Autocrine Growth Factor Regulation of Lysyl Oxidase Expression in Transformed Fibroblasts*

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Amitha H. Palamakumbura‡, Pascal Sommer§, and Philip C. Trackman¶

From the ‡Boston University Goldman School of Dental Medicine, Division of Oral Biology, Boston, Massachusetts 02118 and §Institut de Biologie et Chimie des Protéines, CNRS UMR 5086, Université Claude Bernard, 69677 Lyon Cedex 07 France

Lysyl oxidase catalyzes oxidative deamination of peptidyl-lysine and hydroxyllysine residues in collagens and lysine residues in elastin to form peptidyl aldehydes that are required for the formation of covalent cross-links in normal extracellular matrix biosynthesis. Lysyl oxidase in addition has tumor suppressor activity, and phenotypic reversion of transformed cell lines is accompanied by increased lysyl oxidase expression. The mechanism of low expression of lysyl oxidase in tumor cells is unknown. The present study investigates the hypothesis that autocrine growth factor pathways maintain low lysyl oxidase expression levels in c-H-ras-transformed fibroblasts (RS485 cell line). Autocrine pathways were blocked with suramin, a general inhibitor of growth factor receptor binding, and resulted in more than a 10-fold increase in lysyl oxidase expression and proenzyme production. This regulation was found to be reversible and occurred at the transcriptional level determined using lysyl oxidase promoter/reporter gene assays. Function blocking anti-fibroblast growth factor-2 (FGF-2) antibody enhanced lysyl oxidase expression in the absence of suramin. Finally, the addition of FGF-2 to suramin-treated cells completely reversed suramin stimulation of lysyl oxidase mRNA levels. Data support that an FGF-2 autocrine pathway inhibits lysyl oxidase transcription in the tumorigenic-transformed RS485 cell line. This finding may be of therapeutic significance and, in addition, provides a new experimental approach to investigate the mechanism of the tumor suppressor activity of lysyl oxidase.

Lysyl oxidase catalyzes the final step of collagen and elastin cross-linking by oxidative deamination of the ε-amino group of hydroxyllysine and lysine residues (1). The resulting peptidyl aldehyde products undergo spontaneous condensations with unmodified lysine residues or with other peptidyl aldehyde residues to generate covalent cross-links required for the biosynthesis of mature functional extracellular matrices. Lysyl oxidase is synthesized as a 50-kDa proenzyme, secreted into the extracellular environment, and then processed by proteolytic cleavage, resulting in a functional 30-kDa enzyme and an 18-kDa propeptide. Evidence supports that 30-kDa lysyl oxidase is active and that the 50-kDa proenzyme is enzymatically inactive (2–4). Abnormally increased lysyl oxidase expression and enzyme activity can lead to excessive accumulation of insoluble collagen fibers. A direct relationship is found between fibrotic diseases and increased lysyl oxidase activity (5–8).

Lysyl oxidase has tumor suppressor activity (9–11). Diminished levels of lysyl oxidase are consistently found in cancer cell lines as well as in transformed cell lines (12–17), and restoration of normal expression levels is associated with the return of lysyl oxidase expression to normal levels (15, 16, 18). In particular, lysyl oxidase expression is diminished in cell lines transformed with ras or ras-dependent oncogenes. NIH3T3 cells transformed by overexpression of c-H-ras (RS485 cells) are tumorigenic and have low levels of lysyl oxidase expression (14). Long-term treatment of RS485 cells with interferon-α/β resulted in the isolation of stable phenotypic revertant cell lines (19) that express normal increased levels of lysyl oxidase and are not tumorigenic (9, 14). Transfection of one of these stable phenotypic revertant cell lines (PR4 cells) with an antisense lysyl oxidase expression vector returned cells to a transformed and tumorigenic phenotype and established lysyl oxidase expression as a contributing factor in maintaining a normal cellular phenotype (9–11,14).

As noted above lysyl oxidase is consistently down-regulated in cancer cells and in transformed cell lines, and studies support that lysyl oxidase transcription is diminished in transformed cells (17, 20). It is important to realize, however, that the mechanisms responsible for diminished lysyl oxidase transcription in transformed cells are not well understood. Epigenetic regulation of methylation of the lysyl oxidase promoter may play a role (21, 22), and the participation of an interferon regulatory factor 1 cis-acting element in regulating lysyl oxidase transcription in tumor cells has been reported but has not yet been confirmed (21–23). In the present study, we investigate the hypothesis that autocrine growth factor pathways diminish lysyl oxidase transcription in tumor cells. Autocrine growth factor pathways occur in certain tumors and in tumor cell lines and can contribute to the transformed phenotype and tumorigenicity. For example, malignancy is maintained by autocrine pathways that, respectively, include elevated production and responsiveness in different tissues to FGF-2, epidermal growth factor, or platelet-derived growth factor (24–26). Previous studies show that lysyl oxidase is down-regulated by FGF-2 and up-regulated by several other factors including transforming growth factor-β, interleukin-1β, platelet-derived growth factor, and connective tissue growth factor (27–33). Our working hypothesis is that autocrine growth factor pathways that include FGF-2 down-regulate lysyl oxidase and...
are responsible for the low levels of lysyl oxidase expression observed in the c-H-ras-transformed RS485 cell line.

Suramin is a polysulfonated naphthylurea and was initially used in the treatment of trypanosomiasis and onchocerciasis (34). Its anticaner activity was later identified, and suramin has been introduced into clinical trials for adrenal, lung, and prostate cancer (35–39). Moreover, suramin is active against human immunodeficiency virus-1 (40). Suramin inhibits the binding of growth factors to their respective receptors, and this mechanism is believed to primarily contribute to its therapeutic effects (24, 34, 41–43).

Thus, we investigated whether suramin regulates lysyl oxidase in RS485 cells. Results indicate that suramin increases lysyl oxidase expression more than 10-fold in RS485 cells and that regulation occurs at the transcriptional level. Treatment of RS485 cells with a function blocking FGF-2 antibody in the absence of suramin increases lysyl oxidase mRNA expression. Exogenously added FGF-2 prevented suramin stimulation of lysyl oxidase expression. Data taken together support the hypothesis that an autocrine FGF-2 pathway contributes to lysyl oxidase down-regulation in RS485 cells. Down-regulation of lysyl oxidase via autocrine growth factor pathways is likely to be an important contributing mechanism in tumorigenesis. In addition, normalization of lysyl oxidase expression may be a beneficial outcome of cancer therapeutic drugs, whose mechanism is to block autocrine growth factor pathways.

MATERIALS AND METHODS

Chemicals—Suramin was either provided by the Division of Cancer Treatment Diagnosis and Centers, National Cancer Institute, and Parke-Davis or was purchased from Sigma. Anti-FGF-2-neutralizing and anti-IGF-1 clone bFM-1 was purchased from Upstate Biotechnologies, Waltham, MA. FGF-2 was purchased from PeproTech, Rocky Hill, NJ. All other chemicals were of the highest quality available and were purchased from Sigma.

Cell Culture—RS485 cells were plated onto 100-mm tissue culture dishes in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum plus 1% nonessential amino acids, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cultures were maintained at 37°C in a fully humidified atmosphere of 5% CO₂ in air, and cultures were refed every 2 days. For experiments in which the effects of suramin were determined, cells in logarithmic growth phase were dissociated with trypsin/EDTA and inoculated at 250,000 cells/plate. After 24 h, media were then changed to contain up to 150 μM suramin and cultured as required for the experimental design.

RNA Isolation and Northern Blot Analysis—Total RNA from RS485 cells was isolated using RNAsasy RNA isolation kit (Qiagen). Ten μg samples of denatured RNA were electrophoresed on a 1% agarose gel containing 18% formaldehyde. Gels were transferred in 10× SSC (1× SSC = 0.15 M NaCl and 0.015 M sodium citrate) by capillary blotting overnight to Gene Screen (Du Pont) nylon membranes (15). Labeled probe of mouse lysyl oxidase pc31b (14) was prepared by random primer labeling (44) and was hybridized at 42°C as previously described (27). For normalization and as a measure of constant loading of gels, blots were stripped and rehybridized with a radiolabeled 18 S RNA probe (45). Autoradiograms were assessed and normalized by densitometric scanning using the Versa Doc Model 3000 Imaging System (Bio-Rad).

Pulse Labeling and Immunoprecipitation—RS485 cells were grown in 100-mm plates with 0 or 150 μM suramin as described above. At visual confluence, cultures were refed and incubated for 20 min with 7 ml plate of serum-free and methionine-free Dulbecco’s modified Eagle’s medium containing 1% nonessential amino acid, 100 units/ml penicillin, and 100 μg/ml streptomycin with or without suramin (150 μM). Cultures were then placed in fresh media with or without suramin (150 μM) supplemented with 50 μCi/ml [35S]methionine (1175 Ci/mmol, PerkinElmer Life Sciences). After 5 h, media were harvested and prepared for immunoprecipitation (2). Constant counts/min were used for immunoprecipitation with lysyl oxidase anti-serum and was determined with preimmune serum as control. Samples were subjected to SDS-PAGE and autoradiography.

Lysyl Oxidase Enzyme Activity—RS485 cells cultured with or without 150 μM suramin were grown as described above. At visual confluence, cells were refed with serum-free, phenol red-free media and further cultured in the presence or absence of suramin. After 24 h, the media were collected and centrifuged for 10 min at 10,000 × g at 4°C. Lysyl oxidase activity was determined using a horseradish peroxidase-coupled fluorescent assay method based on the oxidation of Amplex Red and using 1,5-diaminopentane as the substrate for lysyl oxidase (46). Solutions were prepared in a final volume of 2 ml containing 1.2 μM urea, 0.05 M sodium borate, pH 8.2, 1 unit/ml horseradish peroxidase, 10 μM Amplex Red, 10 mM 1,5-diaminopentane, and 500–1000 μM of NaCl. A parallel set of solutions was prepared with 500 μM β-aminopropionitrile to completely inhibit the activity of lysyl oxidase. After incubation of all the samples at 37°C for 30 min, the difference in fluorescence intensity was recorded at an excitation wavelength of 563 nm and an emission wavelength of 597 nm. The rate of the production of hydrogen peroxide was calculated by comparison of fluorescence changes to that of a standard curve prepared using hydrogen peroxide. The units of enzyme activity are defined as the amount of n mole of hydrogen peroxide produced/min above the β-aminopropionitrile controls. Determinations of DNA to normalize enzyme activity measurements were performed from each cell layer (47).

Transient Transfection and Luciferase Assays—Subconfluent RS485 cells were co-transfected with pSV2β-gal and with firefly luciferase reporter gene constructs at a mass ratio of 1:19. The luciferase constructs were (a) the pGL2 basic plasmid (Promega, Madison, WI), which contains no promoter driving the luciferase gene, (b) the pGL2 control plasmid, which contains luciferase driven by the SV40 early promoter, and (c) the lysyl oxidase promoter construct (pPL1), which contains luciferase driven by the mouse lysyl oxidase promoter (nucleotides −2079 to +435) cloned into the KpnI and BgII sites of pGL2 basic (22). Cells were transfected using the calcium phosphate precipitation method (48). After 24 h, cells were treated with 0 or 150 μM suramin and cultured for an additional 24 h. Cells were then lysed in reporter lysis buffer (Promega), and the luciferase activity in the cell lysate was measured immediately after addition of the substrate (luciferase assay system; Promega) using a TD-20/20 luminometer (Turner Designs). β-Galactosidase activity in the lysate was measured according to the manufacturer’s protocol (β-galactosidase enzyme assay system; Promega), and the values were used to normalize the efficiency of transfection.

RESULTS

Dose-dependent Regulation of Lysyl Oxidase by Suramin—RS485 cells were plated as described under “Methods and Materials.” After 24 h, the sub-confluent cultures were then treated continuously with different concentrations of suramin up to 150 μM, with media changes every 2 days. At visual confluence (after 2–5 days in culture), total RNA was then isolated and subjected to Northern blot analysis. As shown in Fig. 1, a dose-dependent up-regulation in the steady state lysyl oxidase mRNA level was found. At least a 10-fold increase was observed in lysyl oxidase mRNA levels in cells treated with 150 μM suramin.

Reversibility of the Regulation of Lysyl Oxidase—If suramin
blocks autocrine growth factor pathways that control lysyl oxidase expression, then lysyl oxidase regulation by suramin should be reversible. Thus, the reversibility of suramin-induced up-regulation of lysyl oxidase mRNA levels was examined. RS485 cells were treated with suramin (0–150 μM) and cultured for 2 days. The medium was then replaced with suramin-free medium for different time intervals (36 and 48 h). RNA was isolated from cells before and after removal of suramin and subjected to Northern blot analysis. Fig. 2 shows that lysyl oxidase mRNA levels increase with suramin treatment as expected (lanes 1–4). After removal of suramin, lysyl oxidase mRNA levels were decreased at both time points (lanes 5–10). Maintenance of RS485 cells in the continuous presence of suramin for the entire experimental period results in persistent high elevation of lysyl oxidase mRNA levels (lanes 11–12). Thus, data support that suramin-stimulated lysyl oxidase expression is reversible. This is consistent with the notion that suramin increases lysyl oxidase mRNA levels by reversibly inhibiting autocrine growth factor pathways.

Lysyl Oxidase Biosynthesis—We next investigated whether increased lysyl oxidase protein production and processing accompanies the suramin stimulation of RS485 cells. Cells were treated with (control) or 150 μM suramin as described under “Materials and Methods,” and the synthesis of lysyl oxidase protein was determined by pulse labeling with [35S]methionine for 5 h followed by immunoprecipitation of secreted and radio-labeled lysyl oxidase from media. These conditions of pulse labeling allow the detection of all forms of lysyl oxidase (2). The samples were subjected to SDS-PAGE and autoradiography. Fig. 3 shows the increased levels of pro-lysyl oxidase enzyme (47–50 kDa) in media samples from suramin-treated cells (lane 4), indicating that suramin increased the biosynthesis and secretion of pro-lysyl oxidase. Only a small proportion of immunoprecipitated lysyl oxidase was fully processed (~30 kDa) lysyl oxidase enzyme. No immunoprecipitated lysyl oxidase protein bands could be detected or identified from control RS485 cells not treated with suramin (lane 1), as expected due to the very low expression of lysyl oxidase in these tumor cells.

Effect of Suramin on Active Lysyl Oxidase Production—To determine whether lysyl oxidase enzyme activity increased as a result of suramin treatment, RS485 cells were first treated in complete medium in the presence or the absence of 150 μM suramin as described under “Materials and Methods.” Cells were then cultured for an additional 24 h in serum-free medium with or without suramin. Accumulated enzyme activity was then measured in these media samples and was normalized to the amount of DNA in the cell layer. As shown in Table
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I, suramin increased lysyl oxidase activity in RS485 cell media by a factor of 2.2. This relatively small increase in enzyme activity in suramin-treated RS485 cells compared with mRNA increases is consistent with our finding of predominant production of unprocessed 50-kDa pro-lysyl oxidase summarized in Fig. 3 and reflects deficient proenzyme processing to active enzyme in suramin-treated cells. Note that phenotypically normal NIH3T3 cells constitutively express more than 10-fold higher levels of lysyl oxidase enzyme activity than RS485 cells (Table I), consistent with previous data (9).

**Time-dependent Up-regulation of Lysyl Oxidase by Suramin**—To investigate mechanisms of regulation of lysyl oxidase by suramin, we next determined the time-dependent up-regulation of steady state levels of lysyl oxidase mRNA by suramin. RS485 cells were treated with 0 or 150 μM suramin. Total RNA was isolated at different time intervals and was then subjected to Northern blot analysis. As shown in Fig. 4, suramin increased the steady state lysyl oxidase mRNA levels within 4 h of treatment, increasing to a maximum level at 16–20 h.

**Lysyl Oxidase Promoter Activity in Luciferase Reporter Constructs**—Because of the rapid and highly significant up-regulation of lysyl oxidase mRNA levels by suramin, we suspected that observed regulation could be at the transcriptional level. Assay of transcriptional activity was accomplished using a murine lysyl oxidase promoter reporter construct. Cells were transfected with lysyl oxidase promoter-driven luciferase gene construct (pPL1) that contained 2 kbp of lysyl oxidase promoter. Control plasmids were the SV40 promoter-driven luciferase gene construct (pGL2 control) and pGL2 basic containing no promoter. Each luciferase construct was co-transfected with pSVβ-gal to allow for normalization. Cells were then treated with 0 or 150 μM suramin for 24 h, and the luciferase activity was measured in cell lysates. The 24-h time point was chosen based on the time study (Fig. 4). Fig. 5 shows the values of luciferase activity normalized to β-galactosidase activity. Suramin increased the luciferase activity of RS485 cells transfected with pPL1 by 2–4-fold in four different experiments (p < 0.01). By contrast and as expected, luciferase activity in cells transfected with pGL2 control was unaffected by suramin (control cells, 264.6 ± 25.3 luciferase units/milliliters of β-galactosidase activity; suramin-treated cells, 268.2 ± 37.0 luciferase units/milliliters of β-galactosidase activity). There was essentially no luciferase activity in control and suramin-treated cells transfected with the pGL2 basic vector, as predicted (Fig. 5).

**Effects of Function-blocking FGF-2 Antibody**—To investigate the presence and role of autocrine growth factor pathways in RS485 cells in lysyl oxidase regulation, we next determined the effect of function blocking FGF-2 antibody on lysyl oxidase expression in RS485 cells. Our working hypothesis is that RS485 cells produce and respond to FGF-2, resulting in low lysyl oxidase expression. If true, then interference of this autocrine growth factor pathway with suramin or, alternatively, with an FGF-2-blocking antibody, should up-regulate lysyl oxidase expression. Subconfluent RS485 cells were treated with 1) no additions, 2) 1 μg/ml anti-FGF-2 antibody, 3) 2.5 μg/ml anti-FGF-2 antibody, and 4) 10 μg/ml anti-FGF-2 antibody. Total RNA was isolated after 24 h and subjected to Northern blot analysis. As shown in Fig. 6, a dose-dependent increase in lysyl oxidase expression up to 2.5-fold was found in the cells treated with anti-FGF-2 antibody under the conditions of this experiment. These results indicate the presence of FGF-2-dependent autocrine pathways in RS485 cells, and interference with those pathways resulted in up-regulation of lysyl oxidase expression.

**Effect of Exogenous FGF-2 on Lysyl Oxidase Up-regulation by Suramin**—If suramin effects occur as a result of inhibition of autocrine pathways, then these effects should be reversed by the exogenous addition of the functional growth factor (49). Thus, to further investigate whether suramin inhibits FGF-2-
FIG. 6. Regulation of lysyl oxidase by FGF-2 blocking antibody. A. Northern blot showing mRNA levels of lysyl oxidase in RS485 cells grown for 24 h in the presence of FGF-2 function blocking antibody; lane 1, no antibody; lane 2, 1 μg/ml; lane 3, 2.5 μg/ml; lane 4, 5 μg/ml. 10 μg aliquots of RNA samples were subjected to Northern blot analysis. B, quantitation of lysyl oxidase levels normalized to 18 S rRNA. Values represent the mean ± S.D. obtained from three scanning densitometry determinations.

dependent pathways in RS485 cells, the effect of exogenous FGF-2 on suramin-induced regulation of lysyl oxidase was determined. Cells were cultured with 1) no additions, 2) 150 μM suramin, 3) 10 ng/ml FGF-2, and 4) both 150 μM suramin and 10 ng/ml FGF-2. Total RNA was isolated at intervals and was subjected to Northern blot analysis. As shown in Fig. 7, in the absence of suramin, FGF-2 alone did not further down-regulate the already low lysyl oxidase mRNA levels. Suramin treatment alone increased lysyl oxidase mRNA levels, as expected. The simultaneous presence of FGF-2 and suramin prevented the suramin-induced up-regulation of mRNA level consistently at every time point (Fig. 7). Data in Figs. 6 and 7 together support autocrine FGF-2 down-regulation of lysyl oxidase in RS485 cells.

DISCUSSION

Although the relationship between low lysyl oxidase expression and a transformed phenotype has been confirmed in independent studies (13, 16, 17), there is surprisingly little information regarding the mechanism of down-regulation of lysyl oxidase in tumor cells. Lysyl oxidase promoter/reporter gene assays have so far not reproducibly identified transcriptional elements that confer clear tumor cell-specific effects on lysyl oxidase transcription (21–23). The present study offers support for the notion that autocrine growth factor pathways play an important role in the low expression of lysyl oxidase in tumor cells. The experimental approach chosen takes advantage of the general inhibitory activity of growth factor receptors by the experimental cancer drug suramin. Treatment of RS485 cells with suramin resulted in more than 10-fold increased lysyl oxidase expression, and data support inhibition of FGF-2 autocrine growth factor pathways by suramin as a major contributing factor to this dramatic increase in lysyl oxidase expression. Autocrine growth factor pathways occur in tumors and in certain tumor cell lines including human colon carcinoma, glioma and glioblastoma, malignant fibrous histiocytomas, melanoma, testicular cancer, rhabdomyosarcoma cells, and small cell lung carcinoma cell lines (24–26, 34, 50–53).

It is interesting that FGF-2 appears to be the only polypeptide growth factor reported so far to decrease lysyl oxidase expression. Interferon-γ, a cytokine produced principally by macrophages, is the only other polypeptide factor reported to diminish lysyl oxidase expression (54). Transforming growth factor-β1 increases steady state lysyl oxidase mRNA levels about 8-fold in murine osteoblast-like MC3T3-E1 cells, whereas FGF-2 decreases lysyl oxidase expression in these cells (27, 28). FGF-2 decreases the steady state mRNA levels in human gingival fibroblasts (29), whereas connective tissue growth factor increases lysyl oxidase enzyme activity about 2-fold in these cells (32). In human lung fibroblasts, transforming growth factor-β and interleukin-1β up-regulate lysyl oxidase expression (55). Platelet-derived growth factor has been reported to stimulate lysyl oxidase levels in vascular smooth muscle cells (33). FGF-2 has been found to act as an autocrine factor, stimulating proliferation of certain tumor cell lines (24, 56–58). Our findings suggest that FGF-2-dependent growth factor pathways are largely responsible for the low levels of lysyl oxidase present in RS485 cells. As noted, data presented in the current study show that FGF-2 prevents suramin-dependent up-regulation of lysyl oxidase in RS485 cells. In addition, FGF-2-blocking antibody in the absence of suramin stimulates lysyl oxidase expression and confirms the involvement of FGF-2 in lysyl oxidase regulation. FGF-2 has a complex extracellular and intracellular distribution, and the sub-optimal stimulation of lysyl oxidase expression by anti-FGF-2 function blocking antibody compared with suramin itself is likely to be related to the lack of accessibility of extracellular exogenously added antibody to biologically active intracellular-sequestered FGF-2 (59–61).

The results presented in this report show that lysyl oxidase expression is dramatically up-regulated by suramin in RS485 cells. The knowledge that lysyl oxidase expression is a potential target for this experimental drug may be of importance in understanding the results of animal and clinical studies. For example, the effect of suramin on an animal model of lung fibrosis was evaluated, testing the hypothesis that inhibition of
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the action of pro-inflammatory cytokines by suramin might have beneficial effects on this fibrotic disease. No improvement in fibrosis was actually observed (62). Lysyl oxidase is elevated in fibrotic disease and renders extracellular matrices less susceptible to proteolysis (63). If lysyl oxidase expression were increased in the lungs of suramin-treated animals, this treatment could actually exacerbate rather than prevent lung fibrosis. By contrast, due to the tumor suppressive activity of lysyl oxidase, beneficial effects of suramin on different forms of cancer may be in part due to increased expression of lysyl oxidase (37, 39, 64–66).

Mechanisms by which lysyl oxidase functions as a tumor suppressor are not clearly identified. Mature lysyl oxidase enzyme has been reported to be taken up by cells and translocated to the nucleus of some cells, where it influences the transcription of type III collagen (67–69). It has been suggested that lysyl oxidase could interact with histones (70), known in vitro substrates of purified lysyl oxidase (71), and thereby possibly directly or indirectly regulate gene transcription. Cell transformation and increased ras activity and decreased cell adhesion all result from antisen lysyl oxidase transfection of NRK-49F cells (72, 73). A recent interesting study indicates that ectopic pro-lysyl oxidase expression in ras-transformed fibroblasts diminishes NF-κB activation and growth in soft agar by a mechanism that includes diminished phosphatidylinositol 3-kinase activity (74). Future studies will determine the mechanisms by which lysyl oxidase regulates signaling pathways that are functionally important in determining the cell phenotype.

It is important to realize that suramin “normalizes” the phenotype of some tumor cell lines (25, 26, 43). Work now in progress is focused on determining the effect of suramin on the phenotype of tumor cells in an effort to understand whether lysyl oxidase mediates normalization of the cell phenotype by suramin, perhaps by somehow regulating phosphatidylinositol 3-kinase localization and activity. Thus, the suramin-induced lysyl oxidase expression reported here will serve as a new experimental tool to help investigate mechanisms by which lysyl oxidase contributes to the normal cell phenotype.

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REFERENCES

1. Kagan, H. M., and Trackman, P. C. (1991) Am. J. Respir. Cell Mol. Biol. 5, 206–210
2. Trackman, P. C., Bedell-Hogan, D., Tang, J., and Kagan, H. M. (1992) J. Biol. Chem. 267, 8666–8671
3. Panchenkov, M. V., Stetler-Stevenson, W. G., Trubetskoy, O. V., Gacheru, S. N., and Trackman, P. C. (1996) J. Biol. Chem. 271, 7113–7119
4. Uzel, M. I., Scott, I. C., Babalkhanov-Chase, H., Palamakumbura, A. H., Pappano, W. N., Hong, H. H., Greenpan, D. S., and Trackman, P. C. (2001) J. Biol. Chem. 276, 25237–25243
5. Kagan, H. M. (2000) Acta Trop. 77, 147–152
6. Trivedy, C., Warnakulasuriya, K. A., Hazarey, V. K., Tarassoli, M., Sommer, P., and Johnson, P. N. (1999) J. Oral Pathol. Med. 28, 246–251
7. Sommer, P., Gleyzal, C., Racurt, M., Delbourgo, M., Serrar, M., Jazaire, P., Peyrol, S., Kagan, S., Trackman, P. C., and Grimaud, J. A. (1993) Lab. Invest. 69, 460–470
8. Jourdan-Le Saux, C., Gleyzal, C., Garnier, J. M., Peraldi, M., Sommer, P., and Peyrol, S. (1999) Cancer Res. 59, 3312–3316
9. Song, S., Wientjes, M. G., Gan, Y., and Au, J. L. (2000) Mol. Cell. Biol. 20, 341–345
10. Song, S., Wientjes, M. G., and Au, J. L. (2001) Cancer Res. 61, 1615–1620
11. Small, E. J., Meyer, M., Marshall, M. E., Reyno, L. M., Meyers, F. J., Natale, R. B., Leshan, P. F., Chen, J. Z., Slichenmyer, W. J., and Eisenberger, M. (2000) J. Clin. Oncol. 18, 1440–1450
12. Garcia-Schurmann, J. M., Schulze, H., Haupt, G., Pastor, J., Allolio, B., and Senga, T. (1999) Urology 53, 545–546
13. Dhah, S., Gulbo, J., Cooja, K., Eriksson, N., Nilsson, K., Nickel, P., Larsson, R., and Nygren, P. (2000) Eur. J. Cancer 36, 803–809
14. Zangh, S. A., Benner, W. A., Bailey, E., and Fussinger, A. (2000) Biochem. Biophys. Acta 1466–1467
15. Miett, H., Chevez-Barrios, P., Feldman, R. M., and Lieberman, M. W. (1998) Br. J. Ophthalmol. 82, 816–820
16. Cottii, R. J., Jr., Leif, E. B., Shipley, G. D., and Moses, H. L. (1987) J. Cell. Physiol. 132, 143–148
17. Feinberg, A. P., and Vogelstein, B. (1983) Anal. Biochem. 132, 6–13
18. Hils, D. M., and Dixon, M. T. (1991) J. Biol. Chem. 266, 411–415
19. Palamakumbura, A. H., and Trackman, P. C. (2002) Anal. Biochem. 309, 245–251
20. Vytassek, R. (1982) Anal. Biochem. 120, 243–248
21. Saper, P. J., and Berg, P. (1982) J. Mol. Appl. Genet. 1, 327–341
22. Cirillo, A., Chifflet, S., and Villar, B. (2001) Cell Tissue Res. 304, 323–331
23. De Giovanni, C., Melani, C., Nanni, P., Landuzzi, L., Niccoli, G., Frabetti, F., Griffoni, C., Colombo, M. P., and Lollini, P. L. (1995) Br. J. Cancer 72, 1224–1229
24. Hsu, S., Huang, H., Hafes, M., Winawer, S., and Friedman, E. (1994) Cell Growth Differ. 5, 267–275
25. Vassobts, P. G. O., Amant, A., Langeland, N., Holmsen, H., Westermark, B., Heldin, C. H., and Nister, M. (1994) J. Cell. Physiol. 158, 381–389
26. Meier, F., Nesbit, M., Hsu, M. Y., Martin, B., Van Belle, P., Elder, D. E., Schaumburg-Lever, G., Garbe, C., Witz, M. J., Donati, P., Croibble, H. M., and Herlym, H. (2000) Am. J. Pathol. 165, 195–200
27. Song, Y. L., Ford, J. W., Gordon, D., and Shanley, C. J. (2000) Artrotiserol. 32, 1999–2002
28. Boak, A. M., Roy, R., Berk, J., Taylor, P., Polgar, P., Goldstein, R. H., and Kagan, H. M. (1994) J. Am. Respir. Cell Mol. Biol. 11, 751–755
29. Song, S., Wientjes, M. G., and Au, J. L. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 8658–8663
30. Murphy, P. R., and Klee, R. S. (1995) Mol. Cell. Endocrinol. 114, 193–203
31. Nesbit, M., Nesbit, H. K., Bennett, J., Andl, T., Hsu, M. Y., Dejesus, E., McKean, M., Gupta, A. R., Eck, S. L., and Herlym, H. (1999) Oncogene 18, 6469–6476
32. Yawen, A., and Kilbrags, M. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 5436–5439
33. Mignatti, P., and Rifkin, D. B. (1991) J. Cell. Biol. 115, 201–207
34. Arnaud, E., Touriol, C., Boutonnet, C., Gensac, M. C., Vagner, S., Kagan, H. M., and Prats, A. C. (1999) Mol. Biol. Cell. 20, 505–514
35. Lossos, I. S., Ishibiki, G., Or, R., Goldstein, R. H., and Breuer, R. (2000) Life Sci. 67, 2873–2881
36. Kagan, R. (1986) in Cancer Biology and Regulation of Extracellular Matrix: Regulation of Matrix Accumulation (Mecham, R. P., ed) Vol. 1, pp. 321–398, Academic Press, Inc., Orlando
37. Mirza, M. R., Jakobsen, E., Pfeiffer, P., Lindeberg-Clasen, B., Bergh, J., and

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65. Davol, P. A., Garza, S., and Frackelton, A. R., Jr. (1999) *Cancer* **86**, 1733–1741.
66. Ryan, C. W., Vokes, E. E., Vogelzang, N. J., Janisch, L., Kobayashi, K., and Ratain, M. J. (2002) *Cancer Chemother. Pharmacol.* **50**, 1–5.
67. Nellaiappan, K., Rizzitano, A., Liu, G., Nicklas, G., and Kagan, H. M. (2000) *J. Cell. Biochem.* **79**, 576–582.
68. Li, W., Nellaiappan, K., Strassmaier, T., Graham, L., Thomas, K. M., and Kagan, H. M. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 12817–12822.
69. Giampuzzi, M., Botti, G., Di Duca, M., Arata, L., Ghiggeri, G., Gusmano, R., Ravazzolo, R., and Di Donato, A. (2001) *J. Biol. Chem.* **276**, 29226–29232.
70. Giampuzzi, M., Oleggini, R., and Di Donato, A. (2003) *Biochim. Biophys. Acta* **1647**, 245–251.
71. Kagan, H. M., Williams, M. A., Calaman, S. D., and Berkowitz, E. M. (1983) *Biochem. Biophys. Res. Commun.* **115**, 186–192.
72. Giampuzzi, M., Botti, G., Cilli, M., Gusmano, R., Borel, A., Sommer, P., and Di Donato, A. (2001) *J. Biol. Chem.* **276**, 29226–29232.
73. Giampuzzi, M., Oleggini, R., and Di Donato, A. (2003) *Biochim. Biophys. Acta* **1647**, 239–244.
74. Jeay, S., Pianetti, S., Kagan, H. M., and Sonenshein, G. E. (2003) *Mol. Cell. Biol.* **23**, 2251–2263.
75. Liu, G., Nellaiappan, K., and Kagan, H. M. (1997) *J. Biol. Chem.* **272**, 32370–32377.
76. Cronshaw, A. D., MacBeath, J. R., Shackleton, D. R., Collins, J. F., Fethergill-Gilmore, L. A., and Hulmes, D. J. (1993) *Matrix* **13**, 255–266.
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