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An immunohistochemical analysis of antioxidant and glutathione S-transferase enzyme levels in normal and neoplastic human lung

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Summary. Samples of normal human lung and six major types of human lung carcinomas were immunostained for antioxidant enzymes (manganese and copper, zinc superoxide dismutases, catalase, and glutathione peroxidase) and six isoenzymes of glutathione S-transferase staining was generally low in tumor cells compared with the high level of staining noted in respiratory epithelium. A notable exception was heterogeneity in immunostaining for manganese superoxide dismutase in lung adenocarcinoma, which showed both positive and negative cells in the same tumor. Tumor stromal cells (fibroblast-appearing cells) often showed strong immunostaining for manganese superoxide dismutase, while stromal cells were negative for other antioxidant and glutathione S-transferase enzymes. None of the carcinomas studied had significant levels of catalase or glutathione peroxidase; this finding has potential clinical relevance since it indicates that these tumors cannot detoxify hydrogen peroxide. The low levels of antioxidant and glutathione S-transferase enzymes in tumor cells is consistent with the hypothesis that these enzymes are markers of cell differentiation.

Key words: Antioxidant enzymes, Lung cancer

Introduction

Antioxidant enzymes detoxify reactive oxygen species generated under physiologic and pathologic conditions (Heffner and Repine, 1989; Sun, 1990). Superoxide dismutase dismutates superoxide to produce hydrogen peroxide. Both catalase and glutathione peroxidase can further reduce hydrogen peroxide, and glutathione peroxidase can also reduce organic hydroperoxides. Antioxidant enzymes are often present in unique locations within cells; for instance, manganese superoxide dismutase is found in mitochondria, and catalase is present in high levels in peroxisomes. The various cell types that constitute the lung have unique antioxidant enzyme profiles (Coursin et al., 1992). Differences in antioxidant enzyme levels have been suggested to determine susceptibility of different lung cell types to free-radical mediated injury and the development of neoplasia (Oberley and Buettner, 1979; Sun, 1990). Overexpression of manganese superoxide dismutase has been shown in an *in vitro* cell culture system to suppress radiation-induced malignant transformation (St. Clair et al., 1992). Further, overexpression of manganese superoxide dismutase in cultured human melanoma cells has been shown to cause resistance to radiation and certain chemotherapeutic agents (Hirose et al., 1993).

Glutathione S-transferase activity is expressed by a family of isoenzymes that utilize the sulfur atom of glutathione for the reduction of electrophilic substrates, a reaction central to the metabolism and deactivation of xenobiotics (Mannervik, 1985). Certain isoenzymes of glutathione S-transferases may be considered to be antioxidant enzymes since they detoxify lipid hydroperoxides. The isoenzymes of glutathione S-transferase show different species and tissue specificities such that the glutathione S-transferase isoenzyme profile of a tissue, as with the classic antioxidant enzymes, defines characteristic phenotypes for specific cell types (Oberley et al., 1995b). The complement of glutathione S-transferases in a neoplastically transformed cell population may be of considerable significance to clinical disease. Maintained or elevated enzyme activity may promote increased metabolism of chemotherapeutic agents, thus forming a biochemical basis for resistance to certain antineoplastic agents (Di Ilio et al., 1988; Cole et al., 1990; Ozols et al., 1990; Singhal et al., 1992).

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Conversely, low glutathione S-transferase activity could result in increased sensitivity to treatment (Carmichael et al., 1988a; Koberda and Hellman, 1991).

In a previous study, we described the light and electron microscopic localization of antioxidant enzymes and two general classes of glutathione S-transferases in normal rat lung (Coursin et al., 1992). The work presented in the present study extends these findings to normal human lung in a light microscopic immunohistochemical study of autopsy and surgical samples. This work was further expanded by the use of several new antisera for isoenzymes of glutathione S-transferase. Six major types of primary human lung tumors were then assessed for immunolocalization of the antioxidant enzymes and glutathione S-transferases.

Previous studies in our laboratories have demonstrated in a large number of *in vivo* and *in vitro* animal systems that antioxidant enzymes are in general lowered in tumor cells compared with their normal cell counterparts (reviewed in Oberley and Oberley, 1993, 1994). Recently, we have documented low levels of antioxidant and glutathione S-transferase enzymes in common human kidney cancers compared with levels in normal kidney cell types (Oberley et al., 1994, 1996). The present study analyzes human lung tumors to determine whether antioxidant and glutathione S-transferase enzymes are also lowered in human lung cancer.

Materials and methods

Whole rabbit antisera to the antioxidant enzymes were produced and characterized as previously described (Oberley et al., 1990). These antisera have previously been shown to be specific in that antigen (purified individual antioxidant enzymes) blocked immunostaining with the appropriate antibody, and Western blot analysis demonstrated bands of the expected molecular weight when tissues were analyzed. Further, Western blot analysis of antibody reaction with purified proteins showed bands of appropriate molecular weights. Whole rabbit antisera to rat glutathione S-transferase isoenzymes were obtained from Biotrin International (Dublin, Ireland). The antisera for glutathione S-transferase Ya, Yc, and Yk were shown to cross react with human alpha enzymes, the Yb_1 and Yb_2 antisera cross reacted with human mu enzymes, and the Yp antisera cross reacted with human pi enzymes. Further, biochemical studies using high pressure liquid chromatography techniques have demonstrated a high correlation between isoenzyme levels in tissues and staining intensities with immunoperoxidase techniques (Moser et al., 1995; Oberley et al., 1995a). An LSAB®2 kit was used for streptavidin-biotin linked immunostaining (Dako Corporation, Carpinteria, CA). All additional reagents were obtained from Sigma Chemical Company (St. Louis, MO).

Protocols for the procurement and use of human surgical and autopsy samples was approved by the University of Wisconsin Human Subjects Committee. Samples used as examples of normal lung are described in Table 1. For the surgical samples, samples of adjacent normal tissue were taken from lobectomy samples that also contained tumor; these samples were obtained within 20 minutes of surgical removal. A single autopsy sample was collected within 5 hours of death from a patient who had a previous history of normal lung function and had no known exposure to pulmonary toxicants. For all of these samples, the large airways and vessels were perfused with neutral buffered formalin. Tissue was cut to smaller blocks and immersion fixed for a total fixation time of one hour. Samples were then subjected to routine histological processing and embedding.

In addition, human lung tumor samples were obtained from the William S. Middleton Memorial Veteran's Administration Hospital archives for autopsy and surgical pathology following approval of a use protocol by the University of Wisconsin Human Subjects Committee. These samples had been subjected to routine fixation of unknown duration with neutral buffered formalin followed by routine histologic processing and embedding, then stored as paraffin blocks. Samples were selected that represented the major primary lung carcinomas: squamous, adeno-, bronchioloalveolar, small cell, and undifferentiated large cell carcinoma. One sample of adenosquamous carcinoma was also studied. Selection was based on light microscopic diagnosis, the presence of normal tissue adjacent to the tumor, and the quality of immunostaining in the adjacent normal tissue. Samples were rejected if immunostaining of the adjacent normal tissue was markedly diminished or not apparent, indicating suboptimal tissue preservation. The cases selected by these criteria for this study are summarized in Table 2. Confirmation of the light microscopic diagnoses was obtained by electron microscopy. Briefly, the electron microscopic diagnosis was dependent on the following ultrastructural features: adenocarcinoma (cells with intracellular lumens lined by microvilli and tight junctions at the cell surface); squamous cell carcinoma (tonofilaments converging on tight junctions at the cell surface); bronchioloalveolar carcinoma (lamellar bodies in the cell cytoplasm, and

Table 1. Normal human lung tissue samples.

SAMPLE	SEX	AGE	SOURCE	DIAGNOSIS OR CAUSE OF DEATH[a]
1	M	68	Surgical	Poorly differentiated squamous cell carcinoma of lung
2	M	63	Surgical	Undifferentiated large cell carcinoma of lung
3	M	34	Surgical	Thymoma
4	F	14	Autopsy	Motor vehicle accident

[a]: blocks of histologically normal lung tissue were used from these cases.

tight junctions and microvilli at the cell surface); small cell carcinoma (tight junctions at the cell surface, and neurosecretory granules in the cell cytoplasm); undifferentiated large cell carcinoma (tight junctions at the cell surface, large nuclei, and a lack of glandular or squamous differentiation). Adenosquamous carcinoma showed single tumor cells with microvilli and tonofilaments converging on tight junctions at the cell surface. Immunostaining results of these samples were included with the appropriate tumor group based on the ultrastructural diagnosis as shown in Table 2.

For all lung samples, four-microns sections were cut and immunostained as previously described (Coursin et al., 1992). For a given antisera, all sections were immunostained simultaneously using the same reagent preparations. Antisera dilutions ranged from 1:100 to 1:200. Identically treated sections were not exposed to primary antisera but rather normal rabbit serum diluted to the same protein concentrations as each antisera; these sections served as controls.

Immunostaining was assessed by two observers in a blinded fashion using a semi-quantitative grading system described in Table 3. Immunostaining of normal tissue was always assessed relative to the staining reaction of normal rabbit serum controls. The intensity of staining in tumor samples was assessed relative to the ability of adjacent normal tissue to stain in a manner similar to that of normal lung samples.

Table 2. Case descriptions.

DIAGNOSIS AND SAMPLE	EM DX[a]	SEX	AGE	SOURCE
Adenocarcinoma				
1	+	M	74	Surgical
2	N.D.	M	69	Surgical
3	+	M	78	Surgical
Squamous cell carcinoma				
1	+	M	62	Surgical
2	+	M	72	Surgical
3	+	M	58	Surgical
4	N.D.	M	68	Surgical
5	+	M	77	Surgical
6	+	M	55	Surgical
Adenosquamous carcinoma				
1	+	M	66	Surgical
Brochioloalveolar carcinoma				
1	+	M	71	Surgical
2	+	M	58	Surgical
3	N.D.	M	70	Surgical
Small cell carcinoma				
1	N.D.	M	70	Surgical
2	+	M	66	Autopsy
3	+	M	59	Autopsy
Large cell carcinoma				
1	+	M	64	Surgical
2	+	M	72	Surgical
3	N.D.	M	72	Autopsy

a: +, diagnosis of tumor type by electron microscopy; N.D., not determined.

Results

Normal lung

Predominant staining of antioxidant enzymes in lung parenchyma was found in the respiratory epithelium (Table 3), with cells lining the alveoli showing less labeling. Essentially all cells of the lungs showed some degree of staining, and staining was consistent for a cell type. However, alveolar macrophages demonstrated variable staining for manganese superoxide dismutase and catalase, with alveolar macrophages often showing intense labeling for manganese superoxide dismutase. Manganese superoxide dismutase and catalase antisera produced granular cytoplasmic immunostaining, while anti-copper, zinc superoxide dismutase and anti-glutathione peroxidase diffusely stained the cytoplasm. Bronchiolar cilia showed marked to intense immunostaining for copper, zinc superoxide dismutase, catalase, and glutathione peroxidase.

The localization of glutathione S-transferase isoenzymes was similar to that of the antioxidant enzymes with predominant staining found in respiratory epithelium (Fig. 1), while other cell types in the lung showed less labeling (Table 4). For all of the glutathione S-transferase isoenzymes, staining was predominantly cytoplasmic, although nuclear staining was also noted, most readily in the respiratory epithelium. Bronchiolar cilia stained densely for Yb_2 and Yc. Staining was consistent for all cell types.

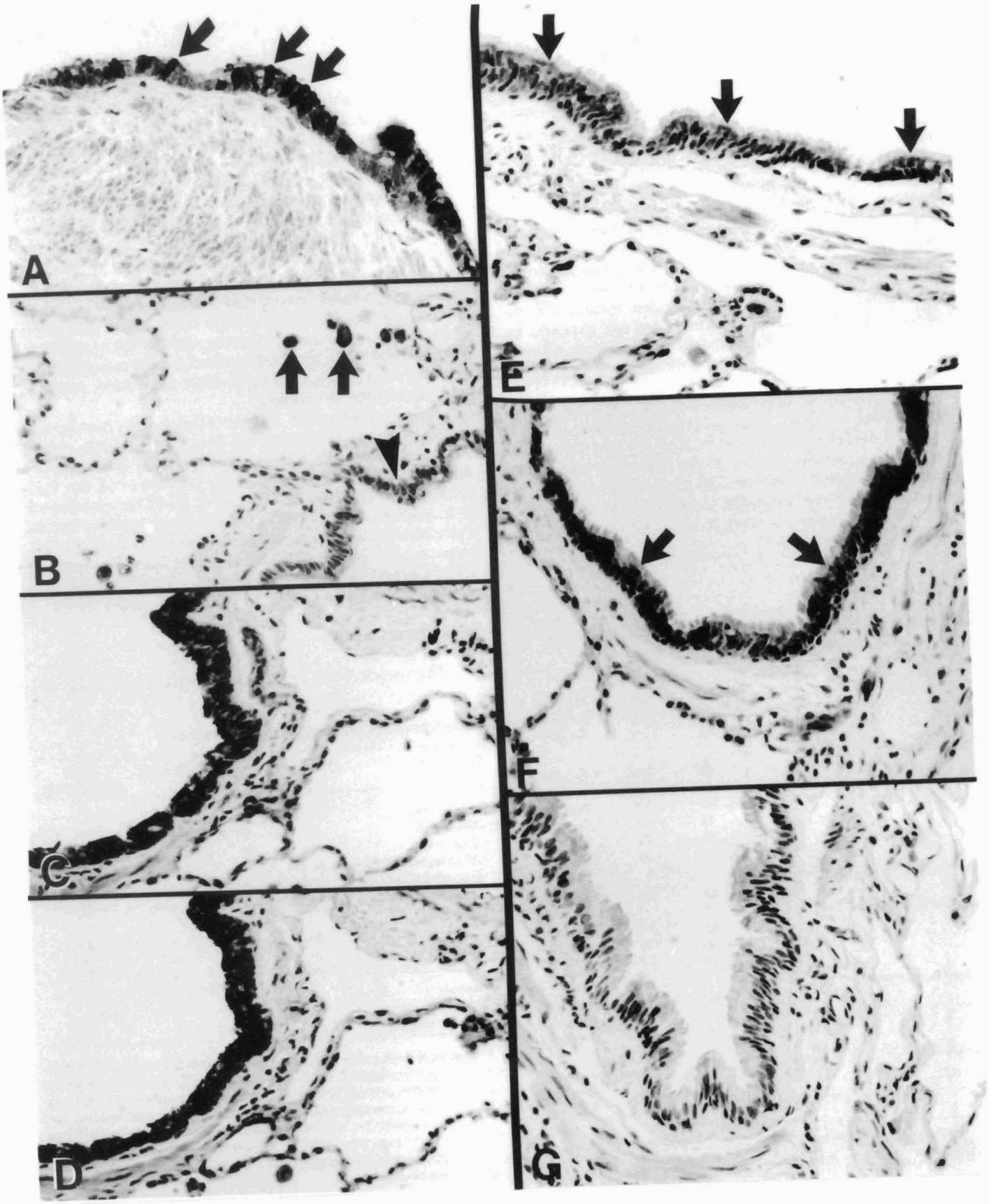
Human lung tumors

The tumor cells of squamous cell, small cell, and

Table 3. Normal human lung antioxidant enzymes[a].

CELL TYPE	MnSOD	CuZnSOD	CATALASE	GSH PEROXIDASE
Parenchyma				
Type I cell	2+	+	+	+
Type II cell	3+	2+	2+	+
Alveolar macrophage	- to 3+	+	- to +	+
Endothelial cell	2+	+	+	+
Interstitial cell	2+	tr	+	+
Bronchiole				
Ciliated epithelial cell	3+	3+	+	2+
Clara cell	3+	3+	+	+
Smooth muscle	2+	tr	+	2+
Arteriole				
Endothelial cell	+	+	+	+
Smooth muscle	2+	+	+	2+

a: immunohistochemical staining of a cell was always assessed relative to staining of a similar or serial section treated with normal rabbit serum instead of the primary antisera. The grading system used was as follows: -, no appreciable staining; tr, trace staining; +, mild staining; 2+, moderate staining; 3+, intense staining. Abbreviations used in this and subsequent tables are: MnSOD, manganese superoxide dismutase; CuZnSOD, copper, zinc superoxide dismutase; and GSH peroxidase, glutathione peroxidase.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undifferentiated large cell carcinomas were consistently negative for manganese superoxide dismutase (Table 5). Some bronchioloalveolar carcinoma samples showed small areas of cells with slight labeling. Adenocarcinoma showed much more variability in manganese superoxide dismutase immunostaining, ranging from negative to strongly positive reactions (Fig. 2). Cells along the periphery of tumor masses tended to show the greatest intensity of staining. Although most tumor cell types were negative for manganese superoxide dismutase, the stroma of all tumor types generally showed intense labeling with anti-manganese superoxide dismutase (Fig. 3).

Copper, zinc superoxide dismutase showed variability in immunostaining, with all tumor cell types except small cell carcinoma showing examples with mild to moderate labeling. Squamous cell carcinoma showed the greatest range in immunostaining intensity, from a negative reaction to moderately positive (Fig. 4). Small cell carcinoma did not show significant immunolabeling for copper, zinc superoxide dismutase. Stromal cells showed trace labeling of fibroblastic cells.

Catalase was negative for the neoplastic cells of all tumor types, and fibroblast stroma was also negative.

All tumors except small cell carcinoma showed trace to mild labeling with anti-glutathione peroxidase. Small cell carcinoma did not exhibit significant immunolabeling for glutathione peroxidase. The fibroblast stroma of all tumors demonstrated trace labeling for glutathione peroxidase. Smooth muscle cells in small

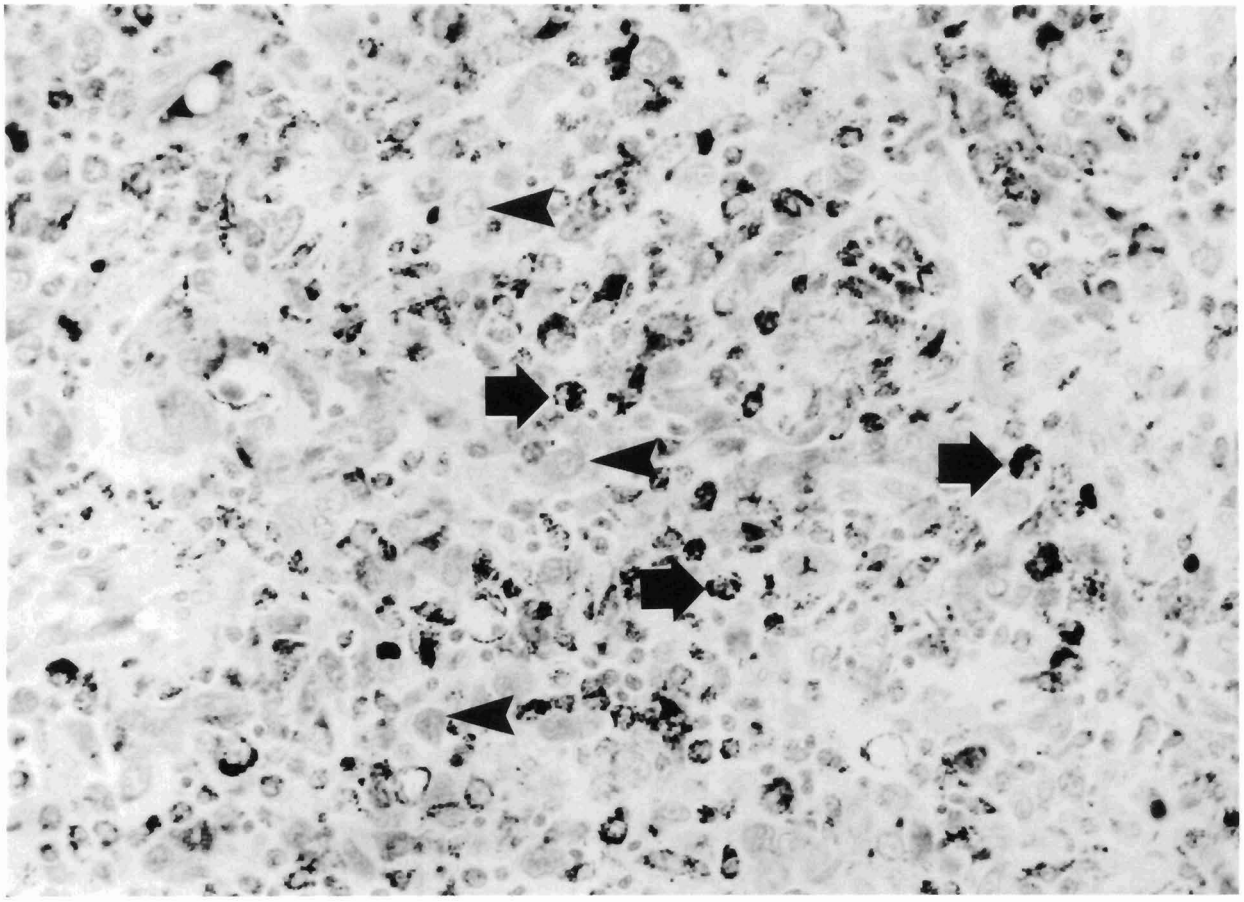

Fig. 2. Adenocarcinoma immunostained for manganese superoxide dismutase. Large variation in immunolabeling in lung adenocarcinomas is observed between tumor samples and within given tumors. This high power view demonstrates the range of immunostaining observed in tumor cells, from negligible (arrowheads) to intense (arrows). x 260

Fig. 1. Glutathione S-transferase (GST) isoenzyme immunolocalization in normal human lung. **A.** GST-Ya. Focal staining is present in the respiratory epithelium (arrows). **B.** GST-Yb_1. This isoenzyme antisera mainly stains alveolar macrophages (arrows), slightly stains the respiratory epithelium (arrowheads), but produces little other labeling. **C.** GST-Yb_2. A more generalized immunostaining occurs with this antisera with marked labeling of the respiratory epithelium and its cilia. **D.** GST-Yc. Strong immunostaining of the respiratory epithelium is present. The cilia show marked immunolabeling. **E.** GST-Yk. Moderate immunostaining of the respiratory epithelium is apparent, although the cilia (arrows) do not stain. **F.** GST-Yp. Marked staining of the respiratory epithelium with slight staining of its cilia (arrows) is apparent. **G.** Normal rabbit serum control. Background staining is minimal and contrast is provided by the counterstain. x 260

Table 4. Normal human lung glutathione S-transferases[a].

CELL TYPE	Ya	Yb_1	Yb_2	Yc	Yk	Yp
Parenchyma						
Type I cell	-	-	+	-	tr	+
Type II cell	2+	-	+	+	+	2+
Alveolar macrophage	- to 3+	+ to 3+	+	+	2+	+
Endothelial cell	-	-	+	tr	-	-
Interstitial cell	-	-	+	-	-	-
Bronchiole						
Ciliated epithelial cell	2+	+	2+	2+	+	2+
Clara cell	3+	+	2+	2+	+	2+
Smooth muscle	+	-	tr	tr	tr	tr
Arteriole						
Endothelial cell	-	-	-	tr	-	-
Smooth muscle	tr	-	tr	tr	tr	tr

[a]: immunohistochemical staining of a cell was always assessed relative to staining of a similar or serial section treated with normal rabbit serum instead of the primary antisera. The grading system used was as follows: -, no appreciable staining; tr, trace staining; +, mild staining; 2+, moderate staining; 3+, intense staining.

Table 5. Human lung tumor antioxidant enzymes[a].

TUMOR TYPE	MnSOD	CuZnSOD	CATALASE	GSH PEROXIDASE
Adenocarcinoma	- to 3+	- to +	-	+
Squamous cell carcinoma	-	- to 2+	-	tr
Adenosquamous carcinoma	3+	+	-	+
Bronchioloalveolar carcinoma	- to +	tr to 2+	-	+
Small cell carcinoma	-	-	-	-
Large cell carcinoma	-	tr to 2+	-	tr

[a]: immunohistochemical staining of a cell was always assessed relative to staining of a similar or serial section treated with normal rabbit serum instead of the primary antisera. The grading system used was as follows: -, no appreciable staining; tr, trace staining; +, mild staining; 2+, moderate staining; 3+, intense staining. The grading reported in this and the following table describe immunostaining in tumor cells and not accompanying tumor stroma or vasculature; see text for a description of these latter results

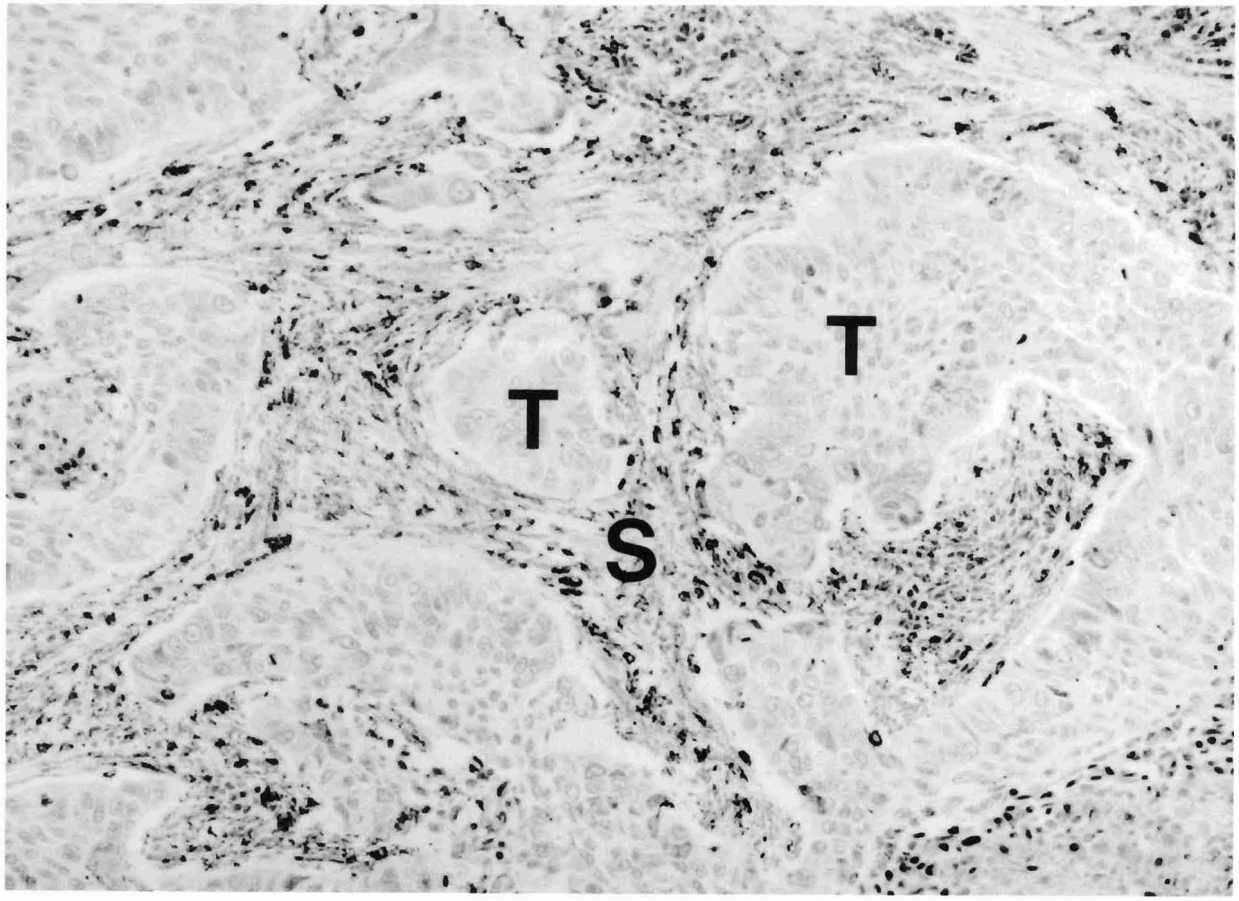

Fig. 3. Squamous cell carcinoma immunostained for manganese superoxide dismutase.Islands of neoplastic squamous cells (T) do not demonstrate any greater staining than the background level observed in control slides treated with normal rabbit serum, such that the tumor cells are graded as negative for staining. However, the connective tissue stroma (S) demonstrates moderate to intense immunostaining. x 130

arteries in tumor stroma showed intense immunostaining.

None of the tumors examined showed strong immunolabeling for glutathione S-transferase isoenzymes. Adenocarcinoma demonstrated very little labeling for the glutathione S-transferase isoenzymes while small cell carcinoma demonstrated no immunostaining (Table 6). Tumor cells in squamous cell carcinomas immunostained with considerable variability for all glutathione S-transferase isoenzymes, with some tumors showing moderate labeling. Bronchioalveolar and large cell carcinomas showed less marked staining intensity than squamous cell carcinoma for glutathione S-transferase isoenzymes. The immunostaining reaction for stroma was negative for the glutathione S-transferase isoenzymes.

Table 6. Human lung tumor glutathione S-transferases[a].

TUMOR TYPE	Ya	Yb_1	Yb_2	Yc	Yk	Yp
Adenocarcinoma	-	-	tr	-	tr	-
Squamous cell carcinoma	- to 2+	- to 2+	- to 2+	- to 2+	- to +	- to 2+
Adenosquamous carcinoma	-	-	tr	-	tr	-
Bronchioloalveolar carcinoma	tr	- to tr	+	- to +	+ to 2+	tr to 2+
Small cell carcinoma	-	-	-	-	-	-
Large cell carcinoma	- to +	- to tr	- to +	- to +	tr	- to 3+

[a]: immunohistochemical staining of a cell was always assessed relative to staining of a similar or serial section treated with normal rabbit serum instead of the primary antisera. The grading system used was as follows: -, no appreciable staining; tr, trace staining; +, mild staining; 2+, moderate staining; 3+, intense staining.

Discussion

Normal and neoplastic human lung were immunohistochemically assessed for the localization of antioxidant enzymes and isoenzymes of glutathione S-transferase. It is important to note a limitation to this type of study; lack of staining does not mean an enzyme is not present, but that it is not present at that level of detection. The immunohistochemical grading system, however, does provide a measure of the relative levels of an enzyme within the cells of a given tissue. A second

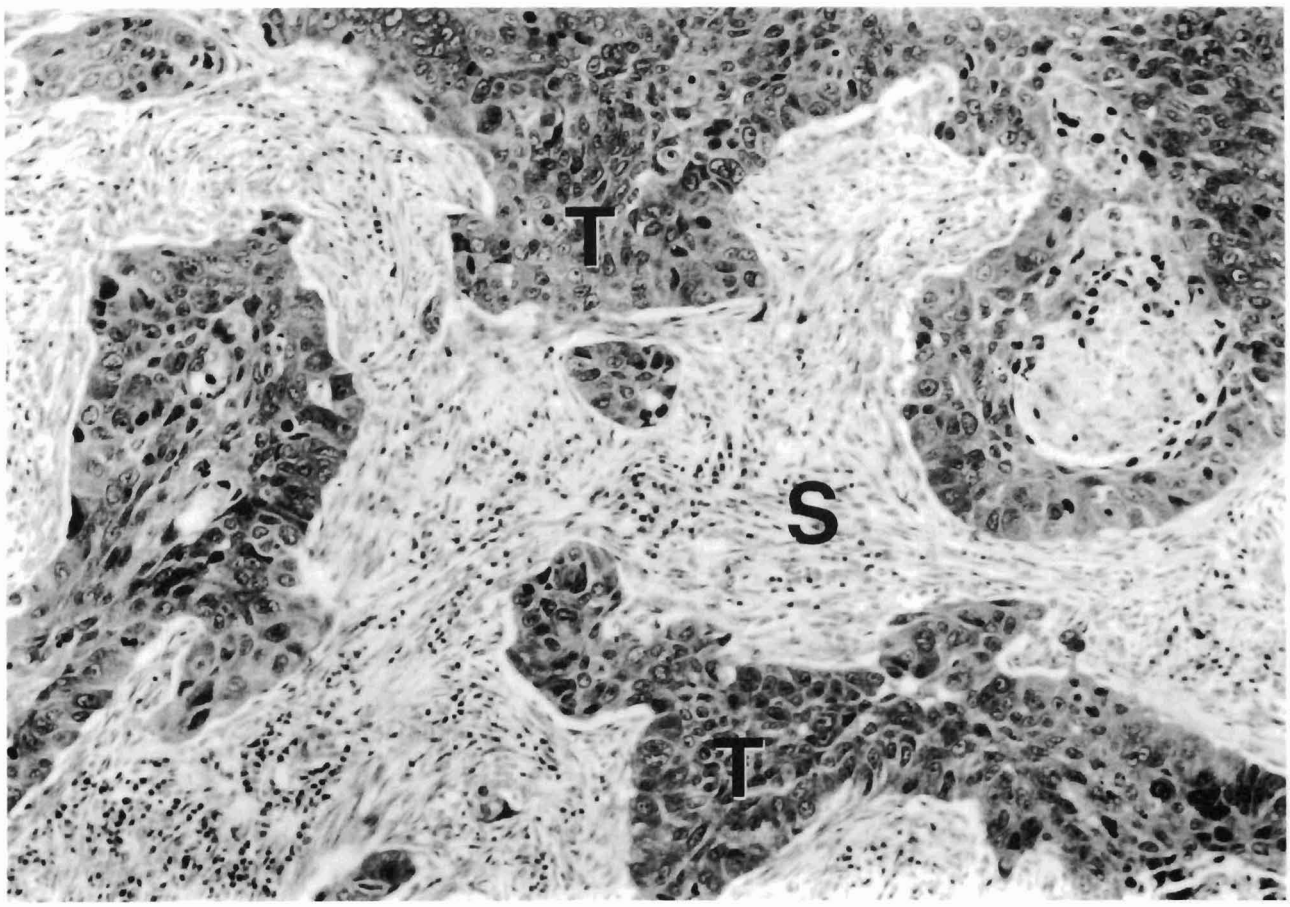

Fig. 4. Squamous cell carcinoma immunostained for copper, zinc superoxide dismutase. The tumor cells (T) demonstrate moderate staining for copper, zinc superoxide dismutase, while stromal cells (S) show only trace staining. x 130

caveat of the present study is that metastatic lesions were not examined; it is possible that the biochemical profile of these more aggressive cell types would be different from the profiles described herein.

Previous studies in our laboratories have documented localization of antioxidant and glutathione S-transferase enzymes in normal rat lung (Coursin et al., 1992); the present study of normal human lung is in general agreement with this previous study. The present study indicates that human respiratory epithelium has high levels of antioxidant and glutathione S-transferase enzymes compared with other lung cell types. However, activity studies have shown that airway epithelium has, in general, low antioxidant enzyme activities compared with other organs such as kidney or liver (Erzurum et al., 1993; Yoo et al., 1994). The present study also documents high levels of antioxidant and glutathione S-transferases in human respiratory cilia, a finding similar to that observed in the rat (Coursin et al., 1992). The high level of these enzymes is probably necessary to protect these cells and their cilia against the high levels of oxygen to which they are exposed. It is not currently known whether the antioxidant enzymes localized to the cilia are actually a component of the cilia or are proteins found in the epithelial lining fluid (Cantin et al., 1987, 1990).

In general, tumor cells demonstrated low levels of immunostaining for the antioxidant enzymes. The significance of decreased antioxidant enzymes in tumor cells is not certain. Studies in our laboratories have demonstrated that antioxidant enzymes are induced during development of the hamster kidney (Oberley et al., 1990, 1995b), and studies of organ cell renewal systems in the adult hamster show lower levels of antioxidant enzymes in precursor compared with differentiated cells (for instance, in the crypt versus the villus of the small intestine) (Oberley et al., 1990, 1991). Since cancer often involves proliferation of precursor cells (Sell and Pierce, 1994), it seems likely that the lack of staining in cancer cells reflects their origin in precursor cells.

Lung adenocarcinomas often showed a mixture of cells that were positive and negative for manganese superoxide dismutase, a mitochondrial enzyme. One possible explanation of this result is that negatively staining tumor cells are precursor cells in which mitochondria are not yet differentiated, whereas positively staining cells have undergone partial cell differentiation with mitochondria now expressing manganese superoxide dismutase. Previous immunogold analysis of human renal adenocarcinomas at the ultrastructural level using antibody to manganese superoxide dismutase has shown some tumor cells with a few very small lightly labeled mitochondria and other cells with a large number of medium-sized heavily labeled mitochondria (Oberley et al., 1994). Thus, in human renal cell carcinoma, there appears to be an increase in the number and differentiation of mitochondria in some cells. However, these latter cells are different from the situation in the cell of origin, the proximal tubule, in that mitochondria in the proximal tubule are larger, have more cristae, and are branching. Thus, while mitochondria are increased in number in some renal cell carcinoma tumor cells, the mitochondria are not as well differentiated as in a normal cell. Future studies at the ultrastructural level will be necessary to determine if the situation is the same in lung adenocarcinoma cells. A second explanation of positive staining is induction of manganese superoxide dismutase by cytokines; it has been well documented that cytokines can regulate manganese superoxide dismutase expression in human lung cells (Kinnula et al., 1994). Future studies will be necessary to distinguish between these two possibilities.

The results of the present study demonstrate very low levels of catalase and glutathione peroxidase within lung carcinoma cells. It has been demonstrated that many tumor cells generate high levels of hydrogen peroxide (Szatrowski and Nathan, 1991); the present study suggests that any hydrogen peroxide produced by lung cancer cells cannot be detoxified, since catalase and glutathione peroxidase are present only in very low amounts. Recently, it has been demonstrated that overexpression of manganese superoxide dismutase sensitizes tumor cells to radiation therapy in an animal experimental model system (Urano et al., 1995); overexpression of manganese superoxide dismutase could result in increased hydrogen peroxide production since hydrogen peroxide is a product of the enzyme. The present results demonstrating low levels of catalase and glutathione peroxidase in lung carcinomas suggest that therapeutic modalities that increase hydrogen peroxide production may have therapeutic benefit in treatment of human lung carcinomas.

Among the most significant of findings from this study is the immunostaining for antioxidant enzymes of the stromal elements in lung tumors, the tumor cells of which may show no immunostaining. The present study demonstrates significant levels of manganese superoxide dismutase in tumor stromal cells and high levels of glutathione peroxidase in tumor vasculature. Biochemical or immunological assays of tumor samples that demonstrate changes in enzyme levels relative to normal tissue may actually be measuring activity in non-tumor stromal cells or tumor vasculature (Bartoli et al., 1980; Marklund et al., 1982; Iizuka et al., 1984; Di Ilio et al., 1987, 1988; Jaruga et al., 1994), which must be considered in interpreting results. One study showed an increase in immunoreactive protein for manganese superoxide dismutase in lung adenocarcinomas using an enzyme-linked immunosorbent assay (ELISA) and suggested therefore that manganese superoxide dismutase was elevated in lung cancer (Iizuka et al., 1984); this would be true only if it could be demonstrated that tumor cells, separate from stromal cells, had high enzyme activity. In fact, the present study demonstrates that both tumor cells and stromal cells show positive staining for manganese superoxide

dismutase in lung adenocarcinomas. The study of Iizuka et al. (1984) further showed low manganese superoxide dismutase levels by ELISA assay in other lung carcinomas, including squamous cell, small cell, adenosquamous, and undifferentiated large carcinoma, results in agreement with the immunoperoxidase results of the present study.

Glutathione S-transferase isoenzymes were present at low levels in adenocarcinoma, not detectable in small cell carcinoma, and variable for squamous and large cell carcinomas. For small cell carcinoma, previous studies have demonstrated quite variable results with glutathione S-transferase pi found in two of three cell lines (Awasthi et al., 1988), low glutathione S-transferase activity in small cell versus non-small cell lines (Carmichael et al., 1988b), and low glutathione S-transferase activity in small cell tumor correlating with sensitivity to chemotherapy (Ogawa et al., 1993). This last finding is emphasized in light of a study demonstrating high glutathione S-transferase activity in a small cell line with correlation to increased resistance to chemotherapy (Cole et al., 1990). Our study did not demonstrate any immunostaining in three samples of small cell carcinoma; this supports findings of low glutathione S-transferase levels in this tumor type and the suggestion that the high susceptibility of this tumor type to certain therapies may be related to an inability of the tumor cell to detoxify these agents. However, adenocarcinoma also demonstrated little glutathione S-transferase immunostaining, in agreement with previous studies (Yamamoto et al., 1988), but it is markedly more resistant to chemotherapy (Ogawa et al., 1993). It is apparent that several forms of drug resistance exist and that one clearly is mediated by glutathione-dependent processes (Beck, 1990; Meijer et al., 1990; Ozols et al., 1990). Adenocarcinomas may resist drugs by other mechanisms. In this respect, it would be interesting to determine if glutathione S-transferase negative, drug-resistant adenocarcinomas demonstrated expression of metallothionein, P-glycoprotein, or DNA topoisomerase II, for example (Beck, 1990; Kasahara et al., 1991), in a study of these combined markers.

The present study documents low levels of antioxidant and glutathione S-transferase enzymes in most human lung carcinomas. Further, the absence of catalase and glutathione peroxidase suggests these tumors may be susceptible to therapies that elevate intracellular hydrogen peroxide. The marked staining of connective tissue in tumors for manganese superoxide dismutase and tumor vasculature for glutathione peroxidase suggests caution must be exercised in interpreting the results of biochemical or immunoblotting studies using "tumor" tissue in comparison with normal tissue. Finally, these results support previous suggestions that low or deficient glutathione S-transferase isoenzymes in certain tumors may help define cell populations susceptible to chemotherapeutic agents.

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