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Lysyl Oxidase and *rrg* Messenger RNA

Several of us recently reported that the *rrg* gene was a putative tumor suppressor gene or anti-oncogene of *ras* (1). Mouse NIH 3T3 cell lines transformed by *ras* expressed almost no *rrg*, in contrast with nontransformed NIH 3T3 cell lines and interferon-reverted cell lines. Revertants continued to express *ras* p21 and mRNA in amounts comparable to those expressed by transformed cell lines. A revertant cell line transfected with an *rrg* antisense expression construct became retransformed. These data

Table 1. Lysyl oxidase activity in cell lines. Lysyl oxidase activity was assayed in both media and cell extract, summed to give total activity, and normalized against cell number and cell protein. In all cell lines, more than 90% of the total lysyl oxidase activity was in the media. ND, not detectable. AS, antisense cell lines, PR4 cells with *rrg* antisense expression construct; S, sense cell lines, PR4 cells with *rrg* sense expression construct; AS3BT1, cell line cultured from tumor induced in a nude mouse by subcutaneous injection of cell line AS-3B. Tumors grew to ~1 cm in diameter within 7 days of first appearance (+++), within 7 to 14 days of first appearance (++) , or 14 days or more after first appearance (+); or they did not grow (-).

Cell line	Total lysyl oxidase activity (11)		Tumor growth (1)
	Cpm/10 ⁶ cells	Cpm/ μ g protein	
NIH 3T3	11,945	22.1	-
PR4	5,260	20.9	-
RS485	620	2.7	+++
AS-3B	1,486	9.2	+++
AS-3BT1	ND	ND	-
AS-30	1,744	11.5	+++
AS-4	4,065	12.3	+
S-10	17,927	38.5	-
S-16	6,267	33.3	-

suggested that the regulated expression of *rrg* product forms a part of the pathway of cell transformation by *ras*.

A search (2) of GenBank Release 65.0 and PIR Release 26.0 revealed a match between *rrg* cDNA sequences (3) and a 2672-bp cDNA of rat lysyl oxidase (4). The two nucleotide sequences were 92% identical, but the protein sequences were only 79% similar with two blocks of nonhomologous sequences interrupting the alignment. Frame shifts at four locations restored identity; each shift location was rich in GC. GC-rich areas are often compressed on sequencing gels. The sequences of *rrg* and rat lysyl oxidase cDNA in these areas were verified with the dGTP analog dITP, which prevents compressions (5). Several base insertions in the rat sequence restored exact alignment with *rrg* (6). Therefore, *rrg*, a regulator of *ras* expression, encodes lysyl oxidase.

Determinations of lysyl oxidase activity in the culture media of NIH 3T3 and derived cell lines (Table 1) indicated a direct correlation between lysyl oxidase activity and *rrg* mRNA expression (Fig. 1). Nontransformed cell lines exhibited high lysyl oxidase activity and large amounts of mRNA, while the transformed lines had low lysyl oxidase activity and small amounts of mRNA.

The down-regulation of lysyl oxidase expression in transformation and the induction of lysyl oxidase in interferon-mediated reversion of transformed cells suggests that this enzyme plays a role in tumor suppression. Lysyl oxidase is a copper-dependent amine oxidase that catalyzes the oxidative deamination of peptidyl lysine in elastin and collagen; intra- and intermolecular conden-

sations then form covalent cross-linkages that insolubilize these matrix proteins (7). The cDNA sequences each predict signal peptide sequences and cleavage sites as well as potential sites for N-glycosylation (4, 6). This is consistent with the known secretory fate of lysyl oxidase in cultured cells (Table 1) (8). Thus, if catalytic activity is essential to the reversion process, it likely occurs in extracellular space.

There is ample evidence for the modulation of cell phenotype by the extracellular matrix (9). Intracellular communication with extracellular cross-linked collagen or elastin may be critical to the present observation. Alternatively, as lysyl oxidase can act on proteins other than elastin and collagen in vitro (10), it may oxidize other accessible proteins such as membrane-bound receptors that are capable of transducing signals through *ras*, or it may oxidize other matrix components to modulate matrix-cell communication, which is important to the non-transformed phenotype.

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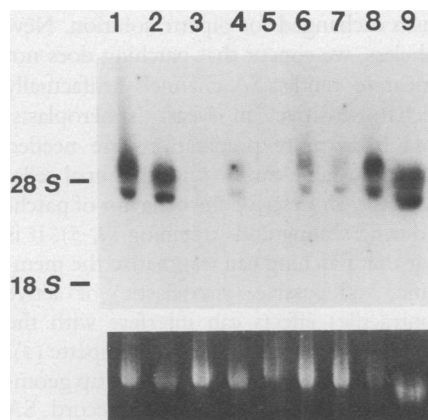


Fig. 1. RNA blot hybridized with rat lysyl oxidase cDNA (4). Total cellular RNA (15 μ g) was separated by electrophoresis on a 1% agarose-formaldehyde gel and transferred to a nitrocellulose membrane. Cell lines as in Table 1. Lanes: 1, NIH 3T3; 2, PR4 (revertant of RS485); 3, RS485 (*ras*-transformed NIH 3T3); 4, AS-3B; 5, AS-3BT1; 6, AS-30; 7, AS-4; 8, S-10; 9, S-16. Bottom panel shows an ethidium bromide staining of 28S ribosomal RNA.

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Lysyl oxidase and rrg messenger RNA

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