Cloning of Genes Expressed in Cell Quiescence: A New Function of the *ras*-Recision/Lysyl Oxidase Gene

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Abstract

The mechanism governing cell quiescence remains to be elucidated, albeit some tumor suppressor genes are known to be involved in this process. If more genes belonging to this regulatory circuit are identified, we will have a better understanding on cell quiescence. For this purpose, the present study was designed to clone genes preferentially expressed in cell quiescence. Using the method of differential display, we cloned *ras*-recision gene (*rrg*), also known as lysyl oxidase gene (*lox*), from BALB/c 3T3T cells, which were rendered quiescent by serum deprivation. Northern blot analysis showed that the induction of *rrg/lox* gene could be detected as early as 12 h following serum deprivation and it was dramatically elevated from 24 hours on after serum starvation. Induction of *rrg/lox* was also observed in cells rendered quiescent by contact inhibition, indicating that *rrg/lox* is induced by cell quiescence in general rather than specific to serum deprivation. Because *rrg/lox* gene products are known to be involved in extracellular matrix maturation, and function as tumor suppressors against *ras* oncogene, our finding suggests that quiescence-associated cell physiology is partly mediated by induction of *rrg/lox*.

Key Words: *ras*-recision gene, lysyl oxidase gene, cell quiescence

Introduction

Quiescent cells are in the nonproliferative state, yet preserve the potential to reinitiate cell cycle. The quiescent cells serve as reserve cells that can either proliferate to replenish cell population or differentiate to acquire new phenotype. Although it may be debatable whether cell quiescence induced by serum-deprivation or contact-inhibition is "out-of-cycle" or can be defined as an arrested point in the G1 phase (8), it has been reported that approximately 3% of the mRNAs expressed in the quiescent cells were not present in the proliferating cells (41).

So far, at least 40 quiescence-specific genes have been identified (1, 5, 11, 18, 23, 28, 31, 39, 40, 44). Some of these genes appear to be important in regulating cell proliferation. For example, *EQ*-1 (mouse homolog of human dermatopontin), a quiescence-inducible gene, could induce cell arrest after being transfected into cells (39), suggesting that *EQ*-1 may play a role of autoregulation for growth inhibition. Similarly, decorin could be induced by cell quiescence (23) and, when overexpressed, could also inhibit proliferation of Chinese hamster ovary cells (42) by blocking the activity of transforming growth factor (43). In addition, the colon carcinoma cells lost their transformed phenotypes, such as tumorigenecity, after being stably transfected with an

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exogenous decorin-expressing gene (30).

Although how these quiescence-specific genes are induced in the environmental cue remains largely unknown, transcriptional regulatory control is involved in some quiescence-inducible genes, such as SV40 viral early promoter (38). The fact that there are genes induced by cell quiescence indicates that it is an active process when cells exit from the cycle into the reversible growth arrest state. The demonstration of tumor suppressor activity in some of these quiescent genes, such as decorin, has made this kind of study attractive and, therefore, prompted us to search for other genes that are induced by cell quiescence.

Materials and Methods

Cell Cultures

3T3T cells are nontransformed mesenchymal stem cells derived from A31 BALB/c 3T3 cells (36). Stock cells were cultured at low density in Dulbecco's modified Eagle's medium (DMEM; Sigma, St. Louis, MO, USA) containing 10% bovine serum (HyClone, Logan, UT, USA). Cells were incubated in a humidified 5% CO_2 incubator at 37°C. Medium was changed three times weekly and stock cells were passed once a week at 1:20 with 0.1% trypsin and 1 mM ethylenediaminetetra-acetic acid (EDTA) in phosphate-buffered saline lacking divalent-cations. Tests for mycoplasma were routinely negative throughout the entire experiment.

The general protocol of the cell culture was similar to the procedures described previously (36). In brief, serum deprivation-induced quiescence was achieved by culture of cells in DMEM containing 0.5% bovine serum for 3 days. Contact-inhibited quiescent cells were prepared by culture of post-confluent cells in 10% serum-containing DMEM. Determination of the quiescent state of cultured cells was performed by standard ^{3}H-thymidine incorporation assays to measure DNA synthesis.

Differential Display Procedure

Total RNA was extracted according to previously described procedure (37). RNAs isolated from the serum-deprived quiescent cells and from the exponentially proliferating cells were subjected to the method of differential display according to the manusfacture's protocols (GenHunter Co., Nashivelle, TN, USA). A set of anchored primers, oligo-dT of 13 nucleotides ($T_{11}M$; M represents A, C, or G.) was used for reverse transcription. The cDNAs were then PCR-amplified by adding an arbitrary primer of 10 nucleotides of defined sequence to the reverse transcriptase mixture along with Taq polymerase. The randomly amplified cDNAs were resolved by a denaturing polyacrylamide gel electrophoresis. DNA fragments that were present in the quiescent cells and absent in the exponentially proliferating cells were determined by inspecting the suitably exposed films. The selected bands were then eluted from the gel and re-amplified.

Northern Blot Analysis

For Northern blot analyses, 30 μg total cell RNA were denatured and fractionated electrophoretically using a 1.2% agarose gel containing 3% formaldehyde, and then transferred by blotting to nitrocellulose filters. Blots were prehybridized for 24 h at 42°C with 5 × standard saline citrate (SSC), 5 × Denhardt's solution, 25 mM potassium phosphate, 50% formamide, and 100 μg/ml denatured salmon sperm DNA. Hybridization was carried out overnight at 42°C in the same solution containing ^{32}P-labeled DNA probes. The DNA probes for *rrg/lox*, β-actin, tubulin, and GAPDH (American Type Cell Culture Collection) were labeled by using a standard nick translation procedure according to the manufacture's instructions (New England Nuclear, Du Pont Co., Boston, MA, USA). Blots were washed with two changes of 1 × sodium chloride-SSC/0.1% sodium dodecyl sulfate (SDS) for 15 min, followed by a final wash in 0.25 × SSC/0.1% SDS for 10 min at room temperature. After the final wash, the filters were autoradiographed with intensifying screens at -70°C.

Results

Identification of the Quiescence-Specific Genes by Differential Display

Murine 3T3T cells exponentially proliferate at low cell density when the culture medium contains adequate growth factors, such as 10% serum. 3T3T cells become quiescent when the culture medium contains serum less than 0.5% regardless of the cell density, a phenomenon referred to serum deprivation or serum starvation. 3T3T cells can also become quiescent after the cell density reaches confluence for 3 days even though the culture medium contains abundant growth factors, a culture condition namely contact inhibition.

The serum-deprived quiescent 3T3T cells confirmed by ^{3}H-thymidine incorporation were used to clone the quiescence-specific genes. Total RNAs isolated from actively proliferating cells and serum-deprived (0.5% calf serum for 72 h) quiescent cells were subjected to PCR differential display. Ten PCR-products were verified to be relatively specific to quiescent cells (Fig. 1a). These quiescence-specific

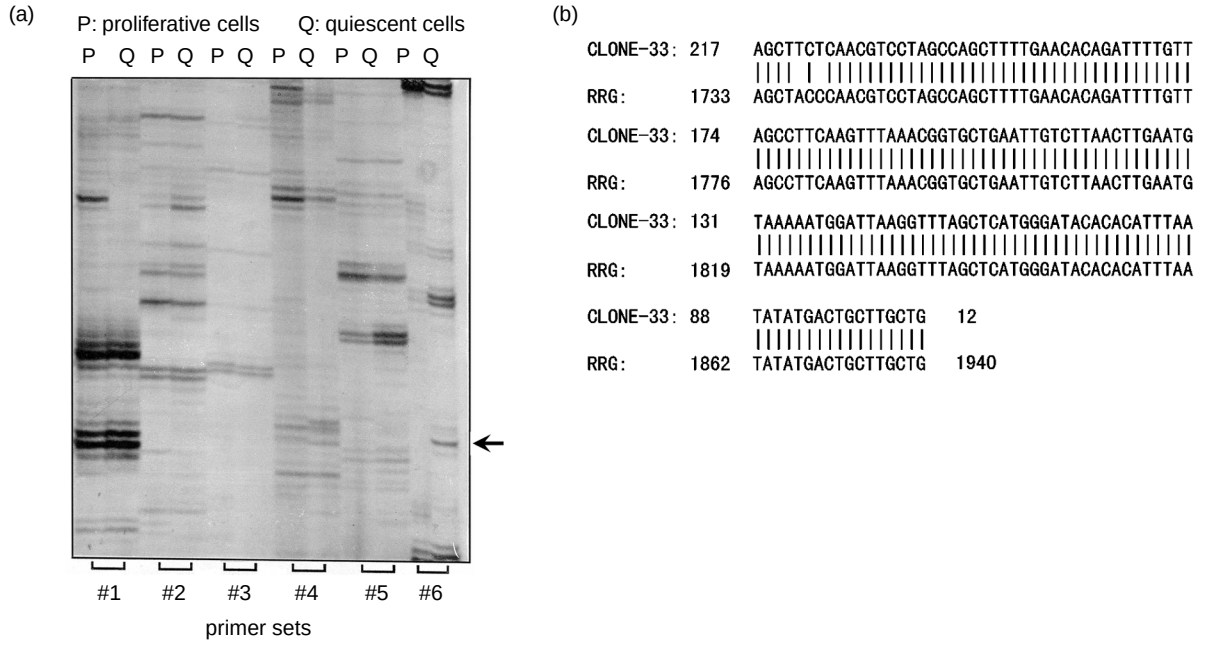

Fig. 1. Identification of *ras*-recision gene from the serum-deprived cells by the differential display. (a) This polyacrylamide gel shows the clone-33 (arrow) preferentially expressed in quiescent cells. (b) The partial cDNA sequences of the clone-33 reveal 98% (144/146) identity to *ras* recision gene (*rrg*).

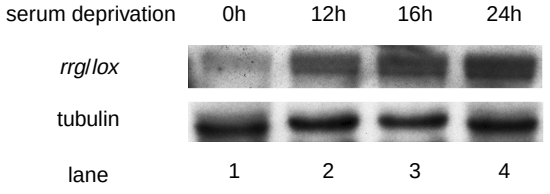

Fig. 2. The kinetics of lysyl oxidase expression after serum deprivation. Northern analysis shows a marked induction the *rrg/lox* expression during the first 12 h following serum deprivation, whereas the expression of tubulin (internal control) remains unchanged.

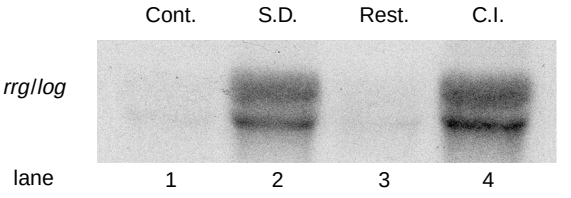

Fig. 3. The expression of lysyl oxidase gene following serum restimulation or contact inhibition. Filters containing RNAs isolated from proliferating cells (lane 1), serum-deprived quiescent cells (lane 2), quiescent cells following 24 h restimulation by medium containing 10% calf serum (lane 3), and cells rendered quiescent by contact inhibition (lane 4) were hybridized with the clone-33 probe.

PCR-products were eluted from the gel for re-amplification and then sequenced. The clone-33 was found to be identical to *ras*-recision gene (*rrg*) or lysyl oxidase gene (*lox*) analyzed by "Blasting" comparison with NCBI/B\GenBank (Fig. 1b).

Modulation of rrg/lox Expression Following Serum Deprivation and Contact Inhibition

To further characterize the effects of serum deprivation on the expression of *rrg/lox*, Northern blot analyses were performed to measure the expression levels in cells at various time points during the course of serum deprivation. As shown in the Fig. 2, the *rrg/lox* was barely expressed in cells at the proliferating phase but was dramatically induced by serum deprivation for 24 h. The clone-33 hybridized with two mRNAs at 4.5 and 5.5 kbs. Such a doublet was also found in the previously published study (20) and was due to alternate polyadenylation signals (13).

On the contrary, the *rrg/lox* gene expressed quiescent 3T3T cells were restimulated by changing medium to 10% serum containing DMEM. Following stimulation for 24 h, the cells were harvested for RNAs isolation. The Northern blot analysis showed that the expression of *rrg/lox* in the reactivated quiescent cells declined to the same levels as that of proliferating cells (Fig. 3).

Because *rrg/lox* was cloned from cells rendered quiescent by serum deprivation, it was intriguing to examine if the *rrg/lox* could be induced by other

means without serum deprivation. For this purpose, we examined the *rrg/lox* level in contact-inhibited quiescent cells, and the results showed that *rrg/lox* expression was also induced in contact-inhibited cells to the level equivalent to that of the serum-deprived cells (Fig. 3). Because cell quiescence mediated by contact inhibition occurs in the presence of sufficient serum, the *rrg/lox* gene expression is induced by cell quiescence rather than serum deprivation.

Discussion

In this study, we used the differential display technique to clone quiescent genes from BALB/c 3T3T cells and thereby rediscovered *rrg/lox*, which is known to convey tumor suppressor effect on *ras* oncogene.

Lysyl oxidase (protein-lysine 6-oxidase, EC 1.4.3.13) is a secreted copper-containing amine oxidase that catalyzes the oxidative deamination of hydroxylysine and peptidyl lysine in tropocollagen and tropoelastin (16). It generates aldehyde residues that spontaneously undergo condensation, converting the soluble monomers into insoluble fibers in the extracellular matrix. Cross-linking of these deaminated lysine residues is critical for the stability of the extracellular matrix. Therefore, LOX is essential to maintain the connective tissue protein, and a deficiency in LOX activity has been reported in patients with Menkes syndrome, X-linked Cutis Laxa, and Ehlers-Danlos syndrome (32). In contrast, increased LOX activity is related to organ fibrosis (24, 33). The distribution of LOX in normal tissue has been reported in aorta, lung, vascular smooth muscle, placenta, and prostate (9, 20, 29, 35). Although LOX is known to be an extracellular protein, some observations have shown that processed LOX is also localized within the cells and nucleus (6, 22, 26). In addition, *lox* was found to be highly expressed in smooth muscle cells and fairly expressed in fibroblasts, vascular endothelium, keratinocytes, myofibroblasts, and myoepithelial cells (14, 27, 33, 34).

The inhibitory effect of *rrg/lox* on cell proliferation is consistent with its association with the tumor suppression. For examples, LOX activity is significantly reduced in the culture medium of a number of malignant cell lines (21) and in cells transformed by *ra*s oncogene (17). The decreased activity of LOX in malignant cells was due to diminished transcription of the *rrg/lox* gene by DNA methylation (4). From a clinical point of view, the absence of LOX favored the stromal invasion by breast cancer cells, whereas LOX produced by myofibroblasts resulted in a scar-like barrier around the *in situ* and invasive ductal breast carcinoma (27). In addition, loss of *rrg/lox* expression was observed during the progression of primary prostate carcinoma to metastasized tumor (29).

LOX was first purified more than two decades ago (15, 25). The gene encoding this enzyme was initially cloned from the rat aorta (35) and then repeatedly cloned from a number of laboratories for different purposes. First, *lox* had been reported as a tumor suppressor gene for three times (3, 12, 19). It was first recloned from cells that were initially transformed by *ras* oncogene and then reverted to non-transformed state following a long-term treatment of interferon (3). The term *rrg* was coined by Friedman *et al*. for its inhibition on the neoplastic transformation induced by *ras* oncogene (3, 17). Along the same line, this gene was verified from revertants of *ras*-transformed NIH 3T3 cells following azatyrosine treatment (19), and also from spontaneous revertants of H-*ra*s-transformed rat fibroblasts (12). A silence of the *rrg/lox* expression in normal rat kidney fibroblast cells by antisense method made these cells neoplastically transformed and associated with constitutive activation of *ras* proto-oncogene (10).

On the other hand, *rrg/lox* was identified to involve in the negatively regulated process of adipocyte differentiation (7). In that study, the expression of the *rrg/lox* gene was found to be enhanced in 3T3-L1 preadipocytes following the treatment of retinoid acid, an inhibitor of adipocyte differentiation. It was, therefore, suggested that the down-regulation of *rrg/lox* was prerequisite for adipocyte differentiation. Recently, this gene was recloned from murine fetal lungs following dexamethasone treatment, indicating that dexamethasone enhances *rrg/lox* gene expression (2). Surprisingly, the *rrg/lox* gene was verified to be highly expressed in quiescent cells in this study.

It is intriguing that *rrg/lox* gene has been repetitively discovered in different experimental approaches in the last 15 years. The common characteristics among these studies appeared to be cell quiescence or slow growing, i.e., flat cells reverted from *ras*-transformation (3, 12, 19), growth-arrested cells prior to adipocyte differentiation (7), mature lung cells following dexamethasone stimulation (2), or non-proliferating cells following contact inhibition or growth factor deprivation. Taken together these results, *rrg/lox* product may play a key function leading to cell quiescence.

In summary, *rrg/lox* is a multifunctional protein involving the extracellular matrix maturation, tumor suppression, adipocyte differentiation, and cell quiescence control.

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References

1. Buckbinder, L., Talbott, R., Seizinger, B.R. and Kley, N. Gene regulation by temperature-sensitive p53 mutants: identification of p53 response genes. *Proc. Natl. Acad. Sci. USA* 91: 10640-10644, 1994.
2. Chinoy, M.R., Zgleszewski, S.E., Cilley, R.E. and Krummel, T.M. Dexamethasone enhances *ras*-recision gene expression in cultured murine fetal lungs: role in development. *Am. J. Physiol. (Lung Cell. Mol. Physiol.)* 279: L312-L318, 2000.
3. Contente, S., Kenyon, K., Rimoldi, D. and Friedman, R.M. Expression of gene *rrg* is associated with reversion of NIH 3T3 transformed by LTR-c-H-ras. *Science* 249: 796-798, 1990.
4. Contente, S., Kenyon, K., Sriraman, P., Subramanyan, S. and Friedman, R.M. Epigenetic inhibition of lysyl oxidase transcription after transformation by *ras* oncogene. *Mol. Cell. Biochem*. 194: 79-91, 1999.
5. Coppock, D.L., Kopman, C., Scandalis, S. and Gilleran, S. Preferential gene expression in quiescent human lung fibroblasts. *Cell Growth Differ*. 4: 483-493, 1993.
6. Di Donato, A., Lacal, J.C., Di Duca, M., Giampuzzi, M., Ghiggeri, G. and Gusmano, R. Micro-injection of recombinant lysyl oxidase blocks oncogenic p21-Ha-Ras and progesterone effects on Xenopus laevis oocyte maturation. *FEBS Lett*. 419: 63-68, 1997.
7. Dimaculangan, D.D., Chawla, A., Boak, A., Kagan, H.M. and Lazar, M.A. Retinoic acid prevents downregulation of *ras* recision gene/lysyl oxidase early in adipocyte differentiation. *Differentiation* 58: 47-52, 1994.
8. Epifanova, O.I. and Polunovsky, V.A. Cell cycle controls in higher eukaryotic cells, resting state or a prolonged G1 period? *J. Theor. Biol*. 120: 467-477, 1986.
9. Gacheru, S.N., Thomas, K.M., Murray, S.A., Csiszar, K., Smith-Mungo, L.I. and Kagan, H.M. Transcriptional and post-transcriptional control of lysyl oxidase expression in vascular smooth muscle cells: effects of TGF-beta 1 and serum deprivation. *J. Cell. Biochem.* 65: 395-407, 1997.
10. Giampuzzi, M., Botti, G., Cilli, M., Gusmano, R., Borel, A., Sommer, P. and Di Donato, A. Down-regulation of lysyl oxidase-induced tumorigenic transformation in NRK-49F cells characterized by constitutive activation of *ras* proto-oncogene. *J. Biol. Chem.* 276: 29226-29232, 2001.
11. Gustincich, S. and Schneider, C. Serum deprivation response gene is induced by serum starvation but not by contact inhibition. *Cell Growth Differ*. 4: 753-760, 1993.
12. Hajnal, A., Klemenz, R. and Schafer, R. Up-regulation of lysyl oxidase in spontaneous revertants of H-*ras*-transformed rat fibroblasts. *Cancer Res*. 53: 4670-4675, 1993.
13. Jourdan-Le Saux, C., Gleyzal, C., Garnier, J.M., Peraldi, M., Sommer, P. and Grimaud, J.A. Lysyl oxidase cDNA of myofibroblast from mouse fibrotic liver. *Biochem. Biophys. Res. Commun*. 199: 587-592, 1994.
14. Jourdan-Le Saux, C., Gleyzal, C., Raccurt, M. and Sommer, P. Functional analysis of the lysyl oxidase promoter in myofibroblast-like clones of 3T6 fibroblast. *J. Cell. Biochem*. 64: 328-341, 1997.
15. Kagan, H.M., Sullivan, K.A., Olsson, T.A. and Cronlund, A.L. Purification and properties of four species of lysyl oxidase from bovine aorta. *Biochem. J*. 177: 203-214, 1979.
16. Kagan, H.M. and Trackman, P.C. Properties and function of lysyl oxidase. *Am. J. Respir. (Cell Mol. Biol.)* 5: 206-210, 1991.
17. Kenyon, K., Contente, S., Trackman, P.C., Tang, J., Kagan, H.M. and Friedman, R.M. Lysyl oxidase and *rrg* messenger RNA. *Science* 253: 802, 1991.
18. Kozian, D.H. and Augustin, H.G. Rapid identification of differentially expressed endothelial cell genes by RNA display. *Biochem. Biophy. Res. Commun*. 209: 1068-1075, 1995.
19. Krzyzosiak, W.J., Shindo-Okada, N., Teshima, H., Nakajima, K. and Nishimura, S. Isolation of genes specifically expressed in flat revertant cells derived from activated *ras*-transformed NIH 3T3 cells by treatment with azatyrosine. *Proc. Natl. Acad. Sci. USA* 89: 4879-4883, 1992.
20. Kuivaniemi, H., Savolainen, E.R. and Kivirikko, K.I. Human placental lysyl oxidase. Purification, partial characterization, and preparation of two specific antisera to the enzyme. *J. Biol. Chem.* 259: 6996-7002, 1984.
21. Kuivaniemi, H., Korhonen, R.M., Vaheri, A. and Kivirikko, K.I. Deficient production of lysyl oxidase in cultures of malignantly transformed human cells. *FEBS Lett.* 195: 261-264, 1986.
22. Li, W., Nellaiappan, K., Strassmaier, T., Graham, L., Thomas, K.M. and Kagan, H.M. Localization and activity of lysyl oxidase within nuclei of fibrogenic cells. *Proc. Natl. Acad. Sci. USA* 94: 12817-12822, 1997.
23. Mauviel, A., Santra, M., Chen, Y.Q., Uitto, J. and Iozzo, R.V. Transcriptional regulation of decorin gene expression, induction by quiescence and repression by tumor necrosis factor-alpha. *J. Biol. Chem*. 270: 11692-11700, 1995.
24. Murawaki, Y., Kusakabe, Y. and Hirayama, C. Serum lysyl oxidase activity in chronic liver disease in comparison with serum levels of prolyl hydroxylase and laminin. *Hepatology* 14: 1167-1173, 1991.
25. Narayanan, A.S., Siegel, R.C. and Martin, G.R. Stability and purification of lysyl oxidase. *Arch. Biochem. Biophys*. 162: 231-237, 1974.
26. Nellaiappan, K., Risitano, A., Liu, G., Nicklas, G. and Kagan, H.M. Fully processed lysyl oxidase catalyst translocates from the extracellular space into nuclei of aortic smooth-muscle cells. *J. Cell. Biochem*. 79: 576-582, 2000.
27. Peyrol, S., Raccurt, M., Gerard, F., Gleyzal, C., Grimaud, J.A. and Sommer, P. Lysyl oxidase gene expression in the stromal reaction to *in situ* and invasive ductal breast carcinoma. *Am. J. Pathol*. 150: 497-507, 1997.
28. Pignolo, R.J., Cristofalo, V.J. and Rotenberg, M.O. Senescent WI-38 cells fail to express EPC-1, a gene induced in young cells upon entry into the G0 state. *J. Biol. Chem.* 268: 8949-8957, 1993.
29. Ren, C., Yang, G., Timme, T.L., Wheeler, T.M. and Thompson, T.C. Reduced lysyl oxidase messenger RNA levels in experimental and human prostate cancer. *Cancer Res.* 58: 1285-1290, 1998.
30. Santra, M., Skorski, T., Calabretta, B., Lattime, E.C. and Iozzo, R.V. De novo decorin gene expression suppresses the malignant phenotype in human colon cancer cells. *Proc. Natl. Acad. Sci. USA* 92: 7016-7020, 1995.
31. Schneider, C., King, R.M. and Philipson, L. Genes specifically expressed at growth arrest of mammalian cells. *Cell* 54: 787-793, 1988.
32. Siegel, R.C. Lysyl oxidase. *Int. Rev. Connect. Tissue Res.* 8: 73-118, 1979.
33. Sommer, P., Gleyzal, C., Raccurt, M., Delbourg, M., Serrar, M., Joazeiro, P., Peyrol, S., Kagan, H., Trackman, P.C. and Grimaud, J.A. Transient expression of lysyl oxidase by liver myofibroblasts in murine schistosomiasis. *Lab. Invest.* 69: 460-470,1993.
34. Song, Y.L., Ford, J.W., Gordon, D. and Shanley, C.J. Regulation of lysyl oxidase by interferon-gamma in rat aortic smooth muscle cells. *Arterioscler. Thromb. Vasc. Biol.* 20: 982-988, 2000.
35. Trackman, P.C., Pratt, A.M., Wolanski, A., Tang, S.S., Offner, G.D., Troxler, R.F. and Kagan, H.M. Cloning of rat aorta lysyl oxidase cDNA, complete codons and predicted amino acid sequence. *Biochemistry* 29: 4863-4870, 1990.
36. Tzen, C.Y., Filipak, M. and Scott, R.E. Metaplastic change in

mesenchymal stem cells induced by activated *ras* oncogene. *Am. J. Pathol*. 137: 1091-1102, 1990.
37. Tzen, C.Y., Cox, R.L. and Scott, R.E. Coordinate induction of IκBα and NFκB genes. *Exp. Cell Res*. 211: 12-16, 1994.
38. Tzen, C.Y., Scott, R.E. and Robinson, F.D. Serum deprivation induces SV40 early promoter activity. *Cell Prolif*. 30: 53-60, 1997.
39. Tzen, C.Y. and Huang, Y.W. Cloning of murine early quiescence-1 gene: the murine counterpart of dermatopontin gene can induce and be induced by cell quiescence. *Exp. Cell Res*. 294: 30-38, 2004.
40. Weir, L., Chen, D., Pastore, C., Isner, J.M. and Walsh, K. Expression of *gax*, a growth arrest homeobox gene, is rapidly down-regulated in the rat carotid artery during the proliferative response to balloon injury. *J. Biol. Chem*. 270: 5457-5461, 1995.
41. Williams, J.G. and Penman, S. The messenger RNA sequences in growing and resting mouse fibroblasts. *Cell* 6: 197-206, 1975.
42. Yamaguchi, Y. and Ruoslahti, E. Expression of human proteoglycan in Chinese hamster ovary cells inhibits cell proliferation. *Nature* 336: 244-246, 1988.
43. Yamaguchi, Y., Mann, D.M. and Ruoslahti, E. Negative regulation of transforming growth factor-beta by the proteoglycan decorin. *Nature* 346: 281-284, 1990.
44. Zhan, Q., Lord, K.A., Alamo, I. Jr., Hollander, M.C., Carrier, F., Ron, D., Kohn, K.W., Hoffman, B., Liebermann, D.A. and Fornace, A.J. Jr. The *gadd* and *MyD* genes define a novel set of mammalian genes encoding acidic proteins that synergistically suppress cell growth. *Mol. Cell. Biol*. 14: 2361-2371, 1994.