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The Skinny on Slug

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Abstract

The zinc finger transcription factor Slug (Snai2) serves a wide variety of functions in the epidermis, with roles in skin development, hair growth, wound healing, skin cancer, and sunburn. Slug is expressed in basal keratinocytes and hair follicles where it is important in maintaining epidermal homeostasis. Slug also helps coordinate the skin response to exogenous stimuli. Slug is rapidly induced by a variety of growth factors and injurious agents and Slug controls, directly or indirectly, a variety of keratinocyte responses, including changes in differentiation, adhesion, motility, and production of inflammatory mediators. Slug thus modulates the interactions of the keratinocyte with its environment and with surrounding cells. The function of Slug in the epidermis appears to be distinct from that of the closely related Snail transcription factor.

Keywords

Skin; Snail family transcription factors; Ultraviolet rays; Wound healing; Skin neoplasms

INTRODUCTION

The first member of the Snail family of transcription factors, Snail (Snai1) was described in *Drosophila* [1]. Since this initial discovery, additional Snail superfamily members, including Slug (Snai 2), have been identified [2–4]. Snail factors play important roles in development, particularly in formation of mesoderm and initiation of epithelial-mesenchymal transformation (EMT), the process by which sessile epithelial cells assume a migratory mesenchymal phenotype [5–7]. During EMT, junctional structures anchoring epithelial cells to the basement membrane and to one another are lost, cytokeratin intermediate filaments are replaced by vimentin, and cells acquire invasive properties due to enhanced secretion of proteases and increased motility [5,8–10]. EMT occurs prominently during gastrulation, as mesoderm is formed by invagination, and during nervous system development, as cells emigrate from the neural crest. Several processes occurring in the adult strongly resemble the EMT seen during development, and Snail family transcription factors also drive some of these processes [7]. For instance, epithelium at healing wound margins undergoes a partial and transient EMT [11]. In addition, as carcinomas evolve into more aggressive tumors, neoplastic cells may assume a spindled shape, lose desmosomes and adherens junctions, begin expressing vimentin, and secrete increased amounts of proteases [8,12,13]. In this review, we focus on the distinctive contributions of the Slug transcription factor to skin biology, including the EMT-like processes of wound healing and skin tumor progression, as

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well as epidermal development and differentiation, hair growth, and cutaneous inflammation.

THE SLUG TRANSCRIPTION FACTOR

The Slug gene is located on human chromosome 8 and on mouse chromosome 16 [14]. In man, the Slug transcription factor consists of 268 amino acids and has a molecular weight of approximately 30 KDa (Figure 1); the mouse protein contains one additional amino acid [15,16]. Slug belongs to the Snail family of C2H2-type zinc finger transcription factors. Slug contains five tandemly arrayed zinc finger domains in the carboxy terminus (amino acids 128–150, 159–181, 185–207, 213–235, and 241–264 in the human protein) of the molecule [12,17]. The zinc finger region of the Slug protein is largely homologous to that of other members of Snail family, but the amino terminus is somewhat more divergent. The first 20 amino acids of the amino terminus constitute the SNAG (Snai/Gfi) domain and are conserved among vertebrate Snail family and Gfi-1 proteins. In the Gfi-1 protein, the SNAG sequence has been shown to be essential for mediating transcriptional repression and nuclear localization [12,17,18]. In addition, the Slug protein contains one stretch of 29 amino acids (amino acids 95–122 in the human protein) of unknown function that is unique to Slug proteins [12,17]. Like Snail, Slug possesses a C-terminal binding protein (CtBP) interaction motif (amino acids 91–97 in the human protein) [17,19].

Both Snail and Slug are localized to the nucleus by importins, which bind to nuclear localization signals, small clusters of basic residues within the zinc finger domains of the proteins [20,21]. Three additional putative nuclear localization motifs in Snail lying outside the zinc finger domain have been identified; similar motifs have not been recognized in Slug [20,22]. While both Slug and Snail may be localized to the nucleus, only Snail is generally found in the cytoplasm. Within the nucleus, both Slug and Snail are localized to discrete foci believed to represent sites of active transcription. Slug and other Snail family members repress transcription by recognizing and binding to E box sequences in the promoters of target genes [23]. The consensus binding site for Slug contains a core of six bases, CAGGTG [24–26]. This motif is identical to the E box of basic helix-loop-helix transcription factor binding sites, suggesting that Slug might compete with these for the same binding sequences [27]. Slug repression of transcription depends on the C-terminal DNA-binding zinc fingers, as well as on a separate repression domain in the amino terminus [17]. The first 32 amino acids of Slug, which are highly conserved among vertebrate Snail and Slug proteins, contain the major amino terminal repressor activity. Transcriptional repression is mediated through the recruitment of the histone deacetylases (HDACS), HDAC1, HDAC2, and mSin3A, which complex with the SNAG domain [17,28]. In addition, Slug can recruit the CtBP co-repressor to promoter sites [29,30], although Slug does not appear to bind directly to CtBP itself [31].

Snail and Slug are highly unstable proteins [32]. The Snail coding sequence contains a GSK-3 β destruction motif allowing the protein to associate with β -Trcp and thus to be targeted for destruction [33–35]. Because Slug lacks this motif, it is unlikely that Slug stability is directly regulated via the GSK-3 β pathway. However, Slug stability is regulated in part through ubiquitin-mediated, proteasome-dependent mechanisms. The E3 ligase, MDM2, is involved in degradation of Slug in lung cancer cells [36]. Snail protein can also be stabilized to prolong its half-life. For example, LOXL2 stabilizes Snail to promote EMT in carcinoma cells [37,38]. Snail, but apparently not Slug, function can also be regulated by intracellular localization, as described above; for example, alcohol stimulates nuclear localization of Snail, leading to EMT in colon and breast cancer cells [39].

SLUG EXPRESSION IN THE SKIN

Slug expression is seen in the epidermis and undifferentiated dermal mesenchyme of the mouse embryo as early as E10 (Figure 2) [40]. Slug is also expressed in the developing hair follicle before and after birth. In the adult, Slug continues to be expressed strongly in a subpopulation of basal keratinocytes of the skin, in regions of the hair follicle, in a variety of other stratified and pseudostratified epithelia, and in some cartilage [41]. The pattern of Snail expression in developing and adult skin remains somewhat unclear. One report [42] indicates that Snail expression in the skin is limited to hair bud cells during the period E15.5 days to birth, with no expression of Snail in mesodermal skin cells. In contrast, other investigators [43] have observed Snail expression only in mesenchymal cells of the skin, particularly in the mesenchymal cells clustered below the hair buds of embryonic skin and in the dermal papillae of adult hair follicles. The discrepancy between the two studies has not been resolved, although it has been suggested, albeit without experimental evidence, that Snail-positive mesenchymal cells of the skin might arise from epithelial cells by EMT [43].

In Slug null mice, skin and hair develop relatively normally; however, the epidermal thickness of Slug null mice is reduced compared to that of wild type mice [44] and the onset of hair growth in neonates is delayed by approximately two days [40]. Because the delay in hair growth is evident very shortly after birth, it is likely that the defect lies in hair follicle morphogenesis. Mice that overexpress an inducible Slug transgene are also viable and appear phenotypically normal; although many of these mice die from cardiac hypertrophy as adults [45]. Snail null embryos die at gastrulation [46]. Mice transgenic for Snail expressed from the keratin 14 promoter are viable, but show epidermal abnormalities, including hyperproliferation and reduced intracellular adhesion [42]. Our *in vitro* studies in keratinocytes expressing exogenous Slug do not reveal any alteration in cell proliferation as determined by BrdU incorporation [47]. Nor are differences in the proliferation-associated antigens keratin 6 and Ki-67 observed adjacent to wound margins of Slug null compared to wild type mice [48]. However, overexpression of a GFP-Slug-ER fusion protein has been shown by other investigators to inhibit keratinocyte proliferation and colony formation *in vitro* [49]. The role of endogenous Snail in keratinocyte proliferation has not been examined.

Because Slug null mice have abnormalities in pigmentation, demonstrate impaired hematopoiesis, and are infertile, it has been proposed that Slug is important for maintenance of melanocyte stem cells, hematopoietic stem cells, and germ cells [50]. Although the role of Slug in keratinocyte stem cells has not been explored, the delayed first hair cycle and susceptibility to development of chronic wounds shown by Slug knockout mice suggests the possibility of a stem cell deficit in this model [40,48].

SLUG REGULATION IN KERATINOCYTES

A wide variety of growth factors and cytokines induce Snail family transcription factors. Epidermal growth factor (EGF) strongly induces Slug in keratinocytes, while insulin-like growth factor-1 and basic fibroblast growth factor are less potent [51]. Expression of Slug during reepithelialization of wounds driven by EGF is Erk5-dependent and Erk1/2-independent [52]. Microarray studies indicate approximately five-fold induction of Slug in the HaCaT keratinocyte cell line one hour following transforming growth factor- β (TGF- β) treatment, although TGF- β does not appear to induce Snail in this cell type [53,54]. In Smad2-negative mouse epidermis, Snail is activated via Smad3/Smad4 pathways and Smad2-null skin tumors in mice undergo accelerated progression, suggesting that Smads 3 and 4 induce, while Smad2 represses Snail [55]. Ligand activation of the aryl hydrocarbon receptor induces Slug expression in HaCaT keratinocytes, resulting in EMT [56]. Interestingly, aryl hydrocarbon receptor accumulates in the nucleus and activates Slug

transcription only under conditions of low cell density. This is in keeping with our findings that basal levels of Slug expression in HaCaT and SCC13 keratinocyte cell lines are much lower in confluent than in subconfluent cells, and the lowest basal levels of Slug are seen under conditions of serum deprivation (unpublished observations). The complete carcinogen ultraviolet radiation (UVR) transiently increases Slug and Snail expression in keratinocytes (Figure 3) [57]. UVR induction of Slug in keratinocytes occurs, at least in part, through the ERK and p38 MAPK pathways [57]. The carcinogen methylcholanthrene also induces Slug expression in cultured keratinocytes [56]. The tumor promoter 12-O-tetradecanoylphorbol 13-acetate (TPA) enhances Slug expression in mouse epidermis (unpublished observations) (Figure 3). Thus, levels of Slug and Snail expression can be rapidly modulated by a variety of factors in the keratinocyte environment, including growth factors, cell density, and environmental challenges. This suggests additional roles for Snail family transcription factors beyond the initiation of EMT.

SLUG TARGET GENES

The canonical Snail family target gene is E-cadherin, an important component of intercellular adherens junctions and loss of E-cadherin expression in epithelial cells is perhaps the most universally accepted hallmark of EMT [5]. Moreover, decreased E-cadherin expression in epithelial tumors is associated with malignant transformation, metastatic dissemination, and unfavorable tumor prognosis [58–60]. Both Slug and Snail bind to E-box elements in the E-cadherin promoter to repress its transcription [12,61,62]. However, Slug expression is not always associated with loss of E-cadherin. While some investigators have reported an inverse correlation between Slug and E-cadherin expression in epithelial tumors [24,25,62–68], others have not seen this association [64,69–72]. A possible explanation is that Slug may also regulate subcellular localization of E-cadherin. Elevated expression of Slug in HaCaT cells correlates with redistribution of E-cadherin from adherens junctions into cytoplasmic compartments rather than with downregulation of expression [53,73].

Slug also appears to regulate desmosome density. Human keratinocytes transfected with Slug expression vectors show desmosomal dissolution [44,47,52], and ectopic Slug reduces expression of the desmosomal markers desmoplakin and desmoglein in urinary bladder cells [73]. In keratinocytes, Slug appears to regulate the desmoplakin component of desmosomes [47]. Slug also regulates expression of the $\beta 4$ integrin component of hemidesmosomes and binds to an E-box in the $\alpha 3$ integrin gene promoter in keratinocytes to repress its transcription [49].

A variety of studies have identified the simple epithelial keratin 8 and its binding partners keratins 18 and 19 as important targets of Slug repression [74,75]. In the adult, keratin 8 is not normally detectable in either intact epidermis or at wound margins [76–78]; however, in Slug knockout mice, keratin 8 is constitutively expressed in the unperturbed epidermis and expression is particularly prominent at wound margins [74]. Although migratory keratinocytes at wound margins have decreased expression of the differentiation associated keratins 1 and 10 and *de novo* production of the injury associated keratins 6, 16, and 17 [79–85], there is no difference in expression of keratins 10, 14, or 6 at wound margins in Slug null mice compared to wild type mice, suggesting selective dysregulation of keratin 8. Slug repression of keratin 8 indicates a role for Slug in keratinocyte differentiation and may be related to the role of Slug in cell adhesion and wound healing, as appropriate interaction between keratins and desmosomes is required for the maintenance of skin integrity and effective wound healing [86,87].

To better understand the role of Slug in the epidermis, we conducted a microarray study comparing basal levels of gene expression in unperturbed epidermis of wild type and Slug null mice [74]. As revealed by this study, differences in expression of over 200 genes are detectable and these genes are related to a wide variety of processes, including epidermal differentiation, proliferation, apoptosis, adhesion, and motility, as well as angiogenesis and response to environmental stimuli (Table 1) [74]. Altered expression of several of these genes, Gli-1, Gli-2, keratin 8, keratin 18, angiomin, and tenascin C, has been confirmed in Slug null epidermis by quantitative RT-PCR or immunohistochemistry. Somewhat surprisingly, expression of E-cadherin, a known target of Slug regulation does not appear to differ between wild type and Slug knockout epidermis. These findings indicate an important role for Slug in maintaining keratinocyte differentiation and homeostasis, a role distinct from that of regulating EMT.

SLUG AND WOUND REEPITHELIALIZATION

A crucial aspect of wound repair is reepithelialization, a process that begins within hours after skin injury to cover the tissue defect and reestablish barrier function [88–92]. Keratinocytes at wound margins become migratory, displaying changes in cell-cell and cell-matrix adhesion and reorganization of the cytoskeleton [88,90,91]. Given the shared features of wound reepithelialization and EMT, we and others have investigated the role of Slug and Snail in the process. Slug expression is elevated in keratinocytes bordering cutaneous wounds in mice *in vivo* (Figure 4), in keratinocytes migrating from mouse skin explants *ex vivo*, and in human keratinocytes at wound margins *in vitro* [47,48,56]. Moreover, antimicrobial peptides that enhance wound healing *in vitro* concurrently induce Slug at wound margins [93]. Although Slug mRNA expression is elevated in the first three layers of cells adjacent to *in vitro* wound margins compared to the level within confluent cells sheets, Snail mRNA expression is not elevated in these cells [47].

Epithelial outgrowth is impaired in tissue isolated from Slug null mice in an *ex vivo* explant model of reepithelialization [51], and the reepithelialization component of excisional wound healing is reduced almost two-fold in Slug null skin [48]. Interestingly, closure of 3–3.5-mm excisional wounds on the dorsum of Slug knockout mice does not appear to be delayed relative to wild type mice; in both genotypes, wounds appear to close completely in four to five days [47]. This is likely due to fact that contraction of the panniculus carnosus rather than reepithelialization accounts for almost all wound closure in the dorsal skin of mice [94]. The EGF receptor is upregulated during reepithelialization [95,96] and activation of this receptor contributes significantly to the migratory and invasive potential of keratinocytes [97–102]. In the absence of Slug expression, EGF does not stimulate outgrowth of skin explants, thus Slug is a downstream mediator of EGF receptor signaling in this process [51]. EGF-dependent expression of Slug at wound margins is modulated via Erk5 [52]. As further evidence that deficiency in Slug expression compromises wound healing, forty per cent of Slug null mice but no wild type mice develop nonhealing cutaneous ulcers in response to chronic UVR exposure [48].

At least in part, Slug regulates cell motility during wound reepithelialization by repressing expression of E-cadherin, thus reducing intercellular adhesion and facilitating cell separation [103]. Slug also drives dissolution of desmosomes, a process that is particularly pronounced at wound margins; indeed Slug-dependent changes in desmosomal proteins at wound margins are more prominent than changes in E-cadherin [47]. Moreover, Slug may also regulate composition of the ECM and receptors for ECM components, as well as the composition and organization of the actin cytoskeleton and intermediate filaments [103–105].

SLUG AND SKIN CARCINOGENESIS

Squamous cell carcinoma (SCC) of the skin in experimental animals develops in a stepwise fashion, evolving through epidermal hyperplasia and premalignant exophytic papillomas into malignant and increasingly invasive endophytic SCC. Ultimately, SCC undergo EMT to form spindle cell tumors that resemble fibrosarcomas [106,107]. Our studies have shown that Slug expression is equally high in UVR-induced SCC with epithelial morphology and mesenchymal (spindle cell) morphology (Figure 5), but Snail mRNA expression is considerably higher in spindle cell than in epithelial SCC, suggesting that increased Slug expression precedes increased Snail expression during the development of SCC and that Snail rather than Slug drives the SCC to spindle cell conversion, as discussed below [44]. The UVR-induced tumor burden is reduced in Slug knockout mice compared to wild type mice and Slug null mice develop fewer spindle cell tumors than wild type mice. Moreover, spindle cell tumors in Slug knockout mice show impaired EMT as evidenced by decreased vimentin and increased E-cadherin and keratin expression compared to spindle cell tumors from wild type mice. Matrix metalloprotease (MMP)-2 expression is greater in spindle cell than in epithelial skin tumors of both wild type and Slug-null mice; however, MMP-2 expression is significantly higher in wild type spindle cell tumors than in Slug-null tumors, suggesting that Slug regulates this gene in keratinocytes. Thus, while Slug can enhance UVR carcinogenesis, Slug is not required for the development or progression of SCC or for conversion of SCC into spindle cell tumors. Interestingly, Snail is not detected by immunohistochemistry in basal cell carcinomas [108].

Other studies in keratinocytes support a role for both Slug and Snail in the EMT-like conversion of carcinomas to spindle cell tumors. TGF- β , a potent inducer of Slug and Snail, stimulates transformation of mouse keratinocytes from a squamous to a spindle morphology, both *in vivo* and *in vitro* [109–112]. Moreover, both invasive and noninvasive mouse epidermal keratinocyte cell lines express Slug, while only the invasive cell lines express Snail [25]. In accordance with this finding, the Snail promoter is hypermethylated during early stages of chemically induced mouse skin tumorigenesis; however, the promoter is demethylated as epithelial SCC undergo EMT to become spindle cell tumors [113]. In SCC arising in mice lacking Smad 2 expression in the epidermis and in Smad2-negative human SCC, enhanced EMT is observed and this is associated with increased Snail expression [55]. *In vitro* and xenograft studies employing murine SCC cell lines show that knocking down Snail expression restores E-cadherin expression, reduces expression of the EMT markers vimentin and fibronectin, suppresses MMP-9 production, and inhibits invasive and metastatic behavior [114,115]. Under the same experimental conditions, abrogating Slug expression alone has minimal effects on E-cadherin, vimentin, fibronectin, or MMP-9 levels, but knocking down Slug markedly enhances the effect of Snail abrogation on mesenchymal markers, MMP-9 production, *in vitro* and *in vivo* invasiveness, and xenograft metastasis. Surprisingly, knocking down expression of Slug, Snail or both under these conditions does not alter the organization of F-actin or microtubules.

In part, the enhanced invasiveness of cells expressing elevated Slug or Snail is due to increased expression of matrix metalloproteinases [72,116–119]. Our microarray studies [44] reveal that MMP-2 expression is greater in spindle cell than in epithelial skin tumors of both wild type and Slug-null mice; however, MMP-2 expression is significantly higher in wild type spindle cell tumors than in Slug-null tumors, suggesting that Slug regulates this gene in keratinocytes. In contrast to their role as upstream modulators of protease expression, Snail family transcription factors may also be targets of protease-dependent processes. In TGF- β -treated renal tubular and A431 epidermal carcinoma cells, MMPs disrupt adherens junctions, resulting in nuclear translocation of β -catenin and enhanced expression of Slug [120,121].

SLUG AND SUNBURN

During our studies of SCC induction in wild type and Slug knockout mice, we were surprised to discover that Slug knockout mice are remarkably resistant to sunburn and UVR-induced cutaneous inflammation [44]. Further studies have revealed that three minimal erythral doses of UVR causes severe sunburn, with patchy epidermal necrosis and marked inflammatory infiltrate, in wild type mice but only mild skin thickening in Slug knockout mice (Figure 6) [122]. Histologically, the skin of UVR-exposed Slug knockout mice shows markedly reduced neutrophil influx, decreased epidermal T cell loss, delayed keratin 6 induction, and decreased epidermal hyperplasia compared to the skin of similarly exposed wild type mice. In Slug knockout mice there is reduced induction of proinflammatory cytokines and chemokines, including fractalkine (Cx3cl1), Cxcl2, interleukin-1 β , and macrophage migration inhibitory factor, all of which are known to play a role in leukocyte chemotaxis, by UVR-exposed Slug knockout compared to wild type epidermis. Our studies do not indicate differences between Slug null and wild type epidermis in p53 induction or caspase-3 staining following UVR exposure, suggesting that altered apoptosis does not underlie the reduced sunburn susceptibility of Slug null mice [122].

Other studies have shown additional links between Snail family transcription factors and inflammation. For example, Snail and Slug appear to act downstream of the EGF receptor and in concert with class I HDACs to repress transcription of 15-hydroxyprostaglandin dehydrogenase, the enzyme responsible for degradation of prostaglandin E2, in colorectal and lung cancer cells, respectively [123–125]. Thus in cancer cells that express high levels of Snail or Slug, prostaglandin E2 levels are elevated, leading to increased inflammation and enhanced tumorigenesis even in the absence of elevated cyclooxygenase-2 expression.

THE SLUG-SNAIL RELATIONSHIP

Studies of Slug and Snail expression in any tissue must be interpreted with caution. First, since both proteins are subject to extensive post-translational processing, mRNA levels may not accurately reflect protein levels. A second consideration is that careful validation of antibodies used to detect Slug and Snail is essential. To date, many of the commercially available polyclonal antibodies do not detect Slug and Snail with sufficient specificity to be useful, especially for immunohistochemistry. Of the many commercial antibodies against Slug that we have tested, only a single antibody, a rabbit polyclonal antibody supplied by Cell Signaling reliably detects Slug in both murine and human tissue by immunohistochemistry and Western blotting. We have yet to identify a commercially available Snail antibody of similar specificity and sensitivity. Thirdly, the effects of Slug and Snail are tissue-specific and their relative contributions to cell function vary among cell types. Finally, studies performed in cell lines may be misleading, especially since basal levels of Slug and Snail expression are highly dependent on cell density and the presence of growth factors in the medium, as described above. It is expected that *in vivo* studies in genetically engineered mice will provide more relevant physiologic information about Slug and Snail function in the skin.

Despite these caveats, the results of Slug and Snail studies to date clearly show that the two genes are not functionally equivalent. They are induced by overlapping but not identical stimuli and may have different targets. It has been suggested that Slug and Snail act sequentially to induce EMT, with Slug acting early to trigger EMT by inducing desmosome dissociation, cell spreading, and partial cell separation and Snail acting later to complete the process by enhancing cell motility and initiating the switch from cytokeratin to vimentin expression [73]. Studies comparing Slug and Snail expression in a variety of tissues support this suggestion. In our studies of UVR skin carcinogenesis, we observed that Slug

expression is increased in both squamous and spindle cell SCC, while Snail expression is elevated primarily in spindle cell tumors [44]. Our findings are in keeping with other reports of non-redundant roles for Slug and Snail in SCC. Snail is reported to be the primary regulator of EMT and invasiveness in mouse keratinocyte cell lines, while Slug apparently has milder effects; however, Slug works synergistically with Snail to enhance its effects [115]. In breast carcinoma cells, Snail induces a more pronounced form of EMT than Slug [126]. Furthermore, canine kidney epithelial cells (MDCK cells) overexpressing Snail or Slug have different morphologies when transplanted into nude mice. Snail-expressing cells form undifferentiated spindle cell tumors with no evidence of epithelial differentiation, whereas Slug-expressing cells form carcinosarcomas characterized by foci of glandular differentiation surrounded by undifferentiated malignant spindle cells [127]. Although ectopic expression of Snail in epithelial cells drives EMT-like changes, a number of recent reports suggest that endogenous Snail expression is largely localized to the stroma supporting normal and neoplastic epithelial cells, while endogenous Slug expression is seen primarily in epithelial cells [43,64,128,129]. Thus the relative contribution of the two transcription factors to the morphology and behavior of both normal and transformed epithelial cells remains to be fully elucidated.

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Abbreviations

CtBP	C-terminal binding protein
EGF	epidermal growth factor
EMT	epithelial-mesenchymal transformation
HDAC	histone deacetylase
MMP	matrix metalloprotease
SCC	squamous cell carcinoma
SNAG	Snai/Gfi
TGF-β	transforming growth factor- β
TPA	12-O-tetradecanoylphorbol 13-acetate
UVR	ultraviolet light

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Figure 1. Structure of the Slug transcription factor. Five zinc fingers are located in the carboxy portion of the Slug protein. The amino half of Slug contains a SNAG domain, a CtBP motif, and a Slug-specific sequence. Repressor activity resides in both amino and carboxy regions of the protein, and multiple nuclear localization signals are distributed within and adjacent to the zinc fingers.

Figure 2.

Slug and keratin 14 expression in embryonic skin. Slug and keratin 14 was detected in CD1 mouse embryo sections (Zyagen, San Diego, CA) using previously described techniques [40]. During skin development, Slug expression becomes progressively localized to basal keratinocytes and parallels keratin 14 expression. Note clear nuclear localization of Slug at all time points. Scale bar; 50 μ m.

Figure 3.

Slug induction by UVR and TPA. UVR: SKH-1 hairless mice were exposed to 2800 J/m² UVR from a solar simulator; unexposed mice provided control skin. TPA: DBA/2 mice were shaved and treated once with 6.8 nm TPA in acetone; control mice were treated with acetone only. Skin was collected 4, 24, and 48 hours after UVR exposure or TPA application. Immunohistochemistry for Slug was performed as previously described [40]. Both UVR and TPA induced Slug expression in basal keratinocytes; however, Slug induction peaked at different time points: 48 hours after UVR exposure and 24 hours after TPA application. Scale bar; 50 μ m.

Figure 4.

Slug induction during reepithelialization of cutaneous wounds. Wild type 129 mice were shaved and depilated. Two days later, excisional wounds were introduced using sterile skin biopsy punches. Wounds were harvested at 6, 24, 48, and 72 hours after wounding. Immunohistochemistry for Slug was performed as previously described [40]. At 6 hours post-wounding, occasional basal keratinocytes have nuclei immunopositive for Slug. Increased Slug expression is seen in migrating cells at wound margins at 24 and 48 hours after wounding; Slug expression becomes maximal 72 hours post-wounding. In all panels, the wound edge is indicated with an arrowhead. Scale bar; 100 μ m.

SCC

Spindle cell

Figure 5.

Slug expression in UVR-induced epithelial and spindle cell tumors of mouse skin. The typical SCC in the SKH-1 hairless mouse was induced by chronic exposure to one minimal erythemal dose of UVR; the spindle cell tumor in the 129 wild type haired mouse was induced by chronic exposure to two minimal erythemal doses of UVR.

Immunohistochemistry for Slug was performed as previously described [40]. Note that many nuclei in both tumor types are immunopositive for Slug. Scale bar; 100 μ m

Wild type

Slug null

Figure 6.

Decreased sunburn response in Slug null mice. Wild type and Slug null littermates on a 129 background were shaved and exposed to three minimal erythral doses of UVR. Skin was sampled 48 hours later. Note the marked inflammatory infiltrate in the dermis and epidermal hyperplasia in the wild type mouse. These changes are much milder in Slug null skin. Scale bar; 100 μ m.

Table 1Genes with Altered Expression in Slug Null Epidermis ^a

Gene	Process/Location	Fold change in Slug Null Epidermis ^{b,c}	
		Microarray	qRT-PCR
Gli1	Differentiation	8.5	5.4
Gli2	Differentiation	3.4	7.0
Keratin 8	Differentiation	35.6	1052
Keratin 18	Differentiation	22.9	1024
Homeo box D9	Differentiation	2.1	ND
Brain expressed gene 1	Differentiation	5.9	ND
SNF-1-like kinase	Differentiation	-4.8	-1.2
Epithelial mitogen	Proliferation/apoptosis	5.7	ND
Insulin-like growth factor binding protein 2	Proliferation/apoptosis	2.1	ND
PMA-induced protein 1	Proliferation/apoptosis	2.9	ND
Insulin-like growth factor binding protein 3	Proliferation/apoptosis	-2.6	ND
Phospholipid scramblase	Proliferation/apoptosis	2.0	ND
CD44 antigen	Adhesion	2.1	ND
CD7 antigen	Adhesion	-2.3	ND
Tenascin C	Extracellular matrix	6.1	9.2
Periostin	Extracellular matrix	5.2	ND
Tenascin XB	Extracellular matrix	-2.4	ND
Agrin	Extracellular matrix	-2.1	ND
Rho GTPase activating protein 25	Motility	-2.0	ND
Angiomotin	Angiogenesis	5.6	8.0
Connective tissue growth factor	Angiogenesis	2.9	ND
Angiopoietin 1	Angiogenesis	2.1	ND
SPARC related modular calcium binding 2	Angiogenesis	-3.8	ND
C-fos induced growth factor	Angiogenesis	-3.2	ND
IL-20 receptor	Inflammation	-3.7	ND
IL-22 receptor	Inflammation	-3.3	ND
Heat shock protein 4 like	Stress response	2.9	ND
Dual oxidase 1	Stress response	2.2	ND
Cytochrome P450 2B10	Detoxification	2.0	ND
Sulfotransferase 5A1	Detoxification	-4.3	ND
Alcohol dehydrogenase 6B	Detoxification	-2.0	ND

^aThis list includes a selection of genes with expression altered at least two-fold in the epidermis of Slug null mice.

^bValues shown are the ratio of Slug null/wild type expression. A positive value indicates that expression was increased in the Slug null relative to the wild type epidermis, while a negative value indicates that expression in Slug null epidermis was decreased relative to wild type.

^cND indicates that the assay was not performed.