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Enhanced Expression of a Glyceraldehyde-3-phosphate Dehydrogenase Gene in Human Lung Cancers[1]

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ABSTRACT

We have shown that a M_r 37,000 protein whose expression is strongly enhanced in human lung cancer tissues is the subunit of glyceraldehyde-3-phosphate dehydrogenase (GAPDH, EC 1.2.1.12). We have isolated a GAPDH complementary DNA from human lung cancer cells and deduced the complete amino acid sequence of the encoded protein. The structure of the lung cancer GAPDH is identical to that of liver GAPDH. In addition, we have found that GAPDH mRNA expression is markedly increased in human lung cancer tissues. These results disclose a molecular basis of increased glycolysis in cancer cells and reveal an important role of energy creating reaction in cancer cell growth.

INTRODUCTION

Studies of cancer-associated proteins are one of the most important areas of cancer research, since they provide not only effective diagnostic indicators for human cancers but also crucial clues to reveal the mechanisms of carcinogenesis and cellular growth control (1).

We have been studying a human lung cancer-associated protein (p37).[4] The expression of this protein is consistently enhanced in human lung cancer tissues of all histological types (2). Based on the agreement of biochemical characters including partial peptide sequences, we have suggested a strong similarity between p37 and the subunit of a glycolytic key enzyme, GAPDH (3).

In this paper, we show that p37 mRNA is arrested by a GAPDH cDNA confirming its identity with GAPDH. We describe the isolation of a GAPDH cDNA from human lung cancer cells and the identity of the encoded protein with liver GAPDH. We also show that GAPDH mRNA expression is strongly elevated in human lung cancer tissues.

MATERIALS AND METHODS

Proteins and RNAs. Analysis of lung cancer proteins was performed as described (2). Total RNA was extracted from tissue specimens by homogenization in guanidine thiocyanate and precipitation through CsCl (4).

Cell Lines. The human lung cancer cell lines used in this study are as follows: adenocarcinomas: PC-3 (5), PC-8 (5), PC-9 (5), PC-14 (6), A549 (7); squamous cell carcinoma: PC-1 (5); and small cell carcinomas: PC-6 (5), QG-90 (8). Cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum.

Hybrid Selection and Arrest of Translation. Hybrid selection and arrest of translation were done by the method described (9).

Isolation of a GAPDH cDNA from Lung Cancer Cells. A cDNA library was constructed from poly(A)$^+$ RNA of PC-9 cells with λgt11 phage as a vector (10). About 10,000 cDNA clones were screened with nick-translated GAPDH cDNA of human liver cells (11). Among the positive clones, one (λKS321) which has a full-length cDNA insert was chosen for further analysis. The insert of this clone was subcloned in pTZ18R (Pharmacia, Uppsala, Sweden) and designated as pKS321.

Sequence Determination. Nucleotide sequence was determined by the method of Sanger *et al.* (12). Both strands have been independently sequenced using a M13-shotgun library generated by sonication.

Northern Blot Analysis. Denaturing agarose gel electrophoresis and blot hybridization of RNA were done as described (13). Radioactive GAPDH probe was prepared with [α-^{32}P]dCTP by nick-translation of pKS321 DNA. The integrity and quantity of the RNA were controlled by the hybridization of the same filter with 18S rRNA plasmid (14).

RESULTS

Hybridization Arrest of p37 mRNA by GAPDH cDNA. In the previous report (3), we have shown that p37 is indistinguishable from the subunit of GAPDH. To confirm this similarity at the molecular level, we tried hybridization arrest of p37 mRNA with a human liver GAPDH cDNA (11). For this purpose, poly(A)$^+$ RNA extracted from PC-9 cells (5) was used. PC-9 cells express a large amount of p37 which is biochemically identical with that of the lung cancer tissues (data not shown). The GAPDH cDNA arrested the translation of p37 mRNA in rabbit reticulocyte lysates, while control DNA had no effect (Fig. 1). This cDNA also selected p37 mRNA for translation (data not shown). These results further confirm the identity of p37 with the subunit of GAPDH.

Structure of the GAPDH Subunit in Human Lung Cancer Cells. Since the human genome contains multiple GAPDH-related genes (11, 15–18), the information on the multiformity of the human GAPDH subunit is still tentative, and little is known about the lung GAPDH. To examine the precise structure of the subunit of GAPDH expressed in human lung cancer cells, we have isolated a cDNA for this protein. Fig. 2 lists the deduced nucleotide sequence of the isolated cDNA with the encoded amino acid sequence. The amino acid sequence completely matches with that of the subunit of human liver GAPDH which has been deduced from its cDNA (11). The nucleotide sequences of the two cDNAs disagree at eight positions (Table 1). Most of these differences are probably because of the polymorphism among the individuals. This result clearly shows that the GAPDH expressed in human lung cancer cells is identical with liver GAPDH and that it represents neither a cancer-specific form nor a fetal form that has been suggested by Arcari *et al.* (18).

GAPDH mRNA Expression in Human Lung Cancer Cells. To investigate the level of GAPDH mRNA in human lung cancer tissues, we have done Northern blot analyses of human lung cancer RNAs using the isolated GAPDH cDNA (pKS321) as a probe. As shown in Fig. 3*B*, significant expression of 1.3-kilobase mRNA for GAPDH was detected in lung cancer tissue

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[4] The abbreviations used are: p37, M_r 37,000 protein; SDS, sodium dodecyl sulfate; cDNA, complementary DNA; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; poly(A)$^+$ RNA, polyadenylate-containing RNA.

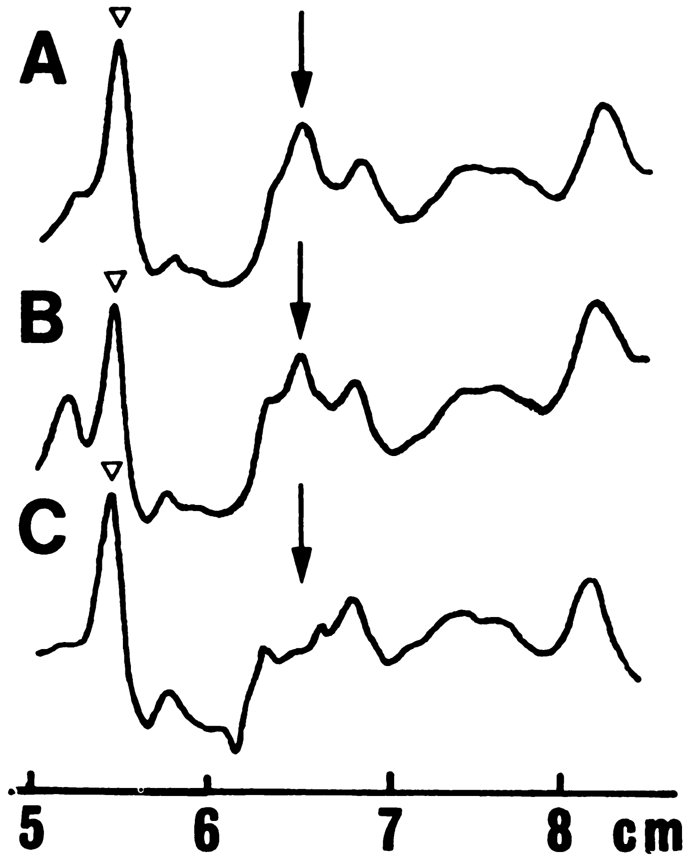

Fig. 1. Hybridization arrest of p37 mRNA by a human liver GAPDH cDNA. *A*, without DNA; *B*, pBR322; *C*, GAPDH cDNA. Poly(A)$^+$ RNA of PC-9 cells was annealed without or with the indicated DNA and translated in rabbit reticulocyte lysates. The translated products were fractionated through a 10% polyacrylamide gel containing 0.1% SDS followed by autoradiography. The X-ray film was scanned with a densitometer. *Arrows*, position of the translation product of p37 mRNA; ∇, cytoskeletal actin peak. Distance from the gel top is scaled below.

RNA with a good correlation with p37 expression which was analyzed side by side through a SDS-polyacrylamide gel (Fig. 3*A*). On the other hand, only a trace amount of GAPDH mRNA of an almost identical size was detected in the normal lung tissue RNA. These results clearly show that the expression of GAPDH is also elevated at the mRNA level.

We further examined the expression of GAPDH mRNA in cell lines of human lung cancers. Strong expression of GAPDH mRNA was detected in all the lung cancer cell lines examined regardless of their histological types (Fig. 4).

Contamination of Erythrocyte Products in the Extracts. Cancer tissues are often accompanied with increased angiogenesis, and erythrocytes are one of the cells rich in GAPDH (19). We examined the possibility of incidental contamination of red cell products in the lung cancer tissue extracts. Neither globin protein nor globin mRNA was detected in the lung cancer extracts (data not shown), indicating that the contamination of erythrocyte products is negligible, if it exists. Furthermore, the strong expression of GAPDH mRNA in established cell lines as described above also excludes the possibility.

DISCUSSION

We have shown that a M_r 37,000 protein whose expression is strongly enhanced in human lung cancers is the subunit of GAPDH. Nucleotide sequence analysis of a cDNA of lung cancer GAPDH has revealed the identity of p37 with liver GAPDH. We have shown that the GAPDH expression is significantly increased in human lung cancer tissues also at the mRNA level.

Since the discovery of Warburg (20) that cancer tissues excrete large quantities of lactate, the activation of the glycolytic cascade has been a well-documented property of a variety of cancers, but the mechanism has yet been obscure. Our observation discloses a molecular basis of this important feature of cancer cells.

Recently, independent of our research, Maehare *et al.* (21) have isolated a partial cDNA corresponding to a 1.5-kilobase mRNA whose expression is enhanced in rat ascites hepatomas. We have examined the nucleotide sequence of this cDNA and noticed that the amino acid sequence encoded by this cDNA agrees well with the carboxyl terminal portion of the GAPDH subunit. Strong expression of GAPDH mRNA has also been detected in cell lines of human hepatocarcinoma and kidney carcinoma (18). These facts indicate that the increased expression of GAPDH mRNA may not be a unique trait of lung cancers.

However, we detected GAPDH expression in normal tissue specimens of colon, kidney, and bladder and did not observe a significant increase in tumor counterparts of these organs (2). It is also difficult to notice significant correlation between the extent of GAPDH elevation and that of malignancy of lung adenocarcinomas (2). Considering these facts, it is important to define the range of cancers to which the enhancement of GAPDH expression is linked. To this goal, the human GAPDH

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                                                                 M  G  K  V  K  V  G  V  N  G  F  G  R  I  G  R  L  V  T  R
GTTCGACAGTCAGCCGCATCTTCTTTTGCGTCGCCAGCCGAGCCACATCGCTCAGACACCATGGGGAAGGTGAAGGTCGGAGTCAACGGATTTGGTCGTATTGGGCGCCTGGTCACCAGG
        10        20        30        40        50        60        70        80        90       100       110       120
 A  A  F  N  S  G  K  V  D  I  V  A  I  N  D  P  F  I  D  L  N  Y  M  V  Y  M  F  Q  Y  D  S  T  H  G  K  F  H  G  T  V
GCTGCTTTTAACTCTGGTAAAGTGGATATTGTTGCCATCAATGACCCCTTCATTGACCTCAACTACATGGTTTACATGTTCCAATATGATTCCACCCATGGCAAATTCCATGGCACCGTC
       130       140       150       160       170       180       190       200       210       220       230       240
 K  A  E  N  G  K  L  V  I  N  G  N  P  I  T  I  F  Q  E  R  D  P  S  K  I  K  W  G  D  A  G  A  E  Y  V  V  E  S  T  G
AAGGCTGAGAACGGGAAGCTTGTCATCAATGGAAATCCCATCACCATCTTCCAGGAGCGAGATCCCTCCAAAATCAAGTGGGGCGATGCTGGCGCTGAGTACGTCGTGGAGTCCACTGGC
       250       260       270       280       290       300       310       320       330       340       350       360
 V  F  T  T  M  E  K  A  G  A  H  L  Q  G  G  A  K  R  V  I  I  S  A  P  S  A  D  A  P  M  F  V  M  G  V  N  H  E  K  Y
GTCTTCACCACCATGGAGAAGGCTGGGGCTCATTTGCAGGGGGGAGCCAAAAGGGTCATCATCTCTGCCCCCTCTGCTGATGCCCCCATGTTCGTCATGGGTGTGAACCATGAGAAGTAT
       370       380       390       400       410       420       430       440       450       460       470       480
 D  N  S  L  K  I  I  S  N  A  S  C  T  T  N  C  L  A  P  L  A  K  V  I  H  D  N  F  G  I  V  E  G  L  M  T  T  V  H  A
GACAACAGCCTCAAGATCATCAGCAATGCCTCCTGCACCACCAACTGCTTAGCACCCCTGGCCAAGGTCATCCATGACAACTTTGGTATCGTGGAAGGACTCATGACCACAGTCCATGCC
       490       500       510       520       530       540       550       560       570       580       590       600
 I  T  A  T  Q  K  T  V  D  G  P  S  G  K  L  W  R  D  G  R  G  A  L  Q  N  I  I  P  A  S  T  G  A  A  K  A  V  G  K  V
ATCACTGCCACCCAGAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATCATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTC
       610       620       630       640       650       660       670       680       690       700       710       720
 I  P  E  L  N  G  K  L  T  G  M  A  F  R  V  P  T  A  N  V  S  V  V  D  L  T  C  R  L  E  K  P  A  K  Y  D  D  I  K  K
ATCCCTGAGCTGAACGGGAAGCTCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCTAGAAAAACCTGCCAAATATGATGACATCAAGAAG
       730       740       750       760       770       780       790       800       810       820       830       840
 V  V  K  Q  A  S  E  G  P  L  K  G  I  L  G  Y  T  E  H  Q  V  V  S  S  D  F  N  S  D  T  H  S  S  T  F  D  A  G  A  G
GTGGTGAAGCAGGCGTCGGAGGGCCCCCTCAAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACACCCACTCCTCCACCTTTGACGCTGGGGCTGGC
       850       860       870       880       890       900       910       920       930       940       950       960
 I  A  L  N  D  H  F  V  K  L  I  S  W  Y  D  N  E  F  G  Y  S  N  R  V  V  D  L  M  A  H  M  A  S  K  E  *
ATTGCCCTCAACGACCACTTTGTCAAGCTCATTTCCTGGTATGACAACGAATTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACATGGCCTCCAAGGAGTAAGACCCCTGGACC
       970       980       990      1000      1010      1020      1030      1040      1050      1060      1070      1080
ACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAGACCCTCACTGCTGGGGAGTCCCTGCCACACTCAGTCCCCCACCACACTGAATCTCCCCTCCTCACAGTTGCCATGTAGACCCCTT
      1090      1100      1110      1120      1130      1140      1150      1160      1170      1180      1190      1200
GAAGAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACCATCAATAAAGTACCCTGTGCTCAACCAAAAAAAAAAAAAAAAAAAAAAAAAAA
      1210      1220      1230      1240      1250      1260      1270      1280      1290
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Fig. 2. Nucleotide sequence of a GAPDH cDNA isolated from human lung cancer cells. Encoded amino acids are listed above the sequence in one-letter form.

Table 1 *Differences in the nucleotide sequence between human lung cancer GAPDH cDNA and liver GAPDH cDNA*

Comparison is to a human liver GAPDH cDNA isolated by Tso *et al.* (11). Nucleotide positions are according to those in Fig. 2. The difference at position 264 does not change the encoded amino acid (valine: GTC and GTG).

Position	Lung cancer cDNA	Liver cDNA
264	C	G
1198	C	—[a]
1222	A	C
1243	A	—
1257	C	—
1260	T	G
1264	C	T
1265	A	—

[a] —, deletion of the corresponding nucleotide.

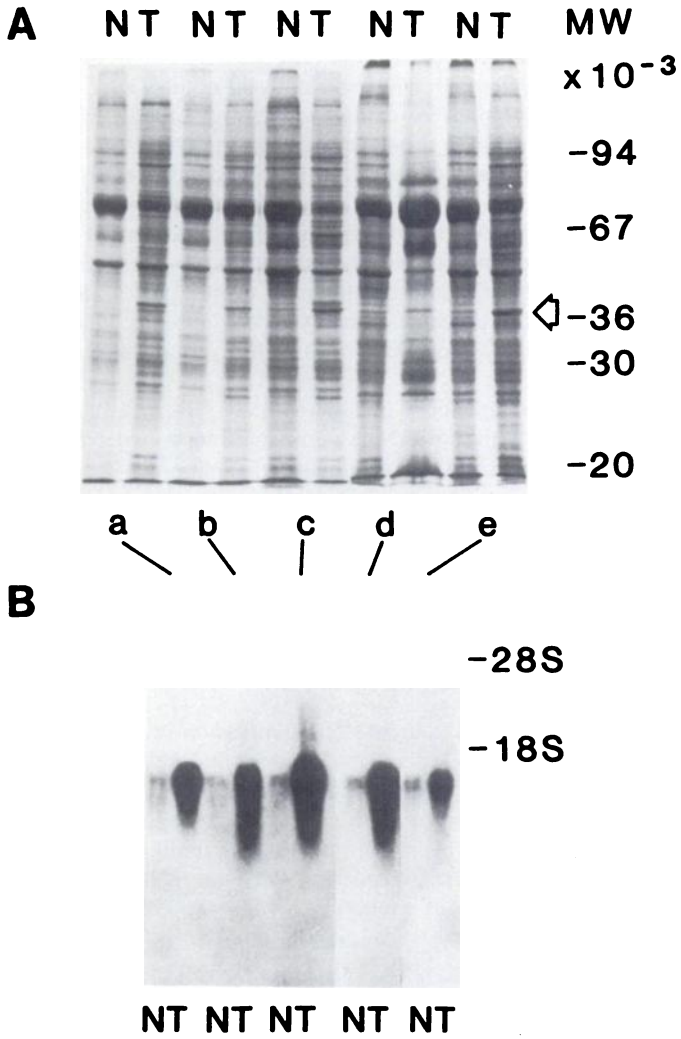

Fig. 3. Expression of p37 and GAPDH mRNA in human lung cancer tissues. In *A*, proteins extracted from normal or tumor lung tissues were fractionated through 10% polyacrylamide gel containing 0.1% SDS and stained with Coomassie blue. The position of p37 is indicated by an *arrow*. In *B*, total RNAs extracted from the same tissues analyzed in *A* were fractionated through 1% agarose gel containing formaldehyde, transferred to a nylon filter, and hybridized with a ^{32}P-labeled GAPDH cDNA (pKS321; 1×10^8 cpm/μg). Filter was exposed to X-ray film overnight. *N*, normal part; *T*, tumor part. Histological classifications of cancers are the following: *a*, small cell carcinoma; *b*, moderately differentiated squamous cell carcinoma; *c*, well-differentiated squamous cell carcinoma; *d*, poorly differentiated squamous cell carcinoma; *e*, squamous cell carcinoma.

cDNA isolated in this work will allow specific detection of GAPDH expression in various cancer tissues.

Matrisian *et al.* (22) have isolated cDNA clones whose mRNA level is increased by the stimulation of serum or epidermal growth factor. They have found that these clones include

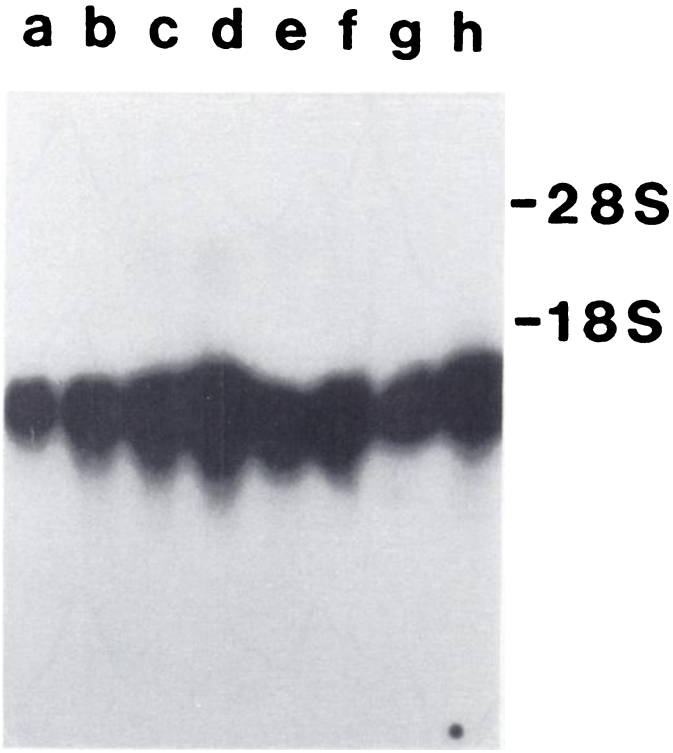

Fig. 4. Expression of GAPDH mRNA in established cell lines derived from human lung cancers. Total RNAs extracted from the indicated cultured cells were fractionated through 1% agarose gel containing formaldehyde, hybridized with ^{32}P-labeled GAPDH cDNA (pKS321; 1×10^8 cpm/μg), and exposed to X-ray film overnight. Adenocarcinomas: *a*, PC-3; *b*, PC-8; *c*, PC-9; *d*, PC-14; *e*, A549. Squamous cell carcinoma: *f*, PC-1. Small cell carcinomas: *g*, PC-6; *h*, QG-90.

cDNAs of four glycolytic enzymes: lactate dehydrogenase; enolase; triosephosphate isomerase; and GAPDH. Our preliminary results have shown that phosphoglycerate kinase mRNA is also increased in human lung cancers,[5] suggesting the existence of a common mechanism which controls the expression of mRNAs of glycolytic enzymes. Relating to this point, it is noteworthy that Alexander *et al.* (23) have reported the induction of GAPDH mRNA in differentiated 3T3 adipocyte by insulin.

A trace amount of GAPDH mRNA of the same mobility as observed in cancer tissues was detected in normal lung tissues, suggesting that the GAPDH mRNA expressed in both tissues could be identical. However, it is also possible that these two mRNAs differ in their precise molecular structures. To clarify this point, the elucidation of the structure of GAPDH cDNA of normal human lung tissues will be necessary.

The increased expression of GAPDH described here could be used for diagnosis of human lung cancers as a novel tumor marker at both the protein and mRNA levels. For this purpose, studies of GAPDH expression in precancerous tissues will provide valuable information. In addition, if the dramatic increase in the expression of GAPDH could be coupled with the rapid growth of lung cancer cells, artificial suppression of the key reaction in glycolysis would have a therapeutic importance as a novel strategy to control human lung cancers.

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[5] K. Tokunaga, unpublished observation.

λgt11. Cell lines were kindly provided by Dr. M. Taniguchi (PC-6, PC-14), Dr. S. Adachi (PC-3, QG-90), and Dr. S. Yasuda (PC-1, PC-8, PC-9, A549).

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