Correlation of Polyamine and Growth Responses to \(N^1, N^{11}\)-Diethylnorspermine in Primary Fetal Fibroblasts Derived from Transgenic Mice Overexpressing Spermidine/Spermine \(N^1\)-Acetyltransferase*

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A recently generated transgenic mouse line having activated polyamine catabolism due to systemic overexpression of spermidine/spermine \(N^1\)-acetyltransferase (SSAT) was used to isolate primary fetal fibroblasts as a means to further elucidate the cellular consequences of activated polyamine catabolism. Basal levels of SSAT activity and steady-state mRNA in the transgenic fibroblasts were about 20- and 40-fold higher than in nontransgenic fibroblasts. Consistent with activated polyamine catabolism, there was an overaccumulation of putrescine and \(N^1\)-acetyl-spermidine and a decrease in spermidine and spermine pools. Treatment with the polyamine analogue \(N^1, N^{11}\)-diethylnorspermine (DENSPM) increased SSAT activity in the transgenic fibroblasts 380-fold, whereas mRNA increased only 3-fold, indicating post-mRNA regulation. SSAT activity in the nontransgenic fibroblasts increased 200-fold. By Western blot, enzyme protein was found to increase 46 times higher in the treated transgenic fibroblasts than nontransgenic fibroblasts: a value comparable to 26-fold differential in enzyme activity. With DENSPM treatment, spermidine pools were more rapidly depleted in the transgenic fibroblasts than in nontransgenic fibroblasts. Similarly, transgenic fibroblasts were much more sensitive to DENSPM-induced growth inhibition. This was not diminished by co-treatment with an inhibitor of polyamine oxidase, suggesting that growth inhibition was due to polyamine depletion per se as opposed to oxidative stress. Since the two fibroblasts were genetically identical except for the transgene, the various metabolic and growth response differences are directly attributable to overexpression of SSAT.

Deregulated polyamine biosynthesis as indicated by overexpressed ornithine decarboxylase (ODC)† activity is a well recognized characteristic of animal and human cancers. In at least one tumor type, the genetic basis for this has been attributed to a point mutation leading to stabilization of the enzyme protein (1). Although several groups have reported that ODC may play a causative role in the process of cell transformation in cultured systems (2–4), that function appears to be attenuated in vivo. Transgenic mice that systemically overexpress native ODC by at least 20-fold fail to show an increased incidence of tumors in any of their tissues (5). More likely, the enzyme interacts with other genes to fulfill a facilitating role in tumorigenesis not unlike that seen with oncogene cooperativity, a phenomenon that has been previously demonstrated between ODC and ras (6). This potential is clearly indicated in mouse skin carcinogenesis models where induction of ODC activity represents an early response to tumor promoters (7) and is known to be critical to tumor formation (8, 9). In this regard, O'Brien's group (10) recently reported that transgenic mice that overexpress a stabilized form of ODC in the skin are much more susceptible to 7,12-dimethylbenz[a]anthracene-induced carcinogenesis. In addition, their findings strongly suggest that among the various components affected by tumor promoter such as 12-O-tetradecanoylphorbol-13-acetate, induction of ODC alone is sufficient to activate cells to expand clonally and form tumors.

As an alternative to transgenic studies examining the biological consequences of increased polyamine biosynthetic activity, we have attempted to genetically alter whole animal polyamine pools by generating a transgenic mouse line that overexpresses the polyamine catabolic enzyme, spermidine/spermine \(N^1\)-acetyltransferase (SSAT). Together with polyamine oxidase, SSAT catalyzes the back-conversion of spermine to spermidine and the latter to putrescine, a function that is presumed to lower polyamine pools by facilitating their catabolism and excretion (12). Consistent with this expectation, transgenic mice overexpressing the SSAT gene displayed significantly altered tissue polyamine pools characterized by decreases in spermidine and/or spermine and by an overaccumulation of putrescine and \(N^1\)-acetylspermidine (11). They also displayed a distinctive phenotype, which, ironically, closely resembled that of animals which overexpress a stabilized form of ODC in their skin (5). In both transgenics, hair development is interrupted, giving rise to follicular cysts in the dermis, permanent hair loss, and excessive wrinkling of the skin (5, 11). Interestingly, the common polyamine disturbance is elevated putrescine pools.

DENSPM, \(N^1, N^{11}\)-diethylnorspermine; SSAT, spermidine/spermine \(N^1\)-acetyltransferase; GST, glutathione S-transferase.
Although we have reported on the polyamine pool and enzyme changes in transgenic SSAT mice (11), interpretation of these studies is complicated by the uptake of dietary polyamines, tissue redistribution of polyamines, and an inability to clearly link SSAT overexpression and/or induction to cell growth. Thus, we have isolated fetal fibroblasts from transgenic animals and their nontransgenic littermates for study in vitro. As described here, they appear to be unique among cultured systems in their ability to stably overexpress SSAT. Previous attempts to constitutively overexpress the enzyme in mammalian cells have been relatively unsuccessful, due apparently to poor translation of the SSAT message in the absence of elevated levels of intracellular polyamines (13, 14). The gene has been transiently expressed in COS cells leading to an accumulation of putrescine and N\textsuperscript{-1}-acetylsermidine, a decrease in spermidine and spermine pools, and enhanced biosynthetic enzyme activities (15). However, due to the transient nature of the effect, the relationship of increased SSAT gene expression to cell growth was not clearly resolved. Human SSAT cDNA has been also been expressed in Escherichia coli using a inducible system leading to the conversion of the spermidine pool to N\textsuperscript{-1}-acetylsermidine and a reduction in bacterial growth rate (16). Again, this represents an inducible system.

Most of what is known regarding the biological and metabolic consequences of SSAT overexpression is based on studies with polyamine analogues such as N\textsuperscript{2},N\textsuperscript{3}-diethylnorspermine (DENSPM), which potently induce the enzyme (17–19). Such analogues however, impact on a variety of other systems including the polyamine biosynthetic enzymes and transport, as well as a somewhat paradoxical inhibition of the SSAT enzyme itself (20). Although a large number of studies suggest that depletion of polyamines and growth inhibition by polyamine analogues are closely related to induction of SSAT (17, 19, 21, 22), this correlation is complicated by the pleiotropic drug action of analogues and by the comparison of paired cell lines having different genetic backgrounds. Since, as will be reported here, fibroblasts from nontransgenic and transgenic fetuses differentially express and induce SSAT and since they are genetically identical except for SSAT gene copy number, these cells would seem to offer a meaningful system for further studying the biological significance of the enzyme and the cellular consequences of its overexpression. Our present findings with these cells clearly indicate that stable overexpression of SSAT is capable of profoundly altering polyamine pools despite activation of various homeostatic responses. High levels of enzyme also sensitize cells to the antiproliferative activity of DENSPM.

EXPERIMENTAL PROCEDURES

Materials—The spermine analogue N\textsuperscript{3}-N\textsuperscript{3}-diethylnorspermine was kindly provided by Warner Lambert Parke-Davis (Ann Arbor, MI). The polyamine oxidase inhibitor MDL 72527 was kindly provided by Dr. N. Seiler. The members of the transgenic line (UKU 165b) harbored more than 20 transgene copies in their genome (11).\textsuperscript{2} The antibody raised against full-length SSAT was initially used for production of SSAT-specific polyclonal antibodies (24).

Generation of Transgenic Mice—The transgenic mice were generated using the standard pronuclear microinjection techniques as described in detail elsewhere (11).\textsuperscript{2} The members of the transgenic line (UKU 165b) harbored more than 20 transgene copies in their genome (11).\textsuperscript{2} The members of the transgenic line (UKU 165b) harbored more than 20 transgene copies in their genome (11).\textsuperscript{2}

Isolation of Primary Fibroblasts from Fetuses—Fetuses were taken on day 13 of pregnancy. Organs (liver, heart, etc.) and other viscera were removed and used for the detection of transgeneosity. The remainder of the carcass was minced with scissors and incubated in trypsin EDTA (0.25% trypsin, 1.1 mm EDTA, 1% chicken serum; Life Technologies, Inc.) in phosphate-buffered saline for 15 min at 37°C. Tryptsin was neutralized with 5 ml of growth medium. Cell suspensions were centrifuged for 5 min at 150 × g and the supernatant fractions discarded. Cells were transferred into gelatin-coated tissue plates.

Cell Cultures—The cells were cultured in minimum essential medium (Life Technologies, Inc.) supplemented with 2 mm glutamine, 10% fetal bovine serum (Life Technologies, Inc.), penicillin (50 units/ml), and streptomycin (50 μg/ml) at 37°C in the presence of 5% CO\textsubscript{2}. The cells were grown at a density of 2 × 10\textsuperscript{5} cells/T-75 flask. For 12 h in 10% CO\textsubscript{2} and incubated for 24 h before any treatments. Cells were harvested by trypsinization and counted using a Coulter model ZM electronic cell counter (Coulter Electronics, Hialeah, FL). Only early passage cells were used for the experiments.

Production of SSAT-specific Polyclonal Antibodies—In our experience this analogue is available at the time of these experiments satisfactorily recognized mouse-derived SSAT protein (either in tissues or cells). New polyclonal antibodies were generated against full-length SSAT and the C-terminal region of recombinant human SSAT. Primers 5\textsuperscript{\textprime}a (5′-ACA AGG ATC CAA ATG GCT AAA TTC GTG ATC GCG CCA-3′), 5\textsuperscript{\textprime}b (5′-ACA AGG ATC CTG ATG AGT GAT TAT AGA AGC-3′), and 3\textsuperscript{\textprime}a (5′-ACA AGT CGA CCT CCT CTG TTG CCA TTT TTA GCA A-3′) were used for amplification of full-length human SSAT (primers 5\textsuperscript{\textprime}a and 3\textsuperscript{\textprime}a) and C-terminal region of SSAT coding for amino acids 97–171 (primers 5\textsuperscript{\textprime}b and 3\textsuperscript{\textprime}b) by polymerase chain reaction using Vent polymerase (New England Biolabs, Beverly, MA). The polymerase chain reaction products were cloned in pGEM-T vector, and the correct sequences were verified by sequencing with the Pharmacia ALFexpress sequencing system (Pharmacia Biotech, Uppsala, Sweden). The full-length SSAT cDNA was digested with BamHI and SalI and cloned into the corresponding sites of pGEX-5X-1 (Pharmacia Biotech) and pQE-32 vectors (Qiagen, Hilden, Germany). BamHI-SalI-digested C-terminal sequence was cloned into the corresponding sites of pGEX-5X-1. The N-terminally His\textsubscript{6}-tagged full-length SSAT protein was expressed from SSAT-pQE vector in E. coli strain M15 (Qiagen). The cells were grown in the presence of 20 μM putrescine. The expressed protein was purified from bacterial cells by Ni\textsuperscript{2+}-NTA affinity chromatography (Qiagen). Purified His\textsubscript{6}-SSAT was used for raising antibodies in rabbits (50 μg of protein per immunization at 4-week intervals). The full-length SSAT and the C-terminal portion of SSAT were expressed from pGEX-5X-1 vector in E. coli strain BL21 (DE3) by growing the cells in the presence of 20 μM putrescine. The glutathione S-transferase (GST) fusion proteins were purified by glutathione-Sepharose (Pharmacia Biotech) affinity chromatography. GST-full-length SSAT and GST-C-terminal SSAT were immobilized on HiTrap NHS-activated Sepharose (Pharmacia Biotech) columns (1 ml) according to the manufacturer’s instructions. These matrices were used for purification of antibodies specific for full-length SSAT and the C-terminal portion of SSAT, respectively. The antibody raised against full-length SSAT was used for immunoprecipitation of the samples before Western blot analysis, and C-terminal antibody was used for the detection of SSAT in Western blots. However, because the C-terminal antibody readily recognized SSAT in Western blots without a prior immunoprecipitation and because quantitation of the signals was more reliable without the immunoprecipitation, the subsequent samples were analyzed using only the C-terminal antibody. Western blotting was carried out essentially as described previously (24) using the C-terminal antibody in dilution of 1:200.

Analytical Methods—Total RNA was extracted with guanidine isothiocyanate and purified by CsCl gradient centrifugation as described elsewhere (24). Human SSAT cDNA probe (25) was used in Northern blot analyses. The ODC and SSAT activities were assayed as described previously (19). Polyamines, their acetylated derivatives, and DENSPM were measured by high pressure liquid chromatography as described by Kramer et al. (26).

RESULTS

Transgenic versus Nontransgenic Fibroblasts—Basal SSAT activity in transgenic fibroblasts was ~20 times higher than that seen in the nontransgenic cells (Table 1), whereas steady-state mRNA was 40 times higher as seen in Northern blots for the two cell types (Fig. 1). Transgenic fibroblasts displayed characteristic signs of activation of polyamine catabolism, including a modest decrease in spermidine and spermine pools and elevated putrescine and N\textsuperscript{3}-acetylsermidine pools. In addition, large amounts of exogenous polyamines were seen in the media indicating that the higher levels of SSAT activity facilitated polyamine excretion out of the cell (18, 19). Putrescine and N\textsuperscript{3}-acetylsermidine were 2- and 10-fold higher, respectively, in the media of untreated transgenic fibroblast cultures.
TABLE I

Cell growth, SSAT activity, and polyamine pools in primary fetal fibroblasts from nontransgenic and transgenic mice exposed to DENSPM or spermine for 48 h

| Cell type and treatment (48 h) | Relative cell growth | ODC activity | SSAT activity | Put  | AcSpd | Spd | Spm | DENSPM |
|-------------------------------|----------------------|--------------|---------------|------|-------|-----|-----|--------|
| Nontransgenic                 | %                    | nmol/h/mg    | pmol/min/mg   | 11   | 150   | 48  | 3350| 2080   |
| + 10 μM DENSPM                | 100                  | 1.64         | 2125          | 74   | 92    | 78  | 222 | 8150   |
| + 10 μM Spm                   | 92                   | ND           | 10            | −10  | <10   | 616 | 2594|        |
| Transgenic                    | 100                  | 4.24         | 200           | 1520 | 432   | 2830| 1634|        |
| + 10 μM DENSPM                | 39                   | 0.01         | 76990         | 47   | 97    | 76  | 53  | 9085   |
| + 10 μM Spm                   | 80                   | ND           | 184           | 424  | 390   | 440 | 3052|        |

a Put, putrescine; Ac-Spd, N^1^-acetylspermidine; Spd, spermidine; Spm, spermine.

b ND, not determined.

Nontransgenic

Transgenic

SSAT mRNA

mRNA level

SSAT activity

1x

3x

1x

193x

18x

40x

3000x

7000x

18 S rRNA

28 S rRNA

mRNA induced by DENSPM Metabolic Effects—To gain indication of the various metabolic responses of the fibroblasts to DENSPM, initial experiments involved treatment with a fixed standard dose of 10 μM analogue for 48 h. As indicated in Table I, DENSPM dramatically enhanced SSAT activity in both nontransgenic and transgenic cells by 200- and 380-fold, respectively. More impressively, the actual level of induced SSAT activity in transgenic fibroblasts at 48 h was ~77,000 pmol/min/mg as compared with ~2,100 in the nontransgenic cells. Despite this large difference in activity, all polyamine pools were almost totally depleted in both cell types by 48 h (Table I). Analogue down-regulation of ODC undoubtedly contributed to this effect. The finding that DENSPM-treated transgenic fibroblasts contained less N^1^-acetylspermidine than untreated cells is attributable to the lack of available spermidine to acetylate. A time-course analysis of analogue-induced growth inhibition (at 3 or 10 μM DENSPM) illustrates the much greater sensitivity of the transgenic fibroblasts relative to the remarkably resistant normal fibroblasts (Fig. 2), a phenomenon that is investigated in greater detail below.

In comparison with the analogue, the natural polyamine spermine only marginally induced SSAT activity, a finding consistent with earlier evidence that DENSPM but not SPM is capable of markedly stabilizing the enzyme protein (28–32). Spermine pools were markedly reduced ~80% in both transgenic and non-transgenic fibroblasts (Table I). This is probably attributable in part to analogue down-regulation of ODC (and presumably SAMDC) activity. Spermine pools were, not surprisingly, somewhat elevated due to uptake of the exogenous polyamine.

Induction of SSAT mRNA and Protein—Fig. 1 shows the SSAT mRNA levels and activities in untreated and DENSPM-treated cells at 48 h. As described above, steady-state levels of the message were 40 times higher in transgenic than nontransgenic fibroblasts, resulting in a 18-fold difference in basal enzyme activities. Exposure to the analogue elevated mRNA levels only ~3-fold in both cell types. It is noteworthy that in DENSPM-treated nontransgenic fibroblasts, SSAT activity was more than 10 times higher than that in untreated transgenic cells, whereas the message level was less than one tenth that of the untreated transgenic cells (Fig. 1). This supports the view that even high levels of SSAT message are very poorly translated in the absence of analogue or excess polyamines (14).

The amount of SSAT protein was also analyzed. In our experience, previous antibody preparations raised against human SSAT protein reacted poorly with the mouse enzyme in Western blots, a problem that is unexpected since the mouse and human deduced sequences differ in only six amino acids (33). The antibody produced and used here seems to be unique in its ability to sensitively recognize both human and mouse SSAT protein. Fig. 3 shows a Western blot analysis from nontransgenic and transgenic cells exposed to DENSPM for 48 h. The SSAT protein was barely detectable in nontreated samples but was strikingly increased in both nontransgenic and especially in the transgenic cells exposed to the analogue. The amount of SSAT protein was 46 times higher in the analogue-treated transgenic versus nontransgenic cells, which is reasonably close to the corresponding ratio of the enzyme activities (36-fold as shown in Table I).

Time Dependence of DENSPM Effects—Because polyamine pools were found to be totally depleted at 48 h by 10 μM DENSPM (Table I), we examined the temporal development of this effect at a slightly lower analogue concentration, 3 μM. As shown in Fig. 4A, SSAT activity rose rapidly in the transgenic fibroblasts to a maximum of 630-fold at 24 h, while, by contrast, enzyme activity increased steadily and much more slowly in the nontransgenic fibroblasts to reach a maximum of only ~70-fold at 48 h. In Fig. 4B, it can be seen that nearly 90% of spermidine was lost in just 6 h in the analogue-treated transgenic cells and near-total polyamine depletion was achieved by 24 h. Comparable depletion of polyamines in the nontransgenic cells required a treatment of 48 h (Fig. 4B). Cellular uptake of DENSPM appeared to be initially faster in transgenic cells, due presumably to the more rapid loss of the polyamines. After 48 h, analogue levels were comparable in both cell types (Fig. 4B, inset), a finding that is consistent with the similarity in

![Table I](https://example.com/TableI.png)

![Fig. 1](https://example.com/fig1.png)

![Fig. 2](https://example.com/fig2.png)

![Fig. 3](https://example.com/fig3.png)

![Fig. 4](https://example.com/fig4.png)
polyamine depletion at that same time.

Relative Growth Inhibition by DENSPM—The antiproliferative effect exerted by DENSPM in nontransgenic and transgenic cells were studied over a concentration range of 1–100 μM. As shown in Fig. 5, the transgenic cells appeared to be strikingly more sensitive to the antiproliferative action of the analogue than the nontransgenic cells. In fact, 60% inhibition of cell growth was achieved at 3 μM concentration of DENSPM, whereas growth of the nontransgenic cells was largely unaffected even in the presence of 100 μM analogue. The residual growth of DENSPM-exposed transgenic cells is probably attributable to the fact that the cells were initially seeded in the absence of drug and allowed to attach for 24 h, during which time some cell growth apparently occurred. The time course (Fig. 2) indicated very little transgenic cell growth took place after DENSPM addition. It is also remarkable that normal diploid fibroblasts appeared to be fully resistant to the analogue at concentrations that profoundly affected the growth of tumor cells (17, 19, 21, 22).

Combination of DENSPM with a Polyamine Oxidase Inhibitor—We examined the effects the specific inhibitor of polyamine oxidase MDL 72527 (34) on DENSPM cell growth inhibition. As indicated in Table II, exposure of the transgenic cells to the inhibitor resulted, as expected, in a marked decrease in putrescine and a substantial increase in N1-acetylspermidine pool. In addition to the intracellular changes, the inhibitor also markedly enhanced the excretion of N1-acetylspermidine into medium (results not shown). These changes appeared to be associated with a modest growth inhibitory effect, which was not seen in nontransgenic cells (Table II). The use of MDL 72527 in combination with DENSPM failed to decrease growth inhibition in both transgenic and nontransgenic fibroblasts and, in fact, seemed to increase it slightly (Table II).

DISCUSSION

Previous studies investigating the relationship of SSAT to changes in polyamine metabolism and cell growth have been based on the use of analogues having multiple sites of action and genetically dissimilar cell lines. Typically, this involved comparing analogue effects in paired cell lines, which induced very different levels of SSAT activity (17, 19, 21, 22). The present approach minimizes this problem by comparing the effects of SSAT induction in cells that differ only in the number of SSAT gene copies which they contain (11). Relative to nontransgenic fibroblasts, the transgenic cells showed large increases in putrescine pools, an accumulation of the SSAT product N1-acetylspermidine, and modest decreases in spermidine and/or spermine pools: findings that are all consistent with activated polyamine catabolism and similar to those seen in certain tissues of the whole animal. Since these events were achieved in the absence of exogenous polyamines contributed in whole animals by diet or tissue redistribution and since the two fibroblasts are genetically identical except for SSAT gene copy number, the findings clearly indicate the metabolic potential of SSAT to reduce higher polyamine pools via the back-conversion pathway.

The catabolic events described above were greatly exaggerated by treatment with DENSPM. This lead to a striking induction of SSAT enzyme activity and a severe depletion of polyamine pools, both of which occurred more rapidly in transgenic than nontransgenic fibroblasts. It is interesting that, although the induction of SSAT activity by DENSPM in transgenic fibroblasts was ~36-fold higher than that seen in nontransgenic fibroblasts (Table I), both were eventually depleted of intracellular polyamines. Although additional homeostatic responses are undoubtedly also involved in preserving pool sizes (27), the findings support the view that some minimal induction of SSAT may be sufficient to totally deplete polyamine pools and that enzyme induction above that level mainly affects the rapidity by which this occurs. This could explain the previous observation that certain analogues induce 20–100
times more activity than others, yet have similar effects on cell growth (19, 35).

As in other systems (14, 21, 24, 29), the magnitude of the enzyme response to DENSPM far exceeds that of the available SSAT mRNA, indicating that a significant portion of enzyme induction occurs at levels downstream to mRNA accumulation. Natural polyamines have been shown to enhance the translational efficiency of SSAT mRNA (14), whereas analogues increase both translational efficiency and the stability of the enzyme protein (28–32). These findings are supported by the observation that spermine was much less effective than DENSPM at inducing SSAT activity in transgenic fibroblasts (Table I). On the other hand, steady-state levels of SSAT mRNA were higher in the transgenic fibroblasts relative to the nontransgenic cells (i.e. 40-fold) than was basal SSAT activity (i.e. 18-fold), again indicating the need for elevated polyamine pools to maximally translate the message.

It appears that relatively low SSAT overexpression as seen under basal conditions has little impact on cell growth since presumably compensatory homeostatic responses tend to minimize polyamine pool effects (27). During DENSPM treatment, however, transgenic fibroblasts were distinctly more sensitive to growth inhibition than their nontransgenic counterparts (Figs. 2 and 5). In fact, transgenic cells showed growth inhibition at an analogue concentration that was about 2 orders of magnitude lower than that required to achieve a similar level of growth inhibition in nontransgenic cells. The difference between the two fibroblasts is obvious, and although the finding is similar to previous observations obtained using paired tumor

**FIG. 4.** A, SSAT activity in nontransgenic (tg-) and transgenic (tg+) fibroblasts grown in the presence of 3 μM DENSPM for 0–48 h. Untreated activity levels were 0.2 nmol/min/mg for transgenic fibroblasts and 0.01 nmol/mg/min for nontransgenic fibroblasts. B, polyamine pools and concentration of DENSPM (inset) in nontransgenic (tg-) and transgenic (tg+) fibroblasts grown in the presence of 3 μM DENSPM for 0–48 h.

**TABLE II**

| Cell type and treatment (72 h) | Relative cell growth | Put  | Ac-Spd | Spd  | Spm  | DENSPM |
|-------------------------------|----------------------|------|--------|------|------|--------|
| Nontransgenic                 |                      |      |        |      |      |        |
| None                          | 100                  | 106  | 24     | 74   | 2820 | 1990   |
| 50 μM MDL 72527               | 106                  | 74   | 2820   | 1990 |
| 10 μM DENSPM                  | 96                   | 40   | 76     | 114  | 6956 |
| 72527 + DENSPM                | 88                   | 54   | 26     | 118  | 4972 |
| Transgenic                    |                      |      |        |      |      |        |
| None                          | 100                  | 1790 | 240    | 3142 | 1128 |
| 50 μM MDL 72527               | 106                  | 74   | 2820   | 1990 |
| 10 μM DENSPM                  | 24                   | <10  | <10    | <10  | 6970 |
| 72527 + DENSPM                | 16                   | 55   | 51     | <10  | <10  | 7706   |

*Put, putrescine; Ac-Spd, N1-acetylspermidine; Spd, spermidine; Spm, spermine.*
Transgenic Fetal Fibroblast Responses to Polyamine Analogues

A. M. H. and M. O. O. epoxygenes, and the interpretation is, as described above, less complicated. Interestingly, the transgenic animals from which these fibroblasts were derived also showed an enhanced sensitivity to DENSPM. The basis for the remarkable resistance of nontransgenic cells to DENSPM growth inhibition is currently being investigated.

The downstream event(s) responsible for enhanced growth sensitivity to DENSPM appears to be related to the very rapid depletion of polyamine pools resulting from the analogue-induced burst in SSAT activity. As has been previously shown, this occurs mainly via the excretion or degradation of acetylated polyamines, both of which are facilitated by the induced SSAT (18, 19). Although depletion of pools eventually occurs in the non-transgenic fibroblasts, the rapidity of the event in the transgenic cells may preclude activation of adaptive responses able to minimize the impact on cell growth. Thus, without having time to compensate for the rapid polyamine depletion, the transgenic fibroblast may be shocked into growth inhibition and/or cytotoxicity. It is also possible that the massive induction of SSAT may lead to the inappropriate acetylation of alternative substrates such as histones (36) and/or rapid depletion of acetyl-coenzyme A, the acetyl donor in the SSAT reaction.

At least one group has suggested that cytotoxicity associated with SSAT induction in breast carcinoma cell lines involves polyamine oxidase (37), the enzyme that oxidizes acetylated polyamines as part of the back-conversion pathway. The hydrogen peroxide resulting from this reaction could lead to oxidative stress and apoptosis in cells. Our present experiments using an inhibitor of polyamine oxidase MDL-72527 (34) during DENSPM treatment (Table II) tend to exclude this possibility. Rather than decreasing the antiproliferative activity of the analogue, the inhibitor actually increased it in transgenic fibroblasts and had no effect in nontransgenic fibroblasts.

In addition to clearly indicating the metabolic consequences of SSAT overexpression in cultured cells, the present results provide some of the most compelling evidence to date for the role of SSAT induction in DENSPM-mediated growth inhibition. Although the present results suggest that antiproliferative activity is mediated via polyamine depletion as opposed to oxidative stress, this relationship may be very cell-type dependent.

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