SAG/ROC2/RBX2 E3 ligase promotes UVB-induced skin hyperplasia, but not skin tumors, by simultaneously targeting c-Jun/AP-1 and p27.

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Sensitive to apoptosis gene (SAG)/regulator of cullins-2/RING box protein 2 is a stress-responsive RING component of Skp1/Cullins/F-box protein E3 ubiquitin ligase. When overexpressed, SAG inhibits apoptosis induced by reactive oxygen species or hypoxia. Here, we report that SAG overexpression inhibits ultraviolet (UV) B-induced apoptosis in mouse JB6 epidermal cells. Using a transgenic mouse model, in which SAG expression was targeted primarily to epidermis by a K14 promoter, we showed that both early and late stage of UVB skin carcinogenesis were reduced (10 weeks post-UVB exposure), c-Jun, p27, p53, c-Fos and cyclin D1 were strongly induced. While having no effect on UVB-induced p53, c-Fos and cyclin D1, SAG-transgenic expression reduced the levels of c-Jun and p27 and inhibited AP-1 activity. The net outcome of SAG-mediated inhibition of c-Jun/AP-1 (pro-tumor promotion) and of p27 (antiproliferation) increased skin hyperplasia, with no apparent effect on apoptosis, as evidenced by increased skin thickness, and increased rate of DNA synthesis, but hardly any apoptosis. Although skin hyperplasia was promoted, SAG-transgenic expression had no significant effect on tumor formation in the later stage of UVB carcinogenesis. Thus, by simultaneously targeting c-Jun and p27, SAG accelerates UVB-induced skin hyperplasia, but not carcinogenesis.

Introduction

Carcinogenesis, caused by physical (e.g. radiation), chemical or viral (e.g. oncogenic) mechanisms, is a multistage process of coordinated acquisition of favorable genetic lesions and complex interactions between tumor and host tissues, which ultimately leads to an aggressive metastatic phenotype (1). Ultraviolet (UV) light is considered a complete carcinogen, with characteristics of both tumor initiators and promoters. It acts as an initiator by producing irreversible mutagenic damage such as formation of pyrimidine dimers in DNA and acts as a promoter by inducing epigenetic changes that cause expansion of the initiated cell population. Indeed, chronic exposure to UV radiation from sunlight is the primary cause of non-melanoma and melanoma skin cancer in humans (2), which are associated with 

Abbreviations: BrdU, 5-bromo-2'-deoxy-uridine; PFA, paraformaldehyde; ROC/RBX, Regulator of Cullins/RING box protein; SAG, sensitive to apopto-

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In vivo ubiquitination

Human lung H1299 cells were transiently transfected with His-Ub, p27 alone or in combination with psiSAG or psiCont. Cells were harvested 48 h after transfection and split into two aliquots with one for direct western blotting analysis and the other for in vivo ubiquitination assay, as described (16, 22). Briefly, cell pellets were lysed in buffer A (0.6 M guanidinium–HCl, 0.1 M Na2HPO4/NaH2PO4, 10 mM Tris–HCl, pH 8.0, 0.1 M β-mercaptoethanol) and incubated with Ni-NTA beads (Qiagen, Valencia, CA) at room temperature for 4 h. Beads were washed once with each of buffer A, buffer B (8 M urea, 0.1 M Na2HPO4/NaH2PO4, 10 mM Tris–HCl, pH 8.0, 10 mM β-mercaptoethanol) and buffer C (8 M urea, 0.1 M Na2HPO4/NaH2PO4, 10 mM Tris–HCl, pH 6.3, 10 mM β-mercaptoethanol). Proteins were eluted from beads with buffer D (200 mM imidazole, 0.15 M Tris–HCl, pH 6.7, 30% glycerol, 0.72 M β-mercaptoethanol and 5% sodium dodecyl sulfate). The eluted proteins were analyzed by western blotting for polyubiquitination of p27 with anti-p27 antibody.

Immunohistochemistry

After 10 weeks of UVB exposure, the skin tissue was excised and fixed in 4% paraformaldehyde (PFA)–phosphate-buffered saline for 3 days at 4°C, and embedded in paraffin. Four micrometer thick sections were cut for immunostaining with an ABC kit (Vector Labs, Burlingame, CA). The primary antibodies used were c-Jun, p27, c-Fos, p53 and cyclin D1. Negative controls were included for each study by using normal goat serum to replace primary antibodies. The papilloma to carcinoma conversion was determined through histopathological observation after hematoxylin and eosin staining.

Measurement of skin thickness

Skin hyperplasia after 10 weeks of UVB exposure was determined by measurement of the epidermal thickness after hematoxylin and eosin staining. A total of 16 measurements were made for each mouse in a systematic manner under a microscope, starting from the top of the basement membrane to the bottom of the stratum corneum. The skin thickness from each mouse was then averaged within the group, expressed as millimeter after bottom of the stratum corneum. The skin tissue from each mouse was then measured by using a UVB radiometer (Ultra-Violet Products). Tumor development (at both ears and the back of trunk) was recorded weekly throughout 29 weeks. Mice were killed once their tumors reached 7 mm in size, and tumor samples were fixed in PFA, embedded in paraffin and analyzed (24).

BrdU incorporation assay

SAG-Tg(+) and SAG-Tg(−) mice were injected intraperitoneal with undiluted BrdU-labeling reagent, 2 ml/100 g (body weight), 2 h prior to killing, but 22 h after last UVB exposure of 10 weeks. The skin tissue was collected and fixed in 4% PFA–phosphate-buffered saline. The assay was performed with BrdU labeling and Detection Kit II (Roche, Indianapolis, IN). Slides were developed with nitroblue tetrazolium/5-bromo-4-chloro-3’-indolylyphosphate- p-toluidine and counterstained with Eosin (Richard-Allan Scientific, Kalamazoo, MI). For quantification of BrdU-positive cells, two random areas were selected from each mouse and the number of positive cells out of a total of 1000 epidermal cells was counted. Student’s t-test was performed to determine the statistical significance of differences between the two groups.

TUNEL assay

Skin tissue post-UVB exposure was excised and fixed in 4% PFA–phosphate-buffered saline and embedded in paraffin. Four micrometer thick sections were cut and deparaffinized slides were used for the TUNEL assay using the In Situ Cell Death Detection Kit (Roche). The slides were stained with TUNEL reaction mixture, along with label solution for the negative control, and counterstained with propidium iodide counterstaining solution. The slides were mounted and analyzed under a fluorescence microscope. For quantification of TUNEL-positive cells, two random areas were selected from each mouse skin. The number of positive labeling cells (cells in green), out of a total of 1000 cells, were counted in each area. Student’s t-test was performed to determine the statistical significance of differences between the two groups.

UVB exposure and skin carcinogenesis

Eight-week-old female K14-SAG mice (n = 17) and their negative littermates (n = 16) were irradiated on their shaved backs with a bank of Q-Panel Ultra-violet B-313 EL/12 sunlamps (Q-Panel Lab Products, Cleveland, OH) with doses of UVB increasing from 2.5 to 10 kJ/m2 for a total of 29 weeks (three times per week with 2.5 kJ/m2 for 4 week, with 5 kJ/m2 for 4 week and then with 10 kJ/m2 for the remaining weeks) (21). UVB exposure periods were calculated using a UVB radiometer (Ultra-Violet Products). Tumor development (at both ears and the back of trunk) was recorded weekly throughout 29 weeks. Mice were killed once their tumors reached 7 × 7 mm in size, and tumor samples were fixed in PFA, embedded in paraffin and analyzed (24).

Statistical analysis of tumor count and growth experiments: Data were analyzed using SAS v9.1 (SAS Institute). All hypotheses were tested at the 0.05 significance level. Tumor counts were compared using Poisson regression. The product-limit (Kaplan–Meier) estimates of the time to tumor formation functions of the SAG-Tg(+) and SAG-Tg(−) mice were compared by the log-rank test. Tumor growth rates were compared assuming an exponential growth model; the log-transformed volumes of each tumor were regressed on week of measurement using a linear random effects model that
accounts for correlated within-animal and within-tumor measurements. The regression coefficients for week of measurement estimate the exponential growth rate parameters, which were compared between SAG-Tg(þ) and SAG-Tg(C0) mice by an F test.

Results

SAG overexpression inhibits UVB-induced apoptosis

It has been shown previously that UVB induced apoptosis in JB6-Cl.41 epidermal cells (23) and SAG protected cells from apoptosis induced by reactive oxygen species and hypoxia both in vitro and in vivo (4,11,12). We investigated whether SAG overexpression would inhibit apoptosis induced by UVB exposure. A lentivirus-based SAG-expressing construct was made (16) to infect JB6-Cl.41 cells, and a 2- to 3-fold increase of SAG levels was achieved (Figure 1A). This level of SAG overexpression, which is within physiological range (16), rendered epidermal cells significantly more resistant to UVB-induced apoptosis, as demonstrated by reduced dead cells by trypan blue exclusion assay (Figure 1B), reduced sub-G1 apoptotic population in fluorescent activated cell sorting analysis (Figure 1C), and reduced activation of caspase-3 activity (Figure 1D). Thus, SAG overexpression caused a partial protection of JB6-Cl.41 cells from UVB-induced apoptosis.

SAG-transgenic expression inhibited UVB-induced AP-1 binding and AP-1 transactivation in mouse skin tissues

We next determined the potential role of SAG in UVB-induced skin hyperplasia, apoptosis and carcinogenesis, using an in vivo SAG-transgenic model, in which the SAG transgene was targeted to express primarily in skin epidermis by the K14 promoter in inbred FVB/N mice, a strain shown previously to be sensitive to UVB carcinogenesis (21). Two independent lines, 345 and 710, were characterized, with similar results in a 9,10-dimethylbenz(a)anthracene-12-0-tetradecanoylphorbol-13-acetate skin carcinogenesis protocol (24). The 710 line was, therefore, used in this study. We have recently found that SAG E3 ubiquitin ligase could, by promoting c-Jun ubiquitination and degradation, inhibit AP-1 activity in cultured cells (16). We determined if SAG transgene expression inhibited AP-1 activity, which is known to be induced by UVB and to play a promoting role in UVB-induced skin carcinogenesis (20). As shown in a gel retardation assay in Figure 2A, control mice, without exposure to UVB, did not have any AP-1 DNA-binding activity, regardless of SAG-transgenic expression (lanes 1–4, samples 1 and 2). However, 10 weeks of UVB exposure, at a frequency of three times a week, produced significant induction of AP-1 binding in three out of three SAG-Tg(þ) lines tested (lanes 5–11, samples 3–5). Significantly, SAG-transgenic
expression caused a remarkable reduction of UVB-induced AP-1 binding in three out of three SAG-Tg(+) mice (lanes 12–18, samples 6–8). Since AP-1 binding can be supershifted by c-Jun antibody (lanes 6, 8, 10, 13, 15 and 17), and be completely blocked by 50× cold oligonucleotide (lanes 11 and 18), it was concluded that the AP-1 complex contained c-Jun and that the binding was specific. Thus, SAG-transgenic expression inhibits AP-1 binding induced by UVB exposure.

We next studied if UVB exposure would cause any differential induction of p53, which is an UV-inducible gene, but is not subjected to degradation by SAG E3 ubiquitin ligase. The same eight samples of nuclear extracts were subjected to a gel retardation assay using the p53 consensus binding sequence. It has been established previously that in the in vitro gel retardation assay, p53 hardly binds to its consensus sequence, unless a p53 antibody against its C-terminal region is present (25,26). We therefore included p53 antibody (pAb421) in some reactions to visualize the p53 binding. As shown in Figure 2B, little or no binding was found in non-exposed controls, regardless of SAG-transgenic expression (lanes 1–4), but UVB exposure caused a significant induction of p53 activity, which was not affected by SAG-transgenic expression (lanes 5–10 versus 12–17).

Due to tight p53-DNA binding in the presence of p53 antibody, an excess of 50× cold oligonucleotide only partially blocked the binding (lanes 11 and 18). The similar levels of histone H2A among all eight samples, as shown in Figure 2C, indicated equal amounts of the nuclear protein were loaded from each sample. Thus, a short-term UVB exposure caused significant activation of AP-1 and p53, which is, remarkably, inhibited or not affected by SAG-transgenic expression, respectively.

To further determine whether reduced AP-1 binding can be reflected by reduced AP-1 transactivation in vivo, we crossed AP-1 luciferase reporter mice (20) with SAG-Tg(+) mice to generate mice that are heterozygous for the AP-1-Luc reporter in SAG-Tg(+) or SAG-Tg(−) background, respectively. The AP-1 activity, as quantified by luciferase light intensity, was measured in ear-punched tissues 24 h post-UVB exposure. AP-1 transactivation activity was induced up to 100-fold 24 h after exposure, which was significantly inhibited (up to 2.5-fold, P < 0.01) by SAG-transgenic expression (Figure 2D).

Taken together, it is clearly demonstrated that SAG, upon transgenic expression, significantly inhibited UVB-induced AP-1 activities without affecting p53 activity.

Fig. 3. Effect of SAG-transgenic expression on UVB-induced protein expression: (A) SAG expression in primary keratinocytes inhibits UVB-induced accumulation of c-Jun and p27: primary culture were prepared from SAG-Tg(+) and SAG-Tg(−) mice and were subjected to a single UVB exposure at indicated doses. Cells were harvested 24 h after exposure and subjected to western blotting analysis for SAG, c-Jun, p27, cyclin D1 and β-actin. The fold change was calculated after densitometry quantification with β-actin normalization, setting the unexposed control of SAG-Tg(−) mouse as 1. (B) SAG silencing inhibits p27 polyubiquitination: H1299 cells were transiently transfected with His-Ub (lane 2) or p27 (lane 1) alone or His-Ub and p27 in combination with psiSAG (lane 3) or psiCont (lane 4) and subjected to in vivo ubiquitination assay by Ni-bead purification of ubiquitinated p27, followed by detection of polyubiquitinated p27 by p27 antibody. (C) SAG-transgenic expression reduces staining of c-Jun and p27 in epidermal cells: mouse skin tissues after 10 weeks UVB exposure from SAG-Tg(−) and SAG-Tg(+) mice were subjected to immunohistochemistry staining using antibodies against c-Jun, p27, cyclin D1, p53 and c-Fos. Magnification ×400.
SAG-transgenic expression reduced the levels of c-Jun and p27

We next determined the effect of SAG-transgenic expression on growth-regulatory proteins, c-Jun, p27 and cyclin D1, in response to UVB exposure, using primary keratinocytes isolated from 1-day-old postnatal pups from SAG-transgenic and non-transgenic littermates. The primary cells were prepared from two independent pups from each line. First, we confirmed SAG-transgenic expression, which was readily detectable in cells from transgenic lines, but not from controls (Figure 3A, top panel, labeled as FLAG-SAG). Second, we determined the effect of SAG-transgenic expression on c-Jun at both basal and UV-induced levels. As shown in Figure 3A (2nd panel), SAG-transgenic expression caused a 2- to 3-fold reduction of basal c-Jun levels in keratinocytes, probably due to targeted degradation, as described previously (16). UVB exposure induced a 4-fold increase of c-Jun levels in both lines, but the total c-Jun levels after UV exposure were 2- to 3-fold higher in non-transgenic than in transgenic cells, apparently due to their lower basal c-Jun level. This result strongly suggests that the lower c-Jun levels are responsible for the observed decrease of AP-1 activity in SAG-transgenic skin tissues (Figure 2A and D).

Third, we determined the effect of SAG-transgenic expression on p27, a well-known, naturally occurring inhibitor of cyclin-dependent kinases, and a known substrate of SAG–SCF E3 ubiquitin ligase (9,15,27). Interestingly, p27 was also subjected to SAG-mediated degradation and UVB induction, with a pattern similar to c-Jun: lower basal and UVB-induced levels in the SAG-transgenic line and a similar magnitude of UVB induction between the two lines (Figure 3A, 3rd panel). On the other hand, cyclin D1, which is degraded by SCF-Fbx4/αB-crystallin (28), was not induced by single exposure of UVB nor significantly affected by SAG-transgenic expression (4th panel), suggesting a certain degree of SAG targeting specificity. Our results demonstrated that SAG-transgenic expression causes simultaneous reduction of c-Jun/AP-1, a tumor promoting transcription factor and of p27, a tumor suppressor.

We have shown previously that SAG small interfering RNA (siRNA) silencing inhibited c-Jun polyubiquitination (16). We determined whether this is the case for p27 as well. H1299 cells were transiently cotransfected with His-Ub and p27, alone or in combination of plasmid expressing control siRNA or SAG siRNA. Ubiquitinated p27 was purified by Ni-NTA beads and detected by antibody against p27. As shown in Figure 3B, p27 polyubiquitination can be readily detected in cells transfected with His-Ub and p27 and control siRNA (lane 4), indicating that endogenous SCF components are sufficient to promote p27 polyubiquitination upon p27 overexpression via transfection. When cotransfected with plasmid that silenced SAG expression, p27 polyubiquitination was remarkably inhibited (lane 3). The lack of complete inhibition of p27 polyubiquitination upon SAG silencing is due to the presence of ROC1/RBX1, a SAG family member known to promote p27 degradation (9). Thus, SAG contributes to p27 polyubiquitination.

We followed up this interesting observation with in situ immunohistochemical analysis. Expression of transgenic SAG in mouse skin in SAG-Tg mice was demonstrated previously (24). As shown in Figure 3C, neither c-Jun nor p27 was detectable in SAG-Tg(−) mice. In SAG-Tg(+) mice, both the number of cells with nuclear staining of c-Jun and p27 were significantly increased compared to non-transgenic SAG-Tg(−) mice (middle). In SAG-Tg(+) mice, both the number of cells with nuclear staining of c-Jun and p27 were significantly increased compared to non-transgenic SAG-Tg(−) mice (middle). In SAG-Tg(+) mice, both the number of cells with nuclear staining of c-Jun and p27 were significantly increased compared to non-transgenic SAG-Tg(−) mice (middle).
c-Jun and p27 and the staining intensity in c-Jun- and p27-positive cells were reduced (bottom panel). On the other hand, 10 weeks of UVB exposure induced cyclin D1 expression mainly at the basal cells, but SAG-transgenic expression had no effect (middle panel), consistent with observation made in primary keratinocytes. Finally, SAG-transgenic expression had no effect on UVB-induced levels of c-Jun and p27, both in primary cultures and in mouse skin in situ. It is worth noting that unlike c-Jun and p27, a single UVB exposure of primary keratinocytes failed to induce cyclin D1 expression (Figure 3A), whereas multiple UVB exposures of mouse skin caused a significantly induction of cyclin D1, regardless of SAG-transgenic expression (Figure 3C).

**SAG-transgenic expression promoted UVB-induced skin hyperplasia**

During immunohistochemistry analysis of c-Jun and p27 expression, we noticed differences in the thickness of the epidermal layer between SAG-Tg(−) and SAG-Tg(+) mice, suggesting an effect of SAG-transgenic expression in skin hyperplasia. We followed up this observation more precisely using two independent assays. The first was the hematoxylin and eosin staining, followed by the measurement of epidermal layer thickness. Representative areas, shown in Figure 4A (top panels), clearly demonstrated that SAG-transgenic expression remarkably increases the thickness of the epidermis ($P < 0.05$). The second was BrdU incorporation assay to measure cells with active DNA synthesis. BrdU-labeled cells (stained in blue) were mainly localized in the basal cell layer. The number of positive cells was significantly increased upon SAG-transgenic expression (panel c, $P < 0.01$) (Figure 4B). Finally, we excluded the possibility that increased skin thickness in SAG-transgenic mice was due to a reduced rate of apoptosis by the TUNEL assay. Apoptotic cells (green dots) were hardly seen after 10 week UVB exposure, and no difference was observed between the two lines ($P > 0.05$, Figure 4C). IHC using antibody against active caspase-3 also failed to detect apoptotic cells (data not shown). Thus, it appears that SAG-transgenic expression increases the thickness of the epidermis induced by UVB exposure by promoting proliferation, rather than inhibiting apoptosis, which was hardly seen in the epidermis after 10 weeks of UVB exposure.

**SAG-transgenic expression had no significant effects on the formation of UVB-induced skin tumors or on papilloma-to-carcinoma conversion**

We next determined the effect of SAG-transgenic expression on UVB-induced skin carcinogenesis. The tumor appearance and growth rates in ear and dorsal trunk were recorded for 29 weeks and summarized in Table I and Figure 5. Tumor incidence in ear (A) or dorsum (B), tumor growth rate in both locations (C) and mean number of tumors formed

![Fig. 5.](https://academic.oup.com/carcin/article-abstract/29/4/858/2624489)

Fig. 5. UVB-induced skin carcinogenesis is not affected by SAG-transgenic expression: SAG-Tg(−) ($n = 17$) and SAG-Tg(+) ($n = 16$) lines were subjected to UVB exposure three times a week for 29 weeks. The number of papillomas detected by palpation and size of tumor detected by a caliper in each group were recorded weekly after its appearance and plotted. Shown are the tumor incidence in ear (A) and dorsum (B), tumor growth rates in both locations (C, the boxes present the 25th and 75th percentiles. The centerline is the median, the plus is the mean and the cross is an outlier) and mean number of tumor in both locations (D and E).
in both locations (D and E) were not significantly different between SAG-Tg(+) and SAG(−) mice. We also measured the rate of papilloma to carcinoma conversion in UVB-induced tumors in ear and dorsum harvested at the end of experiment. A conversion rate of 66.6% was observed among a total of 15 and 9 ear tumors analyzed from SAG-Tg(−) and SAG-Tg(+) mice, respectively. The conversion rate in dorsal tumors was much lower: 9.1% (one tumor) versus 25% (two tumors) in SAG-Tg(−) and SAG-Tg(+) lines, respectively. However, no statistical difference was observed due to the limited number of dorsal tumors. Thus, SAG-transgenic expression had no effect on promoting tumor formation and papilloma to carcinoma conversion during UVB carcinogenesis.

Discussion

SCF E3 ubiquitin ligases constitute the largest family of E3 ubiquitin ligases that attach ubiquitin to a variety of cell-regulatory proteins for targeted degradation by the 26S proteasome (9). The core SCF E3 ubiquitin ligase is a complex of regulator of cullins-1/RING box protein 1-cullins or cullins that recruit E2 (31), while the substrate specificity of the SCF complex is determined by the F-box proteins that recognize the substrates through their WD40 or leucine-rich repeat (LRR) domains (32). For example, F-box protein Fbw7 recognizes c-Jun, whereas Skp2 recognizes p27 for targeted degradation (for review, see ref. 9). We have recently found that, as a RING component of SCF E3 ubiquitin ligases, SAG promotes ubiquitination and degradation of c-Jun, an essential member of the AP-1 transcription factor. As a consequence, TPA-induced and AP-1-mediated neo-plastic transformation in the JB6 epidermal cell model was inhibited upon SAG expression or enhanced upon SAG siRNA silencing (16). This implies that SAG inhibits neoplastic transformation by targeting c-Jun/AP-1. On the other hand, our earlier study had shown that SAG could promote cell proliferation under serum starvation through inhibition of p27 accumulation (15). It appears that the net outcome of SAG expression for tumor promotion and cell proliferation would most probably be determined by the degree of targeted degradation of the SAG substrate proteins in a tissue-specific and/or cell-content-dependent manner.

Here, we directly addressed this important issue in a SAG-transgenic mouse model during UVB skin carcinogenesis. We found that targeted SAG expression in primary keratinocytes led to lower basal levels of both c-Jun and p27. Although both proteins were induced by UVB to a similar extent, regardless of SAG-transgenic expression, the total levels of induced c-Jun and p27 were lower in SAG-transgenic cells because of the lower initial basal levels (Figure 3A). The difference was more striking in situ mouse skin tissue, where targeted SAG expression blocked UVB induction of both c-Jun and p27 dramatically (Figure 3C). Interestingly, this SAG-mediated reduction of c-Jun level/AP-1 activity (pro-tumor promotion) and reduction of p27 level (anti-proliferation) led to a net outcome of increased skin hyperplasia. This is clearly demonstrated by increased skin thickness and increased DNA synthesis, but not apoptosis (Figure 4). The results suggest that p27 plays a more important role in controlling epidermal hyperplasia under this context of UVB skin proliferation. On the other hand, SAG-transgenic expression had no significant effect on promoting tumor formation, suggesting a neutralizing effect of p27 reduction and AP-1 inhibition. However, we cannot exclude the potential contributions of other growth-regulatory substrates of SAG–SCF E3 ubiquitin ligases in the process.

We have recently determined the role of SAG in skin carcinogenesis induced by DMBA/TPA using the same SAG-transgenic mouse model (24). We found that SAG-transgenic expression regulated skin carcinogenesis by a stage-dependent targeting of c-Jun/AP-1 and Inhibitor xB/Nuclear factor-xB. At the early stage, SAG-transgenic expression inhibited AP-1 activity by targeting c-Jun, leading to a reduced skin hyperplasia (reduced skin thickness) and reduced rate of tumor formation, with a longer latent period. At the later stage, however, SAG-transgenic expression activated Nuclear factor-xB by targeting Inhibitor xB, leading to apoptosis inhibition and larger tumor sizes. This apparent difference was determined by the availability of F-box proteins, Fbx7 (expressed in normal skin to target c-Jun degradation at early stage) and β-TrCP (overexpressed in tumor to target Inhibitor xB degradation at later stage), with SAG playing a rate-limiting factor (24). Thus, SAG-transgenic expression either promotes or inhibits skin hyperplasia induced by UVB (this study) or DMBA/TPA (24), respectively. The opposite effect appears to be carcinogen/tumor promoter dependent with a net outcome largely determined by the levels of SAG substrates that control cell proliferation and tumor promotion.

We showed here that SAG overexpression inhibits UVB-induced apoptosis in JB6 epidermal cells, but not in mouse skin tissues. The major reason for this apparent discrepancy is that in JB6 cells, UVB, with a dose of 400 mI/cm², induced significant levels of apoptosis (>35% population, Figure 1C), whereas in mouse skin tissues, 10 weeks of exposure of UVB (2.5 kJ/m² for the first 4 weeks, 5 kJ/m² for weeks 5–8 and 10 kJ/m² for remaining 2 weeks) induced very minimal levels of apoptosis (~1% population, Figure 4C). Although we observed some SAG protection, it is not statistically significant. Thus, it is very difficult to detect a difference when apoptosis is at background levels.

In summary, when overexpressed in mouse JB6 epidermal culture cells, SAG protects UVB-induced apoptosis. When targeted to express in mouse skin epidermal cells, SAG promotes UVB-induced hyperplasia, but not carcinogenesis. This is through enhanced proliferation, not reduced apoptosis. SAG-mediated degradation of c-Jun and p27 appears to contribute to this process, implying a significant role of p27 in inhibition of UVB skin hyperplasia.

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