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REVIEW ARTICLE

# SATB2: A versatile transcriptional regulator of craniofacial and skeleton development, neurogenesis and tumorigenesis, and its applications in regenerative medicine

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**Abstract** SATB2 (special AT-rich sequence-binding protein 2) is a member of the special AT-rich binding protein family. As a transcription regulator, SATB2 mainly integrates higher-order chromatin organization. SATB2 expression appears to be tissue- and stage-specific, and is governed by several cellular signaling molecules and mediators. Expressed in branchial arches and osteoblast-lineage cells, SATB2 plays a significant role in craniofacial pattern and skeleton development. In addition to regulating osteogenic differentiation, SATB2 also displays versatile functions in neural development and cancer progression. As an osteoinductive factor, SATB2

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holds great promise in improving bone regeneration toward bone defect repair. In this review, we have summarized our current understanding of the physiological and pathological functions of SATB2 in craniofacial and skeleton development, neurogenesis, tumorigenesis and regenerative medicine.


## Introduction

Fractures and bone defects are common and complicated clinical problems that pose a great challenge for the healthcare system and place a huge burden on the affected patients. At present, the most widely used substitute materials for bone reconstruction include autologous bone, allogeneic bone and synthetic bone.[1] Autologous and allogeneic bone grafts may increase donor site morbidity and have the potential risk of disease transmission, respectively.[2] On the other hand, the osteogenic capacity of the synthesized bone substitute materials are not as effective as those of autologous and allogeneic bone grafts.[1] Thus, bone tissue engineering is a promising therapeutic strategy to promote regeneration of bone tissue and repair bone defects. A biological factor that effectively stimulates osteogenic differentiation of mesenchymal stem cells (MSCs) is one of the most important components of successful bone tissue engineering. Therefore, as a potent osteoinductive factor, SATB2 may be a potential factor for bone reconstruction.

Special AT-rich sequence-binding protein (SATB) family proteins are key regulators of gene expression that mediate higher-order chromatin organization.[3–5] The SATB family members SATB1 and SATB2 share considerable sequence homology and play similar roles in transcriptional regulation as both of them are implicated in long-range enhancer function extension of chromatin modifications and dynamic tethering of chromatin loops.[4,6–10] The human *SATB2* gene is located in a gene-poor region of 2q32-q33, which was originally identified as KIAA1034, a near full-length cDNA (AB028957).[6,11] The SATB2 transcript is assembled from 11 exons spanning 191 kb, and encodes a large protein consisting of 733 amino acids with a molecular weight of 82.5 kDa SATB2 shows an extraordinarily high degree of evolutionary conservation, with only three amino acid substitutions between mouse and human.[6] SATB2 protein has 5 domains that are highly conserved with those of SATB1, including a ubiquitin-like domain (ULD), a CUT repeat-like (CUTL) domain, two CUT domains (CUT1 and CUT2) and a homeodomain (HOX) (Fig. 1).[6] As a nuclear matrix protein, SATB2 regulates gene transcription by directly binding to the core unwinding elements of nuclear matrix-attachment regions (MARs) through the CUT1 and CUT2 domains.[4,12,13] MARs have been identified as regulatory DNA sequences and play an important role in higher-order chromatin organization, long-range enhancer function, and extension of chromatin modifications.[14–16] While binding to MARs, SATB2 can modify the chromatin structure to regulate gene expression by interacting with histone deacetylase 1 (HDAC1) and metastasis-associated protein 2 (MAT2), which are members of the nucleosome remodeling and histone deacetylase complex.[17] Moreover, SATB2 also acts as a ''scaffolding'' protein that recruits other DNA-binding proteins to specific sub-nuclear sites and promotes their activities, thus participating in the regulation of gene expression.[18]

In this review, we first present the recent findings relevant to the upstream regulators of SATB2 expression, and then explore the contribution of SATB2 to craniofacial patterning, bone formation, and neural development. Next, we summarize the roles of SATB2 in tumorigenesis and the clinical use of SATB2 as a diagnostic biomarker of cancers. Finally, we outline the potential applications of SATB2 in regenerative medicine.

## Regulators of SATB2 expression and posttranslational modification

SATB2 exerts versatile functions in craniofacial patterning, osteoblast differentiation, cortical neuron differentiation and cancer initiation and progression.[18–20] The diverse biological roles of SATB2 are mediated by the regulation of ligand-receptor signaling. Here, the involved regulators of SATB2 gene expression and posttranslational modification are illustrated (Fig. 2A).

### Growth factors and cytokines secreted by cells

Bone morphogenetic proteins (BMPs) belong to the TGF-β superfamily, with diverse and critical biological functions including mesenchymal stem cell differentiation, bone formation, adipogenesis, angiogenesis, and metabolism.[21–26] The functions of BMPs are classically mediated through the canonical BMPR-Smad-dependent pathway.[27] Binding of BMP ligands to receptors activates receptor kinases, which leads to the phosphorylation of Smad proteins. These Smad proteins are necessary for BMP-induced osteogenic differentiation (Fig. 2A). Normal expression of BMP4 is critical for facial development; deletion or elevation of BMP4 in the cranial neural crest of mouse embryos results in severe defects of facial development.[28] *Satb2* mRNA expression was upregulated in the mandible of the mice with conditional overexpression of *Bmp4* in CNC, while significantly downregulated in the mice with *Bmp4* deletion in CNC, indicating that BMP4 may be an upstream regulator of SATB2.[28] Mechanistically, Smad1/5 directly binds to the conserved Smad recognition elements in the 5′ flanking region of *Satb2* gene to regulate its expression.[28] These

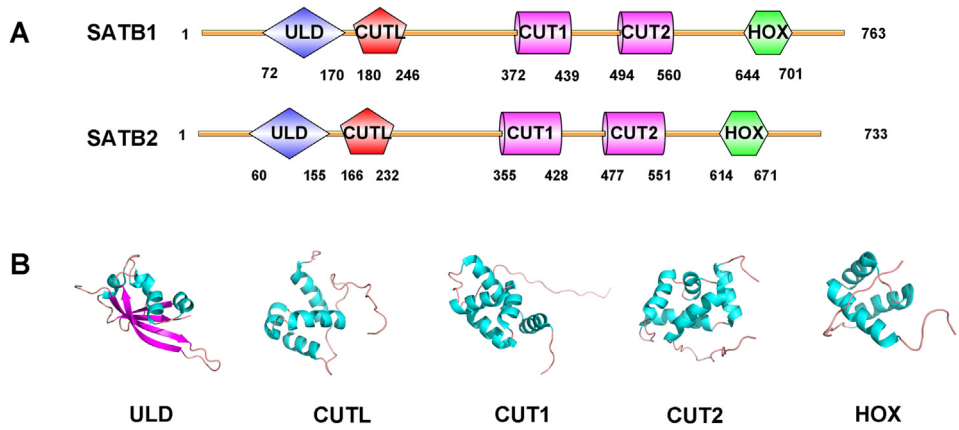


**Figure 1** Domain structure of the human SATB1 and SATB2 proteins. **(A)** Schematic representation of the domain organization of human SATB1 and SATB2. The 5 domains are highly conserved between SATB1 and SATB2, including a ubiquitin-like domain (ULD), a CUT repeat-like (CUTL) domain, two CUT domains (CUT1 and CUT2), and a homeodomain (HOX). **(B)** Cartoon representation of the structures of ULD (Protein Data Bank code 3TUO), CUTL (Protein Data Bank code 2L1P), CUT1 (Protein Data Bank code 1wiz), CUT2 (Protein Data Bank code 2CSF), and HOX (Protein Data Bank code 1WI3) domains.

findings suggest that SATB2 transcription is downstream of the BMP signaling pathway during facial development.

Osterix (OSX), a transcription factor specifically expressed in osteoblasts, is required for osteoblast differentiation and bone formation.[29] In *Osx-null* mice, no bone formation was observed although the cartilage developed normally.[29] Tang et al found that OSX might function as an upstream regulator of SATB2 since SATB2 expression was decreased in the calvaria of *Osx-null* mice embryos.[30] Knockdown of *Osx* expression in MC3T3 osteoblast cells by siRNA resulted in suppression of SATB2 expression.[30] In contrast, the overexpression of *Osx* in the C2C12 mesenchymal cell line enhanced SATB2 expression at both mRNA and protein levels.[30] Mechanistically, OSX can activate SATB2 expression by directly binding to the GC-rich binding sites in the proximal 130 bp of the *Satb2* promoter (Fig. 2A).[30] Taken together, the results of these studies indicate that Osx is an upstream regulator of SATB2 expression during osteoblast differentiation and bone formation.

Additionally, Apostolova et al found that SATB2 was rapidly and strongly induced by members of the neuropoietic cytokines, ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF), in postmitotic sympathetic neurons (Fig. 2A).[31] However, the mechanism by which these two cytokines trigger SATB2 expression in neurons remains unclear.

## Protein modifiers

The small ubiquitin-related modifier (SUMO) proteins are a family of conserved eukaryotic protein modifiers of approximately 100 amino acids.[32] As one of the posttranslational modifications, SUMO modification has been proved to regulate the activity of transcription factors.[4] SUMO covalently conjugates to internal lysine(s) of substrates by SUMO E1, E2, and E3 enzymes.[32] Dobreva et al found that SUMO was conjugated to the lysine residues of the SATB2 protein with the help of SUMO E3 ligase PIAS1.[4] The SUMO modification of SATB2 antagonizes its transcriptional activation and binding ability with MAR sequences, and influences its subnuclear localization (Fig. 2A).[4]

## Non-coding RNA (ncRNA) molecules

Non-coding RNA (ncRNA) molecules play diverse roles in various physiological and pathological processes by regulating target gene expression.[33–36] Long non-coding RNAs (lncRNA) are transcripts that are longer than 200 nucleotides with no protein-coding capacity.[37] LncRNA antisense transcript of SATB2 (SATB2-AS1) is an important regulator of colorectal carcinoma progression; it is expressed in normal colorectal tissues but downregulated in colorectal carcinoma.[20,38] LncRNA SATB2-AS1 might regulate colorectal carcinoma progression by activating SATB2 transcription in colorectal cells.[20,38] Xu et al found that lncRNA SATB2-AS1 cis-activated SATB2 transcription by recruiting WDR5 and GADD45A, and subsequently regulating DNA demethylation and H3K4me3 enrichment of the SATB2 promoter.[20] In another study, Wang et al suggested that lncRNA SATB2-AS1 might upregulate SATB2 expression by serving as a scaffold to recruit p300, thus accelerating histone H3 acetylation at the SATB2 promoter.[38] MicroRNAs (miRNAs) are small ncRNAs that regulate gene expression by binding to the 3′-untranslated region (UTR) of target mRNAs, thereby leading to mRNA degradation or translation inhibition.[39] Hepatocellular carcinoma (HCC) is the sixth most prevalent cancer worldwide, and miR-211 has been shown to be an inhibitor of hepatocellular carcinoma.[40] The expression of miR-211 has been found to be deregulated in HCC cancer tissues compared with adjacent non-neoplastic tissue.[40] Furthermore, luciferase reporter assays have demonstrated that SATB2 is a direct target of miR-211 and SATB2 reportedly rescued the proliferation and invasion ability of HCC cells inhibited by miR-211.[40] In fact, many other miRNAs can inhibit SATB2 expression by binding to its 3′UTR via base-pairing, including miR31, miR-34, miR-166, mi366, and mi383 (Fig. 2A).[41–52] These findings strongly suggest that ncRNAs play an important role in the regulation of SATB2 expression during tumor occurrence and progression.

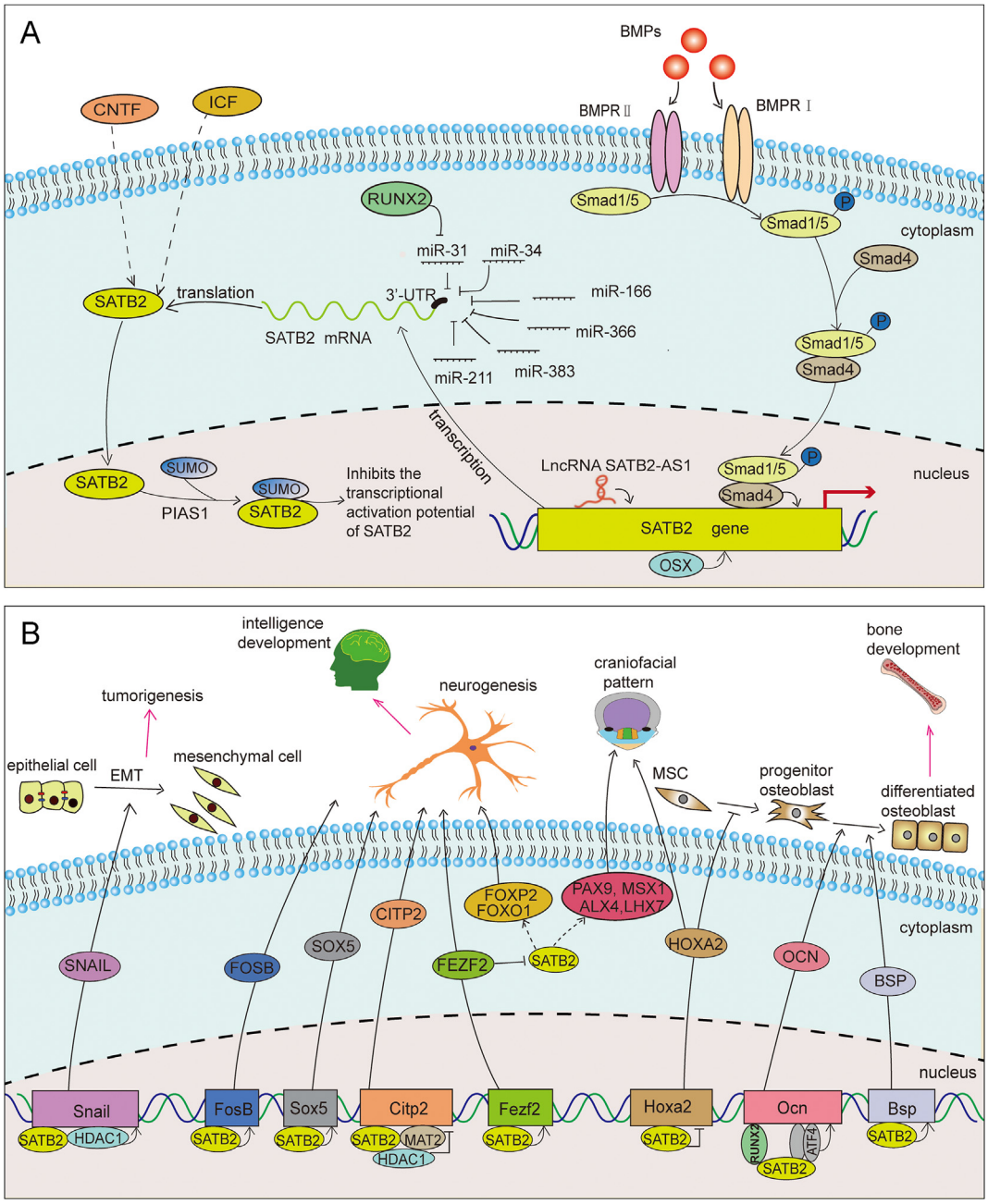


**Figure 2** The regulative mechanism and the function of SATB2. **(A)** SATB2 is regulated by various cellular signaling molecules, including growth factors and cytokines secreted by cells, protein modulators and non-coding RNA molecules. **(B)** SATB2 plays versatile roles in craniofacial pattern and bone development, neurogenesis and tumorigenesis by regulating downstream effectors. The proteins are marked with cycles, and genes are marked with rectangles.

Nonetheless, the regulation of SATB2 expression by various cell signals in different biological processes is complicated. Only a small number of regulators have been identified. Therefore, more research is warranted to gain a better understanding of the underlying mechanisms behind the control of SATB2 expression.

## SATB2 in craniofacial development

Craniofacial development is based on cranial neural crest (CNC) cells that migrate and aggregate at branchial arches and differentiate into multiple cell types to form specific components of the facies cranii.[53–57] In mouse embryos, SATB2 expression is detected in the maxillary component of the first pharyngeal arch and the lateral aspect of the frontonasal process at E10.5.[6] Then SATB2 expression demarcates the region of the medial maxillary process within the primitive oral cavity at E11–11.5. At E12.5, SATB2 expression is seen in the medial edges of the developing palatal shelves and this continues until E13.5 when the strongest expression is in the mesenchyme underlying the medial edge epithelia. At E14.5, the time of palatal shelf

fusion, the SATB2 expression is dramatically down-regulated.[6] The functions of SATB2 in craniofacial development have been uncovered in studies of *Satb2* knockout mice.[18,58] Dobreva et al targeted inactivated the *Satb2* gene in mouse embryonic stem (ES) cells through insertion of the bacterial b-galactosidase (*LacZ* ) gene immediately downstream of the first ATG codon in exon 2.[18] Mice carrying a mutant *Satb2-LacZ* allele were mated to generate heterozygous and homozygous mutant mice. Dobreva et al reported that heterozygous *Satb2*$^{+/-}$ mice were phenotypically normal and fertile, while homozygous *Satb2*$^{-/-}$ mice showed obvious craniofacial defects and died immediately after birth.[18] However, in another study, Britanova et al found that *Satb2* haploinsufficiency caused craniofacial abnormalities and these defects were amplified in *Satb2* homozygotes.[58] The discrepancy between the two studies may have been caused by differences in targeting strategies. Britanova et al constructed *Satb2* deficient mice by elimination of the second exon in the protein-coding region of *Satb2* gene and replacement with a *Cre recombinase*—coding sequence and a *Neo* expression cassette.[58]

The *Satb2*$^{-/-}$ mice showed a shorter lower jaw and were missing the anterior part of the mandible, including the incisors.[18,58] Other craniofacial abnormalities were also observed in *Satb2*$^{-/-}$ mice, including microcephaly, cleft palate, and nasocapsular and premaxillary hypoplasia.[18,58] Furthermore, the horns and the body of hyoid bone which are derived from the second and third branchial arches, were also malformed.[18] These defects in *Satb2*$^{-/-}$ mice are similar to the phenotypes resulting from *SATB2* deletions and translocations in humans.[18,59] Patients with alterations in the *SATB2* gene manifest with cleft palate, microcephaly, facial dysmorphic features, micrognathia, and dental anomalies.[59–62] To uncover the mechanistic basis of the morphologic defects caused by *Satb2* gene mutations, the expression of candidate genes that are correlated with craniofacial development were investigated in *Satb2*$^{-/-}$ mice, including *Pax9, Msx1, Alx4,* and *Lhx7*.[63–73] *Pax9* expression levels in the mandibular incisor field as well as those of *Msx1* and *Alx4* in the developing palate, were markedly decreased in *Satb2*$^{-/-}$ embryos.[58] Additionally, the expression of *Lhx7* was 5-fold decreased in the cells of head and branchial arch regions in *Satb2*$^{-/-}$ embryos compared to that in the wild-type.[18] Both of the *Pax9*$^{-/-}$ mice and *Msx1*$^{-/-}$ mice show a cleft plate and tooth agenesis,[68,74] which is also observed in *Satb2*$^{-/-}$ mice. *Alx4* controls the patterning of the nose and maxillary regions,[69] the abnormal nasocapsular and premaxilla in *Satb2*$^{-/-}$ embryos may owing to the decrease of *Alx4*. Deletion of *Lhx7* in mouse embryos results in a cleft secondary palate but other craniofacial structures appear to be normal that are affected in *Satb2*$^{-/-}$ embryos.[63] The changes of *Pax9, Alx4, Msx1, and Lhx7* expression in *Satb2*$^{-/-}$ mutants suggest that SATB2 may play a role in craniofacial development by regulating the expression of these genes. Furthermore, *Satb2*$^{-/-}$ mice showed enhanced apoptosis in the craniofacial mesenchyme of the mandible, maxillary, and medial frontonasal process regions, where SATB2 is normally expressed in wild-type mice.[58] The results indicate that SATB2 participates in craniofacial mesenchyme cell proliferation and survival, which may also provide a partial mechanistic explanation for the defects in *Satb2*$^{-/-}$ mice. Together, these data suggest that SATB2 may have a profound effect on craniofacial development by regulating gene expression associated with craniofacial development and affecting craniofacial mesenchymal cell survival.

## The essential roles of SATB2 in bone and skeletal development

The function of SATB2 is not restricted to the craniofacial development, in fact, later stages of *Satb2*$^{-/-}$ embryos demonstrate delayed bone formation at E15.5.[18] SATB2 is expressed in the osteoblast lineage of wild-type mouse embryos, and the *Satb2*$^{-/-}$ embryos have demonstrated delayed bone formation, defective extracellular matrix deposition, and shorter and thinner trabecula as compared to those of wild-type.[18] Multiple studies have indicated that SATB2 affects the expression of several key factors that regulate osteoblast differentiation.[18,75–77] It appears that SATB2 acts as a molecular node of a transcriptional network regulating osteoblast differentiation and bone formation. In the following sections, we review the various factors that interact with SATB2 during bone and skeletal development.

### SATB2 inhibits HOXA2-mediated suppression of osteogenic differentiation

HOXA2 is an inhibitor of bone formation and plays a role in branchial arch patterning.[78] The *Hoxa2*$^{-/-}$ mice displayed abnormal craniofacial development, and ectopically activated chondrogenesis.[78,79] In E15.5 *Satb2*$^{-/-}$ mice, HOXA2 expression was markedly increased in the calvarial bones and long bones.[18] qPCR analysis revealed that *Hoxa2* mRNA was 10 times higher in the osteoblasts isolated from *Satb2*$^{-/-}$ mice than that from the *Satb2*$^{+/-}$ group, and it was partially repressed by retrovirus-mediated SATB2 overexpression.[18] These results indicate that SATB2 can inhibit HOXA2 expression in osteoblasts. Additionally, it has been shown that HOXA2 expression is self-regulated by the intragenic EII enhancer in the 3′ region of the *Hoxa2* gene.[80,81] Chromatin immunoprecipitation (ChIP) and electrophoretic mobility shift assay (EMSA) experiments have revealed that SATB2 directly binds to a sequence containing the MAR element of the EII enhancer.[18] Furthermore, SATB2 effectively suppressed the activity of the *Hoxa2* EII enhancer. Finally, the study found that the defects of calvarial bone formation in *Satb2*$^{-/-}$ mice were largely rescued in *Satb2*$^{-/-}$*Hoxa2*$^{-/-}$ mice, indicating that inactivation of the *Hoxa2* gene can effectively overcome the bone defects caused by *Satb2* deletion.[18] Taken together, these results suggest that the inhibition of *Hoxa2* in osteoblasts mediated by the binding of SATB2 to the EII enhancer is necessary for normal osteoblast differentiation.

### SATB2 interacts with RUNX2 and forms a regulatory loop to control osteogenic differentiation

The RUNX family of transcription factors participate in cell differentiation and proliferation, bone formation, and embryonic and organ development.[82–85] RUNX2 is a master regulator of osteoblast differentiation and bone formation as

targeted disruption of *Runx2* in mice results in bone formation defects.[82,86–89] Dobreva et al reported that *Satb2*$^{-/-}$ mice showed defects in osteoblast differentiation.[18] Since RUNX2 is required for normal osteoblast differentiation, Dobreva's group carried out a series of experiments to investigate the possible relationship between SATB2 and RUNX2. Real-time qPCR analysis revealed decreased expression of RUNX2 in calvarial osteoblasts in *Satb2*$^{-/-}$ mice, compared to that of wild-type mice, suggesting that there might be an association between RUNX2 and SATB2. Moreover, molecular analysis has demonstrated that SATB2 physically interacts with the 108 amino-terminal residues of RUNX2 and augments RUNX2-mediated gene activation by enhancing its DNA binding (Fig. 2B).[18] Additionally, the *Satb2*$^{+/-}$*Runx2*$^{+/-}$ mouse embryos demonstrated severe bone defects that were not found in single heterozygous mice, indicating that *Satb2* and *Runx2* genes might interact genetically.[18] Thus, SATB2 may interact with RUNX2 physically and genetically during osteoblast differentiation and bone formation.

Interestingly, emerging evidence indicates that the expression of SATB2 may be regulated by RUNX2 via miR31.[48] miR-31 has been reported to be a pleiotropically acting miRNA, which can inhibit SATB2 expression by binding to the 3′UTR of *Satb2* mRNA.[49] Deng et al reported that miR31 inhibited osteogenic differentiation ability and reduced SATB2 protein level in adipose tissue-derived stem cells (ASCs).[90] Additionally, RUNX2 directly inhibited miR-31 expression by binding to the consensus RUNX2-binding site (AACCACA) of the miR-31 promoter.[90] These studies strongly suggest that RUNX2-mir-31-SATB2 may act as a regulatory loop in the osteogenic differentiation of mesenchymal stem cells.

### SATB2 physically interacts with ATF4 and augments its transcriptional activity

Activating transcription factor 4 (ATF4) is critical for osteoblast differentiation and function by regulating the expression of several osteoblastic genes, including *Ocn*, *Rankl* and *Esp*.[91–94] *Atf4*$^{-/-}$ mice display a phenotype of delayed skeletal development and bone formation, resembling the phenotype of *Satb2*$^{-/-}$ mice,[18] which suggests that there might be physical and/or functional interactions between ATF4 and SATB2. Both *Satb2*$^{+/-}$ and *Atf4*$^{+/-}$ mice displayed normal formation of trabeculae, whereas double heterozygous mice showed a noticeable reduction of bone formation, suggesting that SATB2 might interact genetically with ATF4.[18] The His- and GST-pulldown experiments further demonstrated that the interactions of SATB2 with ATF4 are based on direct physical association. Moreover, EMSA experiments revealed that SATB2 enhanced the DNA binding of ATF4 to OSE1, the binding site of ATF4 to the *Ocn* promoter (Fig. 2B).[18] Thus, these results indicated that SATB2 interacts physically and genetically with ATF4, and hence augments DNA binding and transactivation mediated by ATF4.

### BSP and OCN are important downstream targets of SATB2 during osteogenic differentiation

Bone sialoprotein (BSP) is an early maker of osteoblast differentiation, which contributes to the development and maturation of preosteoblasts to osteoblasts and the activation of bone formation (Fig. 2B).[95,96] Dobreva et al have demonstrated that *Bsp* is one of the most significantly down-regulated genes in the bones of *Satb2*$^{-/-}$ embryos.[18] *Bsp* mRNA expression was about 12-fold decreased in the osteoblasts isolated from *Satb2*$^{-/-}$ mice than that from the *Satb2*$^{+/-}$ group, whereas it was rescued by transduction of *Satb2*$^{-/-}$ cells with a SATB2-expressing retrovirus.[18] ChIP and EMSA analysis revealed that SATB2 directly binds to the *Bsp* promoter region that includes three osteoblast-specific sequence elements.[18] These results suggest that BSP is a direct target of SATB2 during osteogenesis (Fig. 2B).

Osteocalcin (OCN), a marker of osteoblast terminal differentiation, is synthesized by osteoblasts.[97] As the most abundant non-collagenous protein in the extracellular matrix of bone, OCN is closely related to mature bone matrix mineralization.[98,99] Similar to *Bsp*, the expression of *Ocn* is also regulated by SATB2.[18] OCN protein and mRNA expression were significantly decreased by *Satb2* deletion in both mouse embryos and osteoblasts. Mechanistically, overexpression of SATB2 in *Satb2*$^{-/-}$ osteoblasts enhanced the acetylation of histone H3 and the binding of RNA polymerase II in the *Ocn* promoter.[18] Several studies have shown that the *Ocn* promoter is regulated by RUNX2 and ATF4, which bind the nonadjacent sequence elements OSE2 and OSE1, respectively.[100,101] It is conceivable that SATB2 may couple ATF4 and RUNX2 through physical interaction to enhance their DNA binding to the *Ocn* promoter and then to promote the expression of OCN in an indirect fashion (Fig. 2B). Taken together, SATB2 plays a role in osteogenic differentiation partially by regulating the expression of BSP and OCN.

## Essential roles of SATB2 in neural development

Numerous studies have focused on the spatiotemporal expression and possible functions of SATB2 in the nervous system.[102–105] In mouse embryos, SATB2 expression was detected in the rhombomere region of the hindbrain at E8.5, in the cerebral cortex at E12.5, and in the spinal cord at E15.5.[18] In adult mouse, SATB2 is widely expressed in the cerebral cortex, hippocampus, bed nucleus of the stria terminalis, horizontal limb of the diagonal band, spinal cord, lateral hypothalamic area, arcuate nucleus, hypothalamic paraventricular nucleus, and so on.[106] However, the potential functions of SATB2 in neural development remain yet to be fully elucidated.

In the developing cerebral cortex, SATB2 is expressed in cortical neurons that extend axons across the corpus callosum.[102] However, the corpus callosum was absent in *Satb2*$^{-/-}$ mice, and pyramidal neurons extended axons subcortically instead of projecting callosally.[102,103] CTIP2, a transcription factor that is critical for the extension of subcerebral projections by cortical neurons, was found ectopically expressed in the upper-layer neurons in the absence of *Satb2*.[102,103,107] Conversely, overexpression of SATB2 in neural stem cells inhibited CITP2 expression.[102] Mechanistically, SATB2 binds to the upstream promoter region of *Ctip2* gene and interacts with histone deacetylase HDAC1 and MTA2 to repress *Ctip2* expression.[103,108] The results indicate that SATB2 is necessary for normal

development of callosal projection neurons by acting as a repressor of CTIP2 (Fig. 2B).

On the other hand, SATB2 is also required for establishing subcerebral projection neuron identity and corticospinal tract (CST) formation.[19,109] The Forkhead box transcription factors FOXO1 and FOXP2, which are normally expressed in subcerebral projection neurons (SCPN), were lost in Layer 5 and 6 in *Satb2*$^{-/-}$ mice,[109] indicating that SATB2 might be important for the differentiation of subcerebral projection neurons. Moreover, the *Satb2*$^{-/-}$ mice caused a failure of the corticospinal tract (CST) formation.[109] McKenna et al found that SATB2 directly activated transcription of *Fezf2* and *Sox5*, transcription factors critical for subcerebral neuron development.[110–115] Furthermore, FEZF2 in turn inhibited high-level SATB2 expression in subcerebral neurons (Fig. 2B).[19] Thus, these data indicate that the feedback regulatory loop between SATB2 and FEZF2 may be essential for the fate determination of subcerebral projection neurons.

SATB2 may also play a role in intelligence development. In humans, alterations of the *SATB2* gene result in developmental delay and intellectual disability.[59,116,117] The *Satb2* mutant mice also displayed manifestations similar to those of human *SATB2*-mutated patients. Spatial and episodic memory are important cognitive abilities that have been linked to the hippocampus.[118] SATB2 was detected in the hippocampal CA1 region and activation of hippocampal neuronal activities increased the expression level of SATB2.[119,120] Li et al revealed that both of the *Satb2*$^{+/-}$ mice and the mice conditional knockout the *Satb2* in pyramidal neurons were defective in long-term spatial memory and short-term spatial working memory, which might be correlated with decreased neuronal spine density and dendrites in the hippocampus due to *Satb2* deletion.[119] Mechanistically, SATB2 has been shown to regulate *FosB* expression by binding to its promoter.[119] *FosB* is one of the immediate early genes (IEGs), which play an important role in synaptic plasticity and long-term memory formation.[121] Thus, SATB2 may regulate working memory and spatial memory through the orchestration of IEGs-mediated hippocampal synaptic plasticity. In another study, Jaitner et al found that the deletion of *Satb2* in the forebrain of mice resulted in defective long-term memory, and impaired the stabilization of synaptic long-term potentiation.[120] They have further demonstrated that SATB2 plays a role in synaptic plasticity and memory formation by controlling the expression of genes and miRNAs related to learning and memory in the CA1 hippocampal field.[120]

Additionally, expression of SATB2 in neurons in the parabrachial nucleus is critical for encoding sweet taste.[122] SATB2 also acts as a regulator of sympathetic neurons which switch their neurotransmitter phenotypes from noradrenergic to cholinergic.[31] Taken together, SATB2 plays an essential role in neural development and normal function of the nervous system, although the underlying mechanisms remain to be understood.

## Paradoxical roles of SATB2 in the development and progression of human cancer

Recent studies have demonstrated that SATB2 may play an important role in cancer progression by regulating cell proliferation, apoptosis, metastasis and invasion (Table 1).[123,124] Here, we briefly review the current findings of SATB2 in the development and/or progression of human cancer.

### SATB2 suppresses the stemness and epithelial-to-mesenchymal transition of cancer stem cells in colorectal cancer

Colorectal cancer (CRC) is the third most common cancer worldwide, with approximately 600,000 cancer-associated mortalities per year.[125] Numerous studies have demonstrated that SATB2 is a biomarker for CRC diagnosis and prognosis, as well as a sensitive marker to distinguish CRC from other cancer types.[41,126,127] SATB2 is highly expressed in the epithelium of the lower gastrointestinal tract.[126] Wang et al reported that both mRNA and protein levels of SATB2 were significantly reduced in CRC tissue, compared to those in the normal colon tissue samples.[123] They further demonstrated that low SATB2 levels were associated with tumor invasion and metastasis and poor survival in patients with CRC. *In vitro*, silencing of *Satb2* promoted migration, colony formation, and adhesion of CRC cells, while overexpression of SATB2 repressed migration and proliferation of the CRC cells, as well as their self-renewal ability.[127] Cancer stem cells represent a small number of cancer cells with stem cell-like properties that can promote tumor-initiating, cancer relapse, and metastasis.[128,129] Knockdown of SATB2 in colorectal cancer

**Table 1** Correlation of SATB2 expression with patient survival across different cancer types.

| Cancer type | Expression level of SATB2 compared with normal tissue | High expression of SATB2 Poor prognosis | High expression of SATB2 Good prognosis |
|---|---|---|---|
| Colorectal cancer | Lower | | Yes |
| Osteosarcoma | Higher | Yes | |
| Hepatocellular carcinoma | Higher | Yes | |
| Breast cancer | Higher | Yes | |
| Head and neck squamous cell carcinoma | Higher | Yes | |
| Non-small-cell lung carcinoma | Lower | | Yes |
| Laryngeal carcinoma | Lower | | Yes |
| Esophageal squamous cell carcinoma | Lower | | Yes |
| Ovarian endometriod carcinoma | Lower | | Yes |

cells led to an increase in the expression of key cancer stem cell markers, including CD133, CD44, MEIS2, and AXIN2.[127] Furthermore, SATB2 was shown to affect the expression of the cancer stem cells marker genes by binding to their regulatory elements.[127] The above-mentioned findings strongly suggest that SATB2 may exhibit an inhibitory function in colorectal cancer progression via downregulation of the stemness of colorectal cancer stem cells by binding to the regulatory elements of certain stem cell markers.

The epithelial-to-mesenchymal transition (EMT), a dynamic process through which epithelial cells acquire mesenchymal features, is crucial for tumor initiation and progression (Fig. 2B).[130–132] Gu et al found that the expression of the epithelial marker E-cadherin was decreased in CRC cells when *Satb2* was silenced, whereas the mesenchymal markers N-cadherin and vimentin were increased.[50] Conversely, overexpression of SATB2 in CRC cells resulted in increased expression of E-cadherin and decreased expression of N-cadherin and vimentin. These results indicate that SATB2 may suppress EMT in colorectal cancer. SNAIL is a key transcription factor of EMT and can activate the process of epithelial-to-mesenchymal transition.[133–135] Wang et al found that overexpression of SATB2 attenuated the mRNA and protein levels of SNAIL in CRC cells.[38] Subsequently, they identified three potential SATB2-binding regions within an AT-rich sequence in the *Snail* promoter and further demonstrated that SATB2 inhibited *Snail* transcription by recruiting HDAC1 to the *Snail* promoter (Fig. 2B). Taken together, these results suggest that SATB2 inhibits the progression of colorectal cancer mainly by inhibiting the stemness of cancer stem cells within the tumor and suppressing epithelial-to-mesenchymal transition.

## SATB2 promotes the growth and progression of human osteosarcoma

Osteosarcoma (OS) is the most common primary malignant bone tumor in children and young adults with a poor prognosis due to early metastasis and intrinsic therapeutic resistance.[136] As an important player in bone development, SATB2 has been implicated in osteosarcoma development and progression.[137–140] SATB2 has been shown to be highly expressed in osteosarcoma and associated with a poorer clinical outcomes (Table 1).[138,139] Seong et al established *Satb2* knockdown osteosarcoma (OS) cell lines and found that knockdown of *Satb2* markedly decreased migration and invasion ability of osteosarcoma (OS) cells.[138] Gene expression profiling analysis of the *Satb2* knockdown OS cells identified that the actin-binding protein, epithelial protein lost in neoplasm (EPLIN), is one of the most unregulated genes in *Satb2* knockdown OS cells.[138] Downregulation of the expression of EPLIN partially rescued the inhibited invasion phenotype in *Satb2* knockdown OS cells.[138] These findings indicate that SATB2 may modulate the invasion phenotype of OS, partially mediated by affecting EPLIN. It has also been reported that N-cadherin is an essential target gene of SATB2, and that SATB2 promotes osteosarcoma growth via the N-cadherin/NF-Kb pathway.[139] These results suggest that SATB2 plays an important role in osteosarcoma progression and is a potential target for future treatments.

## SATB2 as a prognostic marker for many types of human cancer

Increased expression of SATB2 has been shown in hepatocellular carcinoma tissue compared to that in normal liver tissues and SATB2 promotes the epithelial–mesenchymal transition in hepatocellular carcinoma cells (Table 1).[40,141,142] It has also been reported that miR-34a and miR211 inhibits hepatocellular carcinoma progression by downregulating SATB2.[40,142] Regarding breast cancer, SATB2 induces normal human breast mammary epithelial cells to transform into progenitor-like cells leading to a malignant phenotype. The high expression of SATB2 is correlated with shorter overall survival in breast cancer.[143,144] Furthermore, SATB2 enhanced chemoresistance of head and neck squamous cell carcinoma, and higher expression of SATB2 showed a significant association with poorer prognosis.[145]

On the contrary, the expression of SATB2 was found to be decreased in non-small-cell lung carcinoma, and the downregulation of SATB2 was related to poor prognosis.[146–148] Furthermore, silencing SATB2 in non-small-cell lung carcinoma cells induced EMT by upregulating EMT-inducing transcription factors including *Slug, Twist, and Zeb1*.[147] In laryngeal carcinoma, esophageal squamous cell carcinoma, and ovarian endometrioid carcinoma, the expression of SATB2 protein in carcinoma tissues was much lower than that in paracarcinoma tissues. As expected, the overall survival rate of patients with high SATB2 expression was higher than those with low SATB2 expression in these cancers.[149–151]

Taken together, these results indicate that the expression of SATB2 and the correlation of SATB2 expression with patient survival vary significantly across cancer types as summarized in Table 1, suggesting that SATB2 may play complex roles in cancer development and progression, and its functions may be cell- or context-dependent. Thus, more research is required to gain a better understanding of the molecular mechanisms of SATB2 in tumorigenesis.

# Potential applications of SATB2 in regenerative medicine

Given the fact that SATB2 plays an important role in regulating osteoblast differentiation and skeletogenesis, it is conceivable that SATB2 may be used as a promising osteogenic factor for bone regeneration (Fig. 3). Titanium implants are widely used for restoring missing teeth in dental clinics. Osseointegration, also called direct bone-to-implant contact (BIC), is achieved by the bone regeneration around the implant and plays a key role in successful dental implants.[152,153] Yan et al investigated the function of SATB2 in promoting osseointegration of dental implants in a mouse model.[154] The retroviral vectors (pBABE-*Satb2* and pBABE-hygro) were locally applied to the bone defects on the femurs of mice, and then the implants were placed. Three weeks after implantation, local application of SATB2 augmented new bone formation around the implants and

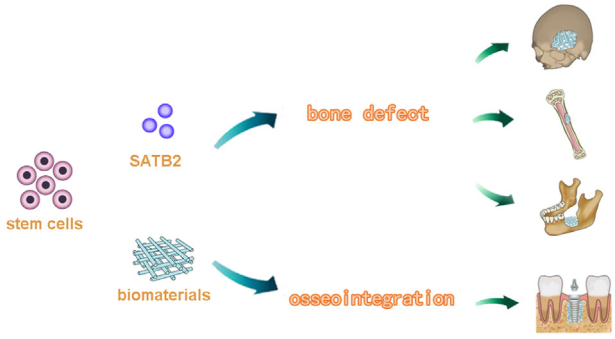


**Figure 3** Potential use of SATB2 in regenerative medicine. SATB2-modified stem cells can be transplanted on or in biomaterial scaffolds to promote tissue regeneration. For example, application of SATB2 protein and stem cells loaded on scaffolds to repair bone defects of the calvaria, long bone, and mandible as well as to enhance the osseointegration of dental implants.

promoted osseointegration on the bone-implant interface by upregulating the expression of osteogenic transcription factors and bone matrix proteins.[154] Another study from the same group used pBABE-*Satb2* retroviral vector to transduced bone marrow mesenchymal stem cells (BMSCs), and then administered the BMSCs to the bone defects in the mouse femurs prior to titanium implant placement.[155] They found that overexpression of SATB2 in BMSCs significantly enhanced osseointegration of dental implants. These findings demonstrated that SATB2 effectively enhanced the osseointegration of dental implants.

Bone defects are a common and frequent disease in the clinic, and the major goal of bone defects treatment is bone regeneration. Ye et al demonstrated the potency of SATB2 to repair calvarial defects in nude mice.[156] In their studies, induced pluripotent stem cells (iPSCs) were transduced with SATB2 overexpressing plasmid (pBABE-hygro-*Satb2*) and they found that the mRNA levels of several key osteogenic genes, including *Osx, Runx2, Bsp and Ocn,* were increased in *Satb2*-transduced iPSCs while the levels of *Hoxa2*, which inhibits osteogenic differentiation were decreased.[156] The *Satb2*-transduced iPSCs and control iPSCs were seeded onto a silk scaffold and then transplanted into the surgically induced calvarial defects in nude mice. Five weeks after transplantation, both micro-computed tomography and histological analysis revealed that the group that received *Satb2*-transduced iPSCs in silk scaffolds had better regeneration of new bones in the calvarial defects compared to that in the control group.[156]

Another compelling study demonstrated the clinical applicability of SATB2 in the repair of alveolar bone defects. Zhang et al found that transduction of adult stem cells (dental follicle cells and bone marrow stromal cells) with *Satb2* retroviral vectors promoted osteogenic differentiation and angiogenic activity *in vitro*.[77] They further showed that more transplanted cells underwent osteogenic differentiation in the *Satb2*-overexpressing group than those of the control group, and new bone formation in alveolar bone defects was consequently accelerated in the *Satb2*-overexpressing groups.[77] These results provide promise of SATB2 induction and stem cell transplantation as a therapeutic strategy to repair bone defects.

In addition to repairing bone defects, SATB2 has also been shown to be effective in preventing alveolar bone loss in the ovariectomized rat model.[157,158] The alveolar bone of ovariectomized rats displayed decreased bone volume, sparser trabecular bone, and fewer osteoblasts compared to those of the sham-controlled group.[158] Moreover, BMSCs originated from the alveolar bone of ovariectomized rats displayed senescent phenotypes such as low proliferation, diminished stemness and osteogenic capacity.[158] However, overexpression of SATB2 could rejuvenate these senescent BMSCs from OVX rats, and systemic injection of SATB2-overexpressing BMSCs into ovariectomized rats markedly attenuated alveolar bone loss.[158] These results not only demonstrate the potential of the clinical application of SATB2, but also the promise of using BMSCs in preventing alveolar bone loss.

## Conclusion and future directions

SATB2 is a nuclear matrix protein that orchestrates many aspects of physiological and pathological processes by regulating gene transcription. As a molecular node in a transcriptional network regulating osteoblast differentiation, SATB2 plays a crucial role in craniofacial pattern and skeletogenesis. Increasing evidence has demonstrated that SATB2 is an effective osteogenic factor and may be utilized as a regenerative agent in osseous defects. In addition to the osteoinductive properties, SATB2 also has implications for neural development and cancer progression. Thus, SATB2 may be explored as a potential diagnostic and/or prognostic biomarker for the clinical management of neural disorders and cancer. However, the molecular mechanisms through which SATB2 functions in osteogenesis and other biological processes remain unclear and warrant further investigation to explore the potential of SATB2 in clinical treatment.

## Conflict of interests

The authors declare no competing conflict of interest.

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