Immunogold analysis of antioxidant enzymes in common renal cancers

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Summary. Immunogold studies of normal human kidney and common human kidney cancers were performed using polyclonal antibodies to antioxidant enzymes, including antibodies to copper, zinc and manganese superoxide dismutases, catalase, glutathione peroxidase, and glutathione S-transferases and their subunits. Normal tissue adjacent to human renal tumors had the same antioxidant enzyme immunoreactive protein profiles as normal human kidney, thus establishing that the presence of tumor does not alter the levels of antioxidant enzyme immunoreactive proteins in adjacent kidney tissue. Levels of immunoreactive protein for antioxidant enzymes were determined in four common types of malignant renal cancer. In general, tumors had low levels of antioxidant enzymes; however, certain histologic types of renal tumors had high levels of immunoreactive protein for glutathione S-transferase subunits, which could affect their susceptibility to chemotherapy. Studies of transitional carcinoma of the renal pelvis were especially informative since it was possible to compare levels of antioxidant enzyme immunoreactive protein with adjacent normal transitional epithelium; the majority of antibodies resulted in lower levels of immunoreactive protein in transitional cell carcinoma than in adjacent normal transitional epithelium. Our results are discussed in relation to the response of renal tumors to therapy.

Key words: Immunogold techniques, Antioxidant enzymes, Kidney cancer

Introduction

Reactive oxygen species produced during aerobic metabolism can be injurious to cells; antioxidant enzymes are a primary defense against reactive oxygen species. The primary antioxidant enzyme system consists of three enzymes: superoxide dismutases, catalases, and peroxidases, of which glutathione peroxidase is the most common in mammalian cells. The basic function of the antioxidant enzymes is to convert reactive oxygen species produced during cellular metabolism into water (Halliwell and Gutteridge, 1985). Certain of the glutathione S-transferases may also be considered primary antioxidant enzymes since they catalyze the descomposition of hydroperoxides; for the sake of convenience, all glutathione S-transferase isoenzymes are designated as antioxidant enzymes in this paper.

Studies of experimental animal tumor models in our laboratories have prompted us to conclude that antioxidant enzymes are generally lower in cancer cells than in their normal cell counterparts (Oberley and Oberley, 1986). However, data from recent human studies have challenged this concept, since at least one antioxidant enzyme, manganese superoxide dismutase, has very high enzymatic activity in certain human tumors (Westman and Marklund, 1981; Yang et al., 1987). In addition, several tumors show elevated levels of immunoreactive protein for manganese superoxide dismutase (Iizuka et al., 1984; Karamura et al., 1992; Nakata et al., 1992).

The question of whether antioxidant enzymes are altered in cancer is of importance since depression of antioxidant enzymes results in elevated levels of reactive oxygen species, which can result in cell damage (Sies and Cadenas, 1985), mutation (Moraes et al., 1991), and cell proliferation (Shibanuma et al., 1988). A recent study has demonstrated that transfection of the cDNA for manganese superoxide dismutase suppressed the malignant phenotype of human melanoma cells (Church et al., 1993). Recent studies suggest that manganese superoxide dismutase is a tumor suppressor gene in SV-40 transformed human fibroblasts (Bravard et al., 1992a,b). A recent study in an experimental animal model system has demonstrated that mouse fibrosarcoma cells transfected with the cDNA for manganese superoxide dismutase were sensitized to subsequent radiation therapy (Urano et al., 1995). Thus, a clearer

understanding of the biology of antioxidant enzyme expression may lead not only to a better understanding of the process of carcinogenesis, but it may ultimately lead to more rational therapy of human cancer.

Elevation of antioxidant enzymes in tumors can have clinical significance since such elevation may cause tumor cells to become resistant to certain chemotherapeutic agents. It has recently been shown in tissue culture that human melanoma cells overexpressing manganese superoxide dismutase are resistant to selected chemotherapeutic agents (Hirose et al., 1993). Therefore, determination of the level of antioxidant enzymes in human tumors may have predictive value in the response to chemotherapeutic regimens. The above results suggest that either over- or underexpression of antioxidant enzymes may dramatically alter tumor cell behavior.

Recent studies have suggested that altered glutathione S-transferase levels could affect tumor susceptibility to chemotherapeutic agents. Elevated enzyme activity may promote increased metabolism of cancer chemotherapeutic agents, thus forming a biochemical basis for resistance to treatment using certain antineoplastic agents (Di Ilio et al., 1988; Cole et al., 1990; Coles and Ketterer, 1990; Singhal et al., 1992). Conversely, low glutathione S-transferase activity would result in increased susceptibility to treatment (Carmichael et al., 1988; Koberda and Hellmann, 1991).

The present study was initiated in order to determine the antioxidant enzyme profiles of selected common renal cancers. The results are discussed in terms of possible biologic and clinical relevance.

Materials and methods

Tissue procurement

Tissues (archival paraffin blocks, fresh tumor and adjacent tissue from surgical nephrectomies, and autopsy kidney) for light microscopic immunogold studies were obtained from the Department of Surgical Pathology at the University of Wisconsin Center for Health Sciences, the combined autopsy facilities at the University of Wisconsin Medical School and VA Hospital, Madison, WI, and the Pathology and Laboratory Medicine Service at the VA Hospital.

Tissue diagnosis

Light microscopic diagnosis was performed by a team of surgical pathologists at the VA Hospital, Madison, WI and the University of Wisconsin Center for Health Sciences. Multiple sections were examined from each tumor so that they could be correctly classified.

Antibody production and characterization

Polyclonal antisera to human kidney manganese superoxide dismutase, human liver copper, zinc superoxide dismutase, bovine liver catalase, human erythrocyte glutathione peroxidase, human placenta

glutathione S-transferase, and rat liver glutathione S-transferase were employed for these studies. Western blot analysis has previously confirmed the specificity of these antibodies (Oberley et al., 1990, 1991, 1993; Coursin et al., 1992; McCormick et al., 1992). Commercial antibodies to rat glutathione S-transferase subunits were also used; the specificity of these antibodies has been rigorously demonstrated by comparison with biochemical analysis using high pressure liquid chromatography techniques (Johnson et al., 1993a,b; Moser et al., 1995; Oberley et al., 1995a) and by immunochemical analysis by the manufacturer (Biotrin, Dublin, Ireland). The antibody to glutathione S-transferase-placental isolate produced a single band, molecular weight 25,800 daltons, on Western blot analysis of hamster kidney tissue; since human placenta contains primarily glutathione S-transferase pi class subunits (Sato and Tsuchida, 1991), this antibody should give results similar to those obtained with Biotrin Yp subunit antibody. The antibody to glutathione S-transferase-liver isolate produced three bands of molecular weights 29,100, 28,600, and 27,000 on Western blot analysis of hamster kidney tissue; since rat liver contains Ya, Yb, and Yc subunits in large amounts (Sato and Tsuchida, 1991), it is presumed that the anti-glutathione S-transferase-liver isolate antibody is recognizing these three classes of glutathione S-transferases.

Light microscopy immunogold techniques

For immunogold analysis, 36 renal tumors were analyzed; 18 renal cell carcinomas (13 clear cell type, 2 granular cell type, and 3 mixed clear and granular cell type), 4 papillary carcinomas, 10 transitional cell carcinomas of the renal pelvis, and 4 Wilms' tumors; these tumors were chosen because they are common and thus readily available in our archival material. Tumors selected for this study were analyzed using immunogold methods since we have demonstrated that this method is more sensitive than immunoperoxidase techniques and therefore of more utility when examining fixed archival material (unpublished observation).

Immunohistochemical studies were restricted to cases for which an appropriate tissue block was available. This was defined as a block containing tumor and adjacent normal renal parenchyma. This served two purposes; first, the normal kidney provided an internal control for all sections stained, and second, staining intensity was assessed semi-quantitatively by comparing tumor reactivity with normal tissue (Table 1). The latter was important since minor variability in intensity of staining of adjacent normal tissue was seen between cases, presumably related to fixation artifacts.

Four micrometer sections were cut from paraffin blocks, mounted on slides coated with 3-amino-propyltriethyloxysilane to insure adhesion during processing, and dried overnight. The slides were then placed in an oven at 56 °C for 60 minutes to melt the paraffin. Removal of paraffin was accomplished by

placing the slides sequentially in xylene four times (10 minutes each), 100% ethanol two times (2 minutes each), 95% ethanol, 70% ethanol, 50% ethanol, and distilled water (2 minutes each). The slides were then washed in Tris-buffered saline (0.05M Tris, 2.5% NaCl, pH 7.4). They were incubated with 100 microliters of a 1:10 dilution of normal goat serum for 15 minutes. After removing the serum, 100 microliters of rabbit primary antibody (1:200 dilution) was added to the tissue sections, which were then incubated overnight at 4 °C. The sections were rinsed in Tris-buffered saline with 0.1% bovine serum albumin for 5 minutes. The buffer was removed, and then secondary antibody conjugated to 5 nanometer colloidal gold particles (Goldmark Biologicals, Phillipsburg, New Jersey, USA) (1:100 dilution) was added for 60 minutes at room temperature. The sections were again rinsed in Tris-buffered saline with 0.1% bovine serum albumin for 5 minutes. Subsequently, they were fixed in 2.5% glutaraldehyde in Tris-buffered saline for 15 minutes and washed thoroughly in distilled water. Silver enhancer solution was added (Sigma Chemical, St. Louis, Missouri, USA). The sections were developed in the enhancer until the desired staining intensity was reached (5-10 minutes). The sections were rinsed in distilled water and placed in 2.5% aqueous thiosulphate for 2 minutes. They were washed in distilled water, counterstained with hematoxylin, dehydrated with ethanol, cleared in xylene, and coverslipped. Controls utilized saline or normal rabbit serum (Sigma Chemical) in place of the primary antibody.

Results

Immunoperoxidase analysis of normal human kidney cells

Three normal human kidneys were evaluated by immunoperoxidase techniques for levels of antioxidant enzyme immunoreactive protein. Four representative cell types were examined: proximal tubular, glomerular epithelial, arterial smooth muscle, and transitional epithelial cells. The results presented in Table 1 are the average of the scores obtained from the 3 samples; there was very little variability between samples examined. Each cell type showed unique antioxidant enzyme immunoreactive protein profiles. Proximal tubular cells showed moderate to heavy labeling for all antioxidant enzymes examined except glutathione S-transferase subunits Yb_1 and Yb_2. Glomerular epithelial cells showed very little staining with antibodies to antioxidant enzymes, with the exceptions of strong staining with antibodies to glutathione S-transferase Yc and Yb_2 subunits. Smooth muscle cells also exhibited very little labeling with antibodies to antioxidant enzymes; however, anti-glutathione peroxidase antibody resulted in strong immunostaining, and these cells demonstrated moderate labeling with antibody to Yc subunit. In contrast, transitional epithelial cells showed moderate to strong labeling with antibodies to all antioxidant enzymes, with the exceptions of manganese superoxide dismutase and glutathione S-transferase Ya subunit, which showed only light staining.

Tissue adjacent to renal tumors were also evaluated for labeling with antibodies to antioxidant enzymes. Thirty six such samples were evaluated. There was very little variability between tissues for each antibody tested, with the exception of glutathione S-transferase-liver isolate, which varied from negative to moderate staining in transitional epithelium. The average of these 36 samples is reported in Table 1, and the results were the same as those in the 3 normal kidneys, with the exception of variability of immunostaining with antibody to glutathione S-transferase-liver isolate in transitional epithelium.

Table 1. Immunogold analysis of antioxidant enzyme immunoreactive protein in selected human kidney cell types[a].

CELL TYPE	ANTIBODY [b]											
	αMnSOD	αCuZnSOD	αCAT	αGPX	αGST-L	αGST-P	αYa	αYc	αYk	αYb$_1$	αYb$_2$	αYp
Normal human kidney												
Proximal tubular cell (n=3)	3	3	2	2	3	2	3	2	2	1	1	2
Glomerular epithelial cell (n=3)	tr	1	tr	tr	tr	tr	tr	3	tr	tr	3	tr
Smooth muscle cell (n=3)	tr	tr	tr	3	tr	tr	tr	2	1	tr	1	tr
Transitional epithelial cell (n=3)	1	3	2	3	2	3	1	3	3	3	3	3
Renal parenchyma adjacent to tumor												
Proximal tubular cell (n=36)	3	3	2	2	3	2	3	2	2	1	1	2
Glomerular epithelial cell (n=36)	tr	1	tr	tr	tr	tr	tr	3	tr	tr	3	tr
Smooth muscle cell (n=36)	tr	tr	tr	3	tr	tr	tr	2	1	tr	1	tr
Transitional epithelial cell (n=10)	1	3	2	3	tr-2	3	1	3	3	3	3	3

[a]: each cell type was graded on a scale of 0 to 4, where 0 was negative staining, tr was a trace of staining, 1 was weak staining, 2 was moderate staining, 3 was heavy staining, and 4 was intense staining. [b]: abbreviations used are: αMnSOD, anti-manganese superoxide dismutase; αCuZnSOD, anti-copper, zinc superoxide dismutase; αCAT, anti-catalase; αGPX, anti-glutathione peroxidase, αGST-L, anti-glutathione S-transferase-liver isolate; αGST-P, anti-glutathione S-transferase-placental isolate; αYa, anti-glutathione S-transferase Ya subunit; αYc, anti-glutathione S-transferase Yc subunit; αYk, anti-glutathione S-transferase Yk subunit; αYb$_1$, anti-glutathione S-transferase Yb$_1$ subunit; αYb$_2$, anti-glutathione S-transferase Yb$_2$ subunit; αYp, anti-glutathione S-transferase Yp subunit.
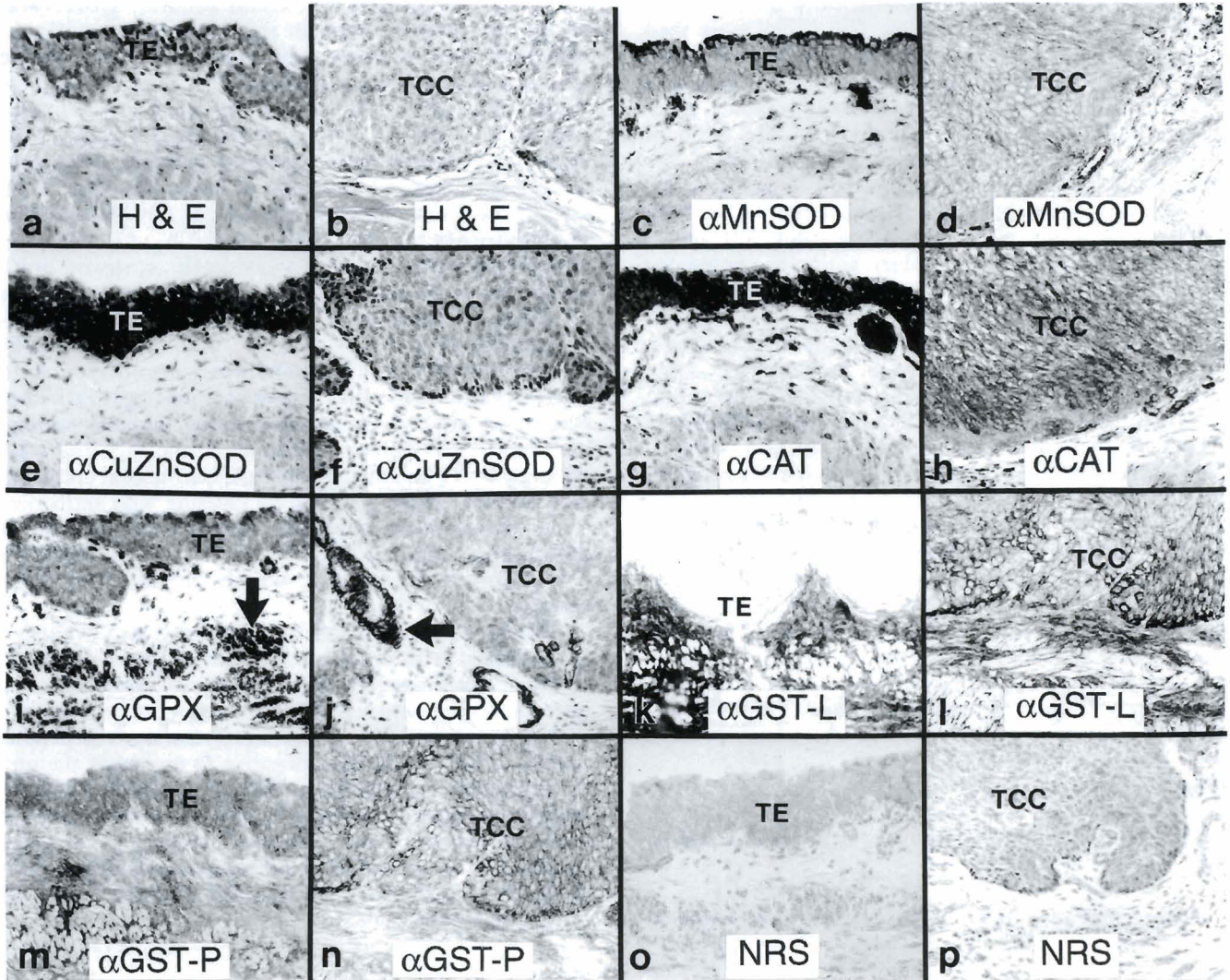
Fig. 1. Immunogold analysis of antioxidant enzymes in transitional cell carcinoma of the renal pelvis and adjacent normal transitional epithelium.
a. Normal transitional epithelium: hematoxylin and eosin stain. Normal transitional epithelium consists of a few cell layers of uniform epithelial cells.
b. Transitional cell carcinoma: hematoxylin and eosin stain. Transitional cell carcinoma shows a multilayered group of nonuniform appearing epithelial cells, with variation in both nuclear and cytoplasmic size and staining characteristics. **c.** Normal transitional epithelium: anti-manganese superoxide dismutase antibody. There was light staining of transitional epithelial cells, with superficial cells showing moderate staining. **d.** Transitional cell carcinoma: anti-manganese superoxide dismutase antibody. Staining was only light in amount. **e.** Normal transitional epithelium: anti-copper, zinc superoxide dismutase antibody. Staining was heavy in amount. **f.** Transitional cell carcinoma: anti-copper, zinc superoxide dismutase antibody. Staining of tumor was weak, although basal cells showed more labeling. **g.** Normal transitional epithelium: anti-catalase antibody. Epithelium showed moderate to heavy labeling. **h.** Transitional cell carcinoma: anti-catalase antibody. Tumor exhibited light labeling. **i.** Normal transitional epithelium: anti-glutathione peroxidase antibody. Moderate labeling was observed in the epithelium. Smooth muscle cells beneath the epithelium showed heavy staining. (arrow). **j.** Transitional cell carcinoma: anti-glutathione peroxidase antibody. Light staining was present in the tumor. Smooth muscle cells of arteries beneath the epithelium showed heavy labeling (arrow). **k.** Normal transitional epithelium: anti-glutathione S-transferase antibody-liver isolate. Epithelium exhibited no significant labeling. However in other cases there was moderate labeling of the epithelium with this antibody. **l.** Transitional cell carcinoma: anti-glutathione S-transferase antibody-liver isolate. Light labeling was present in the tumor. **m.** Normal transitional epithelium: anti-glutathione S-transferase antibody-placental isolate. Epithelium showed moderate staining. **n.** Transitional cell carcinoma: anti-glutathione S-transferase antibody-placental isolate. Light staining was observed in the tumor. **o.** Normal transitional epithelium: normal rabbit serum control. No staining was observed in the epithelium. **p.** Transitional cell carcinoma: normal rabbit serum control. No labeling was present in the tumor. Abbreviations used in this Figure are: TE: transitional epithelium; TCC: transitional cell carcinoma; H&E: hematoxylin and eosin stain; αMnSOD= anti-manganese superoxide dismutase antibody; αCuZnSOD: anti-copper zinc superoxide dismutase antibody; αCAT: anti-catalase antibody; αGPX: anti-glutathione peroxidase antibody; αGST-L: anti-glutathione S-transferase-liver isolate antibody; αGST-P: anti-glutathione S-transferase-placental isolate antibody; NRS= normal rabbit serum. x 525

Immunoperoxidase analysis of selected human kidney tumors

Selected renal cancers were evaluated by immunogold techniques for levels of immunoreactive proteins for antioxidant enzymes. Tumors examined included renal cell carcinoma (clear [n=13], granular [n=2], and mixed [n=3] cell types), papillary carcinoma (n=4), transitional cell carcinoma of the renal pelvis (n=10), and Wilms' tumor (n=4). The results presented in table 2 are the averages of scores obtained from each category of tumor; there was very little variability between samples examined. Each type of renal tumor had a unique antioxidant enzyme profile, although in general,
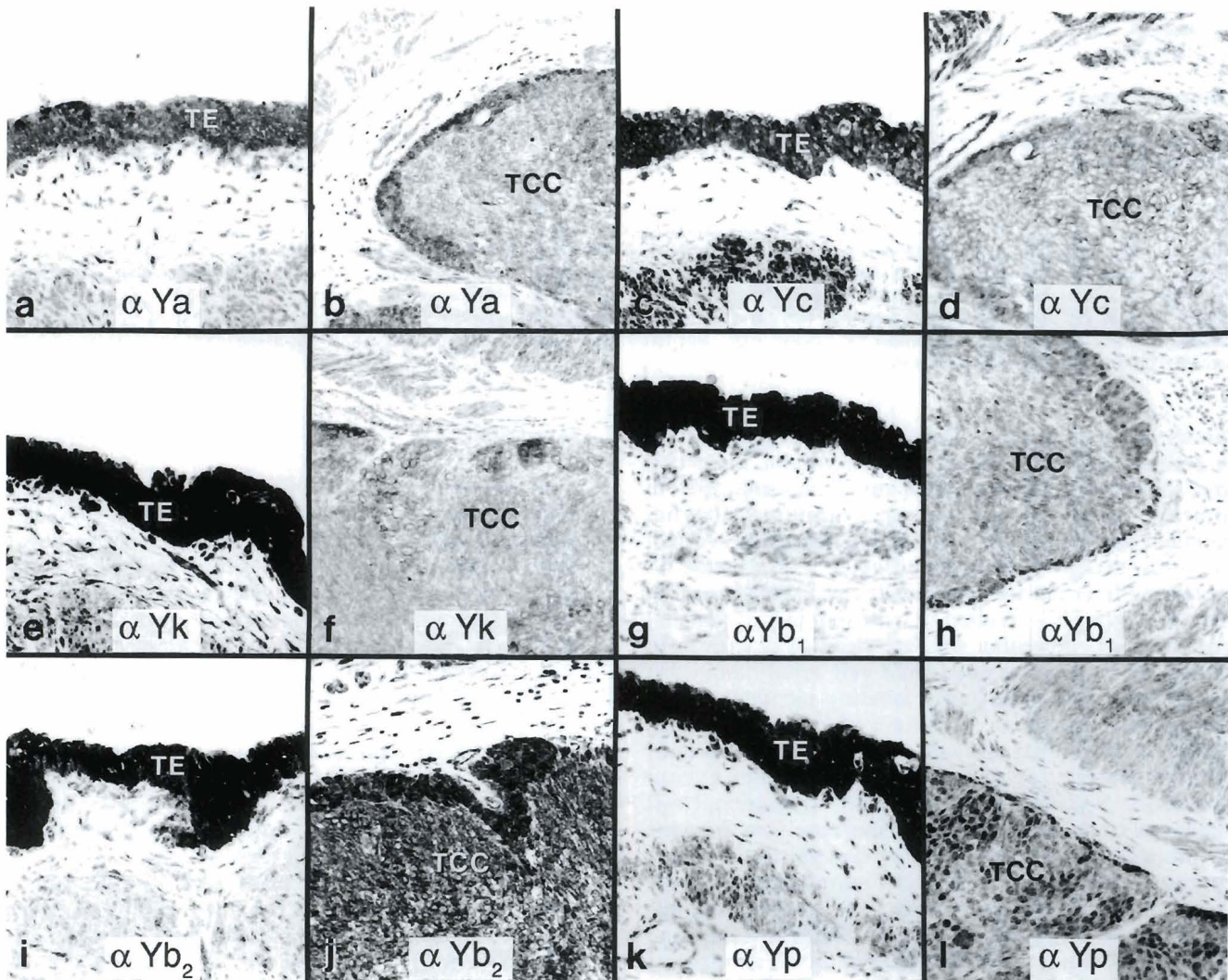

Fig. 2. Immunogold analysis of glutathione S-transferase subunits in transitional cell carcinoma and adjacent normal transitional epithelium. **a.** Normal transitional epithelium: anti-glutathione S-transferase Ya subunit antibody. Epithelium exhibited moderate staining. **b.** Transitional cell carcinoma: anti-glutathione S-transferase Ya subunit antibody. There was light labeling in the tumor. **c.** Normal transitional epithelium: anti-glutathione S-transferase Yc subunit antibody. Epithelium exhibited heavy labeling. **d.** Transitional cell carcinoma: anti-glutathione S-transferase Yc subunit antibody. Tumor demonstrated light immunolabeling. **e.** Normal transitional epithelium: anti-glutathione S-transferase Yk subunit antibody. There was intense staining in the epithelium. **f.** Transitional cell carcinoma: anti-glutathione S-transferase Yk subunit antibody. Tumor exhibited light labeling. **g.** Normal transitional epithelium: anti-glutathione S-transferase Yb_1 subunit antibody. There was intense immunolabeling in the epithelium. **h.** Transitional cell carcinoma: anti-glutathione S-transferase Yb_1 subunit antibody. Tumor showed light staining. **i.** Normal transitional epithelium: anti-glutathione S-transferase Yb_2 subunit antibody. Epithelium demonstrated intense staining. **j.** Transitional cell carcinoma: anti-glutathione S-transferase Yb_2 subunit antibody. Tumor had moderate to heavy staining. **k.** Normal transitional epithelium: anti-glutathione S-transferase Yp subunit antibody. Epithelium showed intense immunostaining. **l.** Transitional cell carcinoma: anti-glutathione S-transferase Yp subunit antibody. Tumor showed moderate labeling. Abbreviations used in this Figure are: TE: transitional epithelium; TCC: transitional cell carcinoma; αYa: anti-glutathione S-transferase Ya subunit antibody; αYc: anti-glutathione S-transferase Yc subunit antibody; αYk: anti-glutathione S-transferase Yk subunit antibody; αYb_1: anti-glutathione S-transferase Yb_1 subunit antibody; αYb_2: anti-glutathione S-transferase Yb_2 subunit antibody; and αYp: anti-glutathione S-transferase Yp subunit antibody. x 525

Table 2. Immunogold analysis of antioxidant enzyme immunoreactive protein in selected human renal tumors[a].

CELL TYPE	ANTIBODY [b]											
	αMnSOD	αCuZnSOD	αCAT	αGPX	αGST-L	αGST-P	αYa	αYc	αYk	αYb$_1$	αYb$_2$	αYp
Renal cell carcinoma												
Clear cell type (n=13)	1	1	1	1	1	tr	1	1	1	tr	1	tr
Granular cell type (n=2)	4	2	3	2	1	2	1	2	3	2	2	2
Mixed (n=3)												
Clear cell	1	1	1	1	1	tr	1	1	1	tr	1	tr
Granular cell	3	2	3	2	1-2	2	1-2	3	3	3	3	3
Papillary carcinoma (n=4)	1	1	1	1	tr	1	tr	1	3	1	1	1
Transitional cell carcinoma of renal pelvis (n=10)	1	1	1	1	1	1	1	2	2-3	2	2	2
Wilm's tumor (n=4)	1	tr	1	1	0	1	0	0	1	tr	tr	tr

[a]: each cell type was graded on a scale of 0 to 4, where 0 was negative staining, tr was a trace of staining, 1 was weak staining, 2 was moderate staining, 3 was heavy staining, and 4 was intense staining. [b]: abbreviations used are: αMnSOD, anti-manganese superoxide dismutase; αCuZnSOD, anti-copper, zinc superoxide dismutase; αCAT, anti-catalase; αGPX, anti-glutathione peroxidase, αGST-L, anti-glutathione S-transferase-liver isolate; αGST-P, anti-glutathione S-transferase-placental isolate; αYa, anti-glutathione S-transferase Ya subunit; αYc, anti-glutathione S-transferase Yc subunit; αYk, anti-glutathione S-transferase Yk subunit; αYb$_1$, anti-glutathione S-transferase Yb$_1$ subunit; αYb$_2$, anti-glutathione S-transferase Yb$_2$ subunit; αYp, anti-glutathione S-transferase Yp subunit.

tumors had low levels of immunoreactive proteins for antioxidant enzymes. Renal cell carcinoma, clear cell type, showed low amounts of all antioxidant enzymes. In contrast, renal cell carcinoma, granular cell type, exhibited moderate to heavy labeling for all antioxidant enzymes except with antibodies to glutathione S-transferase-liver isolate and glutathione S-transferase Ya subunit, which only resulted in light staining. Mixed cell type of renal carcinoma showed clear cells with light immunolabeling as in pure clear cell renal carcinoma and granular cells with heavy labeling identical to that seen in pure granular cell variant of renal carcinoma. Papillary carcinoma showed low levels of immunoreactive protein for all antioxidant enzymes except glutathione S-transferase Yk subunit. Transitional cell carcinoma of the renal pelvis demonstrated low amounts of immunoreactive protein for several antioxidant enzymes, but showed moderate immunolabeling for glutathione S-transferase Yc, Yb$_1$, Yb$_2$, and Yp subunits and heavy labeling for Yk subunit. Wilm's tumor had low levels of immunoreactive protein for all antioxidant enzymes.

Figures 1 and 2 directly compare immunoreactive protein amounts in adjacent normal transitional epithelium and transitional cell carcinoma in a representative case. Normal transitional epithelium has higher antioxidant enzyme levels than transitional cell carcinoma for all antibodies except glutathione S-transferase-liver isolate, which was present in transitional cell carcinoma in low amounts but did not significantly stain adjacent transitional epithelium in this representative case. However, in other cases, antibody to glutathione S-transferase-liver isolate stained normal adjacent transitional epithelium greater than transitional cell carcinoma (compare results in Tables 1, 2).

Discussion

The present study was initiated in order to document levels of immunoreactive proteins for antioxidant enzymes in selected renal tumors and to compare levels of antioxidant enzymes in these tumors with levels in the cell of origin. In order to properly evaluate our results, we first examined immunoreactive proteins for antioxidant enzymes in selected normal kidney cell types. Each cell type had a unique antioxidant enzyme profile. Proximal tubular and transitional epithelial cells had large amounts of many different antioxidant enzymes, while glomerular epithelial and arterial smooth muscle cells exhibited low levels of immunoreactive proteins for the majority of antioxidant enzymes. Similar results were obtained from normal adult kidneys compared to renal parenchyma adjacent to renal tumors.

In general, renal tumors had low levels of immunoreactive proteins for antioxidant enzymes compared to adjacent normal renal parenchymal cells, with exceptions being noted in the Results section. Immunogold studies of transitional cell carcinoma of the renal pelvis and adjacent normal transitional epithelium was especially interesting since a direct comparison of staining properties was possible. With all but one antibody, normal transitional epithelium was found to have more immunoreactive protein than adjacent renal carcinoma. The only exception was using antibody to glutathione S-transferase-liver isolate, and it is possible that the variability in this antibody was due to occasional loss of antigenicity in archival tissue, since this variability was not observed in fresh tissue. While the cause of the decrease in antioxidant enzymes is not clear, we have recently demonstrated that immunoreactive proteins for antioxidant enzymes increase during cell

differentiation (McCormick et al., 1992; Oberley et al., 1995b); therefore, the staining pattern observed may reflect the fact that transitional cell carcinoma cells are less differentiated that normal transitional epithelium. It seems most likely that the low antioxidant enzyme levels observed in most tumors reflect their lack of cell differentiation.

The present study did not show demonstrable variability in the tumors studied for levels of immunoreactive proteins for antioxidant enzymes, except in the case of renal cell carcinoma, to be discussed below. This finding was of interest since it suggests that each tumor has a characteristic antioxidant enzyme phenotype. More cases will have to be studied to determine if this is always true. Further, we have not studied metastatic lesions; these more malignant cells may show more variability than the primary tumors.

Recent studies in our laboratories have demonstrated variable expression of one antioxidant enzyme, manganese superoside dismutase, in human renal carcinoma (Oberley et al., 1994). However, our results in this previous study suggested that the variability was due to the variable numbers of clear and granular cells in various renal cell carcinomas, since we have demonstrated that granular cells have very high levels of superoxide dismutase. The present study confirms these previous findings and extends the results to many other antioxidant enzymes in this tumor. Our present findings demonstrate low levels of immunoreactive protein for antioxidant enzymes in clear cells, with granular cells having moderate to high levels of immunoreactive proteins for many of these same enzymes.

Theoretically, tumors with low levels of antioxidant enzymes should be sensitive to chemo- and radiation therapy. This is certainly true for Wilms' tumor (Johnson et al., 1988). Transitional cell carcinoma is also responsive to chemo- and radiation therapy, though certainly less than for Wilms' tumor (Johnson et al., 1988); the question arises as to whether the greater level of glutathione S-transferases in transitional cell carcinoma than Wilms' tumor could explain the difference in sensitivity between these two tumors. From these theoretical considerations, one would predict that renal carcinoma, clear cell variant, would also be sensitive to chemo- and radiation therapy; however, this is clearly not the case (Johnson et al., 1988). One possibility, therefore, is that levels of antioxidant enzymes have little to do with susceptibility to therapy. However, an alternate possibility is that the variability of renal carcinoma is responsible for treatment failure, with granular cells having high antioxidant enzymes causing the tumor cells to be resistant to chemo- and radiation therapy. Perhaps if all renal cell carcinomas were thoroughly sectioned and analyzed, they would be found to have a proportion of granular cells. Future studies using biochemical analysis of antioxidant enzymes in human renal tumors will be performed to determine if antioxidant enzyme activities correlate with prognosis. This approach should be of great interest since a recent

report has documented greater survival of patients with glutathione S-transferase pi positive renal cell carcinomas than glutathione S-transferase pi negative tumors (Grignon et al., 1994).

In summary, using immunogold techniques and antibodies specific for antioxidant enzymes, common renal cancers were analyzed and each morphologic type was found to have a unique antioxidant enzyme profile. Compared with adjacent normal renal parenchyma, antioxidant enzyme levels were generally low in all cancers examined except the granular cell variant of renal cell carcinoma. It seems most likely that antioxidant enzyme levels are low in most renal cancers as a reflection of the lack of cell differentiation observed in the cancer cell.

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