Snail1 links transcriptional control with epigenetic regulation

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Epithelial-mesenchymal transition (EMT) is a remarkable example of cell plasticity during embryonic development, tissue fibrosis and malignant tumour progression. The loss of epithelial, E-cadherin-mediated cell–cell adhesion appears to be one critical event during EMT, and the search for regulators of E-cadherin gene (*CDH1*) expression has uncovered a number of transcriptional repressors of *CDH1*, of which Snail1 is a prototype. Snail1 gene (*SNAI1*) expression is controlled by a plethora of signalling pathways promoting EMT. In this issue, Lin *et al* (2010) add even more complexity to Snail1 function. Through its N-terminal SNAG domain Snail1 interacts

with the amino oxidase domain of histone lysine-specific demethylase 1 (LSD1) resulting in a stabilization of a ternary Snail1–LSD1–CoREST complex and the repression of specific target genes, such as *CDH1*. Notably, the SNAG domain of Snail1 resembles a histone H3-like structure and serves as a pseudosubstrate to recruit LSD1 to transcriptional target sites where it demethylates lysine 4 on histone 3 and together with Snail1 supports the repression of target genes. These results directly connect Snail1's transcriptional repressor functions with the epigenetic regulation of gene expression, a mechanism obviously critical for EMT.

Figure 1 Model of the regulatory mechanisms modulating Snail1 stability and activity. Snail is either phosphorylated by GSK3β and destined to ubiquitylation by the E3 ubiquitin ligase βTrCP and proteasomal degradation. Alternatively, Snail is stabilized by phosphorylation through protein kinase A (PKA) and casein kinase 2 (CK2), and through its SNAG domain binds LSD1. The ternary Snail1–LSD1–CoREST complex is recruited to E-boxes of Snail target gene promoters, such as E-cadherin, in which LSD1 binds the tail of histone H3 and demethylates lysine 4 of histone H3 (H3K4). Histone deacetylases 1 and 2 (HDAC1/2) deacetylate histones 3 and 4 (H3/H4), and the polycomb repressor complex 2 (PRC2) trimethylates lysine 27 of histone 3 (H3K27). Together, Snail1-targeted chromatin modifications lead to a repression of the E-cadherin gene.

One of the hallmarks of EMT and the concomitant induction of cell migration and invasion during embryonic development and in carcinogenesis is the loss of the epithelial cell-cell adhesion molecule E-cadherin, the major component of epithelial adherens junctions (Thiery *et al*, 2009). Besides E-cadherin gene (*CDH1*) mutations or the proteolytic degradation of E-cadherin protein, transcriptional repression seems to have a predominant role in the loss of E-cadherin function, for example, by the zinc-finger transcription factors Snail1, Snail2 (Slug), ZEB1 (δ EF1), ZEB2 (SIP1; Peinado *et al*, 2007). The best-studied member of these transcription factors, Snail1, represses the expression of many epithelial cell-specific genes, including E-cadherin, cell cycle progression factors and apoptosis genes, while it induces the expression of mesenchymal markers, cell cycle inhibitors and survival factors. Thus, besides being a critical regulator of EMT, Snail1 also has a role in modulating resistance to apoptosis and in tumour recurrence. Snail1 consists of four c-terminal DNA-binding zinc-fingers, and a regulatory region at the N-terminus, comprising an amino-terminal SNAG (Snail/Gfi-1) domain (1–9 amino acids) important for co-repressor interaction, a destruction box (DB) and a nuclear export sequence (NES) recognized by the CRM1 exporter (Figure 1).

Snail1 binds to E-box consensus sequences in the promoters of its target genes concomitant with the recruitment of the co-repressor CtBP, the Sin3A co-repressor complex and the polycomb repressor complex 2 (PRC2) (Peinado *et al*, 2004; Herranz *et al*, 2008). In this issue of *EMBO Journal*, Lin *et al* have identified LSD1 as a novel interacting partner of Snail1. LSD1 is found as a component of the HDAC1/2-containing co-repressor complexes CoREST and mi2/NuRD and removes mono- and dimethyl marks on lysine 4 of histone H3 (H3K4), histone modifications indicative of activated gene expression (Cunliffe, 2008; Wang *et al*, 2009). Snail1 interacts with LSD1 through its SNAG domain in a number of different cancer cell lines and co-localizes with LSD1 in the nucleus. The SNAG domain is required for Snail1 protein stability and transcriptional functions. The SNAG domain, in particular Arg3, Arg8 and Lys9, is critical in interacting with the catalytic amino oxidase domain of LSD1 by mimicking the structure of the *bona fide* substrate of LSD1, the H3 tail (Figure 1). Snail1 mutants lacking the SNAG-LSD1 interaction also lose their ability to suppress *CDH1* promoter activity. The amino oxidase domain of LSD1 is separated by a tower domain into the FAD co-enzyme-binding site and the substrate recognition site. This tower domain is required for binding CoREST, which by allosteric modulation affects FAD and substrate binding and thus enzymatic activity. Formation of a ternary Snail1-LSD1-CoREST complex appears critical for Snail1 stabilization, and the authors speculate that, on failure to form the ternary complex, the N-terminal domain of Snail1 becomes accessible to glycogen synthase kinase 3 β (GSK3 β)-mediated phos-

phorylation, nuclear export and degradation. Consistent with this notion, levels of Snail correlate with levels of LSD1 and CoREST in a number of cancer cell lines and in human breast cancer samples.

The data also show that LSD1 is recruited to the *CDH1* promoter with Snail1. Interestingly, binding the SNAG domain as pseudosubstrate requires LSD1 enzymatic activity: the amino oxidase inhibitors Parnate or Pargyline, as well as SNAG or H3 peptides, repress the interaction between LSD1 and Snail1. Notably, it seems that LSD1 binds the SNAG domain with high affinity and, when recruited to a target gene promoter, the high local concentrations of histone H3 tails compete with SNAG binding and release LSD1 activity to demethylate the *bona fide* substrate H3K4me2. Indeed, chromatin immunoprecipitation experiments and enzyme activity assays document that LSD1 is recruited to the E-cadherin promoter by Snail1 where it is actively demethylating H3K4. Snail1 and LSD1, and with lesser importance CoREST, altogether are required for E-cadherin repression and for cell migration and invasion.

These new insights indicate that the SNAG domain of Snail acts as a pseudosubstrate 'hook' for LSD1 to recruit it together with CoREST to Snail1 target gene promoters. A picture appears in which binding of Snail1 to the *CDH1* promoter leads to epigenetic gene silencing in a multistage process (Herranz *et al*, 2008; Lin *et al*, 2010; Figure 1). First, HDAC1 and 2 within the CoREST-Snail1-LSD1 ternary complex deacetylate histone H3 and H4. Subsequently, PRC2 is drafted to direct trimethylation of H3K27, a histone modification attributed to transcriptional repression. Finally, as reported here, LSD1 removes the activation marks on H3K4. However, how the initial silencing of the E-cadherin gene promoter converts into a long-term repression by DNA hypermethylation remains to be resolved. During TGF β -induced EMT of mammary gland cells a decrease in active histone modifications (H3K9Ac and H3K4me3) and an increase in the repressive histone modification H3K27me3 have been observed concomitant with an increase in DNA methylation of the *CDH1* and the α_4 -integrin gene (*ITGA4*) promoters (Yang *et al*, 2009). The stage has now been set to delineate the concerted actions between transcription factors and chromatin modifiers during malignant tumour progression.

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Conflict of interest

The author declares that he has no conflict of interest.

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