Regulation of Epithelial–Mesenchymal Transition through SUMOylation of Transcription Factors

Maria V. Bogachek1, James P. De Andrade1, and Ronald J. Weigel1,2,3

Abstract

Carcinoma cells can transition from an epithelial-to-mesenchymal differentiation state through a process known as epithelial–mesenchymal transition (EMT). The process of EMT is characterized by alterations in the pattern of gene expression and is associated with a loss of cell polarity, an increase in invasiveness, and an increase in cells expressing cancer stem cell (CSC) markers. The reverse process of mesenchymal-to-epithelial transition (MET) can also occur, though the transitions characterizing EMT and MET can be incomplete. A growing number of transcription factors have been identified that influence the EMT/MET processes. Interestingly, SUMOylation regulates the functional activity of many of the transcription factors governing transitions between epithelial and mesenchymal states. In some cases, the transcription factor is a small ubiquitin-like modifier conjugated directly, thus altering its transcriptional activity or cell trafficking. In other cases, SUMOylation alters transcriptional mechanisms through secondary effects. This review explores the role of SUMOylation in controlling transcriptional mechanisms that regulate EMT/MET in cancer. Developing new drugs that specifically target SUMOylation offers a novel therapeutic approach to block tumor growth and metastasis. Cancer Res; 75(1); 1–5. © 2014 AACR.

Introduction

Epithelial–mesenchymal transition (EMT) is a critical cellular process required for normal organogenesis and for cellular response to stress, inflammation, and hypoxia (1, 2). Cancer cells also use the cellular processes involved in EMT, which are required for invasion and metastasis. Normal epithelial cells demonstrate apical–basal polarity maintained by a cytoskeleton structure and apical tight junctions and basolateral adherens junctions. E-Cadherin plays a central role in maintaining normal epithelial morphology and EMT is characterized by downregulation of epithelial markers (e.g., E-cadherin) and gain of mesenchymal markers (e.g., fibronectin, vimentin, and N-cadherin), with a loss of cellular polarity (1, 2). Transition to a mesenchymal gene-expression pattern is further associated with the acquisition of cancer stem cell (CSC) properties (3–5).

A complex network of transcriptional regulation orchestrates the process of EMT during development and distinct aspects of the physiologic changes characterizing EMT are regulated by the coordinated and overlapping activity of a number of transcription factors (6, 7). The transition in differentiation state characterized by EMT can be induced by a number of transcription factors, including ZEB1/2, TWIST1, and SNAIL1/2, several of the FOX family, GATA4/6 and other basic helix-loop-helix transcription factors (2, 8). In breast cancer, the process of EMT is further characterized by a transition from a luminal gene-expression pattern to a basal-associated pattern of expression. Recent findings have shown that the transcription factor TFAP2C/AP-2γ is required to maintain the luminal pattern of gene expression in normal mammary epithelial cells and in luminal breast cancer (4). Knockdown of TFAP2C in luminal breast cancer cells induced a luminal to basal cell transition associated with the development of a mesenchymal expression pattern characterized by a loss of CDH1/E-cadherin and a gain in VIM/vimentin and CDH2/N-cadherin expression. EMT transition was further associated with enrichment of cells expressing the CD44+/hi/CD24–/low markers of the CSC population. Interestingly, the highly homologous AP-2 family member, TFAP2A/AP-2α, lacked the ability to effect similar changes in luminal gene patterning; however, it was determined that the functional activity of TFAP2A was regulated through SUMOylation (9). Inducing expression of the small ubiquitin-like modifier (SUMO) unconjugated form of TFAP2A by inhibiting critical SUMO pathway enzymes, mutating the SUMO site of TFAP2A or by treating with SUMO inhibitors allowed TFAP2A to acquire TFAP2C-like transcriptional activity. SUMO-unconjugated TFAP2A was able to induce expression of luminal-associated genes, including estrogen receptor-α (ERα), and to repress expression of basal-associated genes, including CD44. Treatment of basal breast cancer cell lines with SUMO inhibitors induced a TFAP2A-mediated repression of CD44 and was associated with a clearing of cells expressing the CSC markers CD44+/hi/CD24–/low and loss of ability for basal cancer cell lines to form tumor xenografts. These findings highlight the ability for the SUMO pathway to regulate the activity of transcription factors mediating the process of EMT.

SUMOylation of Transcription Factors

The SUMOylation pathway results in the reversible binding of SUMO peptide to a lysine residue in the target protein (10). Interestingly, SUMOylation of transcription factors can have a profound effect on functional activity even with an apparently

1Department of Surgery, University of Iowa, Iowa City, Iowa. 2Department of Anatomy and Cell Biology, University of Iowa, Iowa City, Iowa. 3Department of Biochemistry, University of Iowa, Iowa City, Iowa.

Corresponding Author: Ronald J. Weigel, Department of Surgery, University of Iowa, 200 Hawkins Drive, 1509 JCP, Iowa City, IA 52242. Phone: 319-353-7474; Fax: 319-356-8378; E-mail: ronald-weigel@uiowa.edu
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small fraction of the total protein population being SUMOylated (10, 11). The SUMOylation process is mediated through a cascade involving an activating enzyme (e.g., SAE1/2), E2-conjugating enzyme (e.g., UBC9), and E3 ligase (e.g., PIAS family; refs. 10, 11). In addition, specific SUMO-modified proteins can be SUMO deconjugated by a group of Sentrin/SUMO-specific proteases (SENP). In many cases, SUMOylation of transcription factors leads to suppression of their activity, which can occur through a variety of mechanisms. For example, SUMO modification of the ETS domain transcription factor ELK-1 leads to transcriptional repression (12). In this example, SUMO-conjugated ELK-1 recruits histone deacetylase-2 (HDAC-2) to ELK-1 target genes, resulting in chromatin modification that represses transcription. An alternate mechanism of transcriptional repression involves SUMO-conjugated transcription factors initiating the formation of a repressor complex in a SUMO-dependent fashion (11). In other examples, SUMOylation of a transcription factor may influence activation or repression functions at a specific subset of target promoters (13). An additional mechanism regulating the activity of transcription factors may involve SUMO-dependent cell trafficking (11).

Transcription Factors That Induce EMT

There is a growing list of transcription factors that induce EMT in cancer cells that are also regulated by SUMOylation (see Fig. 1). Recently, it was found that stable overexpression of forkhead box transcription factor FOXM1 induced EMT in breast cancer cells, whereas knockdown of FOXM1 inhibited the mesenchymal phenotype (14, 15). FOXM1 induced EMT through repression of...
miR200b and activation of Slug. Interestingly, several studies have demonstrated that SUMOylation regulates FOXM1 activity, potentially involving several mechanisms. In one study, SUMOylation with SUMO-1 was reported to repress the transcriptional activity of FOXM1 by promoting its translocation to the cytoplasm and enhancing APC/Cdh1-mediated ubiquitination and degradation (16). Although this study did not directly examine the role of SUMOylation on FOXM1-mediated EMT, the results demonstrated broad effects of SUMO conjugation on the transcriptional activity of FOXM1. FOXM1 represses a number of genes, including the expression of miR200b/c. The ability of FOXM1 to induce EMT is, at least in part, dependent upon repression of miR200b because forced overexpression of miR200b reversed FOXM1-induced EMT (14). FOXM1b is the transcriptionally active isof orm of FOXM1 that is responsible for repression of miR200b (17). FOXM1b is SUMOylated by SUMO-1 and can be SUMO deconjugated by SENP2. In contrast with miR200b reversed FOXM1-induced EMT because forced overexpression of genes, including the expression of miR200b/c. The ability of FOXM1 to induce EMT is, at least in part, dependent upon repression of miR200b because forced overexpression of miR200b reversed FOXM1-induced EMT. FOXM1b is SUMOylation Regulates EMT in Cancer

Transcription Factors That Induce MET

A number of transcriptional mechanisms have been identified that promote the epithelial differentiation state and MET. One factor that works in concert with TFAP2A/C factors to maintain the epithelial-differentiated phenotype in breast cancer is FOXA1 (28). Similarly, loss of FOXA1 induces EMT in a model of pancreatic adenocarcinoma (29). FOXA1 is SUMO conjugated at lysine residues K6, K267, and K389, and SUMOylation of FOXA1 impairs its direct transcriptional activity and its ability to act as a pioneer factor (30).

The tumor-suppressor p53 promotes the epithelial phenotype through direct transcriptional activation of miR200c (31). Loss of p53 in mammary epithelial cells represses miR200c inducing EMT and increasing the mammary stem cell population. The regulation of p53 by SUMOylation is complex and involves several different mechanisms (32). SENP2 plays a key role in development of the trophoblast by SUMO-deconjugating Mdm2 allowing functional repression of p53 (33). On the other hand, in cancer cells, SKI has been shown to enhance SUMO-conjugating enzyme E2 Ub9 activity, resulting in increased SUMO conjugation of MDM2 (34). In this system, upregulated activity of SUMOylated MDM2 resulted in an overall decreased expression of p53 (34). In addition to activity mediated by MDM2, the function of p53 can be altered by direct SUMO conjugation. PIASy-mediated SUMOylation of p53 can induce cytoplasmic sequestration of p53 (35). SUMOylation of p53 can inhibit its DNA-binding activity and SUMO-mediated inhibition can be blocked by p300-mediated acetylation (36). Hence, there may be competing processes regulated by SUMOylation that affect the transcriptional activity of p53 (32).

In breast cancer systems, TGFβ is a potent inducer of EMT with an associated increase in CSCs (37). The ability for TGFβ to induce EMT in breast cancer cells is mediated, at least in part, through Smad4 (38). A study in SW480 colon cancer cells indicated that Smad4 repressed EMT and invasiveness (39). SUMOylation represses the transcriptional activity of Smad4 and overexpression of UBC9, PIASy, and SUMO-1 leads to repression of TGFβ-responsive promoters (40). In addition, an important feed-forward mechanism has been identified in which TGFβ induces EMT by reducing the level of the SUMO E3 ligase PIAS1; PIAS1 antagonizes the ability for TGFβ to induce EMT through SUMOylation of SnoN (41). In addition, TIF1γ has SUMO E3-ligase activity promoting the SUMOylation of SnoN and suppressing EMT (42). Hence, the data suggest that SUMOylation represses TGFβ-induced EMT through SUMO conjugation of Smad4 and SnoN. However, the effect of SUMO inhibitors based on these mechanisms may be different in epithelial versus mesenchymal cell types.

The expression of the GATA3 transcription factor is associated with the ERα luminal breast cancer phenotype and recent findings showed that GATA-3 promotes the epithelial differentiation state (43). Furthermore, there is evidence that SUMO conjugation of GATA factors repress transcriptional activity (44), suggesting the potential for SUMOylation to regulate the ability for GATA3 to promote the epithelial phenotype.
The protein from the Von Hippel-Lindau (VHL) gene influences EMT and invasion in renal cell and squamous cell carcinomas (45, 46). VHL promotes inhibition of invasion and expression of epithelial markers (E-cadherin), whereas loss of VHL induces EMT. Effects of VHL are mediated through hypoxia-sensitive regulation of HIF. Hypoxic stress represses HIF1α-dependent expression of HIF-target genes. Under conditions of hypoxic stress, PIASy/UBC9 SUMO conjugates VHL, which causes oligomerization and repression of HIF1α function. Loss of VHL results in stabilization of HIF1α-mediated physiologic processes characteristic of EMT.

SIRT1 is a lysine deacetylase that influences many biologic processes through its enzymatic activity on different protein substrates. SIRT1 has been shown to repress EMT and to promote an epithelial phenotype in ovarian and lung carcinomas (47, 48). Hypoxic stress represses SIRT1 expression through SUMOylation-dependent repression of SP1 binding and promoting HIC1 binding, thereby blocking SP1 activation of the SIRT1 promoter (47). UBC9 and PIASy mediates SUMO conjugation of SP1 and HIC1 in these cancer systems. The overall effect of blocking SUMO conjugation to SP1 and HIC1 favors transactivation of the SIRT1 promoter by SP1 binding with the physiologic effect of inhibiting EMT and metastasis.

Conclusion

The differentiation state of carcinomas demonstrates plasticity that can be influenced by the activity of a growing list of transcription factors. Of particular interest is the finding that SUMOylation regulates the activity of transcriptional mechanisms, influencing the transition between epithelial and mesenchymal states of differentiation. Drugs that target the SUMOylation pathway offer the potential of changing the differentiation state of cancers, potentially inducing cytotoxic effects, reducing invasiveness, and eliminating of the CSC population associated with the mesenchymal cell type. The development of new drugs with greater efficacy and specificity for the SUMOylation pathway will be needed to bring this novel approach of cancer therapy to the clinical setting.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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