miRNA expression may alter RUNX2 activity and that epigenetic alterations of SATB2 may affect normal osteogenesis or cell differentiation, thereby resulting in bone dysmorphia, osteosarcoma, or other cancers. Additional evidence is required to confirm the axis connected by miRNA under different cell context.

In addition, many downstream miRNAs regulated by SATB2 are also potential regulators for CBF-β with its 3’UTR directly targeted by miR-143, miR-128, miR-124, miR-125a, miR-381, and miR-326, as searched on the TargetScan. Other than by physically interacting with RUNX2 domains, this provides a new potential mechanism for SATB2 affecting CBF-β binding and cooperating with RUNX2 to regulate downstream genes [Figure 2].

**Conclusion**

It is known that both RUNX2 and SATB2 are key regulators of osteoblast differentiation and development. While many studies tend to place RUNX2 upstream of SATB2, the interaction between the two transcription factors is complex. They are linked by common upstream regulators and osteo-specific markers as well as multiple miRNAs, allowing them to act upstream or
Table 4: Runx-related transcription factor 2 and SATB2 in cancers

| Cancer type       | Gene    | Author                  | Year | Significant finding and conclusion                                                                                                                                 |
|-------------------|---------|-------------------------|------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Osteosarcoma      | SATB2   | Davis and Horva[87]      | 2016 | SATB2 is a sensitive marker for osteosarcoma                                                                                                                         |
|                   |         | Machado et al.[101]      | 2016 | SATB2 immunoexpressing can help to distinguish osteosarcoma from its mimickers, except between chondroblast osteosarcoma and high-grade chondrosarcoma                |
|                   |         | Xu et al.[102]           | 2017 | SATB2 plays an important role in regulating osteosarcoma stem cell-like properties and tumor growth, while metformin reduces the cancer-like phenotypes and tumor growth via inhibition of N-cadherin/NF-kB signaling |
|                   |         | Westendorf et al.[83]    | 2002 | RUNX2 promotes cell proliferation and inhibits cell death by interacting with HDAC6 and repressing p21 promoter in osteosarcoma cells                                 |
|                   |         | Sadikovic et al.[94]     | 2010 | RUNX2 is significantly overexpressed in osteosarcoma and is associated with poor chemotherapy response                                                              |
|                   |         | Galindo et al.[95]       | 2005 | RUNX2 level is not regulated by cell proliferation and remained high in osteosarcoma cells                                                                            |
|                   |         | Pereira et al.[96]       | 2009 | pRB, p53, and RUNX2 form a bone-specific regulatory network that controls normal cell cycle progression in osteoblasts and that is deregulated in osteosarcoma cells  |
| CRC               | SATB2   | Dragomir et al.[90]      | 2014 | SATB2 can be a diagnostic marker for primary and metastatic CRCs                                                                                                       |
|                   |         | Yu et al.[105]           | 2017 | SATB2/β-catenin/TCF-LEF pathway induces cellular transformation by generating cancer stem cells in colorectal cancer                                                       |
|                   |         | Yang et al.[117]         | 2013 | miR-31 mediates colorectal cancer progression by directly inhibiting SATB2 level                                                                                      |
|                   |         | Eberhard et al.[120]     | 2012 | SATB2 expression level can be a prognosis marker for colon cancer                                                                                                     |
|                   |         | Lin et al.[130]          | 2014 | SATB2 and cadherin-17 are sensitive and specific immunomarkers for colorectal carcinomas                                                                               |
|                   |         | Moh et al.[131]          | 2016 | High SATB2 expression can distinguish ovarian metastasis of colorectal origin from primary ovarian tumors                                                              |
|                   |         | Zhang et al.[132]        | 2018 | SATB2 can be a promising biomarker for identifying a colorectal origin from liver metastatic adenocarcinomas                                                           |
|                   |         | Wen et al.[126]          | 2017 | Inhibiting RUNX2 by miR-539 can repress colorectal cancer progression and can be a potential therapeutic approach                                                        |
|                   |         | Wang et al.[127]         | 2016 | CBX4 can suppress metastasis of colorectal carcinoma via inhibiting RUNX2 promoter                                                                                     |
|                   |         | Ji et al.[128]           | 2019 | MALAT1 elevates RUNX2 expression in CRC cells, and they are two biomarkers for predicting the recurrence and metastasis of CRC                                             |
| Breast cancer     | SATB2   | Yu et al.[70]            | 2017 | SATB2 is highly expressed in human breast cancer cell lines and may have a role in regulation of pluripotency, cell survival, and proliferation                         |
|                   |         | Patani et al.[89]        | 2009 | SATB2 mRNA expression is significantly associated with increasing tumor grade and poorer overall survival in breast cancer                                              |
|                   |         | Zuo Z, et al.[142]       | 2019 | Inhibition of RUNX2 by miR-153 can reverse breast tumor growth and metastasis, and miR-153/RUNX2 axis may be used as a potential therapeutic target in breast cancer treatment |
|                   |         | Barnes et al.[90]        | 2003 | RUNX2 expression in metastatic breast cancer cells and may explain the metastasize to the bone                                                                           |
| HNSCC             | SATB2   | Chung et al.[80]         | 2010 | SATB2 expression positively correlates with HNSCC chemoresistance, and knockdown of SATB2 re sensitizes HNSCC to chemotherapy- and γ-irradiation-induced apoptosis    |
|                   |         | Chang et al.[138]        | 2016 | RUNX2 is widely upregulated in HNSCC and downregulation of RUNX2 by miR-376c will suppress metastatic capability                                                        |
| Pancreatic cancer | SATB2   | Yu et al.[89]            | 2016 | SATB2 can induce dedifferentiation by inducing stemness and may have a role in pancreatic carcinogenesis and can be used as a diagnostic biomarker                     |
|                   |         | Kayed et al.[377]        | 2007 | RUNX2 is overexpressed in pancreatic cancer cells and is regulated by cytokines such as TGF-1 and BMP-2 to modulate the expression of extracellular matrix modulators   |
|                   |         | Zhuang et al.[143]       | 2019 | miR-205 is a tumor suppressor by targeting RUNX2 in pancreatic cancer to inhibit cell proliferation and migration                                                        |
| NSCLC             | SATB2   | Ma et al.[133]           | 2018 | SATB2 suppressed lung cancer cell invasion and metastasis and regulated the expression of EMT-related proteins and histone methylation by G9a                           |
|                   |         | Clancy et al.[90]        | 2012 | SATB2 is a commonly increased gene during metal-induced bronchiol epithelial cell transformation                                                                        |
|                   |         | Wu et al.[9]             | 2016 | SATB2 plays a pivotal role in Ni-induced bronchiol epithelial cell transformation                                                                                |
|                   |         | Chen et al.[114]         | 2018 | SATB2 plays an important role in arsenic-induced bronchiol epithelial cell transformation                                                                             |

Contd...
downstream of each other, or function in concert in the network that orchestrates osteogenesis. In this review, we describe how RUNX2 and SATB2 were involved in many cancers and improper expression of these proteins at a wrong time during osteogenesis or wrong place in other organs contributes to tumorigenesis. However, the exact pathway between the two proteins has not been well studied as these bone regulators act

Table 5: The microRNAs connecting Runt‑related transcription factor 2 and SATB2 in carcinogenesis

| miRNA   | Target gene | Regulator | Cancer type | Function/activity                                                                 |
|---------|-------------|-----------|-------------|----------------------------------------------------------------------------------|
| miR‑31[114,117] | SATB2*      | RUNX2     | Lung cancer, CRC           | Inhibition of lung epithelial cell transformation induced by arsenic; regulate CRC cell proliferation, invasion, and metastasis |
| miR‑23a[114]            | SATB2*      | RUNX2*    | Liver cancer               | Increase the metastatic potential of mouse liver cancer cell                        |
| miR‑153[140]            | RUNX2*      | SATB2     | Breast cancer              | Suppress breast cancer cell proliferation, migration, and invasion                 |
| miR‑127[140]            | RUNX2*      | SATB2     | Osteosarcoma               | Inhibit osteosarcoma cell migration, invasion, and induced cell apoptosis           |
| miR‑103[147]            | RUNX2*      | SATB2     | CRC                        | Promote CRC stemness                                                               |
| miR‑34c[141]            | SATB2*      | RUNX2*    | Osteosarcoma               | Regulate cell proliferation                                                        |
| miR‑34c[141]            | SATB2*      | CRC       | REG-3A                    | Regulate cell metastasis                                                           |
| miR‑205[142]            | RUNX2*      | SATB2     | Pancreatic cancer          | Inhibit pancreatic cancer progression                                               |
| miR‑211[143]            | SATB2*/RUNX2 | RUNX2     | Liver cancer               | Reduce cell proliferation and migration                                             |

*Experimentally confirmed. CRC: Colorectal cancer, miRNA: MicroRNA, RUNX2: Runt‑related transcription factor 2

Figure 2: An illustration of a potential pathway that SATB2 mediated core‑binding factor‑β binding with Runt‑related transcription factor 2 by inhibiting QA domain and microRNAs that target core‑binding factor‑β to induce the expression of Runt‑related transcription factor 2 downstream genes

Figure 3: An illustration of a possible SATB2 and Runt‑related transcription factor 2 network in carcinogenesis. SATB2 and Runt‑related transcription factor 2 are linked by multiple microRNAs in the pathways of carcinogenesis. Improper activation of Runt‑related transcription factor 2 might also be induced by SATB2 via β‑catenin/ NF‑κB pathway under a prolonged inflammation condition. Runt‑related transcription factor 2 and SATB2 also share many downstream targets such as B‑cell lymphoma 2, c‑MYC, and vascular endothelial growth factor in mediating cell transformation
in concert, independently, or in opposition depending on cellular conditions and context. This review provides a comprehensive summary of the current studies addressing the relationship between RUNX2 and SATB2 in osteogenesis and sheds light on their roles in carcinogenesis. A summary of RUNX2/SATB2 network involving transcription factors, and roles of different miRNAs in proteins, promoter-binding possibilities between the two proteins, promoter-binding possibilities between the two transcription factors, and roles of different miRNAs in their regulation under different cell context.

Financial support and sponsorship
This study was supported by the following National Institutes of Health grants: ES000260, ES023174, ES029359, and ES026138.

Conflicts of interest
There are no conflicts of interest.

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