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# REVERSION OF MALIGNANT PHENOTYPES OF HUMAN LUNG SQUAMOUS CARCINOMA CELLS BY ORNITHINE DECARBOXYLASE ANTISENSE RNA

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**Abnormally elevated activity of ornithine decarboxylase (ODC), and subsequent polyamine accumulation are intimately associated with the genesis, development and metastasis of cancer. In the present study, to control the growth of tumor cells, ODC antisense RNA was used to transfect human lung squamous carcinoma cell line LTEP-78. Compared with the parental cells, growth of the antisense transfected LTEP-78 cells arrested in G0/G1 phase and colony formation in soft agarose and tumorigenicity in nude mice were significantly reduced. Nucleic acid hybridization demonstrated that the transfectants expressed a high level of ODC antisense RNA and a significantly reduced level of endogenous ODC mRNA. The results suggest that the reversion of malignant phenotypes of human lung squamous carcinoma cells transfected with ODC antisense RNA is associated with the inhibition of polyamine biosynthesis.**

**Key words: Human lung squamous carcinoma cells, Ornithine decarboxylase, Antisense RNA**

Ornithine decarboxylase (ODC, EC 4.1.1.17), a rate-limiting enzyme in polyamine biosynthesis, plays a pivotal role in cell proliferation. Expression of this enzyme is transiently increased upon stimulation by growth factors, but becomes constitutively activated during cell transformation induced by carcinogens, viruses or oncogenes.[1,2] Recent reports also indicate that overexpression of ODC either dramatically increases the transforming activity of a mutated ras gene in R6 fibroblasts or results in the transformation of immortalized NIH3T3 cells.[3,4] Moreover, blocking the endogenous ODC enzyme using its specific inhibitor, difluromethylornithine (DFMO), or antisense ODC RNA prevents transformation of rat fibroblasts by v-src oncogene.[5] The previous studies indicated that the transfection of human lung squamous carcinoma cells with ODC antisense RNA expression plasmid, pCMV/ODCas, elicited cell growth suppression.[6] In this study, we further investigated the reversion of malignant phenotypes of human lung squamous carcinoma cells transfected by ODC antisense RNA.

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## MATERIALS AND METHODS

### Cells

Human lung squamous carcinoma LTEP-78 (L78) cells were obtained from Beijing Institute of Tuberculosis, L78/neo cells derived from transfected L78 cell with plasmid vector pRc/CMV, L78/ODCas1 and L78/ODCas4 derived from transfected L78 cells with ODC antisense RNA expression plasmid pCMV/ODCas.[6]

**Culture Conditions**

All cell lines were maintained in RPMI1640 medium supplemented with 10% fetal calf serum (FCS), a humidified incubator at 37 ℃ with an atmosphere of 95% air and 5% CO2 was used.

**Cell Cycle Analysis**

Approx. $2\times10^6$ cells were trypsin-treated and harvested as a single-cell suspension. Cells were pelleted at 800 rev./min. for 10 min. at 4 ℃, washed twice with ice-cold phosphate-buffered saline, and fixed in 70% ethanol. Then, cell-cycle distribution of transfectants and parental cells was carried out as described in detail by Basu, et al.[7]

**Anchorage-independent Cloning Assay**

$2\times10^2$ cells were suspended in 1 ml of 0.33% agrose solution containing RPMI1640 plus 10% FCS and overlayered onto 1-ml layer of 0.5% agrose gel in 24-well plates (Nunc). All culture were refed once weekly. Colonies greater than 50 cells were scored and the efficiency of colony formation was determined after 2 weeks of culture.

**Tumorigenicity Assay in Nude Mice**

To test for tumorigenicity, $5\times10^6$ cells in 0.2 ml serum-free RPMI1640 medium were injected s.c. into the right groin of 6-week-old male BALB/c nude mice. The formation and growth of tumors in mice were observed.

**Probes and RNA Analysis**

To determine ODC antisense RNA and mRNA, RNA probes were prepared from pCMV/ODCs and pCMV/ODCas respectively. These plasmids were linearized with Not I, and the RNA probes were synthesized by T7 RNA polymerase. Total RNA was extracted from the exponentially growing cells and analyzed by Dot blot analysis with $^{32}$P-labeled RNA probes. Hybridization was performed according to Sambrook J, et al.[8]

## RESULTS

Table 1 showed that the percentage of antisense transfectants arrested in G0/G1-phase increased, while that in s-phase decreased as compared with parental L78 cells.

*Table 1. Cell cycle distribution of antisense transfectants and parental L78 cells*

| Cell Type | Cells in phase (%) | | |
|---|---|---|---|
| | G0/G1 | S | G2/M |
| L78 | 32 | 38 | 30 |
| L78/ODCas1 | 39 | 31 | 30 |
| L78/ODCas4 | 40 | 27 | 33 |

As shown in Table 2, the cells transfected with ODC antisense expression plasmid had a marked inhibition of colony formation in soft agarose, compared to the clonogenic efficiency of parental L78 cells or vector control cells (L78/neo). Moreover, the colony sizes of antisense transfectants in soft agarose were obviously smaller than those of L78 and L78/neo cells (Figure 1).

*Table 2. Efficiency of colony formation of L78 cells and the transfectants in soft agarose*

| Cell Type | Efficiency of colony formation (%) |
|---|---|
| L78 | 27 ± 4. 6 |
| L78/neo | 24 ± 3. 0 |
| L78/ODCas1 | 4. 5 ± 2. 0 |
| L78/ODCas4 | 3 ± 1. 3 |

As shown in Table 3, parental L78 cells and the cells transfected with empty vector gave tumors in all nude mice with a latent period of 12-15 days, whereas the antisense transfectants produced no tumors in any of the five nude mice within 2 monthes after inoculation of these cells.

*Table 3. Growth of L78 cells and the transfectants in nude mice*

| | Animals with tumors* | |
|---|---|---|
| Cell Type | No. of animals | Latent period(days) |
| L78 | 3/3 | 12-15 |
| L78/neo | 3/3 | 12-15 |
| L78/ODCas1 | 0/2 | NA** |
| L78/ODCas4 | 0/3 | NA** |

* shown are the number of animals with tumors detected 2 months postinoculation

**NA, not applicable.

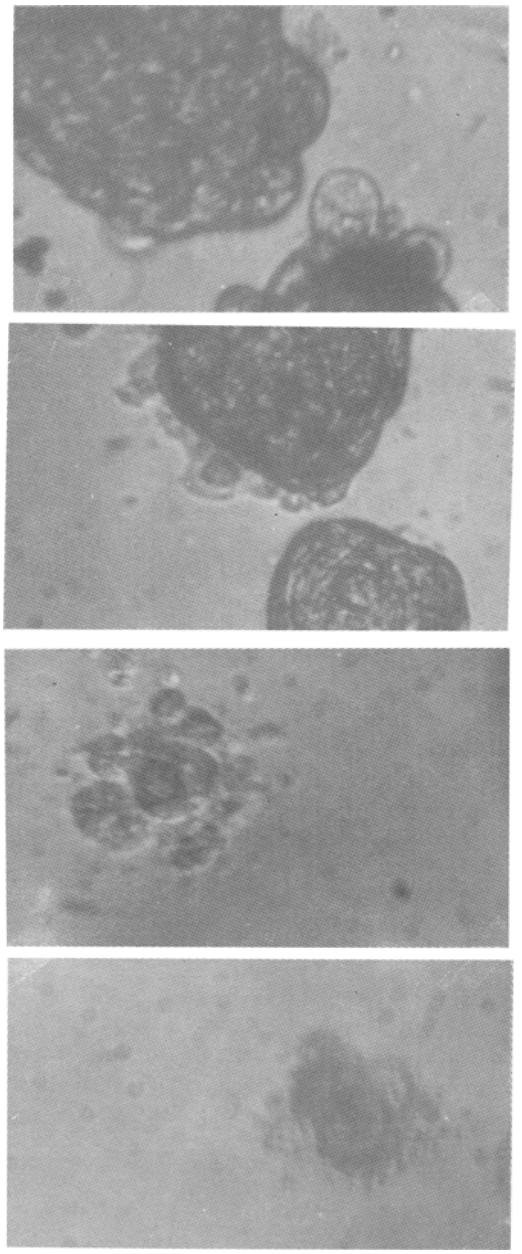

Fig 1. Colony formation of L78 cells and the transfectants in soft agarose . A. L78; B. L78/neo; C. L78/ODCas1; D. L78/ODCas4

As shown in Figure 2, L78/ODCas1 and L78/ODCas4 cells expressed high levels of antisense ODC RNA. Moreover, the two antisense -transfected cell lines had significantly reduced levels of ODC mRNA, compared with the levels of parental L78 cells and the vector control cells (Figure 3).

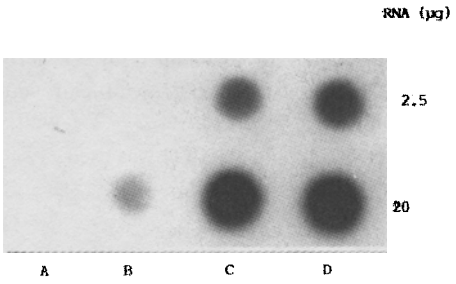


Fig 2. Expression of antisense ODC RNA in cells transfected with pCMV/ODCas.

A. L78; B. L78/neo; C. L78/ODCas1; D. L78/ODCas4.

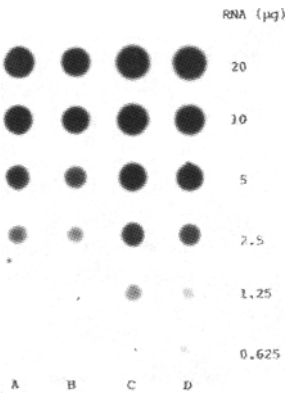


Fig 3. Expression of ODC mRNA in L78 cells and the transfectants.

A. L78/ODCas1; B. L78/ODCas4; C. L78; D. L78/neo

## DISCUSSION

Polyamines, including putrescine, spermidine and spermine, are essential for cellular growth and differentiation. Without polyamines cells stop dividing and cellular metabolism is markedly slowed down. Therefore, the polyamine metabolic pathway is a potential chemotherapeutic target.[9,10] Recent reports have shown that polyamine deprivation represents an important therapeutic tool in cancer treatment.[11] In order to control polyamine biosynthesis at gene level, and further to study the role of ODC in maintaining the malignant phenotypes of cancer, we have constructed previously an ODC antisense RNA expression plasmid containing human ODC cDNA in antisense orientation, with which human lung

squamous carcinoma L78 cells were transfected by the calcium phosphate precipitation method. The growth rate, the biosyntheses of DNA, RNA and proteins, and ODC activities of antisense transfectants were significantly suppressed.[6] In this study, we further observed that the fraction of G0/G1-phase cells increased, while that of S-phase cells decreased in antisense transfectants. it revealed that the transduction of ODC antisense sequence precluded entry of cells into S state; The ability of colony formation in soft agarose of the transfectants was much lower than that of parental cells; No tumors developed in any nude mice inoculated with antisense transfectants, while xenografts did in control mice inoculated with parental cells. Further studies demonstrated that the reversion of malignant phenotypes mentioned above was associated with the expression of ODC antisense RNA and the suppression of endogenous ODC mRNA expression. Our results not only show the important role of ODC in cell proliferation and maintenance of malignant phenotypes of cancer cells, but also provide the strong experiment basis to search new target genes for gene therapy of cancer.

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