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The Role of Snail in EMT and Tumorigenesis

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Abstract

Epithelial-mesenchymal transition (EMT) is a highly conserved process in which polarized, immotile epithelial cells lose adherent and tight junctions, and become migratory mesenchymal cells. As a key transcriptional repressor of E-cadherin expression in EMT, Snail plays an important role in embryonic development and cancer progression. Emerging evidences indicate that Snail confers tumor cells with cancer stem cell-like traits, and promotes drug resistance, tumor recurrence and metastasis. In this review, we summarize recent developments underlying the regulation and functions of Snail in tumor progression, and discuss new approaches against EMT in preventing metastatic cancers.

Keywords

Breast cancer; EMT; metastasis; signaling pathway; Snail

Introduction

Epithelial-mesenchymal transition (EMT) is a profound event for large-scale cell movement during morphogenesis at the time of embryonic development. During this process, epithelial cells loose contact with their neighbors and gain mesenchymal properties, enabling them to break through the basement membrane that separates different tissues within the embryo [1,2]. Because a similar process has observed at the invasive front of metastatic cancer, it suggests that tumor cells usurp the developmental EMT program for their metastatic dissemination. One of the hallmarks of EMT is the functional loss of E-cadherin (encoded by *CDH1*), which is thought to be a metastatic suppressor during tumor progression [3]. Snail is a prominent inducer of EMT and strongly represses E-cadherin expression [4]. In addition to inducing EMT, Snail superfamily members have been implicated in various important developmental processes, including neural differentiation, cell fate and survival

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decisions, as well as left-right identity. Expression of Snail positively correlates with tumor grade, recurrence, metastasis, and poor prognosis in various tumors [5-8]. Therefore, it is important to understand the molecular and cellular mechanisms that control the expression and functions of Snail in tumors.

Structure of Snail

Snail contains various domains and motifs

Snail was first described in *Drosophila melanogaster*, where it was shown to be essential for the formation of the mesoderm [9,10]. Subsequently, Snail homologues have been found in many species from invertebrate to vertebrates, such as nematode, mollusks, and humans [6,11]. Three Snail family proteins have been identified in vertebrates: Snail1 (Snail), Snail2 (Slug), and Snail3 (Smuc). All the family members encode transcriptional repressors and share a similar organization with a highly conserved C-terminal domain, which contains four to six C_2H_2 type zinc fingers and bind to the E-box motif (5′-CANNTG-3′) in target gene promoters [11]. The N-terminal of all vertebrate Snail members contains the evolutionarily conserved SNAG (Snail/Gfi) domain essential for the binding of several transcriptional co-repressor complexes (Figure 1). *Drosophila* Snail does not contain the SNAG domain, but it has a consensus P-DLS-K motif and exerts its repressive function through the interaction with co-repressor CtBP [12]. In the central region of Snail, a serine-rich domain (SRD) and a nuclear export sequence (NES) are found in the regulation of protein stability and subcellular localization of Snail, respectively [13].

Snail interacts with different signaling molecules

The SNAG domain, located at the N-terminus, is required for Snail to interact with several co-repressor complexes, including Sin3A and histone deacetylase 1/2 (HDAC1/2) complex [14]; Polycomb repressive complex 2 (PRC2) [15]; 14-3-3 [16] and protein arginine methyltransferase 5 (PRMT5)/Ajuba complex [17]; lysine-specific demethylase 1 (LSD1)/CoREST complex [18]. Recently, Suv39H1 (suppressor of variegation 3-9 homolog 1), a key component of methyltransferase responsible for H3K9me3, was also identified as a protein that associate with the SNAG domain [19] (Figure 1).

Snail activity is mainly regulated through the central part of the protein which contains most sites for post-translational modifications: serine phosphorylation sites in the SRD, two lysine oxidation sites and the NES for CRM1-dependent nuclear export. Glycogen synthase kinase-3β (GSK-3β) is the major kinase responsible for the phosphorylation at the SRD region [20,21]; whereas small C-terminal domain phosphatase (SCP) interacts with Snail and removes these phosphorylations [22]. Phosphorylated Snail interacts with and is degraded by β-Trcp [21]. Besides β-TrCP1, FBXL14, an F-box E3 ubiquitin ligase, also interacts with Snail and promotes its ubiquitination and proteosome degradation [23]. In addition, oxidase-like 2 (LOXL2) binds to the SNAG domain of Snail and antagonizes the association of FBXL14 or β-TrCP1 to Snail, resulting in Snail stabilization [23]. The C-terminal zinc finger region mediates sequence-specific interactions with DNA. It is also responsible for the repressor activity of Snail. PAK1 phosphorylates Snail on Ser 246 to

promote Snail's nuclear accumulation and consequently enhances its repressor activity in the nucleus [24].

Regulation of Snail

Snail is regulated by various signals from the tumor microenvironment

EMT is a dynamic process triggered by stimuli that emanate from microenvironments, including the extracellular matrix (such as collagen and hyaluronic acid) and many secreted soluble factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), bone morphogenetic proteins (BMPs), transforming growth factor-β (TGF-β), Notch, Wnt, tumor necrosis factor-α (TNF-α), and cytokines [25,26]. Expression of Snail can be found in these EMT processes where they have been studied [11]; many signaling molecules from tumor microenvironment have been shown to induce Snail expression in different cellular contexts (Figure 2).

For example, receptor tyrosine kinases (RTKs) signaling, activated by HGF, FGF, or EGF, acts through the RAS-MAPK or PI3K-Akt pathway and results in the induction of Snail [27,28]. Signaling via MAPK or PI3K has been reported as necessary and sufficient to regulate EMT in collaboration with TGF-β, which is also involved in the induction of Snail [29]. TGF-β is a multifunctional cytokine that regulates cell proliferation, differentiation and apoptosis [30]. It suppresses tumor development at early stages; however, it promotes tumor progression when cells become resistant to TGF-β [31,32]. Snail plays an important role in mediating the escape from the tumor suppressive effects of TGF-β. It confers resistance to TGF-β-mediated apoptosis and switches the response to tumor progression [33]. In the late stages, TGF-β induces EMT by up-regulating Snail via a Smad-dependent manner [6]. It has been demonstrated that TGF-β, via the Smad pathway, induces expression of high mobility group A2 (HMGA2) which regulates expression of many important repressors of E-cadherin [34]. Smads and HMGA2 cooperatively bind to the Snail promoter and induce Snail expression, E-cadherin repression, and the overall EMT phenotype [35].

During TGF-β-induced EMT, it has also been shown that Snail forms a transcriptional repressor complex with SMAD3/4. This complex targets the adjacent E-boxes and Smad-binding elements in genes encoding junction proteins such as E-cadherin, CAR and occludin, resulting in genes repression [36]. In addition, the TGF-β-Smad pathway also cooperates with Ras, Notch and Wnt signaling in inducing Snail expression in development and in tumor metastasis [37]. TGF-β increases Notch activity through Smad3, which up-regulates both Jagged1 and HEY1. Elevated Jagged1 and Notch promote Slug expression, thereby suppressing E-cadherin and leading to an EMT phenotype [38]. Notch controls Snail expression by two distinct but synergistic mechanisms including direct transcriptional activation of Snail and an indirect mechanism operating via lysyl oxidase (LOX). Notch increases LOX expression by recruiting hypoxia-inducible factor 1α (HIF-1α) to LOX promoter, which stabilizes Snail protein and results in up-regulation of EMT and the migration and invasion of cancer cells [39,40].

In the presence of Wnt signaling, GSK-3β is unable to phosphorylate two of its known targets, β-catenin and Snail, and therefore stabilizes these two molecules in the nucleus. β-

catenin, acting as a transcription factor through its interaction with TCF/LEF, is required for EMT both in epithelial cells and during heart cushion development [4,41,42]. In human breast cancer cells, canonical Wnt signaling activates the EMT by inducing the expression of intracellular protein Axin2 to stabilize Snail [43]. Therefore, by blocking the activity of GSK-3β, Wnt can stabilize the level of Snail and β-catenin to induce EMT and cancer metastasis. The synergistic effect of Snail and β-catenin provides cancer cells with the ability to survive during dissemination and invasion. Recently, a new function of Wnt/Snail-mediated tumor progression was found. Through the canonical β-catenin/TCF4/Snail pathway, Wnt signaling suppresses mitochondrial respiration via inhibiting cytochrome C oxidase (COX) activity and induces a glycolytic switch via increased glucose consumption and lactate production [44].

NF-κB pathway regulates Snail expression via transcriptional and post-translational mechanisms. It has been shown that the expression of Snail can be directly activated by NF-κB homologue Dorsal in *drosophila* [45]. NF-κB can also bind to the human Snail promoter at the region between −194 and −78 bp and increase the transcription of Snail [46]. In addition, Akt can activate NF-κB through direct phosphorylation of IKKα [47-49], and thus results in the upregulation of Snail [50]. Our study found that TNF-α is the major inflammatory cytokine to induce Snail stabilization and EMT induction [51]. The TNF-α/NF-κB stabilized Snail is mediated by the transcription induction of COP9 signalosome 2 (CSN2) which inhibits the phosphorylation and ubiquitylation of Snail by disrupting the binding of Snail with GSK-3β and β-Trcp, and results in the Snail stabilization at a non-phosphorylated and non-ubiquitinated functional state. CSN2 is the second and most conserved component of the eight subunits of CSN complex which controls the functional assembly and activity of cullin-RING ubiquitin ligases [52]. Recent studies show that the metastatic suppressor Raf kinase inhibitor protein (RKIP) can inhibit NF-κB activity, and conversely, Snail can repress the expression of RKIP. Therefore, there is a circuitry between RKIP, NF-κB and Snail in which overexpression of Snail inhibits RKIP and induces EMT [53-55].

Snail is modulated at the transcription level

At the transcriptional level, Snail is regulated by many signaling pathways. For instance, there is evidence for the direct regulation of Snail promoter by different growth factors, such as TGF-β, FGF2 and EGF as well as different signaling molecules like integrin linked kinase (ILK), H-ras, v-Akt and NF-κB/p65 [46,56]. ERK pathway is involved in the activation of Snail minimal promoter (−78/+59). NF-κB/p65 also stimulates Snail transcription through a region located immediately upstream the minimal promoter, between −194 and −78. Moreover, in mammary epithelial cells, TGF-β induces expression of HMGA2 via the Smad pathway, and subsequently Smads and HMGA2 cooperatively bind to the Snail promoter and induce Snail expression [35]. HGF is also involved in transcriptional regulation of Snail. HGF-mediated MAPK activation induces the expression of early growth response-1 (Egr-1), which can bind to the Snail promoter and activate its expression; in turn, Snail also binds to the Egr-1 promoter and represses Egr-1 transcription, thereby establishing a negative regulatory feedback loop [57]. Interestingly, recent studies have shown that Snail binds to and represses its own promoter, indicating the existence of an

autoregulatory loop [58]. As for Slug, surprisingly, it has the ability to activate its own promoter during avian neural crest development, although Snail family proteins have been known as a repressor [59]. Comparative analysis of Snail and Slug promoters from different species has identified conserved and functional response elements, including AP1 and AP4 sites, Smad-binding elements, LEF1 binding sites and two conserved E-boxes[6]. In addition, a 3′ enhancer element, conserved throughout the mammalian lineage, has been described to directly control expression of Snail via interaction with the Snail promoter [60].

Snail is also controlled through post-translation modifications

Snail is a labile protein with a short half-life. In the nucleus, its turnover is decreased while in the cytosol it is rapidly degraded by proteasomes [21]. Post-translational modifications, such as phosphorylation, ubiquitination, and lysine oxidation all influence Snail protein stability, subcellular localization and activity. We found that Snail is degraded by proteasome after GSK-3β-dependent phosphorylation and β-TrCP-mediated ubiquitination [21]. The two phosphorylation motifs within Snail, one for protein degradation and the other for subcellular localization, dually regulate the function of this protein. We propose that GSK-3β binds and phosphorylates Snail (at motif 2) and thereby induces its nuclear export through a conformational change mediated by the prolyl isomerase PIN1. Subsequent phosphorylation by GSK-3β (at motif 1) results in the association of Snail with β-Trcp, and thus leads to the degradation of Snail in cytoplasm [21]. The phosphorylation by GSK-3β is counteracted by the action of SCP, which stabilizes Snail in the nucleus [22]. Hyperglycaemia-regulated *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc) modification of Snail at Ser 112 also stabilizes Snail and increases its repressor function by inhibiting GSK-3β-mediated phosphorylation [61]. Post-translational regulation of Snail stability by GSK-3β-independent mechanisms has also been reported. For example, stabilization of Snail is stimulated by LOXL2 interaction and occurs by interfering with FBXL14 binding to Snail [23,62]. Either the phosphorylated or the unphosphorylated forms of the Snail can interact with FBXL14, resulting in Snail ubiquitination and proteasome degradation [23]. The FBXL14 orthologue Ppa has been reported to interact with Slug in *Xenopus*, specifically with a hydrophobic region of Slug located at the N-terminus [63]. Our study shows that Snail is stabilized by the TNF-α/NF-κB pathway through the induction of CSN2, which blocks the phosphorylation and ubiquitination of Snail by disrupting its binding to GSK-3β and β-Trcp [51]. Another proposed modulator of Snail is p21-activated kinase (PAK1), which phosphorylates Snail at Ser 246, resulting in increased protein retention in the nucleus [24]. Moreover, phosphorylation of Snail at Ser 11 and 92, mediated by cAMP-activated kinase PKA and the ubiquitous serine/threonine protein kinase CK2, respectively, positively regulate the function of Snail [64]. In addition, Lats2 kinase interacts with Snail, directly phosphorylates Snail at residue Thr 203, and serves to retain Snail in the nucleus thereby enhancing its stability [65].

Snail controls target-gene expression via epigenetic regulation

In the eukaryotic nucleus, DNA is packaged with core histones and other chromosomal proteins to form chromatin [66-68]. Chromatin harbors not only genetic information encoded in the DNA but also epigenetic information carried by the reversible covalent modifications at the N-terminal tails of histones [69]. Histone modifications, such as

acetylation, phosphorylation and methylation, control gene expression by altering chromatin structure [70-72]. As a transcription factors, Snail contains DNA-binding motifs, however, it does not modify chromatin. On the contrary, chromatin-modifying enzymes contain many histone-binding modules (such as the Bromo, Chromo, and PhD domains) and are capable of changing the chromatin structure through acetylation or methylation although they lack specific DNA-binding motifs [18].

The manner in which Snail recruits specific chromatin-modifying enzymes to its target gene promoters is an interesting area investigation. It has been demonstrated that Snail can interact with chromatin remodeling factors through the SNAG domain to exert its function of transcriptional repression. For instance, Snail interacts with the Sin3A-HDAC1/2 complex through the SNAG domain to deacetylate histone H3 and H4, leading to the repression of E-cadherin expression [14]. In addition, Snail has been shown to interact with Ezh2 and Suz12 of PRC2 and induce the subsequent trimethylation of H3K27 on the E-cadherin promoter [15]. Furthermore, PRMT5 has been identified as an effector recruited to Snail through an interaction with Ajuba on the SNAG domain. The Snail-Ajuba-PRMT5 ternary complex is found at the proximal promoter region of the E-cadherin gene, where it increases arginine methylation of H4R3 [17].

Our study identified LSD1 as a partner of Snail [18]. LSD1 is the first identified histone demethylase that specifically removes methylations of H3K4, a transcriptional mark associated with gene activation [73]. We proposed a model in which the SNAG domain of Snail resembles a histone H3-like structure and functions as a molecular hook for recruiting LSD1 to the E-cadherin promoter for the demethylation on H3K4 [18]. However, H3K4 demethylation is known to be an initial step in gene repression [74], suggesting that an intermediate step is required to bridge H3K4 demethylation to the DNA methylation on the E-cadherin promoter. Recently, we identified that Snail also interacted with G9a and Suv39H1, two major methyltransferases responsible for H3K9 methylation that intimately link to DNA methylation with different mechanisms. G9a interacts with DNA methyltransferases (DNMTs) directly and recruits it to target gene promoters for DNA methylation, whereas Suv39H1 creates an H3K9me3 binding and docking site for the adapter molecule HP1 which in turn recruits DNMT and HDAC to catalyze DNA methylation and histone deacetylation, respectively [19,75,76]. Therefore, demethylation on H3K4 (by LSD1), together with dimethylation and trimethylation on H3K9 (by G9a and Suv39H1), provide synergistic binary switch in gene repression.

Functions of Snail

Snail is an important EMT inducer

Metastasis is responsible for a majority of cancer patient deaths. The metastatic cascade is a complex process divided into a series of steps, including detachment of tumor cells from the primary tumor, invasion, intravasation, survival within the circulation, extravasation and colonization at the secondary site [77]. EMT is involved in the metastatic cascade of many solid tumors and represents as a hallmark of this event. In cancer, EMT entails the molecular reprogramming and phenotypic changes that characterize the conversion of immobile cancer epithelial cells to motile mesenchymal cells. Several transcription factors, including the

Snail/Slug family, Twist, δEF1/ZEB1, SIP1/ZEB2 and E12/E47 respond to different microenvironmental stimuli and function as molecular switches of the EMT program [11,78,79]. As a critical regulator of multiple signaling pathways leading to EMT, the expression of Snail is closely associated with cancer metastasis. It has been demonstrated that Snail is required for lymph node metastasis of human breast carcinoma MDA-MB-231 cells [80]. A set of genes of the “lung metastasis signature” are direct or indirect targets of Snail such as ID1, SPARC or MMP2 [6,81]. It has also been observed that the level of expression of Snail is elevated in metastatic lesions in ovarian cancer [82]. A recent study demonstrated that Snail-induced EMT accelerates metastasis through induction of immune-suppression [83]. Knockdown of Snail significantly inhibits tumor growth and metastasis by increasing tumor-infiltrating lymphocytes and systemic immune responses [83]. Therefore, Snail is an effective target for preventing metastasis.

Slug, another member of the Snail family of transcription factor, is known to be required for neural crest cell migration during development, has been characterized as strong E-cadherin repressor and major EMT inducer and is correlated with distant metastasis [84,85]. It has been shown that Slug is required for the metastasis of the transformed melanoma cells [86]. Slug is an essential mediator of Twist-induced EMT and metastasis. Twist needs to induce Slug to suppress the epithelial branch of the EMT, then Twist and Slug act together to promote EMT and metastasis [87].

Snail increases cancer stem cell (CSC)-like features

Stem cells, which have an unlimited replicative and long life-time potential, are the basis for tissue homeostasis in the adult organism. Increasing evidence suggests that tumors develop and progress from a small subset of cells with the ability to self-renew and produce non-stem differentiated cells [88]. Such CSCs have been identified from several human tumors, including human acute myeloid leukemia malignancies, breast tumors, colon tumors, among others [89-91]. Recently, a relationship between EMT and CSCs was demonstrated. EMT facilitates the generation of CSCs with the mesenchymal traits required for dissemination as well as self-renewal properties needed for initiating secondary tumors [92]. The induction of EMT in immortalized human mammary epithelial cells (HMLE) by ectopic expression of Snail, Twist or exposure to TGF-β leads to an increased ability to form tumorspheres and yield cells enriched with $CD44^{high}/CD24^{low}$ markers. Those cells adopt a mesenchymal phenotype, are greatly enriched in tumor initiating cells, and are akin to breast CSCs [93]. In ovarian cancer cells, Snail and Slug effectively mediate cell survival and are involved in the acquisition of CSC-like traits [94]. These two molecules indirectly increase the activation of a self-renewal program by up-regulating NANOG, HDAC1, TCF4, KLF4, HDAC3 and GPC3, and further induce expression of other pluripotency activators including OCT4, BMI1 and Nestin; as well as increase the number of cells with $CD44^{+}CD117^{+}$, which represent as ovarian CSC markers [94]. In addition, Slug and Sox9 cooperatively determine the mammary stem cell state. Co-expression of both transcription factors confers upon dormant micrometastasis-forming cells extensive self-renewal ability, thereby allowing them to spawn macrometastases [95].

Snail increases tumor recurrence

Advancement in early detection technologies and cancer therapies has greatly improved the survival of cancer patients, however, the majority of patients will ultimately develop refractory or resistant disease because the residual tumor cells have the ability to survive in a dormant state following treatment [92]. Tumor recurrence is a cardinal manifestation of breast cancer progression and represents the principal cause of death from this disease. Recent studies have demonstrated that EMT plays a critical role not only in tumor metastasis, but also in drug resistance and tumor recurrence. As a master regulator of EMT and a core factor linked to the formation of CSCs, expression of Snail has been associated with tumor recurrence and resistance to chemotherapy and radiotherapy. Aberrant expression of Snail and/or Slug promotes resistance to programmed cell death in MCF7 cells elicited by the DNA damaging chemotherapeutic agent doxorubicin accompanied by acquisition of invasive growth properties [96]. Using a conditional transgenic mouse model for the recurrence of HER2/neu-induced mammary tumors, Snail is found spontaneously up-regulated in recurrent tumors and that recurrence is accompanied by EMT. It is further demonstrated that Snail is sufficient to promote mammary tumor recurrence *in vivo* and that high levels of Snail predict decreased relapse-free survival in women with breast cancer [7]. This is further confirmed by clinical data that high expression of Snail is a significant predictor of tumor recurrence in superficial bladder tumors [97].

Snail suppresses estrogen receptor signaling

Breast cancer is the most common malignancy diagnosed among women worldwide and is the leading cause of cancer mortality [98]. About 70% of breast cancers are estrogen receptor (ER)-positive and estrogen dependent [99]. ER is a key regulator of proliferation and differentiation in mammary epithelia and represents a crucial prognostic indicator and therapeutic target in breast cancer [100]. Loss of ERα in breast cancer is correlated with poor prognosis, increased recurrence after treatment, and an elevated incidence of metastasis [101]. Metastasis-associated protein 3 (MTA3), which is directly activated by ERα, is a component of the histone deacetylation Mi-2/NuRD complex in breast epithelial cells. This complex is dedicated to gene repression, and one direct target is Snail [100]. Therefore, the absence of ER or of MTA3 results in aberrant expression of Snail, and loss of expression of E-cadherin. Activated Snail in turn diminishes the ER activity maintaining the cell resistance to tamoxifen [102]. ERα also down-regulates Slug transcription by forming a co-repressor complex consisting of ligand-activated ERα, HDAC1 and nuclear receptor co-repressor (N-CoR) that binds to the estrogen-response elements at the Slug promoter [103,104].

Aromatase, also called estrogen synthetase (encoded by the *CYP19* gene), is responsible for a key step in the estrogen pathway. It converts androgen to estrogen and is expressed at higher levels in breast cancer tissue than normal breast tissues [105]. Snail has been reported to repress the transcription of aromatase gene [106].

Snail controls metabolism

Metabolism plays a fundamental role in essentially every function of a cell, however, little is known about how the cell's metabolism contributes to the morphological and molecular changes in EMT. The best-characterized metabolic phenotype of cancer cells is the Warburg

effect, which describes the shift in ATP generation from energy-efficient oxidative phosphorylation to inefficient glycolysis, even in the presence of ample oxygen [107,108]. This aerobic glycolytic switch substantially enhances glucose uptake and lactate production, increases intermediate metabolites for the synthesis of macromolecules, and reduces the level of cellular reactive oxygen species (ROS) potentially harmful to the genome.

Our recent study shows that Snail controls this glycolytic switch through repressing the fructose-1,6-biphosphatase (FBP1), a rate limiting enzyme in gluconeogenesis [109]. The Snail-G9a-Dnmts complex binds to the FBP1 promoter and silences FBP1 expression in basal-like breast cancer, resulting in enhanced CSC-like traits and tumorigenicity by increasing glycolysis and by reducing oxygen consumption and ROS production in this subtype of breast cancer. Another study shows that Wnt/Snail signaling inhibits mitochondrial respiration and COX activity, and induces the glycolytic switch [44]. Therefore, Snail-mediated metabolic reprogramming intertwines with tumor progression. Understanding the causes and consequences of altered metabolism in EMT may reveal the therapeutic drug targets for metastatic breast cancer.

Therapeutic interventions

Snail is an attractive target for the development of pharmaceutical agents. Blocking Snail function has great potential to prevent tumor cell metastasis by interfering with processes such as EMT, invasion and metastasis. Moreover, the link between Snail and CSCs suggests that Snail inhibitors could prevent tumor recurrence. Recently, a Co (III)-DNA conjugate, Co(III)-E-box, has been developed for selective inhibition of Snail family of transcription factors. This Co(III)-E-box complex was designed to irreversibly bind zinc finger transcription factors through a ligand exchange mechanism on Co(III) and is targeted to Snail family factors through an E-box consensus sequence (CAGGTG) in the oligo. Together, Co(III)-Schiff base and E-box, as two components of Co(III)-E-box, mediate precise inhibition of target zinc finger proteins [110,111]. Another study focused on inhibiting the Snail-p53 binding. As a tumor suppressor gene, p53 induces cell death, growth arrest and suppresses metastasis in response to different types of cellular stress including oncogene activation, DNA damage, hypoxia and so on. However, p53 is frequently mutated by genetic alternation or suppressed by various kinds of cellular signaling pathways in human cancers [112]. Therefore, reactivation of p53 seems to be the most promising strategy for cancer therapy. It has been found that p53 is suppressed and eliminated from K-Ras-mutated cancer cells through direct interaction with Snail [113]. Based on these observations, two specific inhibitors (GN25 and GN29) against the p53-Snail binding are generated to induce p53 expression and functions in K-Ras-dependent manner. However, it does not show cytotoxic effect on normal cells or K-Ras-wild-type cells. Moreover, GN25 can selectively activate wild-type p53 in $p53^{WT/MT}$ cancer cells [114].

Conclusions

In the past decade, the understanding of molecular mechanism of Snail in cancer progression has been greatly appreciated. Accumulated evidence indicates that Snail displays a broad spectrum of biological functions. Elevated expression of Snail not only enhances cell

motility and invasiveness by down-regulating epithelial markers and up-regulating mesenchymal markers, but also confers tumor cells with stem cell-like features and provides them with resistance to various types of therapy. Therefore, Snail promotes metastasis of breast cancer cells and overexpression of Snail is a biomarker of poor clinical outcome for patients with breast cancer. The recent recognition of the regulation and functions of Snail has shed a new light on understanding its potential clinical and therapeutic implications in cancers. Despite these developments, much remains unknown about the role of Snail in metastasis because metastasis is a complex and multistep process and cancer cells hijack the normal developmental networks for tumor progression and metastasis. Within the complex signaling networks, Snail is regulated through signal integration, crosstalk and feedback control. It is often hard to identify whether a particular molecule or pathway under investigation is specific to the Snail-mediated EMT. For example, it has been shown that Snail recruits specific chromatin-modifying and -remodeling complexes to the E-cadherin promoter to silence the expression of E-cadherin and induce EMT during tumor progression, however, how these factors cooperate to induce EMT and whether these factors also control the expression of other genes during EMT remains to be established. Therefore, further investigations to disclose the contribution of various tumor microenvironmental factors to tumor progression will lead to a comprehensive understanding of Snail in cancer and will provide us with effective therapeutic strategies for treating metastatic disease.

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ABBREVIATIONS

BMPs	bone morphogenetic proteins
COX	cytochrome C oxidase
CSCs	cancer stem cells
CSN2	COP9 signalosome 2
DNMTs	DNA methyltransferases
EGF	epidermal growth factor
Egr-1	early growth response-1
EMT	epithelial-mesenchymal transition
ER	estrogen receptor
FBP1	fructose-1,6-biphosphatase
FGF	fibroblast growth factor
GSK-3β	glycogen synthase kinase-3β
HDAC	histone deacetylase
HGF	hepatocyte growth factor

HIF-1α	hypoxia-inducible factor-1α
HMGA2	high-mobility group A2
ILK	integrin linked kinase
LOXL2	lysyl oxidase-like 2
LSD1	lysine-specific demethylase 1
MTA3	metastasis-associated protein 3
NCoR	nuclear receptor co-repressor
NES	nuclear export sequence
NF-κB	nuclear factor κB
***O*-GlacNAc**	*O*-linked β-*N*-acetylglucosamine
PRC2	Polycomb repressive complex 2
PRMT5	protein arginine methyltransferase 5
RKIP	Raf kinase inhibitor protein
ROS	reactive oxygen species
RTKs	receptor tyrosine kinases
SCP	small C-terminal domain phosphatase
SRD	serine-rich domain
Suv39H1	suppressor of variegation 3-9 homolog 1
TGF-β	transforming growth factor-β
TNF-α	tumor necrosis factor-α

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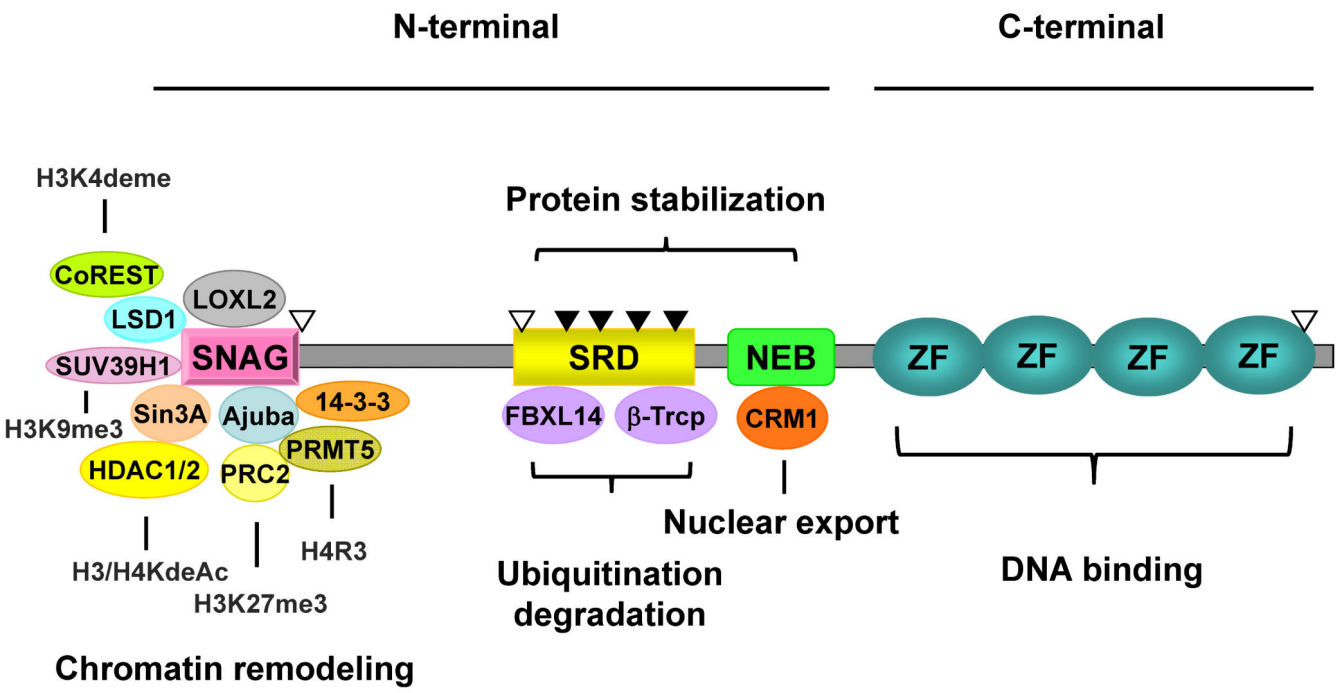

Figure 1. Structure of Snail

Snail contains an N-terminal SNAG domain and C-terminal zinc finger domains. The N-terminal SNAG domain interacts with several co-repressors and epigenetic remodeling complexes, and the C-terminal zinc finger domains are responsible for DNA binding. The serine-rich domain (SRD) and nuclear export sequence (NES) control the Snail protein stability and subcellular localization. Phosphorylation sites are indicated as triangles.

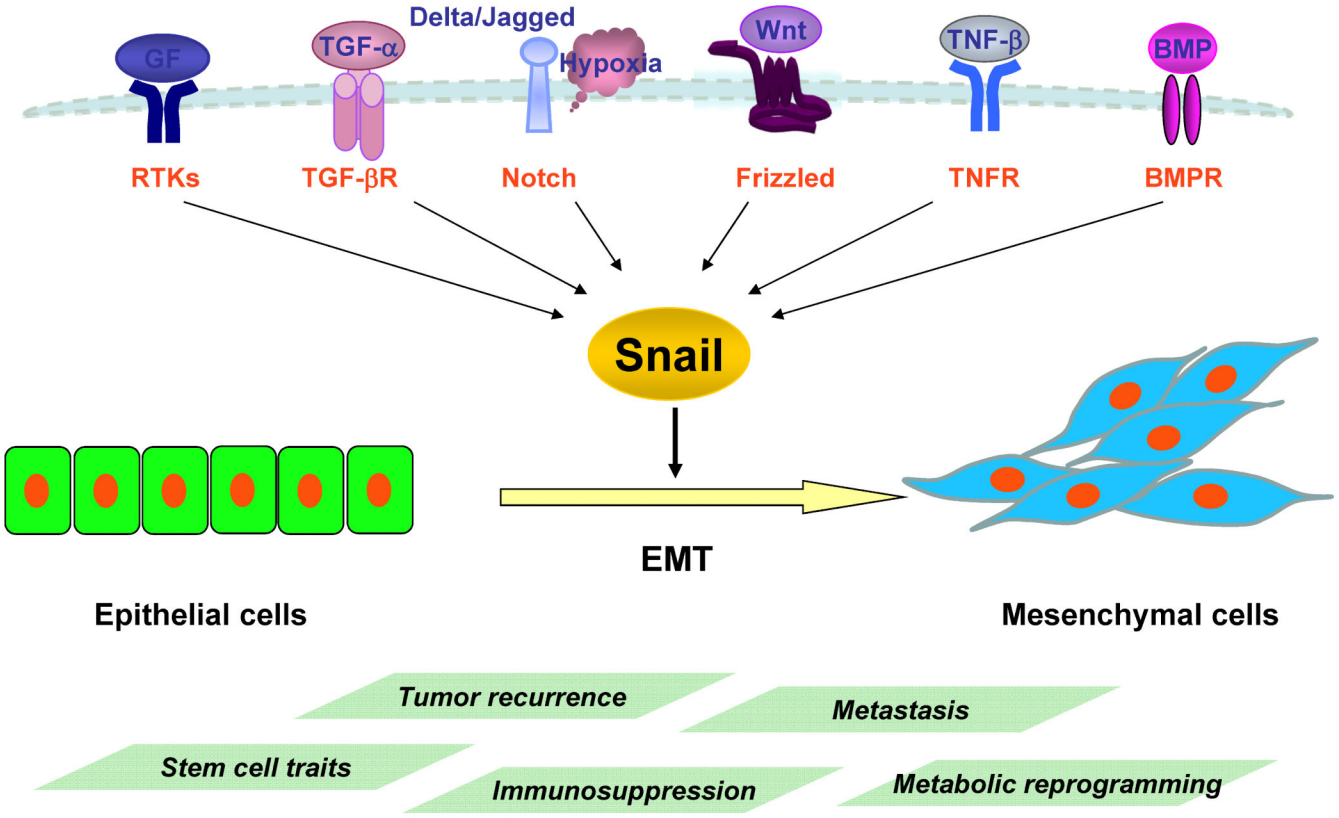

Figure 2. Schematic diagram of the signaling pathways associated with Snail-induced EMT

An integrated and complex signaling network, including RTKs, TGF-β, Notch, Wnt, TNF-α, and BMPs signaling pathways, activate the transcription factor Snail, resulting in the induction of EMT. The expression of Snail causes a metabolic reprogramming, confers tumor cells with stem cell-like traits, resistance to immunosuppression, and promotes tumor recurrence and metastasis.