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REVIEW

Normal and disease-related biological functions of Twist1 and underlying molecular mechanisms

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This article reviews the molecular structure, expression pattern, physiological function, pathological roles and molecular mechanisms of Twist1 in development, genetic disease and cancer. Twist1 is a basic helix-loop-helix domain-containing transcription factor. It forms homo- or hetero-dimers in order to bind the Nde1 E-box element and activate or repress its target genes. During development, Twist1 is essential for mesoderm specification and differentiation. Heterozygous loss-of-function mutations of the human *Twist1* gene cause several diseases including the Saethre-Chotzen syndrome. The Twist1-null mouse embryos die with unclosed cranial neural tubes and defective head mesenchyme, somites and limb buds. Twist1 is expressed in breast, liver, prostate, gastric and other types of cancers, and its expression is usually associated with invasive and metastatic cancer phenotypes. In cancer cells, Twist1 is upregulated by multiple factors including SRC-1, STAT3, MSX2, HIF-1α, integrin-linked kinase and NF-κB. Twist1 significantly enhances epithelial-mesenchymal transition (EMT) and cancer cell migration and invasion, hence promoting cancer metastasis. Twist1 promotes EMT in part by directly repressing E-cadherin expression by recruiting the nucleosome remodeling and deacetylase complex for gene repression and by upregulating Bmi1, AKT2, YB-1, etc. Emerging evidence also suggests that Twist1 plays a role in expansion and chemotherapeutic resistance of cancer stem cells. Further understanding of the mechanisms by which Twist1 promotes metastasis and identification of Twist1 functional modulators may hold promise for developing new strategies to inhibit EMT and cancer metastasis.

***Keywords*:** Twist1; development; differentiation; cancer; epithelial-mesenchymal transition; metastasis
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Introduction

Organogenesis, which involves morphogenesis and cytodifferentiation, requires intricate and delicate interplays among transcription factors, growth factors, cell surface molecules and extracellular matrix proteins. Post-fertilization, the multicellular blastula forms and undergoes gastrulation, resulting in mesoderm formation between the endoderm and the ectoderm. *Twist1* was originally identified in *Drosophila* as one of the zygotic genes essential for mesoderm specification and subdivision into different tissue types and dorsal-ventral patterning during early embryo development [1-3]. The name "*Twist*" was given to this gene on the basis of observations that *Drosophila* embryos lacking the *Twist1* gene failed to gastrulate normally, produced no mesoderm and died at the end of embryogenesis with a 'twisted' appearance [1, 2]. The *Twist1* gene encodes a transcription factor containing a basic helix-loop-helix (bHLH) domain [4] and an amino-acid motif present in a protein family involved in the regulation of organogenesis [5-7]. The critical roles of *Twist1* in mesodermal development have been well illustrated by genetic studies. Human studies have shown that *Twist1* gene mutations cause Saethre-Chotzen syndrome (SCS), an autosomal dominant inheritance disease characterized by a broad spectrum of malformations including short stature, craniosynostoses, high forehead, ptosis, small ears with prominent crus, and maxillary hypoplasia with a narrow and high palate [8-13]. Gene-ablation experiments demonstrated that the heterozygous *Twist1* null mice [14-17] manifest craniofacial and limb abnormalities resembling those in SCS patients. Recent-

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ly, a number of studies have indicated that in addition to its essential roles in the development of multiple organs and systems, Twist1 also plays important roles in cancer metastasis [18-24].

This review will focus specifically on the biological roles of Twist1, with the overall objective of summarizing the remarkable progress toward our understanding of its structure, tissue/cell expression and biological functions. The review will also place particular emphasis on Twist1's roles in tumor initiation and progression, since the data concerning its involvement in cancer are newer and have attracted considerable attention in recent years. It is worth noting that Twist2 or Dermo1 shares many structural and functional similarities with Twist1, so we will also consider Twist2 when it is relevant to the discussion of Twist1 structure and function.

Molecular structures of the *Twist1* gene and protein

Twist1 is a transcription factor that belongs to the bHLH family [3]. Structurally, bHLH proteins are characterized by the presence of a conserved domain containing a stretch of basic amino acids adjacent to two amphipathic α-helices separated by an inter-helical loop [5, 6]. The α-helices mediate the interaction of this protein with a second bHLH factor, leading to the formation of a dimer that binds to CATATG hexanucleotide sequences known as the Nde1 E-box. The E-boxes are present in the regulatory elements of many genes that are essential for various types of organogenesis [6]. The traditional classification categorizes the bHLH family into three subfamilies: class A, class B and class C [5]. The proteins in class A, which include E12, E47, HEB, E2-2 and Daughterless [25], are ubiquitously expressed in mammalian cells. Class B comprises bHLH proteins that have relative specificity in tissue expression and form dimers with class A molecules for binding to E-boxes. Class C molecules, consisting of the Myc proteins, do not form heterodimers with either class A or class B proteins. The Twist family, which has relative tissue specificity and forms heterodimers with E12 and E47, falls into class B [26].

Human *Twist1* gene is mapped to 7q21.2 and contains two exons and one intron [10, 27]. The first exon contains an ATG site followed by an open reading frame encoding 202 amino-acid residues. The open reading frame is followed by a 45-bp untranslated portion in exon 1, a 536-bp intron and a second untranslated exon with two potential polyadenylation sites that are 65 and 415 bp from the 5′ end of exon 2. The molecular mass calculated from the amino-acid sequence of human Twist1 is approximately 21 kDa, with a theoretical isoelectric point of ~9.6. The protein contains relatively more polar amino-acid residues in the region close to the NH_2-terminus and more nonpolar residues at the COOH-terminus where the bHLH domain is located. Thus, the NH_2-teminal portion of Twist1 appears to be more hydrophilic than the COOH-terminus. Human Twist1 protein shares 96% amino-acid sequence identity with mouse Twist1 [28]. It is worth noting that *Drosophila* Twist1 with 490 amino-acid residues is remarkably larger than human, mouse and *Xenopus* Twist1, which have 202, 206 and 166 residues, respectively.

The bHLH domains of Twist1 show a very high degree of conservation among a broad range of species, including human, mouse, frog, *Drosophila*, leech and *Caenorhabditis elegans* (Figure 1). Indeed, the bHLH domain of human Twist1 from residue^{109} to residue^{163} shares 100% homology with that of the mouse Twist1 (Figures 1 and 2). The functional importance of the bHLH domain has been well illustrated by the fact that point mutations in the bHLH domain result in SCS [9, 10]. The basic region, ^{109}Q–T^{121}, of human Twist1 protein is the main domain responsible for binding DNA. The R118C mutation in this region diminishes Twist1 DNA-binding capability, and results in SCS (Figures 1 and 2) [29]. Sequence homology between Twist1 and MyoD basic regions elicits the proposal that the R118C mutation can disrupt Twist1 interaction with the phosphodiester backbone of E-box DNA [29]. In addition, the Loop-Helix II junction region of Twist1 is also involved in DNA binding. In agreement with this notion, human Twist1 with S144R or K145E mutation is unable to bind the CATATG Nde1 E-box [29]. Mutation of S144 or K145 in Twist1 may prevent hydrogen bond formation between the phosphate group and the lateral amino-acid chain of Twist1 protein [29]. In addition to using its bHLH domain to form heterodimers with E12 or E47 for DNA binding, Twist1 also interacts with MyoD, a bHLH transcription factor that regulates muscle differentiation (Figure 2) [30]. The interaction of Twist1 with MyoD results in inhibition of MyoD and MEF2 functions, leading to the inhibition of muscle differentiation [30, 31].

The WR motif, also known as the Twist box [32], which is located between 20 and 55 amino acids COOH-terminal to the bHLH region, is highly conserved among vertebrates; the amino-acid sequences in this domain show 100% homology among human, mouse and *Xenopus* Twist1 (see the region from residue^{180} to residue^{202} in the human Twist1) (Figure 1). However, the WR domain is less conserved in the more ancient species. In fact, *C. elegans* Twist1 does not contain a WR dipeptide (Figure 1). While the function of this WR region is unclear, it is postulated to be required for Twist1 protein folding

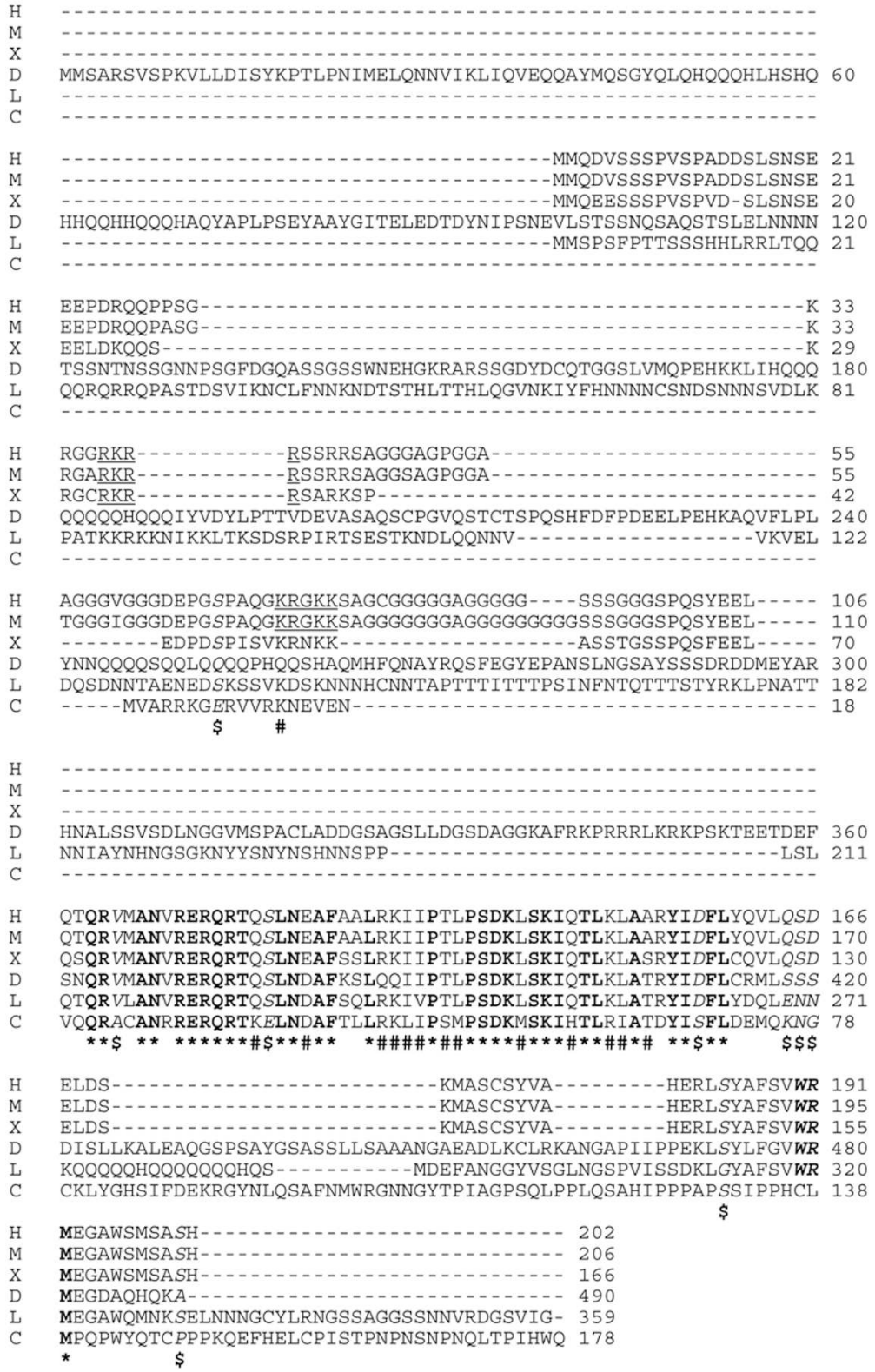

Figure 1 Comparison of amino-acid sequences among human (H), mouse (M), *Xenopus* (X), *Drosophila* (D), leech (L) and *C. elegans* (C) Twist1. The Twist1 amino-acid sequences from human (NP_000465.1), mouse (NP_035788.1), *Xenopus* (NP_001079352.1), *Drosophila* (NP_523816.2), leech (AAL05567.1) and *C. elegans* (AAC26105.1) have been aligned with the aid of the computer program Clustal W2 (Larkin *et al*., 2007). "*" indicates the identical residues in all sequences in the alignment; "#" indicates conserved substitutions; "$" indicates semi-conserved substitutions; "-" indicates gaps in the alignment.

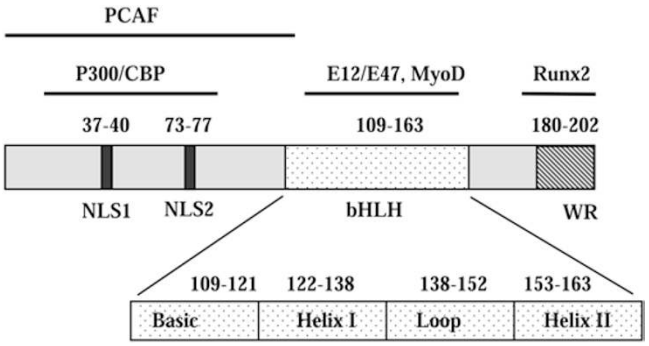

Figure 2 Molecular structure of the human Twist1 protein. The number of amino-acid residues for each structural domain is indicated. The regions that interact with other proteins are also indicated by solid lines. NLS1 and NLS2, nuclear localization signal sequences 1 and 2; bHLH, basic helix-loop-helix domain; WR, the tryptophan and arginine motif; CBP, cAMP-response element binding protein (CREB)-binding protein; PCAF, p300/CBP-associated factor, Runx2, Runt-related transcription factor; E12/E47, a bHLH transcription factor that forms dimer with Twist1; MyoD, a bHLH transcription factor that regulates muscle differentiation.

and activity [12]. It has been demonstrated that the WR domain is required for the transactivation function of Twist1, and genetic mutations in the WR domain are associated with SCS of human patients [33-35].

Twist1 functions as a transcription factor in the cell nucleus. There are two nuclear localization signal (NLS) sequences, ^{37}RKRR^{40} and ^{73}KRGKK^{77}, in the human Twist1 protein (Figures 1 and 2). The Twist1 protein with K76R mutation is capable of translocating into the nucleus by itself, while the Twist1 proteins with K38R, K73R or K77R mutation cannot translocate into the nucleus by themselves. However, the K38R, K73R or K77R Twist1 mutant is fully capable of forming heterodimers with E12 or TCF-4; these heterodimers can translocate into the nucleus despite the NLS mutations [36]. These findings indicate that NLSs are functional and also that Twist1 can translocate into the nucleus with its heterodimer partners without using its own NLSs.

Furthermore, the N-terminus of Twist1 can interact with p300, cAMP-response element binding protein (CREB), CREB-binding protein (CBP) and p300/CBP-associated factor (PCAF) (Figure 2), resulting in inhibition of the acetyltransferase activities of these histone-remodeling enzymes [37]. Since histone acetylation is usually coupled with transcriptional activation, the inhibition of p300, CBP and PCAF activities by Twist1 should repress gene expressions mediated by transcription factors that recruit these histone acetyltransferases. Finally, the C-terminus of Twist1 interacts with the DNA-binding domain of Runx2 to repress Runx2 function (Figure 2) [17]. Runx2 is a necessary transcription factor for osteoblast differentiation. During osteoblast development, relief of Runx2 inhibition by Twist1 is a mandatory event [17]. These findings suggest that Twist1 not only serves as a transcription factor to directly regulate its target genes, but also regulates other transcription factor/coregulator-mediated gene expression through interaction with other transcriptional regulators.

The human Twist2 protein contains 160 amino-acid residues and it shares 68% homology with human Twist1. The amino-acid sequences of their bHLH and Twist box domains are almost identical, which provides a structural base for their partially redundant biological functions.

The expression of *Twist1* in normal tissues

Consistent with its major roles in organogenesis, *Twist1* is primarily expressed in mesoderm-derived tissues. In *Drosophila*, the maternal protein Dorsal (dl) activates *Twist1* transcription in the presumptive mesoderm [4, 38, 39]. Dorsal, a Rel-containing sequence-specific transcription factor and the final maternal morphogen, forms a nuclear gradient that aids *Twist1* expression in the presumptive ventral mesoderm. The expressed Twist1 protein forms a steep gradient across the presumptive mesoderm-neuroectoderm border in the early embryo. Specifically, during *Drosophila* embryogenesis, Twist1 is first detected at the cellular blastoderm stage, in the nuclei of ventral midline cells which are destined for mesoderm and mesectoderm formation [4]. During the early gastrulation period, *Twist1* expression level is highly elevated in the mesodermal layer of the embryos, and is quantitatively similar among all of the cells that participate in ventral furrow invagination [4]. During the germ band elongation stage (the late gastrulation stage), Twist1 can be seen in cells of the whole germ band mesodermal layer and the anterior midgut primordium. During germ band retraction and later stages of *Drosophila* embryogenesis, *Twist1* expression signals, though relatively weak, are seen within the mesodermal layer of the somatopleura and splanchnopleura. After birth, Twist1 can be seen in adult mesenchymal cells such as muscle stem cells, referred to as adult muscle precursors in *Drosophila* [40-43]. In summary, during *Drosophila* embryogenesis, Twist1 accumulates at early stages (from the beginning of cellular blastoderm to germ band extension) in all cells of the presumptive mesodermal layer, but its expression decreases to relatively low levels in the mesodermal layer cells during later stages. This expression pattern is consistent with the critical roles of this zygotic gene in early embryogenesis. It should be noted

that as a nuclear protein, Twist1 is localized in the nuclei at all developmental stages.

During mouse embryogenesis, *Twist1* transcripts are first seen at embryonic day 7.5 (E7.5) in the anterior-lateral mesoderm underlying the head folds [44], in the primitive streak epiblast, and in scattered cells in the amniotic cavity [45]. Then, *Twist1* is predominantly and sequentially expressed in the somites, the neural crest-derived head mesenchyme, the first aortic arches, the lateral mesoderm, the second, third and fourth branchial arches, the anterior limb buds, and finally, the posterior limb buds [28, 44-46]. *Twist1* expression in mouse dental mesenchyme and heart valves remains at least until E18, and the same is true for its expression in the mesenchyme underneath the epidermis and tongue epithelium [44]. *Twist1* expression in the developing limb [44, 47, 48] is uniform in the limb bud mesenchyme at E9.5, but becomes regionalized to a broad anterior (preaxial) and a narrow posterior (postaxial) mesenchymal domain, spanning most of the proximal-distal length of the bud at E10.5. By E11.5, *Twist1* expression becomes confined to the marginal and proximal parts of the limb bud and is down-regulated in the core. *Twist1* expression in the mesenchyme is generally stronger in the preaxial region than in the postaxial region of the E10.5 and E11.5 limb buds. At E12.5-E15.5, *Twist1* expression localizes to the interdigital tissues and then the perichondrium of the phalanges [48]. A general tendency is that *Twist1* expression in the mouse embryo occurs first along a dorso-ventral gradient pattern until the headfold stage, and then moves along the rostro-caudal axis of the embryos as mesoderm cell layer- and neural crest cell-derived tissues develop.

After birth, *Twist1* is expressed in the adult stem cells of the mesenchyme [43, 49]. *Twist1* mRNA has also been detected in primary osteoblastic cells derived from newborn mouse calvariae [50] and in mouse brown and white adipocytes [51, 52]. This mouse *Twist1* expression pattern is consistent with the role of Twist1 as a mesoderm-determining factor that regulates genes involved in the specification and differentiation of mesoderm and in the development of mesenchyme tissues throughout the mouse body.

While the expression patterns of *Twist1* during embryonic development of *Drosophila* and mouse have been well studied, information regarding the expression profile of *Twist1* during human embryogenesis is lacking. Wang *et al*. used various adult human tissues, as well as several cell lines originated from different normal human tissues to examine *Twist1* expression pattern [27]. Among the human tissues tested, the strongest signals for *Twist1* mRNA were observed in the placenta, a tissue containing a large fetal portion and a small maternal portion. In particular, strong Twist1 signals were seen in the fetal portion of the placenta developed from the chorionic sac, which is mostly derived from the mesoderm. Intermediate strength signals were seen in the adult heart and skeletal muscle, which are also mostly derived from the mesoderm. Weak signals were found in the kidney and pancreas, while no signals were observed in the ectoderm-derived cells in the brain and endoderm-derived cells in the lung and liver. These findings indicate that in adult humans, *Twist1* is preferentially expressed in mesodermally derived tissues. Among cell lines derived from different human tissues, *Twist1* expression was found in WI-38 cells (fetal lung-derived fibroblasts), human peritoneal mesothelial cells and endometrial fibroblasts (both of which represent mesodermal cells derived from young adults) and human bone marrow-derived mesenchymal stem cells [27, 53]. Additionally, *Twist1* expression is also observed in human white adipocytes [52]. *Twist1* expression was not observed in human epithelial cells, although its mRNA was detected in both fetal and adult human skin fibroblasts [27].

In summary, as one of the zygotic genes essential for organogenesis, *Twist1* is highly expressed in the mesoderm-derived embryonic mesenchyme. This is consistent with its roles in the development and specification of the tissues with a mesodermal origin. In postnatal tissues, *Twist1* is primarily expressed in relatively quiescent adult stem cells located in mesoderm-derived mesenchymal tissues such as the muscle, adipose tissue and bone marrow.

The function of Twist1 in development and signaling pathways

Twist1 is a bHLH transcription factor essential for mesoderm development [1, 2, 4]. Although especially important to the morphology and behavior of head mesenchyme cells that support morphogenesis of the cranial neural tube [14], Twist1 is also an important regulator of many other biological processes. This is supported by the widespread expression of *Twist1* and the various phenotypes associated with human *Twist1* gene mutations and mouse *Twist1* gene knockout [17]. Twist1 is believed to act upon a set of downstream target genes, through which it controls a variety of cellular events necessary for the proper formation of mesenchyme derivatives [14, 54].

While previous research has shown the cruciality of Twist1 in mesoderm-associated organogenesis, the exact molecular mechanisms by which Twist1 controls mesenchymal tissue formation remain largely undefined [26]. This section focuses on the regulatory roles of Twist1 in

signaling pathways as well as its binding partners.

A number of studies have shown that Twist1 is involved in FGF signaling [55-58]. In *Drosophila* and *C. elegans*, Twist1 induces expression of the FGFR homolog DFR1 and egl-15, respectively [26]. Humans with features of the autosomal dominant SCS can carry mutations in the *TWIST1*, *FGFR2* or *FGFR3* genes [59], supporting the speculation that Twist1 is required for FGF signaling maintenance during morphogenesis progression. Additionally, the observation that calvaria cells isolated from an SCS patient with a *Twist1* gene mutation had decreased FGFR2 levels [60] also lends support to this hypothesis.

Twist1 is postulated to perform its central regulatory roles in organogenesis at least partially via its control over FGF, BMP and perhaps also TGFβ signaling [56]. Unlike most other bHLH proteins, Twist1 can form both functional homodimers (T/T) and heterodimers with E12 (T/E). The T/T homodimers and T/E heterodimers appear to have distinct activities and regulate expression of different gene sets. The relative levels of Twist1 and helix-loop-helix Id proteins determine the ratio between these dimers [56]. Id proteins represent a third class of HLH proteins that lack the basic domain and, therefore, cannot bind to DNA. Id proteins preferentially dimerize with E proteins such as E12 and thus prevent them from forming functional heterodimers with Twist1 [61]. Twist1 forms T/E dimers in the absence of Id proteins and forms T/T dimers with increased Id protein levels [56]. Consistent with this belief, in the osteogenic fronts of the cranial sutures where Twist1 and Id1 are coexpressed, genes regulated by T/T dimers, such as *FGFR2*, are expressed; the Id protein here binds to E proteins, thus favoring the formation of T/T dimers in these places, elevating and expanding the expression of *FGFR2*. T/E-regulated genes, such as thrombospondin 1 (*TSP-1*), are expressed in the mid-sutures where only *Twist1* is expressed; in such places, E proteins are not occupied by Id proteins and are thus available to form T/E heterodimers. In the sutures of Twist1$^{+/-}$ mice, the ratio between these dimers is altered to favor an increase in homodimers. This results in an elevated expression of T/T-regulated genes [57]. Additionally, T/E dimers have recently been shown to inhibit BMP signaling [62], which is predominantly active in the osteogenic fronts of cranial sutures. Thus, the decrease of T/E dimers in the cranial sutures of Twist1$^{+/-}$ mice (or SCS patients) also allows for an expansion of BMP signaling. BMP signaling induces Id expression [55], which would further promote T/T formation. This positive feedback loop is a likely cause for the premature closing of the sutures, leading to craniosynostosis that is one of the major clinical features of SCS. Studies also showed that Twist1 activity may have a similar effect on FGF signaling in the limb bud [15, 55, 63-65]. In its regulation of the growth and differentiation of limb bud tissues, Twist1 appears to be also involved in the sonic hedgehog (SHH) pathways, in addition to the FGF signaling pathways. Along with the altered expression of *FGFR2*, *FGF4*, *FGF8* and *FGF10*, *Twist1* deletion in the limb bud also reduced the overall activities of genes involved in SHH signaling in the limb bud mesenchyme, which include *Shh*, *Gli1*, *Gli2*, *Gli3* and *Ptch* [65, 66]. Twist1 also appears essential to *Bmp4* expression in the apical ectoderm, as well as Alx3, Alx4, Pax1 and Pax3 activities in the mesenchyme [65, 67].

Hand1 and Hand2 are also members of the Class B bHLH subfamily, to which Twist1 belongs. The balance between Twist1 and Hand2 within the developing limb may be critical for normal morphogenesis, and an increase in Hand2 relative to Twist1 may result in polydactyly [68]. This notion is strengthened by the observation that *Twist1-Hand2* double-heterozygous null mice are more phenotypically normal than mice heterozygous for only *Twist1* [68]. These findings suggest that Twist1 and Hand2 may function antagonistically in organogenesis [69]. However, it remains unclear whether Twist1 forms heterodimers with Hand2 and/or Hand1.

Runx2 is a critical osteoblast-differentiation transcription factor essential for bone formation [70]. *Twist1* is expressed in Runx2-expressing cells during early skeletal development, and expression of the osteoblast-specific gene *Runx2* occurs only after *Twist1* expression decreases [17]. Twist1 is believed to maintain mesenchymal cells in an undifferentiated state by negatively regulating *Runx2* [17, 55]. This belief is supported by the following observations: (1) double heterozygotes for *Twist1* and *Runx2* deletion had none of the skull abnormalities observed in *Runx2*$^{+/-}$; (2) Twist1 deficiency in mice led to premature osteoblast differentiation; and (3) *Twist1* overexpression inhibits osteoblast differentiation. Twist1 is speculated to perform its antiosteogenic functions through interaction with the Runx2 DNA-binding domain, and the relief of Runx2 inhibition by Twist1 is a mandatory event preceding osteoblast differentiation [17]. The lack of sufficient Runx2 inhibition by Twist1 in *Twist1*$^{+/-}$ mice is believed to be responsible for the premature differentiation of odontoblasts, leading to the formation of extensive pulp stones in the tooth [71].

TNFα signaling induces both proapoptotic and NF-κB-mediated antiapoptotic pathways. The TNFα-activated NF-κB upregulates both *Twist1* and *Twist2* expression to prevent cells from apoptosis. Interestingly, Twist1 and Twist2, in turn, can interact with the p65 (RelA) subunit of NF-κB to repress NF-κB-mediated

expression of cytokine genes to control inflammatory responses. The importance of this negative feedback loop was demonstrated by the phenotypes that Twist1 and Twist2 heterozygous mutant mice or Twist2 null mice exhibit elevated levels of proinflammatory cytokines and perinatal death from cachexia. These findings suggest that the Twist1 and Twist2-mediated negative feedback regulation plays an important role in preventing overactivation of NF-κB-mediated cytokine expression [72].

In summary, both the pivotal role that Twist1 plays in mesenchymal development and the biological function of Twist1 within mesenchymal cell populations are well established. Although the exact mechanisms by which Twist1 functions in organogenesis are not entirely clear, Twist1 likely plays its critical roles by regulating a set of target genes that include those in the FGF and SHH signaling pathways. Additionally, Twist1 may also modulate the functions of the Hand proteins (Hand 1 and 2), Runx2 and NF-κB. These downstream target genes or interacting proteins of Twist1 are known to be involved in the development of various mesenchymal derivatives and diverse physiological functions.

Regulation of Twist1 protein stability

Regulation of protein stability is an important way to control its function. It has been shown that truncated Twist1 proteins derived from nonsense mutations were unstable, resulting in SCS [73]. Interestingly, formation of Twist1/E47 heterodimers stabilizes Twist1 protein, while formation of Twist1/Id1 heterodimers destabilizes Twist1 protein. Thus, Twist1 overexpression can suppress bone morphogenetic protein (BMP)-induced osteoblast differentiation, and this inhibition can be overcome by Id1 expression through induction of Twist1 degradation [62]. Recently, it was reported that Twist1 protein stability is largely regulated by mitogen-activated protein kinase (MAPK)-mediated phosphorylation on S^{68}. The S^{68} in Twist1 can be phosphorylated by p38, JNK and ERK1/2 MAPKs, and this phosphorylation prevents Twist1 protein from ubiquitination-mediated degradation [74]. Accordingly, activation of MAPKs by an active Ras protein or TGFβ treatment significantly increases S^{68} phosphorylation and Twist1 protein levels without altering Twist1 mRNA expression, while blocking of MAPK activities by either specific inhibitors or dominant-negative inhibitory mutants effectively reduces both S^{68} phosphorylation and Twist1 protein levels [74]. These findings may suggest a positive correlation among active MAPKs, Twist1 protein level, epithelial-mesenchymal transition (EMT) and invasiveness of cancer cells.

The expression and roles of Twist1 in cancer

In addition to its essential role in modulating mesenchymal tissues critical for organogenesis, Twist1 is also expressed in and associated with many types of aggressive tumors, including breast cancer [18], hepatocellular carcinoma [75, 76], prostate cancer [19, 77], gastric cancer [78, 79], oesophageal squamous cell carcinoma [80, 81], bladder cancer [82, 83] and pancreatic cancer [84]. Twist1 plays multiple roles in cancer initiation, progression and metastasis. More specifically, Twist1 can override oncogene-induced cell senescence and apoptosis [85-87], increase cancer cell resistance to chemotherapy [23, 88], enhance cancer stem cell (CSC) population [89-91], and facilitate cancer cell invasion and metastasis [18, 20, 24, 68, 92-95].

Many recent studies have highlighted the role of Twist1 in promoting cancer cell EMT and metastasis. Cancer metastasis consists of several steps: EMT, local invasion, intravasation, transportation in the circulation, extravasation, survival and proliferation at a secondary organ site, and formation of overt metastatic lesions [96]. Twist1 enhances the ability of cells within a primary tumor to undergo a pathological EMT [18, 24, 68], similar to the role of Twist1 in development [97]. EMT allows tumor cells to migrate away from the primary tumor, enter the lymphatic system and/or blood stream, and settle into secondary tumor sites [18].

While Twist1 promotes cancer initiation, progression and metastasis, its expression patterns, functions and molecular mechanisms by which Twist1 affects different types of cancers may vary. In this section, we will discuss Twist1 expression and function in different types of cancers and potential pathways by which Twist1 may participate in the initiation and progression of cancers.

Twist1 and breast cancer

Yang *et al.* [18] observed that four types of mouse-mammary tumor cell lines isolated from the same breast cancer displayed distinct abilities to metastasize in mice. By comparing the gene expression profiles, they discovered that (a) increased *Twist1* expression correlates with breast cancer invasion and metastasis, (b) suppression of *Twist1* expression by siRNA in the metastatic mammary-carcinoma cells specifically inhibits the cells' ability to metastasize from the mammary gland to the lung, and (c) expression of *Twist1* in the epithelial cell lines results in loss of E-cadherin-mediated cell-cell adhesion, activation of mesenchymal markers and induction of cell motility; Twist1 binds to the E-box elements in the promoter region of E-cadherin and represses the transcriptional expression of this cell-cell adhesion molecule. Based on

these findings, along with the known functions of Twist1 as a master regulator of embryonic morphogenesis, the authors postulated that Twist1 contributes to metastasis by promoting EMT in cancer progression [18].

A number of studies have shown the associative relationships among Twist1, EMT, and breast cancer metastasis [22-24, 88, 92, 98-102]. STAT3 is known to be involved in breast cancer progression. Using an RNA interference (shRNA) approach, Ling and Arlinghaus [98] examined the effects of STAT3 knockdown on mammary tumor growth in mice. They found that Twist1 was eliminated in STAT3 knockdown cells; the proliferation rate of these cells remained the same, but the invasive capability of these cells was significantly reduced. These observations suggest that STAT3 enhances *Twist1* expression in its promotion of breast cancer progression.

Subsequently, another study [22] compared low invasive human breast cancer lines with highly invasive human breast cancer lines, and showed that activation of STAT3 (i.e., phosphorylation of Tyr^{705} in the STAT3 amino-acid sequence) increased *Twist1* expression while inhibition of STAT3 significantly reduced *Twist1* expression in the aggressive (more invasive) human breast cancer cell lines. The inhibition of STAT3 reduced migration, invasion, and colony formation of these more invasive cancer cells. This study also found that STAT3 binds directly to the second proximal STAT3-binding site on the human *Twist1* promoter and activates *Twist1* transcription. Based on the strong correlation between the levels of activated STAT3 (i.e., Tyr^{705} p-STAT3) and Twist1 at the late stages of breast cancer, this group postulated that activated STAT3 transcriptionally induces *Twist1* expression, which subsequently promotes the migration, invasion and anchorage-independent growth of breast cancer cells. Together with another observation that Twist1 transcriptionally induces AKT2 (a serine/threonine kinase) to promote oncogenic functions [88], Cheng *et al.* [21] proposed that STAT3, Twist1 and AKT2 form a functional signaling axis to regulate pivotal oncogenic properties of cancer cells. Recently, Eckert *et al*. demonstrated that *PDGFRα* is a direct target gene of Twist1 in breast cancer cells. The Twist1-induced PDGFRα activates the Src kinase to promote formation of invadopodia, which are specialized membrane protrusions for extracellular matrix degradation. Therefore, this invadopodia-mediated matrix degradation induced indirectly by Twist1 is another mechanism by which Twist1 promotes breast cancer metastasis [103].

Some microRNAs may be involved in mediating cancer metastasis. The microRNA-10b (miR-10b) is highly expressed in metastatic breast cancer cells, and it positively regulates cell migration and invasion [102]. The miR-10b transcription is directly regulated by Twist1, and miR-10b in turn inhibits translation of the mRNA-encoding homeobox D10, resulting in increased expression of the pro-metastatic gene *RhoC*. Moreover, the level of miR-10b expression in primary breast carcinomas is associated with clinical cancer progression. These findings suggest that Twist1 indirectly upregulates RhoC expression to increase breast cancer, cell invasion and metastasis.

Steroid receptor coactivator-1 (SRC-1) is a coactivator for nuclear hormone receptors such as estrogen and progesterone receptors and certain other transcription factors such as Ets-2 and PEA3 [104, 105]. In breast cancer, SRC-1 expression positively correlates with poor prognosis [105]. A recent study showed that in the virus-polyoma middle T breast cancer mouse model, SRC-1 specifically promotes breast cancer metastasis without affecting primary tumor growth [106]. A subsequent investigation by the same group found that SRC-1 serves as a coactivator for the transcription factor PEA3 to enhance Twist1 expression, suggesting a molecular mechanism whereby SRC-1 promotes breast cancer invasiveness and metastasis by upregulating Twist1 expression [92].

As already mentioned, MAPKs phosphorylate Twist1 and increase Twist1 protein stability in cultured cells [74]. Examination of the invasive human breast ductal carcinomas further revealed that the levels of S^{68} phosphorylation and Twist protein positively correlate with the JNK MAPK activities, which are significantly higher in progesterone receptor-negative and HER2-positive breast cancers. These findings suggest that JNK activation by multiple signaling pathways may substantially promote breast tumor cell EMT and metastasis via phosphorylation and stabilization of Twist1 [74].

Twist1 and hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is a rapid-growth metastatic tumor. Tissue microarray and immunohistochemical staining of paired primary and metastatic HCC showed that *Twist1* overexpression correlated positively with HCC metastasis and negatively with E-cadherin expression [75]. Further studies on different HCC cell lines revealed that HCC cells with increased levels of Twist1 and decreased levels of E-cadherin have higher metastatic ability. This suggests that Twist1 suppresses E-cadherin expression and induces EMT changes, which are partially responsible for the increased HCC-cell invasiveness [75]. Subsequent investigations also revealed that Twist1 upregulated vascular endothelial growth factor (VEGF) and N-cadherin expression in HCC, suggesting that Twist1 may also play an important role in HCC angiogenesis [107]. More recently, Matsuo *et al.*

[108] showed that *Twist1* overexpression in HCC cells enhanced cell motility, while its knockdown reduced cell migration. Yang *et al*. [76] analyzed three major EMT regulators, Twist1, Snail and Slug, and found that Twist1 and Snail synergistically enhance HCC metastasis: co-expression of *Snail* and *Twist1* reduced E-cadherin levels and worsened HCC prognosis more dramatically than did *Twist1* expression alone. *Slug* expression, however, did not seem to affect the outcome. Additionally, Sun *et al*. [109] demonstrated that Twist1 enhanced the motility, invasiveness and vasculogenic mimicry formation of HCC cells through the suppression of E-cadherin expression and the upregulation of N-cadherin.

Twist1 and prostate cancer

The main cause of prostatic cancer-related death is metastasis. These cancer cells have a particular predilection for metastasis to bones, and androgen-independent metastatic prostate cancer poses an even greater challenge to the treatment of this disease. Kwok *et al*. [19] showed that down-regulation of Twist1 in androgen-independent prostate cancer cells increased their sensitivity to anticancer drugs and suppressed their migration and invasion abilities, suggesting Twist1 inactivation as a potential strategy to control the growth and metastasis of these cells. Subsequent studies by the same group [77, 110] correlated high levels of *Twist1* expression with aberrant E-cadherin expression and bone metastasis. Twist1 might promote bone metastasis by enhancing the osteomimicry of prostate cancer cells and by modulating prostate cancer cell-mediated bone remodeling via regulation of the osteolytic metastasis-promoting factor, DKK-1. These results were in agreement with those from another study [111], which demonstrated that Twist1 knockdown reduced the expression level of N-cadherin and inhibited the migration rate of PC-3 prostate carcinoma cells. The regulation of N-cadherin by Twist1 requires an E-box element located within the first intron of the N-cadherin gene. A more recent study [83] revealed that in addition to promoting the hallmark changes associated with EMT, such as E-cadherin down-regulation and N-cadherin upregulation, Twist1 also enhances VEGF production in prostate and bladder cancers. Since VEGF is an angiogenic factor, its enhanced production may accelerate angiogenesis associated with the metastasis of these tumors.

Twist1 and gastric cancer

Immunohistochemistry and RT-PCR analyses revealed high levels of *Twist1* expression in gastric cancer tissues, and the increase in *Twist1* expression correlated with lymph node metastasis [82]. A subsequent study further demonstrated that gastric cancer cells stably transfected with Twist1 had greater migration and invasion abilities, and formed a greater number of cancer nodules in the abdominal cavity and liver of nude mice inoculated with the transfected cells [78]. Moreover, *Twist1* overexpression in these cells promoted the expression of Tcf-4's downstream target genes cyclin D1 and MMP-2, while its suppression reduced cyclin D1 expression and MMP-2 activity. These results suggest that Twist1 may promote gastric cancer cell migration, invasion and metastasis via the Wnt/Tcf-4 signaling pathway. Microarray analyses revealed that depletion of Twist1 in the HGC-27 gastric cancer cells increased the expression of NF1, RAP1A, SRPX, RBL2, PFDN4, ILK (integrin-linked kinase), F2R, ERBB3, and MYB, and decreased the expression of AKR1C2, FOS, GDF15, NR2F1, ATM, and CTPS, supporting that many Twist1-regulated genes are involved in the differentiation, adhesion and proliferation of gastric cancer cells [112]. The same research group also discovered that the MGC-803 and HGC-27 gastric cancer cells with higher Twist1 levels exhibited higher invasive potential than did the BGC-823 and SGC-7901 gastric cancer cells with lower Twist1 levels. Twist1 overexpression in BGC-823 cells increased their migration and decreased their sensitivity to the arsenic oxide-induced cell death, while Twist1 ablation in MGC-803 and HGC-27 cells suppressed migration ability, increased cell apoptosis in response to arsenic oxide, and inhibited the cell cycle. Furthermore, Twist1 and p53 levels were negatively correlated, further supporting Twist1 as a critical regulator of gastric-cancer cell proliferation and migration [79].

Twist1 and other types of cancer

Immunohistochemical staining and RT-PCR analyses showed upregulation of *Twist1* expression in primary oesophageal squamous cell carcinoma [80, 113], while immunoblotting analysis revealed the elevation of *Twist1* expression in the oesophageal squamous cell carcinoma cell lines [80]. In addition, high *Twist1* expression in oesophageal squamous cell carcinoma was significantly associated with greater metastasis risks in patients with oesophagectomy, suggesting a role of *Twist1* upregulation in the development of distant metastasis of oesophageal squamous cell carcinoma [80]. Another study showed a correlation between high *Twist1* expression and low E-cadherin expression. In the group with preserved E-cadherin expression, the 5-year survival rate was better for patients with low *Twist1* expression than for those with high *Twist1* expression [81].

Immunohistochemical staining analyses of cancerous and non-cancerous bladder tissues revealed significantly higher Twist1 expression in the cancer specimens.

Among the cancer tissues, Twist1 expression was remarkably higher in the metastatic lesions than in the primary tumors [82]. Additionally, bladder cancers also exhibited a correlation between Twist1 elevation and E-cadherin reduction.

Satoh *et al*. observed that the presence of MSX2 correlates with the malignant behavior of pancreatic cancer cells, and that MSX2 enhanced the proliferation, migration and liver metastasis of pancreatic cancer cells via the induction of Twist1. When MSX2 was knocked down in pancreatic cancer cells, Twist1 was down-regulated. These findings indicate that MSX2-induced *Twist1* expression plays a crucial role in pancreatic cancer progression by inducing changes consistent with EMT [84].

Elias *et al*. analyzed *Twist1* expression in human gliomas and normal brains using RT-PCR, Northern blot, *in situ* hybridization and immunohistochemistry. They found *Twist1* expression in a majority of human glioma-derived cell lines and human gliomas. The expression of *Twist1* was also observed in embryonic and fetal human brain neurons, but not in the glia of the mature brain. The increased *Twist1* expression accompanied the transition from low-grade to high-grade gliomas, and *Twist1* overexpression in a human glioma cell line significantly enhanced tumor cell invasion. These findings support the roles for Twist1 in both early glial tumorigenesis and subsequent malignant progression [114].

Epstein-Barr virus (EBV)-associated nasopharyngeal carcinoma is highly metastatic compared to other head and neck tumors. A study showed that the principal EBV oncoprotein, latent membrane protein 1 (LMP1), upregulates Twist1 to induce EMT, suggesting the contribution of Twist1 induction by the human viral oncoprotein LMP1 to the highly metastatic nature of nasopharyngeal carcinoma [115]. Furthermore, the study revealed that LMP1 regulates Twist1 through the NF-κB pathway.

Immunohistochemical staining analysis of head and neck cancer tissues showed that *Twist1* expression was positively associated with cancer progression and lymph node metastasis [116]. Further analysis in this study revealed a positive correlation between *Twist1* expression and the expression of CXCR4 and CCR7, suggesting that Twist1 may regulate CXCR4 and CCR7 expression in these cancer cells, which in turn promotes lymph node metastasis.

Loss of E-cadherin triggers peritoneal dissemination (detachment from the primary lesion, spreading in the abdominal cavity) of epithelial ovarian carcinoma, leading to an adverse prognosis for most patients with this cancer. A study showed that suppression of *Twist1* expression in epithelial ovarian carcinoma cells changes the cellular morphology from a fibroblastic and motile phenotype to an epithelial phenotype, and inhibits their adhesion to mesothelial monolayers [117]. Furthermore, this investigation revealed that Twist1 down-regulation reduced the expression of MMP-2, membrane type 1 MMPs, and adhesion molecules CD29, CD44 and CD54. These findings suggest that reducing *Twist1* expression suppresses the multistep process of peritoneal dissemination and may be a potential therapeutic strategy for the treatment of this carcinoma.

The role of Twist1 in cancer cell survival, immortalization and acquired chemoresistance

Oncogenic insults usually induce p53 and/or retinoblastoma (Rb) expression and result in cell apoptosis or senescence, which is a defensive barrier against cell transformation and tumor progression. Thus, tumorigenesis needs to protect cells from apoptosis or immortalize cells from senescence. Interestingly, both Twist1 and Twist2 were shown to inhibit oncogene-induced and p53-dependent cell death. Further analysis revealed that Twist might affect p53 indirectly through inhibition of ARF expression to modulate the ARF/MDM2/p53 pathway [85]. The same study also demonstrated that Twist could promote colony formation of ras-transformed mouse embryo fibroblasts (MEFs) in soft agar, and Twist overexpression might enhance rhabdomyosarcoma formation by inhibiting myogenic differentiation [85]. Similarly, Twist1 was found to be constantly overexpressed in neuroblastomas with N-Myc amplification, where this Twist1 overexpression was responsible for the inhibition of the ARF/p53 pathway involved in the Myc-dependent apoptotic response [86]. Furthermore, Ansieau *et al*. [87] also demonstrated that Twist1 and Twist2 play a role in preventing H-Ras-induced premature senescence by abrogating the p53- and Rb-dependent pathways. Twist1 or Twist2 works cooperatively with H-Ras to transform MEFs and induce complete EMT and aggressive cell migration and invasion in mammary epithelial cells. These findings suggest that Twist proteins may facilitate potential tumorigenic cells to escape from safeguard programs and acquire invasive features.

Twist1 also plays a role in the acquired resistance of cancer cells to chemotherapy. Twist1 upregulation is associated with cellular resistance to taxol and vincristine, two microtubule-targeting anticancer drugs in nasopharyngeal, bladder, ovarian, and prostate cancers [118]. On the other hand, the chemotherapy-induced cancer cell apoptosis is counter-regulated by a subset of NF-κB-regulated genes. *Twist1* is one of the major targets of NF-κB responsible for antagonizing chemotherapy-induced apoptosis, suggesting an important role of Twist1 in NF-κB-mediated cell survival and chemoresistance [119].

Twist1 and cancer stem-like cells

Multiple lines of evidence have demonstrated a link between Twist1-induced EMT and stem-like cells. Mani *et al*. have shown that induction of EMT by expressing Twist1 or Snail in mammary epithelial cells increases stem-like cell population with high *CD44* and low *CD24* expression, while isolated mammary epithelial stem-like cells express endogenous EMT-inducing factors including Twist1, Snail, SIP1, Slug and FOXC2, and EMT marker genes [89]. Another study has shown that Twist may repress *CD24* expression to increase the CD44-high and CD24-low stem-like cell population [90]. Furthermore, expression of Twist or Snail in HER2-transformed mammary epithelial cells also facilitates EMT and generates cancer stem-like cells that efficiently form mammospheres, soft agar colonies and tumors [89]. Moreover, Battula *et al*. further demonstrated that Twist1- or Snail-induced EMT could convert human mammary epithelial cells to mesenchymal stem-like cells with the capacity to differentiate into multiple cell types, including osteoblasts, adipocytes and chondrocytes. This study also demonstrated that these EMT-derived cells, but not the control cells, have the ability to migrate towards tumor cells and wound sites, as mesenchymal stem cells do [91].

Dysregulation of Twist1 expression and function in cancer

As described in preceding sections, several factors have been shown to upregulate *Twist1* expression in cancers, including NF-κB in nasopharyngeal carcinoma and PEA3 and SRC-1 in breast cancer [92, 115]. Several other factors have also been shown to regulate *Twist1* expression. Firstly, HIF-1α has been shown to mediate *Twist1* expression under hypoxia condition in tumors. Stabilization of the hypoxia-inducible factor-1α (HIF-1α) transcription complex caused by intratumoral hypoxia, promotes tumor progression and metastasis, leading to treatment failure and mortality in several types of human cancers. HIF-1α can bind to the hypoxia-response element in the *Twist1* proximal promoter to upregulate *Twist1* expression, thus promoting EMT and metastatic phenotypes of cancers [120]. This study also revealed that co-expression of HIF-1α, Twist1 and Snail in primary head and neck cancers correlated with metastasis and the worst prognosis. These results suggest a key-signaling pathway involving HIF-1α and Twist1 that promotes metastasis in response to intratumoral hypoxia. Interestingly, SRC-1 has been demonstrated as a coactivator of HIF-1α [121], although it is unclear whether SRC-1 can enhance HIF-1α-mediated upregulation of *Twist1* expression. Secondly, thrombin contributes to the malignant phenotype by promoting tumor metastasis. Thrombin may promote tumor progression by upregulating Twist1 to enhance angiogenesis [122]. Thirdly, Twist1 has been implicated in type I interferon (IFN)-induced suppression of TNFα expression and thus, TNFα-mediated inflammation [123]. Type I IFNs activate Fc receptors and Toll-like receptors, leading to induction and activation of the Axl receptor tyrosine kinase and downstream *Twist1* expression. Twist1 subsequently binds to the E-boxes in the TNFα promoter and represses NF-κB-dependent expression of the *TNFα* gene [123]. Finally, Twist1 has been shown to be involved in ILK-mediated upregulation of HER2 [124]. Overexpression or activation of ILK may upregulate *Twist1* expression, and Twist1 in turn upregulates Y-box binding protein-1 (YB-1) expression to enhance HER2 expression [124].

Twist1 has been shown to upregulate the expression of several target genes important for cancer progression. Twist1 upregulates *Akt2* expression in breast cancer cells, which enhances cell migration, invasion and resistance to chemotherapy [88]. Furthermore, Twist1 directly upregulates *Bmi1*, a polycomb-group protein that maintains stem cell self-renewal and is frequently overexpressed

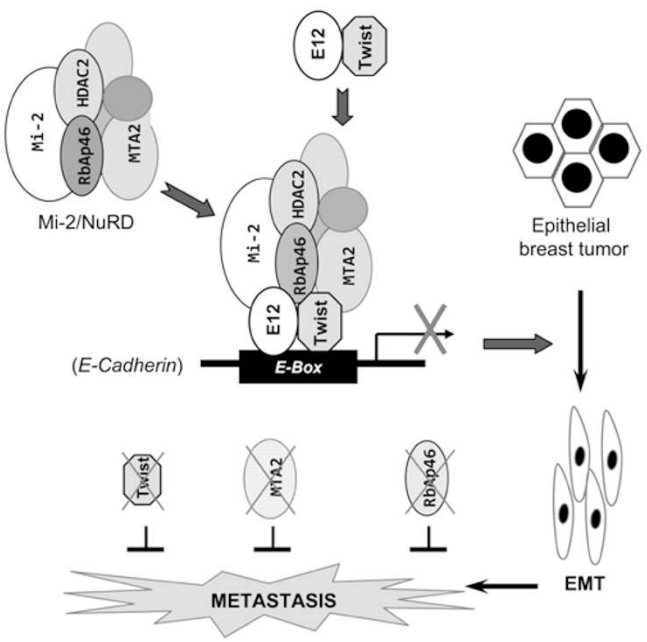

Figure 3 Twist1 recruits Mi-2/NuRD complex to repress E-cadherin expression and promote EMT and metastasis. Twist1 forms heterodimer with E12, and interacts with the components of MTA2 (metastasis-associated protein 2), RbAp46 (Rb-associated protein 46) and Mi-2 in the Mi-2/NuRD protein complex. Consequently, the Mi-2/NuRD complex is recruited to the E-cadherin promoter by Twist1, resulting in suppression of E-cadherin expression and promotion of EMT and metastasis of breast cancer. Knockdown of Twist, MTA2 or RbAp46 in the complex inhibits breast cancer cell metastasis.

in human cancers. Twist1 targets *Bmi1* expression and works with Bmi1 to promote tumor-initiating capability and EMT by repressing E-cadherin expression [125].

In addition to activation of target gene expression, Twist1 also promotes cancer cell EMT, migration, invasion and metastasis by repressing target gene expression. It is well established that Twist1 promotes EMT by repressing E-cadherin expression by associating with the E-cadherin promoter [18, 24, 126]. Twist1 also represses *CD24* expression to help generate CD44-high/CD24-low cancer stem-like cells [90]. Moreover, Twist1 may enhance MMP activities to promote cancer cell invasion, and metastasis by repressing the expression of *TIMP1*, a key inhibitor of MMPs [127].

Molecular mechanisms responsible for Twist1-mediated repression of E-cadherin expression

A recent study has investigated the molecular mechanisms by which Twist1 represses the E-cadherin expression to promote EMT and cancer cell migration, invasion and metastasis [24]. The authors purified and characterized the Twist1-associated protein complex. They discovered that Twist1 either directly or indirectly interacts with several components of the Mi2/nucleosome remodeling and deacetylase (Mi2/NuRD) protein complex, including metastasis-associated protein 2 (MTA2), Rb-associated protein 46 (RbAp46), Mi2 and histone deacetylase 2 (HDAC2). Twist1 recruits this gene repression protein complex to the E-cadherin promoter, resulting in repression of the E-cadherin promoter activity and E-cadherin expression (Figure 3). Among the components of the Mi2/NuRD complex, Mi2 harbors chromatin-dependent ATPase activity and facilitates nucleosome mobility through a sliding mechanism [128-130]. The combined activities of HDAC and ATPase in the Mi2/NuRD complex result in the generation of densely packed, hy-

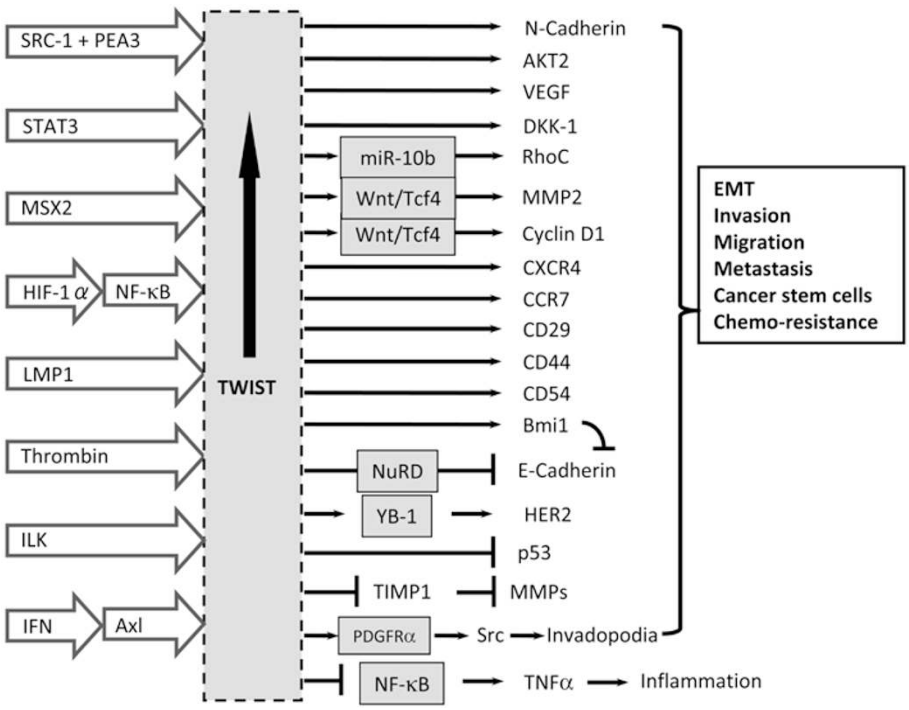

Figure 4 Possible regulatory pathways by which Twist1 is involved in cancer progression. Factors that directly or indirectly upregulate Twist1 are listed on the left side. Targets and cellular functions that are directly or indirectly regulated by Twist1 are listed on the right side. SRC-1, steroid receptor coactivator-1; STAT3, signaling transducer and activator of transcription 3; MSX2, Msh homeobox 2; HIF-1α, hypoxia-inducible factor 1α; NF-κB, nuclear factor kappa B; LMP1, EBV latent membrane protein 1; ILK, integrin-linked kinase; IFN, type I interferon; Axl, Axl receptor tyrosine kinase; miR-10b, micro-RNA 10b; Wnt, wingless and Int; Tcf4, transcription factor 4; Mi-2/NuRD, nucleosome remodeling and deacetylase protein complex; YB-1, Y-box binding protein-1; TIMP1, tissue inhibitor of metalloproteinase-1; VEGF, vascular endothelial growth factor; DKK-1, dickkopt-related protein 1; RhoC, ras homolog C; MMP2, metalloproteinase 2; CXCR4, chemokine (C-X-C motif) receptor 4; CCR7, chemokine (C-C motif) receptor 7; CD29, integrin β1; CD44, CD44 antigen; CD54, inter-cellular adhesion molecule 1; Bmi1, BMI1 polycomb ring finger oncogene; HER2, human epidermal growth factor receptor 2; TNFα, tumor necrosis factor α; EMT, epithelial-mesenchymal transition.

poacetylated nucleosomes for gene silencing [128]. The RbAp46 and/or RbAp48 subunits were originally identified as proteins associated with the Rb tumor suppressor [131], and they may function as structural proteins that provide interactive interfaces for other components of the Mi2/NuRD complex [128, 132, 133]. The Mi2/NuRD complex also contains one of the MTA protein family members, MTA1, MTA2 or MTA3 [128, 134-136]. Each MTA member modifies the functional specificities of the Mi2/NuRD complexes relevant to its upstream and downstream signaling pathways and molecular targets. Although different members of the MTA family direct the Mi2/NuRD complex to play distinct functions, the primary function of the Mi2/NuRD complex is to repress gene expression involved in many biological processes, including cancer initiation and progression. In agreement with the roles of the Mi2/NuRD complex in mediating Twist1-dependent repression of the E-cadherin promoter, knockdown of MTA2 or RbAp46 releases the Twist1-repressed E-cadherin promoter activity and endogenous E-cadherin expression in cancer cells. Knockdown of MTA2 or RbAp46 in the 4T1 mouse mammary tumor cells or the MDA-MB-435 human cancer cells also inhibits their migration, invasion and metastasis, just as knockdown of Twist1 does [24]. These findings not only provide novel mechanistic and functional links between Twist1 and the Mi2/NuRD complex but also establish new essential roles for the components of the Mi2/NuRD complex in cancer metastasis.

In summary, numerous studies have revealed *Twist1* upregulation in a variety of cancers. It is relatively clear that Twist1 promotes cancer development by protecting cells from oncogene- and chemotherapy-induced apoptosis and senescence and enhances cancer invasion and metastasis by promoting EMT. However, the exact mechanistic pathways by which Twist1 regulates cancer initiation and progression remain largely unknown. In this section, we have summarized the possible pathways and molecules via which Twist1 may participate in tumor development, progression and metastasis (Figure 4). This illustration scheme is based on the available data reported in the English literature over the past 7 years. It should be noted that the potential upstream and downstream regulators of pathways in different contexts of cancer cells could vary. Further studies are required to define the nature of Twist1's involvement in a defined cancer type. As the studies on Twist1 continue, more information regarding the specific roles of this molecule and its involvement in signaling pathways during cancer progression will certainly emerge. A better understating of Twist1's roles in cancer progression is likely to have important clinical implications for both prognosis prediction and therapeutic targeting of different cancers.

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References

1 Simpson P. Maternal-zygotic gene interactions during formation of the dorsoventral pattern in *Drosophila* embryos. *Genetics* 1983; **105**:615-632.
2 Nusslein-Volhard C, Wieschaus E, Kluding H. Mutations affecting the pattern of the larval cuticle in *Drosophila* melanogaster. *Roux's Arch Dev Biol* 1984; **193**:267-282.
3 Thisse B, el Messal M, Perrin-Schmitt F. The twist gene: isolation of a *Drosophila* zygotic gene necessary for the establishment of dorsoventral pattern. *Nucleic Acids Res* 1987; **15**:3439-3453.
4 Thisse B, Stoetzel C, Gorostiza-Thisse C, Perrin-Schmitt F. Sequence of the twist gene and nuclear localization of its protein in endomesodermal cells of early *Drosophila* embryos. *EMBO J* 1988; **7**:2175-2183.
5 Murre C, McCaw PS, Vaessin H*, et al.* Interactions between heterologous helix-loop-helix proteins generate complexes that bind specifically to a common DNA sequence. *Cell* 1989; **58**:537-544.
6 Jan YN, Jan LY. HLH proteins, fly neurogenesis, and vertebrate myogenesis. *Cell* 1993; **75**:827-830.
7 Kadesch T. Consequences of heteromeric interactions among helix-loop-helix proteins. *Cell Growth Differ* 1993; **4**:49-55.
8 Reardon W, Winter RM. Saethre-Chotzen syndrome. *J Med Genet* 1994; **31**:393-396.
9 el Ghouzzi V, Le Merrer M, Perrin-Schmitt F*, et al.* Mutations of the TWIST gene in the Saethre-Chotzen syndrome. *Nat Genet* 1997; **15**:42-46.
10 Howard TD, Paznekas WA, Green ED*, et al.* Mutations in TWIST, a basic helix-loop-helix transcription factor, in Saethre-Chotzen syndrome. *Nat Genet* 1997; **15**:36-41.
11 Krebs I, Weis I, Hudler M*, et al.* Translocation breakpoint maps 5 kb 3' from TWIST in a patient affected with Saethre-Chotzen syndrome. *Hum Mol Genet* 1997; **6**:1079-1086.
12 Gripp KW, Zackai EH, Stolle CA. Mutations in the human TWIST gene. *Hum Mutat* 2000; **15**:150-155.
13 Rose CS, Patel P, Reardon W, Malcolm S, Winter RM. The TWIST gene, although not disrupted in Saethre-Chotzen patients with apparently balanced translocations of 7p21, is mutated in familial and sporadic cases. *Hum Mol Genet* 1997; **6**:1369-1373.
14 Chen ZF, Behringer RR. Twist is required in head mesenchyme for cranial neural tube morphogenesis. *Genes Dev* 1995; **9**:686-699.
15 Bourgeois P, Bolcato-Bellemin AL, Danse JM*, et al.* The variable expressivity and incomplete penetrance of the twist-null heterozygous mouse phenotype resemble those of human Saethre-Chotzen syndrome. *Hum Mol Genet* 1998; 7:945-957.
16 Soo K, O'Rourke MP, Khoo PL*, et al.* Twist function is required for the morphogenesis of the cephalic neural tube

and the differentiation of the cranial neural crest cells in the mouse embryo. *Dev Biol* 2002; **247**:251-270.
17 Bialek P, Kern B, Yang X*, et al.* A Twist code determines the onset of osteoblast differentiation. *Dev Cell* 2004; **6**:423-435.
18 Yang J, Mani SA, Donaher JL*, et al.* Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* 2004; **117**:927-939.
19 Kwok WK, Ling MT, Lee TW*, et al.* Up-regulation of TWIST in prostate cancer and its implication as a therapeutic target. *Cancer Res* 2005; **65**:5153-5162.
20 Puisieux A, Valsesia-Wittmann S, Ansieau S. A twist for survival and cancer progression. *Br J Cancer* 2006; **94**:13-17.
21 Cheng GZ, Zhang W, Wang LH. Regulation of cancer cell survival, migration, and invasion by Twist: AKT2 comes to interplay. *Cancer Res* 2008; **68**:957-960.
22 Cheng GZ, Zhang WZ, Sun M*, et al.* Twist is transcriptionally induced by activation of STAT3 and mediates STAT3 oncogenic function. *J Biol Chem* 2008; **283**:14665-14673.
23 Li QQ, Xu JD, Wang WJ*, et al.* Twist1-mediated adriamycin-induced epithelial-mesenchymal transition relates to multidrug resistance and invasive potential in breast cancer cells. *Clin Cancer Res* 2009; **15**:2657-2665.
24 Fu J, Qin L, He T*, et al.* The TWIST/Mi2/NuRD protein complex and its essential role in cancer metastasis. *Cell Res* 2011; **21**:275-289.
25 Murre C, Bain G, van Dijk MA*, et al.* Structure and function of helix-loop-helix proteins. *Biochim Biophys Acta* 1994; **1218**:129-135.
26 Castanon I, Baylies MK. A Twist in fate: evolutionary comparison of Twist structure and function. *Gene* 2002; **287**:11-22.
27 Wang SM, Coljee VW, Pignolo RJ, Rotenberg MO, Cristofalo VJ, Sierra F. Cloning of the human twist gene: its expression is retained in adult mesodermally-derived tissues. *Gene* 1997; **187**:83-92.
28 Wolf C, Thisse C, Stoetzel C, Thisse B, Gerlinger P, Perrin-Schmitt F. The M-twist gene of Mus is expressed in subsets of mesodermal cells and is closely related to the Xenopus X-twi and the *Drosophila* twist genes. *Dev Biol* 1991; **143**:363-373.
29 El Ghouzzi V, Legeai-Mallet L, Benoist-Lasselin C*, et al.* Mutations in the basic domain and the loop-helix II junction of TWIST abolish DNA binding in Saethre-Chotzen syndrome. *FEBS Lett* 2001; **492**:112-118.
30 Hamamori Y, Wu HY, Sartorelli V, Kedes L. The basic domain of myogenic basic helix-loop-helix (bHLH) proteins is the novel target for direct inhibition by another bHLH protein, Twist. *Mol Cell Biol* 1997; **17**:6563-6573.
31 Spicer DB, Rhee J, Cheung WL, Lassar AB. Inhibition of myogenic bHLH and MEF2 transcription factors by the bHLH protein Twist. *Science* 1996; **272**:1476-1480.
32 Spring J, Yanze N, Middel AM, Stierwald M, Groger H, Schmid V. The mesoderm specification factor twist in the life cycle of jellyfish. *Dev Biol* 2000; **228**:363-375.
33 Seto ML, Hing AV, Chang J*, et al.* Isolated sagittal and coronal craniosynostosis associated with TWIST box mutations. *Am J Med Genet A* 2007; **143**:678-686.
34 Laursen KB, Mielke E, Iannaccone P, Fuchtbauer EM. Mechanism of transcriptional activation by the proto-oncogene Twist1. *J Biol Chem* 2007; **282**:34623-34633.
35 Kress W, Schropp C, Lieb G*, et al.* Saethre-Chotzen syndrome caused by TWIST 1 gene mutations: functional differentiation from Muenke coronal synostosis syndrome. *Eur J Hum Genet* 2006; **14**:39-48.
36 Singh S, Gramolini AO. Characterization of sequences in human TWIST required for nuclear localization. *BMC Cell Biol* 2009; **10**:47.
37 Hamamori Y, Sartorelli V, Ogryzko V*, et al.* Regulation of histone acetyltransferases p300 and PCAF by the bHLH protein twist and adenoviral oncoprotein E1A. *Cell* 1999; **96**:405-413.
38 Pan DJ, Huang JD, Courey AJ. Functional analysis of the *Drosophila* twist promoter reveals a dorsal-binding ventral activator region. *Genes Dev* 1991; **5**:1892-1901.
39 Ip YT, Park RE, Kosman D, Bier E, Levine M. The dorsal gradient morphogen regulates stripes of rhomboid expression in the presumptive neuroectoderm of the *Drosophila* embryo. *Genes Dev* 1992; **6**:1728-1739.
40 Bate M, Rushton E, Currie DA. Cells with persistent twist expression are the embryonic precursors of adult muscles in *Drosophila*. *Development* 1991; **113**:79-89.
41 Currie DA, Bate M. The development of adult abdominal muscles in *Drosophila*: myoblasts express twist and are associated with nerves. *Development* 1991; **113**:91-102.
42 Fernandes J, Bate M, Vijayraghavan K. Development of the indirect flight muscles of *Drosophila*. *Development* 1991; **113**:67-77.
43 Figeac N, Daczewska M, Marcelle C, Jagla K. Muscle stem cells and model systems for their investigation. *Dev Dyn* 2007; **236**:3332-3342.
44 Fuchtbauer EM. Expression of M-twist during postimplantation development of the mouse. *Dev Dyn* 1995; **204**:316-322.
45 Stoetzel C, Weber B, Bourgeois P, Bolcato-Bellemin AL, Perrin-Schmitt F. Dorso-ventral and rostro-caudal sequential expression of M-twist in the postimplantation murine embryo. *Mech Dev* 1995; **51**:251-263.
46 Gitelman I. Twist protein in mouse embryogenesis. *Dev Biol* 1997; **189**:205-214.
47 Hebrok M, Wertz K, Fuchtbauer EM. M-twist is an inhibitor of muscle differentiation. *Dev Biol* 1994; **165**:537-544.
48 O'Rourke MP, Tam PP. Twist functions in mouse development. *Int J Dev Biol* 2002; **46**:401-413.
49 Zhao P, Hoffman EP. Embryonic myogenesis pathways in muscle regeneration. *Dev Dyn* 2004; **229**:380-392.
50 Murray SS, Glackin CA, Winters KA, Gazit D, Kahn AJ, Murray EJ. Expression of helix-loop-helix regulatory genes during differentiation of mouse osteoblastic cells. *J Bone Miner Res* 1992; **7**:1131-1138.
51 Pan D, Fujimoto M, Lopes A, Wang YX. Twist-1 is a PPARdelta-inducible, negative-feedback regulator of PGC-1alpha in brown fat metabolism. *Cell* 2009; **137**:73-86.
52 Pettersson AT, Laurencikiene J, Mejhert N*, et al.* A possible inflammatory role of twist1 in human white adipocytes. *Diabetes* 2010; **59**:564-571.
53 Isenmann S, Arthur A, Zannettino AC*, et al.* TWIST family of basic helix-loop-helix transcription factors mediate human mesenchymal stem cell growth and commitment. *Stem Cells* 2009; **27**:2457-2468.
54 Rose CS, Malcolm S. A TWIST in development. Trends Gen-

et 1997; **13**:384-387.
55 Rice DP, Aberg T, Chan Y*, et al.* Integration of FGF and TWIST in calvarial bone and suture development. *Development* 2000; **127**:1845-1855.
56 Connerney J, Andreeva V, Leshem Y, Muentener C, Mercado MA, Spicer DB. Twist1 dimer selection regulates cranial suture patterning and fusion. *Dev Dyn* 2006; **235**:1345-1357.
57 Connerney J, Andreeva V, Leshem Y*, et al.* Twist1 homodimers enhance FGF responsiveness of the cranial sutures and promote suture closure. *Dev Biol* 2008; **318**:323-334.
58 Miraoui H, Severe N, Vaudin P, Pages JC, Marie PJ. Molecular silencing of Twist1 enhances osteogenic differentiation of murine mesenchymal stem cells: implication of FGFR2 signaling. *J Cell Biochem* 2010; **110**:1147-1154.
59 Paznekas WA, Cunningham ML, Howard TD*, et al.* Genetic heterogeneity of Saethre-Chotzen syndrome, due to TWIST and FGFR mutations. *Am J Hum Genet* 1998; **62**:1370-1380.
60 Guenou H, Kaabeche K, Mee SL, Marie PJ. A role for fibroblast growth factor receptor-2 in the altered osteoblast phenotype induced by Twist haploinsufficiency in the Saethre-Chotzen syndrome. *Hum Mol Genet* 2005; **14**:1429-1439.
61 Massari ME, Murre C. Helix-loop-helix proteins: regulators of transcription in eucaryotic organisms. *Mol Cell Biol* 2000; **20**:429-440.
62 Hayashi M, Nimura K, Kashiwagi K*, et al.* Comparative roles of Twist-1 and Id1 in transcriptional regulation by BMP signaling. *J Cell Sci* 2007; **120**:1350-1357.
63 Wilkie AO, Oldridge M, Tang Z, Maxson RE, Jr. Craniosynostosis and related limb anomalies. *Novartis Found Symp* 2001; **232**:122-133; discussion 133-143.
64 Zuniga A, Quillet R, Perrin-Schmitt F, Zeller R. Mouse Twist is required for fibroblast growth factor-mediated epithelial-mesenchymal signalling and cell survival during limb morphogenesis. *Mech Dev* 2002; **114**:51-59.
65 O'Rourke MP, Soo K, Behringer RR, Hui CC, Tam PP. Twist plays an essential role in FGF and SHH signal transduction during mouse limb development. *Dev Biol* 2002; **248**:143-156.
66 Rice DP, Connor EC, Veltmaat JM*, et al.* Gli3Xt-J/Xt-J mice exhibit lambdoid suture craniosynostosis which results from altered osteoprogenitor proliferation and differentiation. *Hum Mol Genet* 2010; **19**:3457-3467.
67 Loebel DA, O'Rourke MP, Steiner KA, Banyer J, Tam PP. Isolation of differentially expressed genes from wild-type and Twist mutant mouse limb buds. *Genesis* 2002; **33**:103-113.
68 Firulli AB, Conway SJ. Phosphoregulation of Twist1 provides a mechanism of cell fate control. *Curr Med Chem* 2008; **15**:2641-2647.
69 Firulli AB. A HANDful of questions: the molecular biology of the heart and neural crest derivatives (HAND)-subclass of basic helix-loop-helix transcription factors. *Gene* 2003; **312**:27-40.
70 Ducy P, Zhang R, Geoffroy V, Ridall AL, Karsenty G. Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. *Cell* 1997; **89**:747-754.
71 Galler KM, Yasue A, Cavender AC, Bialek P, Karsenty G, D'Souza RN. A novel role for Twist-1 in pulp homeostasis. *J Dent Res* 2007; **86**:951-955.
72 Sosic D, Richardson JA, Yu K, Ornitz DM, Olson EN. Twist regulates cytokine gene expression through a negative feedback loop that represses NF-kappaB activity. *Cell* 2003; **112**:169-180.
73 El Ghouzzi V, Legeai-Mallet L, Aresta S*, et al.* Saethre-Chotzen mutations cause TWIST protein degradation or impaired nuclear location. *Hum Mol Genet* 2000; **9**:813-819.
74 Hong J, Zhou J, Fu J*, et al.* Phosphorylation of serine 68 of Twist1 by MAPKs stabilizes Twist1 protein and promotes breast cancer cell invasiveness. *Cancer Res* 2011; **71**:3980-3990.
75 Lee TK, Poon RT, Yuen AP*, et al.* Twist overexpression correlates with hepatocellular carcinoma metastasis through induction of epithelial-mesenchymal transition. *Clin Cancer Res* 2006; **12**:5369-5376.
76 Yang MH, Chen CL, Chau GY*, et al.* Comprehensive analysis of the independent effect of twist and snail in promoting metastasis of hepatocellular carcinoma. *Hepatology* 2009; **50**:1464-1474.
77 Yuen HF, Chua CW, Chan YP, Wong YC, Wang X, Chan KW. Significance of TWIST and E-cadherin expression in the metastatic progression of prostatic cancer. *Histopathology* 2007; **50**:648-658.
78 Luo GQ, Li JH, Wen JF, Zhou YH, Hu YB, Zhou JH. Effect and mechanism of the Twist gene on invasion and metastasis of gastric carcinoma cells. *World J Gastroenterol* 2008; **14**:2487-2493.
79 Feng MY, Wang K, Song HT*, et al.* Metastasis-induction and apoptosis-protection by TWIST in gastric cancer cells. *Clin Exp Metastasis* 2009; **26**:1013-1023.
80 Yuen HF, Chan YP, Wong ML*, et al.* Upregulation of Twist in oesophageal squamous cell carcinoma is associated with neoplastic transformation and distant metastasis. *J Clin Pathol* 2007; **60**:510-514.
81 Sasaki K, Natsugoe S, Ishigami S*, et al.* Significance of Twist expression and its association with E-cadherin in esophageal squamous cell carcinoma. *J Exp Clin Cancer Res* 2009; **28**:158.
82 Zhang Z, Xie D, Li X*, et al.* Significance of TWIST expression and its association with E-cadherin in bladder cancer. *Hum Pathol* 2007; **38**:598-606.
83 Wallerand H, Robert G, Pasticier G*, et al.* The epithelial-mesenchymal transition-inducing factor TWIST is an attractive target in advanced and/or metastatic bladder and prostate cancers. *Urol Oncol* 2009; **28**:473-479.
84 Satoh K, Hamada S, Kimura K*, et al.* Up-regulation of MSX2 enhances the malignant phenotype and is associated with twist 1 expression in human pancreatic cancer cells. *Am J Pathol* 2008; **172**:926-939.
85 Maestro R, Dei Tos AP, Hamamori Y*, et al.* Twist is a potential oncogene that inhibits apoptosis. *Genes Dev* 1999; **13**:2207-2217.
86 Valsesia-Wittmann S, Magdeleine M, Dupasquier S*, et al.* Oncogenic cooperation between H-Twist and N-Myc overrides failsafe programs in cancer cells. *Cancer Cell* 2004; **6**:625-630.
87 Ansieau S, Bastid J, Doreau A*, et al.* Induction of EMT by twist proteins as a collateral effect of tumor-promoting inactivation of premature senescence. *Cancer Cell* 2008; **14**:79-89.
88 Cheng GZ, Chan J, Wang Q, Zhang W, Sun CD, Wang LH.

Twist transcriptionally up-regulates AKT2 in breast cancer cells leading to increased migration, invasion, and resistance to paclitaxel. *Cancer Res* 2007; **67**:1979-1987.
89 Mani SA, Guo W, Liao MJ, *et al.* The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 2008; **133**:704-715.
90 Vesuna F, Lisok A, Kimble B, Raman V. Twist modulates breast cancer stem cells by transcriptional regulation of CD24 expression. *Neoplasia* 2009; **11**:1318-1328.
91 Battula VL, Evans KW, Hollier BG, *et al.* Epithelial-mesenchymal transition-derived cells exhibit multilineage differentiation potential similar to mesenchymal stem cells. *Stem Cells* 2010; **28**:1435-1445.
92 Qin L, Liu Z, Chen H, Xu J. The steroid receptor coactivator-1 regulates twist expression and promotes breast cancer metastasis. *Cancer Res* 2009; **69**:3819-3827.
93 Vernon AE, LaBonne C. Tumor metastasis: a new twist on epithelial-mesenchymal transitions. *Curr Biol* 2004; **14**:R719-721.
94 Karreth F, Tuveson DA. Twist induces an epithelial-mesenchymal transition to facilitate tumor metastasis. *Cancer Biol Ther* 2004; **3**:1058-1059.
95 Yang J, Mani SA, Weinberg RA. Exploring a new twist on tumor metastasis. *Cancer Res* 2006; **66**:4549-4552.
96 Welch DR, Steeg PS, Rinker-Schaeffer CW. Molecular biology of breast cancer metastasis. Genetic regulation of human breast carcinoma metastasis. *Breast Cancer Res* 2000; **2**:408-416.
97 Yu W, Kamara H, Svoboda KK. The role of twist during palate development. *Dev Dyn* 2008; **237**:2716-2725.
98 Ling X, Arlinghaus RB. Knockdown of STAT3 expression by RNA interference inhibits the induction of breast tumors in immunocompetent mice. *Cancer Res* 2005; **65**:2532-2536.
99 Martin TA, Goyal A, Watkins G, Jiang WG. Expression of the transcription factors snail, slug, and twist and their clinical significance in human breast cancer. *Ann Surg Oncol* 2005; **12**:488-496.
100 Mironchik Y, Winnard PT Jr, Vesuna F, *et al.* Twist overexpression induces *in vivo* angiogenesis and correlates with chromosomal instability in breast cancer. *Cancer Res* 2005; **65**:10801-10809.
101 Watson MA, Ylagan LR, Trinkaus KM, *et al.* Isolation and molecular profiling of bone marrow micrometastases identifies TWIST1 as a marker of early tumor relapse in breast cancer patients. *Clin Cancer Res* 2007; **13**:5001-5009.
102 Ma L, Teruya-Feldstein J, Weinberg RA. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. *Nature* 2007; **449**:682-688.
103 Eckert MA, Lwin TM, Chang AT, *et al.* Twist1-induced invadopodia formation promotes tumor metastasis. *Cancer Cell* 2011; **19**:372-386.
104 Xu J, Li Q. Review of the *in vivo* functions of the p160 steroid receptor coactivator family. *Mol Endocrinol* 2003; **17**:1681-1692.
105 Xu J, Wu RC, O'Malley BW. Normal and cancer-related functions of the p160 steroid receptor co-activator (SRC) family. *Nat Rev Cancer* 2009; **9**:615-630.
106 Wang S, Yuan Y, Liao L, *et al.* Disruption of the SRC-1 gene in mice suppresses breast cancer metastasis without affecting primary tumor formation. *Proc Natl Acad Sci U S A* 2009; **106**:151-156.
107 Niu RF, Zhang L, Xi GM, *et al.* Up-regulation of Twist induces angiogenesis and correlates with metastasis in hepatocellular carcinoma. *J Exp Clin Cancer Res* 2007; **26**:385-394.
108 Matsuo N, Shiraha H, Fujikawa T, *et al.* Twist expression promotes migration and invasion in hepatocellular carcinoma. *BMC Cancer* 2009; **9**:240.
109 Sun T, Zhao N, Zhao XL, *et al.* Expression and functional significance of Twist1 in hepatocellular carcinoma: its role in vasculogenic mimicry. *Hepatology* 2010; **51**:545-556.
110 Yuen HF, Kwok WK, Chan KK, *et al.* TWIST modulates prostate cancer cell-mediated bone cell activity and is upregulated by osteogenic induction. *Carcinogenesis* 2008; **29**:1509-1518.
111 Alexander NR, Tran NL, Rekapally H, Summers CE, Glackin C, Heimark RL. N-cadherin gene expression in prostate carcinoma is modulated by integrin-dependent nuclear translocation of Twist1. *Cancer Res* 2006; **66**:3365-3369.
112 Feng MY, Wang K, Shi QT, Yu XW, Geng JS. Gene expression profiling in TWIST-depleted gastric cancer cells. *Anat Rec (Hoboken)* 2009; **292**:262-270.
113 Xie F, Li K, Ouyang X. Twist, an independent prognostic marker for predicting distant metastasis and survival rates of esophageal squamous cell carcinoma patients. *Clin Exp Metastasis* 2009; **26**:1025-1032.
114 Elias MC, Tozer KR, Silber JR, *et al.* TWIST is expressed in human gliomas and promotes invasion. *Neoplasia* 2005; **7**:824-837.
115 Horikawa T, Yang J, Kondo S, *et al.* Twist and epithelial-mesenchymal transition are induced by the EBV oncoprotein latent membrane protein 1 and are associated with metastatic nasopharyngeal carcinoma. *Cancer Res* 2007; **67**:1970-1978.
116 Ou DL, Chien HF, Chen CL, Lin TC, Lin LI. Role of Twist in head and neck carcinoma with lymph node metastasis. *Anticancer Res* 2008; **28**:1355-1359.
117 Terauchi M, Kajiyama H, Yamashita M, *et al.* Possible involvement of TWIST in enhanced peritoneal metastasis of epithelial ovarian carcinoma. *Clin Exp Metastasis* 2007; **24**:329-339.
118 Wang X, Ling MT, Guan XY, *et al.* Identification of a novel function of TWIST, a bHLH protein, in the development of acquired taxol resistance in human cancer cells. *Oncogene* 2004; **23**:474-482.
119 Pham CG, Bubici C, Zazzeroni F, *et al.* Upregulation of Twist-1 by NF-kappaB blocks cytotoxicity induced by chemotherapeutic drugs. *Mol Cell Biol* 2007; **27**:3920-3935.
120 Yang MH, Wu MZ, Chiou SH, *et al.* Direct regulation of TWIST by HIF-1alpha promotes metastasis. *Nat Cell Biol* 2008; **10**:295-305.
121 Carrero P, Okamoto K, Coumailleau P, O'Brien S, Tanaka H, Poellinger L. Redox-regulated recruitment of the transcriptional coactivators CREB-binding protein and SRC-1 to hypoxia-inducible factor 1alpha. *Mol Cell Biol* 2000; **20**:402-415.
122 Hu L, Roth JM, Brooks P, Ibrahim S, Karpatkin S. Twist is required for thrombin-induced tumor angiogenesis and growth. *Cancer Res* 2008; **68**:4296-4302.
123 Sharif MN, Sosic D, Rothlin CV, *et al.* Twist mediates sup-

pression of inflammation by type I IFNs and Axl. *J Exp Med* 2006; **203**:1891-1901.
124 Kalra J, Sutherland BW, Stratford AL*, et al*. Suppression of Her2/neu expression through ILK inhibition is regulated by a pathway involving TWIST and YB-1. *Oncogene* 2010; **29**:6343-6356.
125 Yang MH, Hsu DS, Wang HW*, et al.* Bmi1 is essential in Twist1-induced epithelial-mesenchymal transition. *Nat Cell Biol* 2010; **12**:982-992.
126 Vesuna F, van Diest P, Chen JH, Raman V. Twist is a transcriptional repressor of E-cadherin gene expression in breast cancer. *Biochem Biophys Res Commun* 2008; **367**:235-241.
127 Okamura H, Yoshida K, Haneji T. Negative regulation of TIMP1 is mediated by transcription factor TWIST1. *Int J Oncol* 2009; **35**:181-186.
128 Denslow SA, Wade PA. The human Mi-2/NuRD complex and gene regulation. *Oncogene* 2007; **26**:5433-5438.
129 Brehm A, Langst G, Kehle J*, et al.* dMi-2 and ISWI chromatin remodelling factors have distinct nucleosome binding and mobilization properties. *EMBO J* 2000; **19**:4332-4341.
130 Guschin D, Wade PA, Kikyo N, Wolffe AP. ATP-Dependent histone octamer mobilization and histone deacetylation mediated by the Mi-2 chromatin remodeling complex. *Biochemistry* 2000; **39**:5238-5245.
131 Qian YW, Wang YC, Hollingsworth RE Jr, Jones D, Ling N, Lee EY. A retinoblastoma-binding protein related to a negative regulator of Ras in yeast. *Nature* 1993; **364**:648-652.
132 Zhang Y, Ng HH, Erdjument-Bromage H, Tempst P, Bird A, Reinberg D. Analysis of the NuRD subunits reveals a histone deacetylase core complex and a connection with DNA methylation. *Genes Dev* 1999; **13**:1924-1935.
133 Marhold J, Brehm A, Kramer K. The *Drosophila* methyl-DNA binding protein MBD2/3 interacts with the NuRD complex via p55 and MI-2. *BMC Mol Biol* 2004; **5**:20.
134 Bowen NJ, Fujita N, Kajita M, Wade PA. Mi-2/NuRD: multiple complexes for many purposes. *Biochim Biophys Acta* 2004; **1677**:52-57.
135 Manavathi B, Kumar R. Metastasis tumor antigens, an emerging family of multifaceted master coregulators. *J Biol Chem* 2007; **282**:1529-1533.
136 Yao YL, Yang WM. The metastasis-associated proteins 1 and 2 form distinct protein complexes with histone deacetylase activity. *J Biol Chem* 2003; **278**:42560-42568.