Targeted expression of spermidine/spermine \( N^1 \)-acetyltransferase increases susceptibility to chemically induced skin carcinogenesis

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The bovine keratin 6 gene promoter was used to target expression of spermidine/spermine \( N^1 \)-acetyltransferase (SSAT) to epidermal keratinocytes in the hair follicle of transgenic mice. K6-SSAT transgenic mice appeared to be phenotypically normal and were indistinguishable from normal littermates until subjected to a two-stage tumorigenesis protocol. For such tumorigenesis studies, mice were bred for six generations onto a tumor promoter resistant C57BL/6 background strain. K6-SSAT transgenic mice showed a 10-fold increase in the number of epidermal tumors that developed in response to a single application of 400 nmol of the tumor initiator 7,12-dimethylbenz[a]anthracene followed by twice weekly applications of 17 nmol of the tumor promoter 12-O-tetradecanoylphorbol-13-acetate for 19 weeks. Tumor samples from transgenic animals showed marked elevations in SSAT enzyme activity and SSAT protein levels compared with tumors from non-transgenic littermates, and the accompanying changes in putrescine and \( N^1 \)-acetylspermidine pools indicated activation of SSAT-mediated polyamine catabolism in transgenic animals. An unusually high number of tumors were shown both grossly and histologically to have progressed to carcinomas in this model and these occurred with an early latency and only in mice carrying the K6-SSAT transgene. These results suggest that activation of polyamine catabolism leading to increases in putrescine and \( N^1 \)-acetylspermidine may play a key role in chemically induced mouse skin neoplasia.

Introduction

Putrescine, spermidine and spermine are the natural polyamines present in mammalian cells. Although their precise cellular function is not understood, these polycations play a fundamental role in the regulation of cell growth and differentiation (1,2).

Ornithine decarboxylase (ODC) is the enzyme responsible for the \emph{de novo} synthesis of putrescine, the diamine precursor of spermidine and spermine. Putrescine, however, is also the end product of a polyamine catabolic pathway that is regulated by the action of spermidine/spermine \( N^1 \)-acetyltransferase (SSAT) which catalyzes the \( N^1 \)-acetylation of spermine and spermidine. The corresponding acetyl derivatives formed are either excreted from the cell, or undergo further metabolism by polyamine oxidase to form spermine or putrescine respectively (3).

SSAT is thought to play a key role in maintaining a properly balanced ratio of polyamines in cells and in preventing the overaccumulation of higher polyamines that may become cytotoxic (3,4). Whereas SSAT activity is normally very low in cells and tissues, the enzyme is strongly induced by elevations in intracellular polyamines and in response to terminally alkylated polyamine analogues that are currently undergoing clinical trials as cancer chemotherapeutic agents (5,6). Stabilization of SSAT by elevated polyamines or analogues contributes importantly to the induction of cellular SSAT activity, as the protein undergoes very rapid turnover by ubiquitin-mediated proteolysis in the absence of polyamines (7,8). This is consistent with SSAT being an important homeostatic regulator of the polyamine metabolic pathway.

The classical, two-stage mouse skin model of chemical carcinogenesis can be divided mechanistically into three stages termed initiation, promotion and progression (9). In this model, mice develop lesions on the skin in response to the sequential application of a single, sub-carcinogenic dose of a mutagenic tumor initiator such as 7,12-dimethylbenz[a]anthracene (DMBA) followed by repetitive applications of a non-carcinogenic tumor promoter such as 12-O-tetradecanoylphorbol-13-acetate (TPA). It is well documented that induction of ODC is a key step in the promotion of tumors by TPA (10) and that constitutively elevated polyamine levels are required for both the development and maintenance of the neoplastic phenotype in mouse skin (11). Although large numbers of tumors characterized as benign papillomas can be induced in sensitive mice, relatively few tumors obtained using this protocol ever progress to squamous cell carcinomas (12,13).

Transgenic mouse models have been developed to address the impact of overexpressing candidate genes thought to be involved in defined stages of skin tumor development. Many of these models have successfully targeted expression of the transgene to specific skin cell populations using promoter elements of various cytokeratin genes (14–16). Such a strategy was used to target expression of an ODC transgene to the outer root sheath (ORS) of the mouse hair follicle using a bovine keratin 6 (K6) promoter (17) that produced transgenic mice with severe skin abnormalities that included spontaneous skin tumors (18). Although the latter phenotype was lost on backcrossing onto the TPA promotion resistant C57BL/6 background strain, K6-ODC transgenic mice were shown to be more susceptible to a DMBA/TPA skin tumorigenesis protocol than their non-transgenic littermates. In fact, K6-ODC mice did not require promotion with TPA to develop...
papillomas following DMBA initiation, indicating that ODC overexpression was a sufficient stimulus to promote epidermal tumors in this model (19).

In order to examine further the role of polyamines in chemical skin tumorigenesis, we decided to use the K6 promoter to target expression of SSAT to keratinocytes of the ORS. The end result of activation of either SSAT or ODC activity is to expand intracellular levels of putrescine, which has been suggested to be the important effector molecule in the response of K6-ODC mice to skin tumorigenesis (11). In contrast to ODC, expression of which increases both putrescine and spermidine levels, SSAT mediated elevations in putrescine pools occur at the expense of spermidine and spermine. Therefore this approach can address whether it is the increase in putrescine or the higher polyamines that may play a critical role in tumor development. In the present study, we generated three lines of K6-SSAT transgenic mice that were phenotypically indistinguishable from normal littersates until subjected to a two-stage tumorigenesis protocol. Under these conditions, K6-SSAT transgenic mice showed a greatly increased susceptibility over their normal littersates to a DMBA/TPA protocol. In addition, an unusually high number of tumors characterized grossly and histologically as carcinomas occurred in mice carrying the K6-SSAT transgene, and these formed within 20 weeks on the promotion resistant C57BL/6 background. These results suggest a high level of putrescine and/or N1-acetylsperrimine is a contributing factor in neoplastic development.

Materials and methods

Materials

All reagents were purchased from Fisher Scientific (Pittsburgh, PA) or Sigma Chemical (St Louis, MO) unless stated otherwise. Oligodeoxynucleotides were synthesized in the Macromolecular Core Facility, Hershey Medical Center, PA. Restriction enzymes were from New England Biolabs (Beverly, MA) and Roche Molecular Biochemicals (Indianapolis, IN). DMBA was obtained from Kodak Laboratory Chemicals (Rochester, NY) and TPA was purchased from Calbiochem–Novabiochem (La Jolla, CA).

Generation and identification of K6-SSAT transgenic mice

A plasmid containing the cDNA for SSAT in the Bluescript vector was used as a template for PCR to introduce flanking Cla I restriction sites for cloning into the transgenic vector. The SSAT cDNA retained 26 bp of 5′-UTR and 28 bp of 3′-UTR sequences. The PCR product was digested, purified and the fragment ligated into a unique Cla I restriction site downstream of a K6 promoter/regulatory sequence in a pBR322 based vector described previously (18). The entire SSAT cDNA was sequenced to ensure that no PCR mutations were introduced during preparation of the K6-SSAT transgene. K6-SSAT transgenic mice were generated using the standard technique of pronuclear microinjection of Not I linearized transgene, purified after electroelution using Elutip-d minicolumns (Schleicher and Schuell, Keene, NH). Three founder mice were generated on either the B6D2F2 (Transgenic Core Facility, Hershey Medical Center) or B6C3F2 (Lankenau Institute for Medical Research) hybrid background. Genotyping was performed by PCR amplification of genomic DNA isolated, using a DNAse tissue kit following the manufacturer’s directions (Qiagen, Valencia, CA), from tail clips of 3-week-old offspring. Screening by PCR used a sense primer directed to the K6 promoter region with the sequence 5′-GCAGAAGGGGGAGATTTAGC-3′ and the antisense primer sequence directed to a coding region in the SSAT cDNA was 5′-TGGGCGGATCACGAATTTAGC-3′. Positive founder mice were confirmed by Southern blot analysis of Eco RI digested genomic DNA from the K682 line and detected with a fluorescein-labeled SSAT cDNA probe generated using an ECF random prime labeling and detection system (Amersham Pharmacia Biotech, Piscataway, NJ) (Figure 1).

Tumorigenesis protocol

The dorsal hair of 7–8 week old experimental mice from the K683 transgenic line was shaved 3 days prior to initiation with 400 nmol of DMBA in acetone applied topically to their backs under yellow light. The promotion stage began 1 week later with the topical application of 17 nmol of TPA in acetone and was continued on a twice weekly basis for up to 26 weeks. Treatment groups were assigned as follows: 42 transgenic and 52 normal littersates on the C57Bl/6j background received DMBA and TPA with 17 transgenic and nine normal littersates receiving DMBA alone.

Tumor processing

Mice were monitored every 2 weeks for the development of tumors. Papillomas and carcinomas were excised 1 week after the last TPA treatment and either flash frozen in liquid nitrogen for biochemical analysis or fixed overnight in 10% neutral buffered Fekete’s solution (60% EtOH, 3.2% formaldehyde and 0.75 M acetic acid). The fixed tumors were embedded in paraffin and 5 μm sections were stained with hematoxylin and eosin for histological analysis.

Biochemical analysis

Preliminary biochemical analysis of mouse skin from wild type and K6-SSAT animals used epidermis and dermis that was separated after incubation of excised whole skin at 55°C for 20 s and scraped using a razor blade as described previously (18). Excised frozen tumors were cut in two, each half was then minced with a scalpel and was either homogenized in ice cold buffer A (50 mM Tris HCl pH 7.5, 2.5 mM DTT, 0.1 mM EDTA) containing 1X protease inhibitor cocktail (Calbiochem-Novabiochem, La Jolla, CA) to measure SSAT activity and for Western analysis or, homogenized in 10% TCA for polyamine analysis. SSAT activity was determined by measuring the production of [3H]acetylspermidine in an assay mixture containing 3 mM spermidine and 16 μM [3H]acetyl-CoA (50 μCi/mmol) (21). Polyamines were determined by reverse-phase HPLC using post-column derivatization with phthalaldehyde and fluorescence detection as described previously (22). Aliquots of tumor extracts in buffer A were resolved by SDS–PAGE and transferred to a PVDF membrane using standard techniques. The membrane was probed with a polyclonal rabbit anti-SSAT antibody followed by a secondary alkaline phosphatase-linked antibody. SSAT protein bands were
detected following addition of an ECF substrate (Amersham Pharmacia Biotech) and scanned on a Molecular Dynamics Fluorimagere 595. Protein content was measured by the Bradford assay (23).

Results

Identification and characterization of K6-SSAT transgenic mice

We used the bovine keratin IV* (homologous to human K6) promoter to target expression of a cDNA for human SSAT to the ORS of the mouse hair follicle. This promoter has been used successfully to direct expression of several different genes, including the polyamine biosynthetic enzyme ODC, to the epidermis. Three K6-SSAT founder mice were generated which were designated K681, K682 and K683. Transgenic and wild type siblings were distinguished by PCR amplification of genomic DNA (Figure 1A) and the reproducibility of the PCR screen was confirmed by Southern blot analysis of which an example from the K682 line is illustrated in Figure 1B. All three founder mice were fertile and appeared to be phenotypically normal. Initial experiments used the K681 and K682 transgenic lines housed at the Hershey Medical Center. These mice were maintained in the hemizygous state by crossing with B6D2/JF1 hybrid mice to generate approximately equal numbers of transgenic and control littermates. The K683 transgenic line, backcrossed six generations onto the C56Bl/6J inbred strain and housed at the Lankenau Institute for Medical Research, was selected for the present two-stage tumorigenesis study since breeding onto the inbred background was at a more advanced stage. All experimental K683 transgenic and wild type siblings used in the tumorigenesis experiment were genotyped by PCR.

The SSAT activity measured in extracts from dorsal skin was very low as previously reported (24) and was not increased in the transgenic mice even after treatment with TPA. Also, SSAT protein could not be detected on western blots of skin extracts. Two possible explanations for these negative results are that, expression of the transgene was limited to a discrete population of cells within the skin and/or that an additional stimulus such as an increase in polyamines or other alterations that occur at an early stage of carcinogenesis are needed for a high level of SSAT gene expression and translation.

Response of K6-SSAT transgenic and control littermates to a two-stage tumorigenesis protocol

Despite the lack of SSAT expression in the absence of chronic stimulation described above, a preliminary two-stage tumorigenesis protocol using 31 mice from the K682 line on the B6D2 hybrid background indicated that K6-SSAT transgenic mice developed a higher tumor burden than normal littermates after 16 weeks of promoting DMBA initiated mice with TPA. (Preliminary results using K6-SSAT mice on the B6D2 background were published in abstract form (25).) In addition, the tumors in the transgenic population were considerably larger than those in non-transgenic littermates and a significant number of the tumors progressed to carcinomas and failed to regress after cessation of the TPA treatment. Similar results were found also with the K681 transgenic line (results not shown).

In order to reduce the contribution of differences in strain susceptibility to phorbol ester skin tumor promotion (mice on the DBA/2 background are more sensitive to TPA treatment than the C57BL/6 resistant strain), K6-SSAT transgenic and normal littermates were derived from the K683 line after being crossed six generations onto the C57BL/6 background. The results of a protocol in which the K683 backcrossed mice were initiated with 400 nmol of DMBA followed by twice weekly applications of 17 nmol of TPA are shown in Table I. The data clearly show that K6-SSAT transgenic mice are more susceptible to DMBA/TPA induced tumor development than their normal counterparts with 98% of the transgenic population having an average of 8.9 tumors at 13 weeks of promotion while 40% of the control group were tumor free after 19 weeks of the same treatment schedule (Table I). In addition, K6-SSAT transgenic mice showed a remarkable 10-fold increase in tumor number after 19 weeks of treatment (Table I). A representative comparison of the tumor burden of a wild type and transgenic littermate is illustrated in the photograph shown in Figure 2 that was taken after DMBA initiation followed by 13 weeks of promotion. In addition to the larger size and increased number of papillomas in the K6-SSAT mice, there was a notable difference in the histological features of the papillomas (Figure 3A and 3B). Many of the papillomas from the K6-SSAT transgenic mice showed follicle derived dermal cysts present at the base of the tumor. These were not seen in any papillomas excised from non-transgenic littermates exposed to the same treatment protocol which were typical exophytic, hyperkeratotic squamous papillomas. Microscopically, at high magnification, the cysts in the papilloma sections from the K6-SSAT transgenic mice appeared to be derived from dilated hair follicles, contained some keratin and in some cases, sebaceous cell components and hair remnants. In general, cysts appeared to be located in the proximity of the tumor, becoming less abundant at sites further from the papilloma.

A significant number of the tumors developed into carcinomas in the K6-SSAT mice. No carcinomas were found in the wild type mice, whereas 31% of the K6-SSAT mice had carcinomas after 27 weeks (Table I). A histological section of one such carcinoma is shown in Figure 3C and D. This moderately differentiated squamous cell carcinoma is representative of the malignant tumors that developed in K6-SSAT mice, showing local invasion into and through the dermis and typical squamous features, including keratin pearls.

<table>
<thead>
<tr>
<th>Weeks of promotion</th>
<th>Tumor incidence (%)</th>
<th>Tumor multiplicity (tumors/mouse)</th>
<th>Tumor incidence (%)</th>
<th>Tumor multiplicity (tumors/mouse)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>38</td>
<td>0.4 ± 0.09</td>
<td>98</td>
<td>8.9 ± 0.62</td>
</tr>
<tr>
<td>15</td>
<td>38</td>
<td>0.6 ± 0.12</td>
<td>98</td>
<td>10.1 ± 0.69</td>
</tr>
<tr>
<td>17</td>
<td>50</td>
<td>0.9 ± 0.16</td>
<td>98</td>
<td>10.2 ± 0.56</td>
</tr>
<tr>
<td>19</td>
<td>60</td>
<td>1.2 ± 0.18</td>
<td>98</td>
<td>10.1 ± 0.52</td>
</tr>
<tr>
<td>Carcinomas</td>
<td>0</td>
<td>0/52</td>
<td>31</td>
<td>16/42</td>
</tr>
</tbody>
</table>

The dorsal hair of 42 K683 transgenic and 52 control littermate mice was shaved prior to initiation with 400 nmol of DMBA. The promotion stage was started 1 week later by twice weekly applications of 17 nmol of TPA as described in Materials and methods. Tumors were counted at the times shown (*mean ± SE of 52 normal littermates; †mean ± SE of 42 transgenic mice; statistical significance reached P < 0.001 for both tumor incidence and multiplicity at each time point as determined by Student’s unpaired 2-tailed t-test). The experiment was continued for up to 27 weeks by which time a total of 16 carcinomas was observed in 13 out of 42 mice. No carcinomas were observed in the control group.
SSAT activity and polyamine levels in mouse skin tumors

Tumors produced in response to the DMBA/TPA protocol were harvested at sacrifice 1 week after the last TPA application as described in the Materials and methods section. The data presented in Table II are measurements derived from single tumors that were assayed for both SSAT activity and polyamine content. In contrast to epidermal and dermal skin extracts, SSAT activity was readily measured in all tumors. Notably, there was a 13-fold increase in the SSAT activity measured in tumors from transgenic mice compared with normal littermates. The elevations in SSAT activity observed in tumor extracts were also observed as immunoreactive protein on a western blot probed with a polyclonal antibody to recombinant SSAT (Figure 4). At the same time, no immunoreactive protein was observed in tumor samples from normal mice (Figure 4). The elevation in SSAT activity in the tumors from K6-SSAT mice was also consistent with alterations in polyamine pools (Table II). The overall changes observed in transgenic tumor samples were a 2.5-fold increase in total polyamines of which 77% was in the form of putrescine and N1-acetylspermidine.

When compared with normal mice, this represents more than a 6-fold increase in putrescine pools and >400-fold increase in N1-acetylspermidine which was barely present at the level of detection in non-transgenic tumors (Table II). Although spermine levels remained unchanged, spermidine levels in transgenic tumors were reduced by over a third of the wild type value. Collectively, these results indicate that polyamine catabolism has been activated in tumors from K6-SSAT transgenic mice, or at least in epidermis that has given rise to the tumors.

Discussion

We have investigated the effects of perturbing epidermal polyamine levels in mouse skin using a bovine K6 promoter construct that includes regulatory elements to target expression of a cDNA for SSAT to keratinocytes in the ORS of the hair follicle. This K6 promoter construct has been shown previously to contain the necessary elements to drive both constitutive and inducible expression of ODC and antizyme cDNA transgenes to mouse epidermal keratinocytes (18,27). The K6 promoter responds in an inducible manner to such hyperproliferative stimuli as the application of TPA, that extends transgene expression to cells in the interfollicular epidermis (18,27).

Despite using this strong cytokeratin promoter to target high levels of SSAT mRNA production, there were no obvious phenotypic differences between K6-SSAT transgenic mice and their normal siblings. All three male founder mice were fertile and transgenic progeny were of a size similar to their non-transgenic littermates, appeared to have a regular hair cycle and normal life span. The absence of a phenotype is consistent with the lack of a detectable increase in SSAT activity in the skin and suggests that in vivo expression of a cDNA for SSAT using the K6 promoter is subject to tight regulation, even when challenged acutely with TPA. This finding is consistent with previous attempts to achieve high level expression of
SSAT activity from cDNA constructs in cell culture systems in which high levels of expression only occur after treatment with polyamine analogues (8). It is likely that the increased synthesis of polyamines which occurs at early stages in the carcinogenic process, activates SSAT gene expression and this activation causes a redistribution of the polyamine pool towards putrescine and \( N^1 \)-acetyl spermidine that potentiates neoplastic growth. The up-regulation of expression from the K6 promoter, which is reported to occur in benign and malignant tumors (28), is also likely to contribute to the elevated SSAT expression. An interesting possibility is that the presence of a degradation mechanism that is responsible for the rapid turnover of the SSAT protein in dorsal skin may be absent or impaired in transgenic papillomas, and would contribute to stabilizing the SSAT protein. In fact, in support of such a mechanism, ODC activity was reported to be stabilized in squamous papillomas compared to TPA-stimulated mouse epidermis following cycloheximide injection (29).

An increased tumor burden and an increased progression of tumors to carcinomas was observed in the K6-SSAT transgenic mice after treatment with DMBA/TPA. Overexpression of SSAT was demonstrated in transgenic tumors but not in tumors from normal mice. The accompanying changes in polyamine pools, namely decreased levels of spermidine, the appearance of \( N^1 \)-acetyl spermidine and elevations in putrescine were consistent with activation of SSAT and polyamine catabolism. It has been reported previously that SSAT activity in papillomas from female CD-1 mice was not greatly different from that in epidermis promoted either singly or chronically with acetone or TPA (24). Therefore, the elevated levels of SSAT activity clearly results from expression of the transgene and the resultant changes in epidermal putrescine and \( N^1 \)-acetyl spermidine pools in particular, contribute to the dysregulation of proliferative signalling pathways in this model. In contrast to the results shown here with K6-SSAT transgenic mice, the levels of \( N^1 \)-acetyl spermidine present in 4 papillomas from K6-ODC mice did not exceed 4 nmol/mg of protein (C.S.Coleman and T.G.O’Brien, unpublished results).

Table II. SSAT and ODC enzyme activities and polyamine content of papillomas from wild type and K6-SSAT mice

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>SSAT activity (pmol/min/mg)</th>
<th>ODC activity (pmol/min/mg)</th>
<th>Polyamines (nmol/mg of protein)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Putrescine</td>
<td>( N^1 )-acetyl spermidine</td>
</tr>
<tr>
<td>Wildtype</td>
<td>9.4 ± 1.8</td>
<td>1.5 ± 0.5</td>
<td>5.8 ± 1.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>K6-SSAT</td>
<td>124.8 ± 15.7*</td>
<td>5.5 ± 1.8</td>
<td>38.4 ± 5.9*</td>
<td>45.8 ± 7.7*</td>
</tr>
</tbody>
</table>

Tumors were excised at sacrifice (27 weeks after the start of promotion) from the backs of K683 transgenic and control littermate mice that were subjected to the DMBA/TPA protocol described in the legend to Table I. The frozen tumors were cut in half and processed either for enzyme activity measurements or polyamine analysis as described in Materials and methods. SSAT activity and polyamine content is expressed as the mean ± SE of 18 tumors from each group. ODC activity is expressed as the mean ± SE of seven tumors from each group. Statistical significance was determined by Student’s unpaired 2-tailed t-test; *P < 0.001; **P < 0.05.

There are both similarities and differences to be drawn from the response of the K6-SSAT mice to the two-stage protocol and the previously described K6-ODC model (19). Although a few K6-SSAT transgenic mice developed tumors that became visible after 18 weeks of DMBA treatment alone, these tumors did not persist over the course of 24 weeks (results not shown). Overexpression of ODC on the other hand, was shown to abolish the need for promoting initiated mice with TPA in order to develop benign, but persistent tumors (19). This indicates that the level of expression of the SSAT transgene in the target cells for carcinogen action in skin (ORS keratinocytes) is not sufficient per se to act as a promoting stimulus. It is only after chronic treatment with TPA, which is known to increase cellular polyamines via an increase in ODC, that SSAT expression is sufficiently elevated to accelerate tumor progression.

In addition to the altered pathology of the papillomas induced in K6-SSAT transgenic mice, a second notable phenotypic difference between these mice and the non-transgenic controls was the early development of squamous cell carcinomas in the K6-SSAT mice. A total of 16 out of 42 tumors from the K6-SSAT treatment group were identified as carcinomas using gross appearance and histological analysis as the scoring criteria. This represents a carcinoma incidence of 31% whereas in non-transgenic mice the carcinoma incidence is very low and no carcinomas have arisen in any of our experiments on the C57BL/6 background after <27 weeks of promotion (T.G.O’Brien, unpublished observation). This result suggests that the combination of perturbed polyamine pools due to elevated SSAT, together with the effects of TPA induce conditions that enhance the rate of progression from a pre-malignant to a more advanced tumor phenotype.

Many studies have shown that polyamine analogues such as \( N^4,N^{11}-\text{bis(ethyl)norspermidine} \), which are very powerful inducers of SSAT, are active antitumor agents and act to suppress cell proliferation in cultured tumor cells. Our results showing that inappropriate expression of SSAT can enhance carcinogenesis are not incompatible with these studies. As shown in Table II, the tumors in the K6-SSAT mice had higher levels of ODC (~4-fold greater than those in control tumors, although this did not reach statistical significance). It is likely that this ODC activity (and possibly, additional uptake of extracellular polyamines) maintains the total polyamine content in the skin tumors studied. Even though the presence of SSAT redistributes the polyamines towards a large increase in...
putrescine and $N^1$-acetylsperrmidine and a decrease in sperrmidine pools, there is a net increase in total polyamines. In cultured tumor cells treated with polyamine analogues, there is a decrease in total polyamines and a considerable accumulation of the analogue which may influence cell growth by binding to sites vacated by the natural polyamines.

The increased susceptibility of K6-SSAT mice to a two-stage tumorigenesis protocol is in contrast to the reported resistance to skin carcinogenesis of mice that overexpress SSAT under the control of the MT promoter (31). However, these studies were not carried out on an inbred strain background and used mice which, like other transgenic mice that express SSAT in a wide variety of tissues from ubiquitous promoters (32), have multiple metabolic alterations that could alter responses to carcinogens. These include profound changes in the skin histology (which could affect responses to DMBA and TPA) and in general metabolism, including lipid disposition, reproductive changes and a short lifespan.

In summary, we have described a new transgenic model that can be induced to perturb polyamine metabolism in the mouse epidermis. The K6-SSAT model shows a similar increased susceptibility to chemically induced skin tumorigenesis as the previously described K6-ODC model despite lacking an obvious phenotype in the absence of treatment. A distinct difference between the two models was seen as the development of a more advanced tumor phenotype in the K6-SSAT transgenic mice that occurred with a very early latency. This model will be useful to study further the possible role of putrescine and acetylsperrmidine in the progression of benign papillomas to a more malignant phenotype and to derive a high incidence of such tumors for other experimental purposes.

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