Elevated Expression of the Ornithine Decarboxylase Gene in Human Esophageal Cancer

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ABSTRACT

The mechanisms involved in sustaining the high levels of ornithine decarboxylase (ODC) activity in human cancers are not well defined. We examined the level of expression of ODC mRNA together with ODC activity in surgically excised human cancers, including esophagus, stomach, colon, and liver tumors, the objective being to determine whether the ODC mRNA level correlates with enhancement of ODC activity in these cancers. Among these tumors, the esophageal cancers had the highest ODC activity (120 ± 43.9 pmol of CO₂/h/mg of protein), compared with the stomach (37.6 ± 13.7), colon (22.8 ± 5.9), and liver (10.2 ± 5.6) cancers. A remarkable increase in ODC mRNA was seen in all of the esophageal cancers. The ratio of ODC mRNA in the tumors, relative to the paired normal tissues, was 14.6 ± 3.7. Some increase was noted in some of the stomach (2.9 ± 0.9) and colon (2.1 ± 0.9) cancers, but there was no increase in the liver tumors (0.9 ± 0.2). A significant correlation was noted between ODC activity and mRNA expression in cancerous and noncancerous tissues of the esophagus, stomach, and colon, thereby suggesting that increased steady-state mRNA may be responsible for the high ODC activity in these tumors. Southern blot analysis of the DNA from the esophageal cancers revealed no amplification or significant rearrangement of the gene. Mechanisms sustaining high ODC mRNA levels in esophageal cancers may be an enhancement of the promoter activity of this gene or stabilization of the mRNA.

INTRODUCTION

Ornithine decarboxylase is a key enzyme in the synthesis of polyamines implicated in the process of both normal and neoplastic growth (1, 2). There is considerable evidence that ODC activity is increased in human colon cancer compared with findings in adjacent normal tissue (3–5). Little is known of whether activation of ODC is associated with other malignancies in the digestive system (5–7).

Regulation of ODC activity has been examined in laboratory animals and in cultured cells. The enhanced ODC activity seemed to be primarily caused by the accumulation of ODC mRNA leading to the production of ODC protein (2, 8–10). Other mechanisms such as translational efficacy (11, 12), stabilization of ODC protein and mRNA (12), and feedback inhibition of translation by polyamines themselves (13) have also been discussed. The regulation of ODC activity in human tumors was given less attention.

We examined the level of expression of ODC mRNA together with ODC activity in surgically excised human cancers, including the esophagus, stomach, colon, and liver. The objective of the study was to determine whether ODC activity is generally enhanced in tumors of these organs and to investigate the involvement of modulation of mRNA levels in enhanced ODC activity in these human cancers.

MATERIALS AND METHODS

Tissue Samples. The excised tissue samples were obtained from tumors and adjacent uninvolved tissues, including esophagi, stomachs, colons, and livers. All tissue samples were examined microscopically by a pathologist. The histological diagnoses of all esophageal cancers were squamous cell carcinomas, while those of stomach and colon cancers were adenocarcinomas. Liver tumors include five hepatocellular carcinomas and one cholangiocellular carcinoma. All excised tissues were immediately placed in liquid nitrogen and stored at −80°C until determination of ODC activity and mRNA analysis. Preliminary examinations showed that a 15-min delay before freezing significantly decreased the ODC activity.

ODC Activity. Frozen tissue samples were homogenized 1:10 (w:v) in 50 mM Tris-HCl (pH 7.5), containing 5 mM dithiothreitol, and then centrifuged at 10,000 × g to remove the insoluble fraction. The protein content of the supernatant was determined using a Bio-Rad protein assay kit, with bovine serum albumin as a standard. The enzyme activity was measured using a modification of the method of Russell and Snyder (14). The enzyme assay mixture consisted of 0.4 mM l-ornithine, 0.125 μCi of DL-[1-14C]ornithine (58 mCi/mmol; Amersham), 0.02 mM pyridoxal phosphate, 0.4 mM EDTA, 50 mM Tris-HCl (pH 7.5), and 0.2 ml (1.6 to 8.0 mg of protein) of tissue extract in a total volume of 0.52 ml. Incubations were carried out for 60 min at 37°C. The reaction was stopped by adding 0.8 ml of 2 M citric acid. ODC activity was determined by measuring the release of [14C]CO₂ which was collected in filter paper soaked with 25% phenylethylamine. The results are expressed as pmol of [14C]CO₂ released/h/mg of protein.

mRNA Analysis. Total RNA was isolated from the frozen tissues by the guanidinium isothiocyanate/CsCl method, and Northern blot hybridization was done as described elsewhere (15). The integrity and the quantity of electrophoresed RNA were monitored by ethidium bromide staining. The hybridization probes used were as follows: SauII/PvuII 1.8 kilobases from mouse ODC cDNA (16); v-Ki-ras (oncor); PstI 0.4 kilobase (exon I) from the human c-myc genomic clone (oncor); PstI/PvuII 0.6 kilobase (exons III and IV) from the human c-fos genomic clone; and ClaI 2.4 kilobases from the human EGFR cDNA (17).

DNA Analysis. A high-molecular-weight DNA was isolated from frozen tissues, as described (18). Five μg of DNA digested with EcoRI, HindIII, or BamHI were electrophoresed in 0.8% agarose gels. DNA was denatured in 0.5 M NaOH and 1.5 M NaCl and neutralized in 1 M Tris-HCl (pH 8.0) and 1.5 M NaCl. Blotting and hybridization were carried out as described (19).

RESULTS

ODC Activity. The levels of ODC activity in tumors and adjacent uninvolved tissues are plotted in Fig. 1. The majority of the esophageal cancer tissues had remarkably high levels of ODC activity, as compared with the corresponding uninvolved tissues. The mean value in the esophageal cancers (120 ± 43.9 pmol of CO₂/h/mg of protein) was 3 or 5 times that in stomach (37.6 ± 13.7) or colon (22.8 ± 5.9) cancers, respectively, where an increase in ODC activity was observed in only half the number of specimens examined. The mean value in the liver cancers...
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(10.2 ± 5.6) was much lower than that in the esophageal cancers. Among 6 liver tumors examined, one tumor did have an elevated ODC activity, and histological examination revealed this tumor to be a cholangiocellular carcinoma, whereas the other 5 tumors were hepatocellular carcinomas. Thus, the increase in ODC activity is a common, but not essential, feature in human cancers. In addition, there was no correlation between the levels of ODC activity and staging of the individual tumors (data not shown).

The range of ODC activity in the uninvolved tissues varied from tissue to tissue; the esophagus and liver had narrow ranges (0.4 to 3.4 and 0.3 to 4.7, respectively), whereas the stomach and colon had wide ranges (0.9 to 19.6 and 0.5 to 50.3, respectively). Therefore, although there is a higher level of ODC activity in stomach and colon tissues, the difference in ODC activity between cancers and uninvolved tissues is much more striking in esophageal tissues.

ODC mRNA. To examine the involvement of ODC gene activation in enhancement of ODC activity, the levels of ODC mRNA were examined using Northern blots. A single 2.0-kilobase band was detected when RNAs from human tissues were hybridized with mouse cDNA. Esophageal cancerous tissues exhibited a higher expression of ODC mRNA, in comparison with uninvolved mucosa of the esophagus or stomach (Fig. 2).

Each Northern blot was evaluated by densitometric scanning, and the relative levels of ODC mRNA are shown as ratios compared with the level in an apparently normal esophagus (Fig. 3). The mean ODC mRNA level was highest in the esophageal cancers relative to other tumors in the digestive system. In contrast, uninvolved tissues from the esophagus exhibited lower mRNA levels than stomach and colon tissues, in accord with observations of the ODC activity. Thus, the ODC activity in the uninvolved tissues may at least partly depend on mRNA levels. Moreover, all esophageal cancers exhibited higher levels of ODC mRNA when compared with findings in adjacent uninvolved tissues. In the stomach and colon cancers, there were several cases in which the tumors exhibited lower mRNA levels than seen in the normal mucosa. The finding that both ODC activity and mRNA content are augmented in tumors of the esophagus and colon implies that the high ODC activity in these tumors is also a consequence of increased steady-state mRNA.

A significant correlation (r = 0.588, n = 16, p < 0.05) was found between the ODC mRNA level and the enzyme activity in both cancerous and noncancerous tissues of the esophagus. The correlations were also significant in the stomach (r = 0.711, n = 9, p < 0.05) and colon (r = 0.508, n = 18, p < 0.05), while there was no correlation in the case of liver (r = −0.27, n = 12). Thus, ODC mRNA expression is responsible at least in part for the high ODC activity in the cancerous and noncancerous tissues of the esophagus, stomach, and colon. In particular, a remarkably high ODC activity in esophageal cancers may depend on the massive ODC mRNA accumulation in this type of tumor.

A remarkably high level of ODC gene expression in esophageal cancer raised the question of whether the expression of other growth-related genes is also activated in this tumor. Some of the RNAs tested for ODC gene expression were examined by Northern blot hybridization with probes of v-Ki-ras, c-myc, c-fos, and the EGFR gene. There was no evidence of alteration of v-Ki-ras or c-myc expression in the tumors, as compared with findings in uninvolved tissues, but the levels of c-fos mRNA were unexpectedly low in the tumors (Fig. 4). In two tumors, remarkably high levels of EGFR mRNA were noted. These results are in contrast to the finding that ODC gene expression is activated in all cases of esophageal cancer.

DNA Analysis. We next investigated whether increased steady-state ODC mRNA is due to gene rearrangement or to amplification. DNAs were isolated from one metastatic lymph
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5-T N c-Ki-ras c-myc e-fos EGFR

Fig. 4. Relative levels of oncogene mRNA in cancers (T) and adjacent uninvolved tissues (N). The transcript levels were quantitated by densitometric analysis and shown as ratios to the level in the same uninvolved tissue in Fig. 3 (open circle).

A specific biological marker for esophageal cancer has yet to be found. Hyperproduction of EGFR occurs in half the number of the tumors examined (22). We observed overexpression of EGFR mRNA in only 2 of 10 tumors, in accord with a report in which the overexpression was observed in 3 of 20 cases (23). We found no other oncogene expression to be enhanced in esophageal cancer. Since ODC activation was evident in the esophageal cancers studied and is remarkably high relative to other tumors in the digestive system, ODC activity may possibly serve as a biological marker, as was suggested from our endoscopic examinations. Our preliminary analysis of urinary specimens in cancer patients also suggests the utility of urine polyamine levels for diagnosis of esophageal cancer (data not shown).

ODC activation in esophageal cancer was associated with ODC mRNA expression; hence, ODC activity may be primarily regulated by increased steady-state mRNA in this tumor. Gene amplification was observed in several cell lines resistant to α-fluoromethylornithine, a specific inhibitor of ODC (11, 16, 24). However, gene amplification or rearrangement is not likely to be concerned with the increase in ODC mRNA seen in esophageal cancer. Mechanisms sustaining increased steady-state node and 4 primary tumors in which ODC mRNA expression was 3 to 28 times the level seen in paired adjacent tissues. Southern blot analysis with EcoRI-digested DNA revealed no change in the banding pattern or in the density (Fig. 5). The same samples were also digested with HindIII or BamHI, but neither restriction fragment length polymorphisms nor rearrangement was revealed using these restriction enzymes (data not shown). When the same filter was rehybridized with the EGFR probe, gene amplification was noted in one tumor in which EGFR mRNA expression was augmented 15-fold over that in the adjacent tissue. Thus, the overexpression of EGFR mRNA is probably due to gene amplification. The cause of the increase in steady-state ODC mRNA was not determined.

Endoscopic Examination of ODC Activity. Esophageal cancer can spread to apparently normal mucosa. We examined ODC activity in endoscopic specimens taken from the tumors and from adjacent tissues prior to surgery. As shown in Fig. 6, a high level of ODC activity was detected in specimens from the main tumor. The adjacent suspicious lesions showed a moderate increase in ODC activity. Pathological examinations of the surgically excised specimens revealed these lesions to be dysplasias. Thus, preoperative examination of the ODC activity may prove to be a useful biological marker for determining the oral surgical margin.

DISCUSSION

Among malignant tumors in various organs in the digestive system, esophageal cancer has a remarkably high ODC activity. Since ODC activity is associated with cell proliferation, the esophageal cancer might be the fastest growing tumor among those examined, and the prognosis is poor (20). However, more advanced tumors of the esophagus, as well as in other organs, did not necessarily have a higher ODC activity. In addition, ODC activity was not enhanced in hepatocellular carcinoma, whereas activation of ODC is associated with normal cell growth, such as liver regeneration (8, 21). ODC activation in malignancies may not simply reflect the growth rate, but it is more likely to depend on the histological types.

A case No. 2 7 8

Fig. 5. Southern hybridization of ODC (A) or EGFR (B) cDNA probe to EcoRI digests of DNA from esophageal cancer (T), adjacent uninvolved mucosa (N), and metastatic lymph node (L). Representative bands were examined by densitometric scanning, and the relative levels of DNA content are given under each lane. Relative levels of mRNA content in the tissues subjected to DNA analysis are also shown. kb, kilobase(s).

Fig. 6. ODC activity in endoscopic specimens from cancer (T), dysplasia (D), and uninvolved mucosa (N) of an esophagus. prot., protein.
mRNA may be the enhancement of transcription rate or the stabilization of mRNA.

Analyses of the nucleotide sequence of the 5' flanking region of the ODC gene showed the presence of a TATA box and several SP1 transcription factor binding sites near the transcription starting site (25). This region appears to carry full transcriptional control activity (26). Studies of protein-DNA interactions revealed a cyclic AMP-responsive element-like sequence in the region and a binding protein which differs from well-characterized nuclear cyclic AMP-responsive element-binding protein (27). Activation of the promoter action may be involved in the high ODC mRNA levels seen in esophageal cancer.

ODC activation is not associated with high steady-state mRNA in all cases of stomach cancer. The dissociation of ODC activation and gene expression in colon cancer was also noted in the present study and by Radford et al. (28). Feedback inhibition of ODC activity by polyamines was thought to be a post-transcriptional control (13, 29–31). The predicted structure of the 5'-untranslated region of ODC mRNA may affect the translational efficiency, possibly controlled by polyamines (32–34). A carboxyl-terminal domain is responsible for the rapid intra-cellular degradation of ODC protein (35–37). Antizyme, which noncompetitively inhibits ODC activity, may also accelerate ODC degradation by forming an ODC-antizyme complex (38). These multiple posttranscriptional regulations are probably involved in the high ODC activity, at least in some cases of stomach and colon cancer.

Activated ras gene transformation in NIH 3T3 cells resulted in augmentation of the ODC content which could be largely, but not solely, accounted for by an enhanced accumulation of ODC mRNA. The increased efficacy of translation and stabilization of the product were also associated with the high ODC content (12). Since ras gene mutations frequently occur in the stomach (39, 40) and in colon cancer (41, 42), this multistep mechanism may be involved in the ODC deregulation in these tumors. In contrast, no evidence of ras mutation in human esophageal cancers was noted (43). Dissociation of the steady-state level of ODC mRNA expression and ODC activity noted in stomach and colon cancer is an expected finding, yet esophageal cancer might be a rare model in which the high steady-state level of ODC mRNA directly reflects ODC activation.

ACKNOWLEDGMENTS

We thank Dr. D. Nathans for providing cDNA of mouse ODC. The human EGFR cDNA clone pE7, developed by Dr. Ira Pastan, was obtained from the Cancer Research Resources Bank, Japan. We thank Dr. T. Tatibana and Dr. S. Fujimura for advice and Dr. M. O'Connell and M. Ohara for helpful comments.

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