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Expression of Snail in epidermal keratinocytes promotes cutaneous inflammation and hyperplasia conducive to tumor formation

Fei Du^{1,*}, Yoshikazu Nakamura^{1,*}, Tuan-Lin Tan¹, Pedro Lee¹, Robert Lee², Benjamin Yu², and Colin Jamora^{1,2}

¹Section of Cell and Developmental Biology, Division of Biological Sciences, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093

²Department of Medicine (Division of Dermatology), School of Medicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093

Abstract

Although metastasis is the most lethal consequence of tumor progression, comparatively little is known regarding the molecular machinery governing this process. In many carcinomas, there is a robust correlation between the expression of the transcription factor Snail and a poor prognosis, but the contribution of this protein to the metastatic process remains unresolved. Interestingly, the prolonged expression of Snail in epidermal keratinocytes is sufficient to recapitulate early features of metastasis. However, it does so without inducing a complete epithelial-mesenchymal transition (EMT), a developmental phenomenon mediated by Snail that is extensively invoked as the mechanism fueling tumorigenesis. Instead we found that the local invasiveness of keratinocytes is the consequence of the recruitment and activity of macrophages. Moreover, keratinocyte proliferation is the product of an IL-17/IL-6/Stat3 signaling module initiated by activated resident $\gamma\delta$ T-cells in the transgenic skin. Together these phenotypes prime the transgenic skin for the formation and metastasis of tumors in response to chemically induced carcinogenesis. Thus the contribution of Snail to the progression of carcinomas is largely through the creation of a hyperproliferative and inflammatory niche that facilitates tumor development and dissemination.

INTRODUCTION

A decisive factor in the multistage process of metastasis is the early step of local invasion of carcinoma cells(1). However, the mechanisms coordinating the increased motility of proliferating cancer cells remain elusive. Members of the Snail family of transcription factors have garnered widespread interest in this context as they are expressed in a variety of carcinomas(2) (3) and are associated with recurring or metastasizing tumors(4). Snail proteins have a well-established role in embryogenesis during which they mediate a process known as an epithelial-mesenchymal transition (EMT) to facilitate tissue formation(5). The

Correspondence to: Colin Jamora, Natural Science Building Room 6113, 9500 Gilman Drive, MC 0380, La Jolla, California 92093-0380, Telephone: (858) 822-4709, Fax: (858) 822-5740, cjamora@ucsd.edu.

*Fei Du and Yoshikazu Nakamura contributed equally to this paper

Snail-mediated EMT causes cells to lose their epithelial characteristics such as E-cadherin mediated adhesion and polarity, while adopting phenotypes of mesenchymal cells such as an increased migratory capacity(6). Given the similarities to its effects in development, it is widely extrapolated that Snail functions similarly in the metastasis of somatic cells during tumorigenesis(7). The ability of Snail to induce an EMT in multiple cancer cell lines supports this notion but the absence of in vivo evidence that this likewise occurs in cancerous cells of the body(8) suggests that additional mechanisms are operational in this disease.

We previously found that Snail is expressed during budding morphogenesis of the hair follicle, wherein proliferating keratinocytes in the epidermis invade into the underlying dermal compartment of the skin (9). Moreover, transgenic expression of Snail in the epidermis of young mice leads to features commonly seen in cutaneous cancers(10). This includes the activation of the Ras-MAPK signaling axis, involution of the epidermis, reduction in intercellular adhesion and degradation of the basement membrane separating the epidermis from the underlying dermis (9). These attributes suggest that the Snail transgenic mouse may be a prime system with which to elucidate the regulatory network specifically controlling the critical initial stage of the metastatic cascade (1, 11).

RESULTS

The extensive attention paid to the role of Snail in tumorigenesis is partly due to its expression in numerous carcinomas (3) including the skin (Supplementary Fig. S1). Interestingly, we found that a transgenic mouse engineered to overexpress Snail in epidermal keratinocytes shares some features with these carcinomas including an elevated proliferative index and local invasiveness (9). We therefore investigated the extent to which the Snail transgenic skin recapitulates the biochemical features of the metastatic program. We found that several factors known to promote tumor dissemination are upregulated in the Snail transgenic skin of neonatal mice (Fig. 1A). Among these are the activated Akt kinase, which is necessary for many events of the metastatic pathway (12) and c-Jun/AP-1, which has been linked to invasive properties of aggressive breast cancer cells(13).

The growth of tumors within somatic tissues imposes an increased metabolic burden that is met by an increased supply of nutrients delivered by the targeted growth of new blood vessels to the cancer cells. Moreover, these vessels provide a route for metastatic dissemination by providing a site for entry into the circulation(14). The level of CD31 staining that denotes endothelial vessels is markedly increased in the transgenic dermis indicating augmented vascularization (Fig. 1A). This phenotype is assisted by an increase in the level of the pro-angiogenic protein, vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9), which facilitates remodeling of the local extracellular matrix and directional growth of the blood vessel (Fig. 1B)(15). This restriction of MMP-9 to the dermal compartment of the skin is unexpected as it has previously been shown that introduction of Snail into an epithelial cell line is sufficient to induce expression of MMP-9(16). Additionally, MMP-2, which is also known to facilitate angiogenesis, has relatively low activity in both the wild type and transgenic dermis (Fig. 1B).

Increasing evidence demonstrates that the transcription factor Stat3 plays an indispensable role in various aspects of oncogenesis including keratinocyte proliferation and tumor angiogenesis, invasion and metastasis (17). Consonant with its varied roles in tumor progression, Stat3 (Fig. 1C) and its transcriptional target c-myc (18) (Supplementary Fig. S2) are activated in the transgenic skin. Moreover, c-myc has recently been demonstrated to induce a transcriptional program leading to the propagation of cancer stem cells(19). In line with this hypothesis, keratinocytes expressing the marker keratin 5 coexpress the epithelial stem cell marker CD44(20) in the Snail transgenic epidermis (Fig. 1D) suggesting an expansion of this pool of cells.

Given Snail's ability to singularly induce multiple features of the early stages of metastasis we focused our investigation on elucidating the mechanisms by which it promotes two phenomena of this complex process - keratinocyte proliferation and local invasion. Keratinocytes in the basal layer of the epidermis are cuboidal in shape while suprabasal cells are flattened. In contrast, keratinocytes in the transgenic epidermis display an elongated and spindle-like morphology (Fig. 1A and 2D), which implies an enhanced migratory capacity. Together with our previous finding that transgenic epidermis exhibited lower levels of E-cadherin in regions expressing Snail(9), these results support the notion that Snail induces an EMT for tumor progression (7). However, despite the continual expression of Snail in the transgenic epidermis in neonatal mice, keratin expression is maintained and there is no induction of the mesenchymal marker fibronectin in the epidermal cells (Fig. 2A). This is particularly surprising as constitutive expression of exogenous Snail in epithelial cell lines is sufficient to induce mesenchymal marker expression(6). Moreover, previous reports have also suggested that cells stably transfected with Snail have decreased cell proliferation(21) whereas epidermal keratinocytes expressing Snail were found to be proliferative(9). These results suggest that a complete or permanent EMT, which occurs during development, is not required for the early stages of metastasis of cancer cells derived in vivo.

Accumulating data suggests that secondary inflammatory responses may instead be responsible for the invasiveness of cancer cells(22). The elevated levels of nuclear NF κ B (which is a classic regulator of proinflammatory genes) in the upper layers of the epidermis as well as the dermis (Fig. 2A) suggest that inflammation may contribute to the invasive phenotype of the transgenic skin. Furthermore, Stat3 (Fig. 1C) and CD44 (Fig. 1D) staining in the transgenic dermis are consistent with their expression in infiltrating immune cells. The transgenic dermis indeed has elevated T-cells which is illustrated by the pan T-cell marker CD3 (Fig. 2A) and include CD4⁺, CD8⁺, and $\gamma\delta$ T-cells (Supplementary Fig. S3A). The cytokines secreted by these T-cells are skewed towards the T_H2 factors (IL-4, 13) which promotes an inflammatory microenvironment (23) at the expense of IFN γ , a T_H1 cytokine and tumor antagonist (Fig. 2B). Cells of the innate immune system are also recruited into the transgenic skin including macrophages (Fig. 2C) and granulocytes (Supplementary Fig. S3A). One level of crosstalk between the adaptive and innate immune systems is illustrated by the ability of T_H2 derived cytokines to polarize macrophages along the M2 lineage into tumor associated macrophages (TAMs). Evidence of this is seen in the Snail transgenic skin by the coexpression of the lectin CD206 on a subset of macrophages (Fig. 2C) and the presence of other markers of TAMs (Supplementary Fig. S3B–C). Interestingly, it has been reported that TAMs are a source of MMP-9, which is found exclusively in the transgenic

dermis (Fig. 1B), and this enzyme contributes to their role in the metastatic cascade(24). The importance of these various immune cells in manifesting the changes in the Snail transgenic skin was demonstrated by the ability of an immunosuppressive cocktail to significantly reduce the epidermal involution and hyperplasia (Fig. 2D) and cutaneous inflammation (Supplementary Fig. S4) in the mutant mouse.

Among these infiltrating immune cells, macrophages have garnered extensive attention for their remarkable ability to promote tumor proliferation and metastasis(25). Thus the mechanism by which Snail expressing keratinocytes induces their recruitment into the skin becomes an important problem to resolve. We observed that the transgenic epidermis had an elevated level of monocyte colony stimulating factor-1 (CSF-1), which is a well-known chemoattractant for macrophages (Fig. 3A) (26). The induction of CSF-1 appears to be cell autonomous as transfection of Snail into primary keratinocytes is sufficient to elicit CSF-1 expression (Fig. 3A). Importantly, we found that conditioned medium (CM) from epidermal explants of transgenic mice is capable of recruiting macrophages using a transwell assay (Fig. 3B). An inhibitory antibody against this cytokine demonstrates that CSF-1 is a required component of the CM to stimulate macrophage mobilization. Since the epidermal explants used to condition the media are a heterogeneous population of cells, we tested whether Snail expression in keratinocytes is directly involved in the recruitment of macrophages by reconstituting this process completely in vitro. Primary keratinocytes transfected with Snail are capable of synthesizing and secreting CSF-1 to promote macrophage recruitment (Fig. 3C). These macrophages can, in turn, potentially stimulate invasion of primary keratinocytes through an extracellular matrix (Fig. 3D). Moreover, the cytokine milieu present in the transgenic skin favors the polarization of the macrophages into TAMs (Fig. 2B–C, Supplementary Fig. 3B–C), which have been localized to areas of metastasis and shown to promote tumor cell invasion(27, 28). Consistent with this scenario, we found that TAMs can stimulate keratinocyte invasion at even higher levels than the classically activated macrophages (Fig. 3D).

Given TAMs ability to stimulate proliferation of breast carcinoma cells (29) we hypothesized that they may also be responsible for the elevated keratinocyte proliferation found in the Snail transgenic skin (Supplementary Fig. S5) (9). Surprisingly, we found that both M1 macrophages and M2/TAMs have no effect on the growth rate of keratinocytes (Supplementary Fig. S6). To decipher the mechanism of increased keratinocyte proliferation in the transgenic skin we focused on the activation of Stat3 (Fig. 1C), which is a prerequisite for keratinocyte proliferation during carcinogenesis(17). Profiling of the cytokines released from the transgenic epidermis revealed an increased level in the amount of secreted IL-6, which is able to stimulate the phosphorylation and nuclear translocation of Stat3 (Fig. 4A). Moreover, IL-6 can increase the rate of keratinocyte growth in a Stat3 dependent fashion (Fig. 4A). IL-6 is not normally expressed in wild type epidermis and transfection of Snail into keratinocytes is incapable of inducing its expression (Fig. 4A). However, it is found in inflammatory skin diseases such as psoriasis(30) suggesting that the stimulus may be derived from an activated leukocyte. A clue into this mechanism came from reports that IL-17 can induce IL-6 expression in autoimmune (31) and tumor settings (32). While profiling cytokine expression, we observed that IL-17 was induced in the transgenic skin and that the activated $\gamma\delta$ T-cells in the transgenic dermis are a source of this cytokine (Fig. 4B).

Analysis of the biological activity of this cytokine on epidermal keratinocytes revealed that IL-17 can promote keratinocyte proliferation via IL-6 signaling (Fig 4B) and this cascade is competent to activate Stat3 (Fig 4C). As noted above, c-myc is a target of Stat3 and the increased expression of this proto-oncogene upon treatment of keratinocytes with IL-17 verified that the IL-17/IL-6/Stat3 signaling cascade was operational (Fig. 4D).

In light of the effect of Snail expression in the skin, we investigated whether this transgene renders mice more susceptible to inflammation-driven skin cancer. To test this hypothesis, we subjected wild type and transgenic mice to the two-stage chemical carcinogenesis protocol using DMBA as the mutagen and TPA as the promoter (33). This protocol was facilitated by the fact that the neonatal phenotypes decrease as the mice reach adulthood (Fig. 5A) and Snail protein levels diminish (data not shown), thus allowing us to test whether Snail primes the epithelial cells of the skin for tumor formation. Transgenic mice have a higher frequency and incidence of tumor formation than wild type littermate controls (Table 1 and Supplementary Fig. S7). The transgenic skin responded with a significant epidermal hyperplasia and involution of the tissue reminiscent of migrating cells (Fig. 5A). Moreover, all of the adult transgenic mice displayed a hyperplastic sebaceous gland, an appendage of the epidermis, and treatment with DMBA + TPA led to the development of sebaceous carcinomas. The dermis of the DMBA + TPA treated transgenic mouse verified a substantial increase in the number of lobular acini relative to the wild type skin as marked by Oil Red O staining (Fig. 5B). Histological analysis demonstrates that these sebaceous carcinomas invade the blood vessels (Fig. 5B), induce epidermal ulceration, and invasion into the adipose and stromal tissues of the skin (Fig. 5C). These sebaceous carcinomas were indeed metastatic as 15 out of 16 transgenic mice (Table 1) had sebocytes in their lymph nodes (Fig. 5D) that were positive for keratin 5 expression (Fig. 5E).

DISCUSSION

A model summarizing the epithelial-leukocytic crosstalk stimulated by the expression of Snail in epidermal keratinocytes to promote an early metastatic phenotype is presented in Figure 6. In the aggregate these findings highlight the non-cell autonomous role that Snail has in promoting oncogenesis in vivo. We found no evidence of a complete or permanent EMT but can attribute many of the phenotypes to the recruitment and activity of immune cells recruited to the skin of the Snail transgenic mouse. This is consonant with the lack of reports of definitive evidence of an EMT occurring in any carcinoma cells in vivo (8). The intermediate phenotype of the Snail transgenic mouse may therefore be more appropriately referred to as “EMT-like” (34). Our data suggests that the cell autonomous function of Snail during carcinogenesis in vivo may be to maintain the undifferentiated state of a metastasizing cell (20) (35) as it disseminates to new tissues, thereby contributing to the maintenance of the “cancer stem cell” pool. On the otherhand, the driving force for the local invasion of these cancer cells is the reciprocal interactions between the carcinoma cells and leukocytes. These findings concur with recent reports implicating Snail in mediating inflammation(36, 37). Interestingly, the epidermal hyperplasia and cutaneous inflammation that is prominent in neonatal mice significantly dissipates in the adult mice (Fig. 5). This is likely due to the inherent instability of Snail expression(6) and reduction in protein levels despite the continual transcription driven by the keratin-14 promoter in epidermal

keratinocytes. This reversibility of the phenotype implies that continual intercellular signaling stimulated by Snail is required to preserve the changes we documented in the transgenic skin.

At first glance the activity of resident $\gamma\delta$ T-cells in the Snail transgenic skin appears to contradict their anti-cancer capability(38). However, $\gamma\delta$ T-cells are activated and play a critical role in wound healing(39), which shares many processes in common with tumorigenesis such as inflammation/cytokine signaling and cell proliferation. In fact tumor promotion and development is often found at sites of wounds/tissue damage(40). The activation and function of $\gamma\delta$ T-cells in the transgenic skin likely occurs along the lines of a wound-healing program to elicit a pro-tumor response from these cells. Another important subset of T-cells in this metastatic cascade appears to be T_H2 cells that contribute to the inflammatory microenvironment and polarizes the macrophages into M2/TAMs. These T_H2 cells are probably recruited to the skin by the chemokines CCL18, 22, and 27, which are upregulated in the epidermis of the transgenic skin (data not shown).

We postulate that the sebaceous gland carcinoma induced via chemical carcinogenesis is likely the response to the fact that the alteration in the sebocytes is the strongest remaining phenotype in adult transgenic mice. Moreover, these hyperplastic cells appear to be positive for keratin 15 (data not shown) which is another marker for sebaceous neoplasms as well as the sebaceous carcinoma of Murre-Torre syndrome (MTS)(41). MTS is a subtype of hereditary nonpolyposis colorectal cancer and sebaceous differentiation (Fig. 5B–C) seems to be a strong phenotypic marker of this disease. Other genes associated with hair follicle morphogenesis that were expressed in the basal layer of the epidermis, such as a mutated Lef1, also generated sebaceous skin tumors (42). Altogether, a clearer picture is emerging regarding the mechanism by which genes associated with budding morphogenesis of the hair follicle can be usurped by carcinomas to serve similar, albeit unregulated, roles in tumor development and metastasis.

MATERIALS AND METHODS

Generation of Snail transgenic mice

Mice engineered to express the Snail transgene in the epidermis was previously described (9). All animal work was approved and adhered to the guidelines of IACUC.

Transwell cell migration and invasion assays

For the cell migration assay, 5×10^4 Raw246.7 cells (ATCC) were seeded in Transwell inserts with an 8.0 μ m pore (Corning) with DMEM + 10% heat inactivated FBS. Conditioned medium (CM) from the epidermis of P7 mice was added to the bottom chamber at a 1:2 dilution in medium with or without 1.6 μ g/ml M-CSF neutralizing antibody (R&D Systems) or goat IgG control antibody. After 8 hours incubation, cells were stained with 0.1% Crystal Violet. The membrane inserts were removed and mounted on a slide. For the keratinocyte invasion assay, the Transwell inserts were coated with Collagen I (Sigma) and 1×10^5 HaCat cells (ATCC) were used. CM from thioglycollate elicited peritoneal

macrophages stimulated with (TAM CM) or without (Mac CM) 20ng/ml IL-4 (eBioscience) were collected and added to the bottom chamber and incubated for 14 hours.

Proliferation assays

After overnight starvation, primary mouse keratinocytes were trypsinized and resuspended in mouse keratinocytes medium with 2% FBS. 1500 cells were inoculated in 96 well dishes with or without 10ng/ml IL-6 (eBioscience), 25ng/ml IL-17 (R&D Systems), 2µg/ml IL-6 neutralizing antibody (R&D Systems), or 50µM STAT3 inhibitor peptide (Calbiochem). Cell numbers were measured by Cell TiTer 96 Aqueous One Solution (Promega) as suggested by the manufacturer.

Dexamethasone and indomethacin treatment

Mice were injected subcutaneously with a mixture of 0.05 mg/kg dexamethasone (Sigma–Aldrich) and 0.05 mg/kg indomethacin (Fluka) starting from new born mice for 5 consecutive days. Control mice were injected with vehicle (0.4% ethanol in PBS).

Histology, in situ hybridization and immunohistochemistry

Mouse skin or lymph nodes from wild type and Snail transgenic animals were either frozen in OCT (Tissue-Tek) or embedded in paraffin depending on the application. Paraffin sections were prepared for histology, and counterstained with hematoxylin and eosin-Y (H&E). Antibodies used were anti-phospho-Akt, c-Jun, CD31, phospho-STAT3 (Tyr 705) all from Cell Signaling, CD44, Fibronectin, phospho-NFκB (Ser276, Cell Signaling), CD3 (BD Biosciences), MAC-1 (BD Biosciences) and CD206. For nuclear staining Hoechst 33342 (Calbiochem) was added in a final concentration of 1 mg ml⁻¹ to the secondary antibody dilution. Immunofluorescence was detected using rhodamine-X or FITC conjugated secondary antibodies (Jackson ImmunoResearch) or expression was developed using the Vectastain ABC kit (Vector Labs) according to the manufacturer's instructions. Images were acquired on an Olympus Bx51 microscope with an Olympus DP70 camera. Acquisition were performed using a 40X 1.3 UPlan FL N objective (Olympus).

Quantitative real time–PCR

Total RNA was extracted from whole skin of WT (n=5) and Snail Transgenic (Tg) (n=5) mice at postnatal day 7 (P7) using Trizol reagent (Invitrogen) according to manufacturer's instructions. Similarly, epidermis from P7 WT (n=5) and Tg (n=5) mice was isolated with dispase treatment and total RNA isolated using the Trizol protocol. cDNA was synthesized by reverse transcription using oligo-dT as primers (Superscript III kit, Invitrogen). Real time-PCR analysis was performed with previously described primers(43). Experiments were carried out in triplicate from cDNA isolated from five different animals.

Zymography

Presence of active MMP-9 and MMP-2 were detected using a previously described protocol(44).

Two stage chemically induced skin carcinogenesis

7–8 week old mice were subjected to the two stage skin chemical carcinogenesis protocol as previously described(33) using 400nmol DMBA as the initiating agent and 10nmol TPA as the promoting agent.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Presence of metastasis associated markers in the Snail transgenic skin

(A) Skin sections from wild type (WT; left column) and Snail transgenic (Snail Tg; right column) were subjected to immunofluorescence (IF) with antibodies recognizing keratin 5 (K5) in red and phospho-Akt, c-jun and the blood vessel marker CD31 in green. Dotted lines denote the basement membrane, which separates the epidermis (epi) and hair follicle (hf) from the dermis (der). (B) RT-PCR of VEGF (left column) from RNA extracted from two wild type and three transgenic skin samples with GAPDH as a loading control and zymography of MMP9 and MMP2 activity (right column). (C) Immunohistochemistry of phosphorylated Stat3. (D) IF of CD44 expression. Bars, 30 μ m.

Figure 2. Inflammation in the Snail transgenic skin

(A) Expression of the mesenchymal marker fibronectin (FN), activated NF κ B (p NF κ B), and the pan T-cell marker CD3 in wild type (WT, left column) and Snail transgenic (Snail Tg, right column) skin. (B) Profile of the Th1 cytokine IFN γ and Th2 cytokines IL-4 and IL-13 via RT-PCR with GAPDH as a loading control. (C) Infiltration of macrophages detected by Mac-1 staining in the dermis of WT and Snail Tg skin (top row) and the presence of M2/TAMs (tumor associated macrophages) marked by a subset of macrophages (Mac-1; green) coexpressing the mannose receptor recognized by the CD206 antibody in red. (D) Histological analysis of the effect of PBS vehicle control or dexamethasone-indomethasin (DI) immunosuppressive cocktail on the transgenic phenotype. Bars, 30 μ m.

Figure 3. Recruitment and function of macrophages in the transgenic skin

(A) Analysis of the expression of colony stimulating factor-1 (CSF1) and Snail in wild type (WT epi) or Snail transgenic (Tg epi) epidermis and primary mouse keratinocytes transfected with either empty vector (mKT + vector) or Snail (mKT + Snail) via RT-PCR using GAPDH as a loading control. (B) Migration of macrophages in a transwell assay when treated with conditioned media from wild type epidermis (WT epi CM) or Snail transgenic epidermis (Tg epi CM) and either control immunoglobulin (Con IgG) or a CSF1 inhibitory antibody (CSF1 Inh. Ab). Results are expressed as a fold increase over macrophages treated with normal growth media. (C) Effect of CM from mouse keratinocytes (mKT) transfected with vector or Snail on macrophage migration in the presence of control or CSF-1 inhibitory antibody. (D) Quantification of keratinocyte invasion through extracellular matrix in a transwell assay. Primary keratinocytes were exposed to growth media or conditioned media from macrophages or tumor associated macrophages (Mac CM or TAM CM. respectively).

Figure 4. Leukocyte derived IL-17 stimulates IL-6 production in keratinocytes to promote proliferation

(A) Quantification of IL-6 secreted from wild type (WT) or Snail transgenic (Snail Tg) epidermis (column 1) and nuclear translocation of phosphorylated Stat3 (pStat3) in keratinocytes treated with buffer control or recombinant IL-6 (column 2). Proliferation rate of keratinocytes treated with growth media (blue), recombinant IL-6 (orange), or IL-6 + Stat3 inhibitor (green) using the XTT assay (column 3). IL-6 expression (column 4) detected via RT-PCR of RNA extracted from wild type (WT epi) or transgenic (Tg epi) epidermis and keratinocytes transfected with empty vector (mKT + vector) or Snail (mKT + Snail). (B) Coexpression of $\gamma\delta$ T-cell marker (green) and IL-17 (red) in column 1. Right panel is an enlarged image of the area marked by the arrowhead in the left panel. Column 2 displays the proliferation rate of keratinocytes treated with media (blue), IL-17 (orange), or IL-17 + IL-6 inhibitory antibody (green). (C) Nuclear translocation of phosphorylated Stat3 in keratinocytes treated with buffer, IL-17 + control antibody, or IL-17 + IL-6 inhibitory antibody. (D) Quantitative RT-PCR of c-myc expression in wild type or transgenic epidermis or keratinocytes treated with buffer or IL-17 and/or inhibitors. Note: standard deviation for each triplicate data point in the proliferation assays were less than 3%. Bars, 30 μ m.

Figure 5. Tumor Development and Metastasis in the Snail transgenic mouse

7–8 week old mice were treated with DMBA+TPA unless otherwise noted. (A) Histological staining with hematoxylin and eosin (H&E) of wild type (WT) and Snail transgenic (Tg) $\pm$ DMBA and TPA. (B) Oil Red O staining to detect sebaceous glands in WT(left panel) and transgenic (middle panel) skin. Right panel is a transgenic skin section stained with H&E – arrowheads point to blood vessel. (C) H&E of Snail transgenic skin demonstrating an epidermal ulcer (left panel), invasion into adipose tissue, and stromal tissue (asterisk). (D) Upper panels: H&E of lymph nodes from wild type (left panel) and transgenic (middle panel, 10X; right panel, 40X) mice treated with DMBA and TPA. Lower panels: immunofluorescence of keratin 5 (red) in lymph nodes. Bars, 30 μ m.

Figure 6. Signaling in the Snail transgenic skin

Expression of Snail in epidermal keratinocytes leads to a program that maintains the undifferentiated state of the transgenic cells. Snail also transcriptionally upregulates colony stimulating factor 1 (CSF1) to cause homing of macrophages (M Φ) into the skin. A subset of these macrophages is polarized along the M2/TAM (tumor associated macrophage) lineage. Together these macrophages then stimulate the local invasion of the keratinocytes into the underlying dermis. Snail expressing keratinocytes also leads to the wound-like activation of resident $\gamma\delta$ T-cells that migrate into the dermis and begin secreting IL-17. Epidermal keratinocytes respond to IL-17 by inducing expression of the cytokine IL-6, which works in a paracrine fashion to activate the transcription factor Stat3. Stat3 activation contributes to tumorigenesis by augmenting proliferation, cell survival, and angiogenesis.

Table 1**Incidence and frequency of tumorigenesis**

Quantitation of phenotypes in wild type and transgenic mice with or without chemical carcinogenesis. Data is compiled from mice 8 weeks after the promotion phase of the two step chemical carcinogenesis protocol.

Adult mouse + treatment	# of animals	Incidence of epidermal hyperplasia (%)	Incidence of Sebaceous hyperplasia (%)	Incidence of Sebaceous carcinoma (%)	# Sebaceous carcinoma per mouse	Incidence of lymph node metastasis (%)
Snail Tg	10	0	100	0	0	0
WT + DMBA/TPA	10	30	0	0	0	0
Snail Tg + DMBA/TPA	16	100	100	100	20.56±10.3	93.8