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Antioxidant enzymes in renal cell carcinoma

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Summary. The aim of the study was to estimate the significance of oxidative/nitrosative damage and expression of antioxidant enzymes in renal cell carcinomas (RCC). For this we investigated immunohistochemically six antioxidant enzymes (AOEs) including MnSOD, ECSOD, thioredoxin, thioredoxin reductase, and gammaglutamyl cysteine synthetase heavy and light chain in 138 RCCs. As an indicator of oxidative/nitrosative damage, sections were stained with an antibody to nitrotyrosine. The extent of apoptosis was evaluated by TUNEL method and proliferation by immunohistochemistry to Ki67. Variable expression of all AOEs could be seen in RCC with expression of MnSOD being strongest. Nitrotyrosine was significantly associated with high grade tumors. MnSOD was associated with tumors of a lower stage. Cases showing ECSOD reactivity had higher and cases expressing thioredoxin lower apoptotic index than other tumors. No association with patient prognosis was observed. According to the results renal cell carcinomas show oxidative/nitrosative damage which, according to nitrotyrosine staining, was higher in high grade tumors. Of AOEs, MnSOD was more abundantly expressed in low stage tumors suggesting that its antioxidant function could play a main role to prevent development of oxidative damage leading to more aggressive tumors.

Key words: Oxidative damage, Cancer, Kidney

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Introduction

Reactive oxygen species (ROS) are reactive oxygen molecules which have at least one unpaired electron on their outer orbital (Gutteridge and Halliwell, 1989; Fridovich, 1998). Consequently, they may be harmful to tissues since they may react with different components of cells. They are formed in several pathological conditions and are involved in for instance inflammation and ischemia. Oxidative damage may also cause genomic damage and thus is involved in the development of cancer (Erhola et al., 1997a,b). There are several lines of defence against oxidative damage in tissues. Compounds capable of neutralising free radicals include vitamin E and C, metal binding proteins, such as ceruloplasmin and transferrin, which counteract the ROS formation induced by metal ions. Additionally, there are antioxidant enzymes (AOEs). They are capable of decomposing superoxide radicals, hydrogen peroxide, and a number of reactive oxygen metabolites (Halliwell and Gutteridge 1996).

Superoxide dismutases (SODs) constitute the first line of antioxidant defense against free radicals (Fridovich, 1998) neutralizing superoxide to hydrogen peroxide which is further metabolised to water by peroxidases. There are three different types of SODs, namely manganese superoxide dismutase (MnSOD), copper zinc superoxide dismutase (CuZnSOD) and extracellular SOD (ECSOD) (Zelko et al., 2002; Kinnula and Crapo, 2003). MnSOD is located in mitochondria, while CuZnSOD is cytosolic and ECSOD extracellular (Weisinger and Fridovich, 1973; Slot et al., 1986; Oury et al., 1994). Thioredoxin (Trx) and thioredoxin reductase (TrxR) are involved in regulating the redox balance of the cells (Powis and Montfort, 2001). Thioredoxin influences transcriptional factors, such as p53, c-fos, c-jun and AP-1 by modulating their

attachment to DNA (Bannister et al., 1991; Ueno et al., 1999) and cell proliferation by increasing it (Grogan et al., 2000). Trx is regulated by TrxR (Arner and Holmgren, 2000) and there are three isoforms of this enzyme in mammalian cells (Sun et al., 1999). The function of TrxR is dependent on selenium concentration of the cells (Arner and Holmgren, 2000).

Gammaglutamyl cysteine synthetase i.e. glutamate-L-cysteine ligase (GLCL) is involved in glutathione synthesis (Sekura and Meister, 1977). Glutathione is an important antioxidant during oxidative stress, it plays a role in the development of chemoresistance of cells and regulates intracellular redox balance (Tew, 1994; Hayes and McLellan, 1999; Stover et al., 2000). GLCL consists of two subunits, the heavy or catalytic subunit with a molecular weight of 73 kDA and the light or regulatory subunit with a molecular weight of 30 kDA (Sekura and Meister, 1977; Seelig et al., 1984). The heavy subunit is more important for its function which is shown by the fact that homozygous knockout GLCLc mice do not survive while heterozygous do (Dalton et al., 2000).

Catalase (CAT) has a molecular weight of 240 kDA (Fridovich and Freeman, 1986). It decomposes hydrogen peroxide to water and oxygen (Fridovich and Freeman 1986). It is mainly located in peroxisomes (Radi et al., 1991). However, acatalasemia, in which there is a deficiency of CAT, does not influence life expectancy (Ogata, 1991).

Expression of AOEs may be of relevance in carcinogenesis. Their expression may influence the progression of tumors by defending tumor cells from oxidative damage (Erhola et al., 1997a). On the other hand, some AOEs, such as GLCL, MnSOD or Trx, induce chemoresistance. Our aim was to investigate the expression of AOEs and related proteins MnSOD, ECSOD, Trx, TrxR, GLCLc and GLCLr in a large set of RCCs to see whether their expression influences the biological behaviour of these tumors. To study the extent of oxidative damage in tumors, they were also stained with an antibody to nitrotyrosine. Additionally, three tumors were investigated by western blotting for all AOE antibodies.

Materials and methods

Materials

The study population consisted of 138 consecutive patients who underwent radical nephrectomy for renal cell carcinoma (RCC) between 1994 and 1999 and who were followed according to a protocol including regular visits and examinations at Tampere University Hospital as shown in a previous study (Kallio et al., 2003). The median age of the patients at the time of operation was 64 years (range 35-86 years). Clinical stage was assigned using the TNM Classification of Malignant Tumors (Sobin and Wittekind, 2002). The mean follow-up time was 40 months, 49 months for survivors and 12 months for non-survivors.

As a control we used two sections from a normal renal tissue removed at the operation of a kidney tumor. All the samples had been fixed in 4% buffered formalin and embedded in paraffin. The array blocks were constructed with a custom-built instrument (Beecher Instruments, Silver Spring, MD). The sample diameter of the tissue core in the array block was 3000 μm. The histological classification of the tumors was made according to the WHO classification of renal tumors (Eble et al., 2004). 125 (90.6%)of the cases were conventional clear cell carcinomas, 6 (4.3%) were papillary carcinomas, 3 (2.2%) were chromophobe and 2 (1.4%) collecting duct carcinomas. 2 cases were unclassified. According to WHO classification there were 6 (4.3%) grade I, 93 (67.4%) grade II and 39 (28.3%) grade III tumors. According to nuclear grading by Fuhrman there were 5 (3.6%) grade I, 60 (43.5%) grade II, 59 (42.8%) grade III and 14 (10.1%) grade IV tumors. The TNM status and stage of all tumors was known. There were 58 (42.0%) stage 1, 15 (10.9%) stage 2, 28 (20.3%) stage 3 and 37 (26.8%) stage 4 tumors. The mean diameter of the tumors was 7.0+3.9 cm (range 23 cm, min 2.0 cm, max 25 cm). 52 (37.7%) patients were women and 86 (62.3%) were men. The mean survival of the patients was 37.5±25.2 months. 40 (29.0%) of the patients died of renal cell carcinoma and 17 (12%) of other causes.

Three kidney tumor samples had been obtained from the operating theatre and immediately stored in liquid nitrogen. These samples were used for western blotting experiments.

The study was approved by the Ethics Committee of Tampere University Hospital and oral informed consent was obtained from every patient.

Methods

Immunohistochemistry/antibodies

The immunostaining procedure was as follows. Four-micron thick sections were cut from a representative paraffin block. The sections were first deparaffinized in xylene and rehydrated in descending ethanol series. In order to enhance immunoreactivity, the sections were incubated in 10 mM citrate buffer (pH 6.0), boiled in a microwave oven for 2 min at 850 W, and after that 8 min at 350 W. Endogenous peroxidase activity was eliminated by incubation in 0.1% hydrogen peroxide in absolute methanol for 10 min. The antibodies used in the study were as follows. A polyclonal rabbit anti-human antibody to MnSOD and ECSOD (a gift from Professor J.D. Crapo, National Jewish Medical Center, Denver, Colorado, dilution 1:1000, 1:200, respectively), rabbit polyclonal anti-human antibodies to GLCLc and GLCLr (a gift from Dr Kavanagh, University of Washington, Seattle, dilution 1:1000 for both), a rabbit polyclonal antibody to CAT (a gift from Professor J.D. Crapo, National Jewish Medical Center, Denver, Colorado, dilution 1:200), an affinity

purified goat polyclonal human Trx antibody (American Diagnostica, Greenwich, CT, dilution 1:200), antibody to TrxR (generous gift from Professor Arne Holmgren, Karolinska Institutet, Stockholm, Sweden, dilution 1:1000) was the gammaglobulin fraction of a polyclonal rabbit anti-rat antibody directed against cytosolic TrxR in rat liver (Luthman and Holmgren, 1982). A polyclonal rabbit anti-human antibody to nitrotyrosine (Upstate, cat No 06-284, Lake Placid, NY) was used with a dilution of 1:100 overnight at 4°C.

The immunostainings for MnSOD, ECSOD, GLCL, CAT and TrxR were done using the Histostain-Plus Kit (Zymed Laboratories Inc, South San Francisco, CA) and the chromogen was aminoethyl carbazole (AEC) (Zymed Laboratories Inc.). In negative controls the primary antibodies were substituted with phosphate-buffered saline (PBS) or non-immune rabbit serum. For Trx, a biotinylated secondary anti-goat antibody was applied followed by the avidin-biotin-peroxidase complex (all from Dakopatts, Glostrup, Denmark). The color was developed using 3,3'-diaminobenzidine, and the sections were lightly counterstained with hematoxylin and mounted with Eukitt (Kindler, Freiburg, Germany). Replacement of the primary antibody by phosphate buffered saline (PBS) at pH 7.2 and goat IgG immunoglobulin isotype (Zymed Laboratories, Inc., San Francisco, CA) were used as negative controls.

The immunostaining results were evaluated by two experienced pathologists (YS, PH) semiquantitatively by dividing the staining reaction into four categories: -, no immunostaining present; +, weak (<25%) immunostaining; ++, moderate (25-50%) immunostaining; +++, strong (>50%) immunostaining.

3'-end labelling of DNA in apoptotic cells

In order to detect apoptotic cells, *in situ* labelling of the 3'-ends of the DNA fragments generated by apoptosis-associated endonucleases was performed using the ApopTag in situ apoptosis detection kit (Oncor, Gaithersburg, MD, USA) as previously described (Soini et al., 1996). As a positive control we used tissue sections from hyperplastic lymph nodes showing an increased number of apoptotic B cells within germinal centers and a low number of apoptotic T cells in the interfollicular areas. Since the array sections were filled with tumor tissue apoptotic cells and bodies were counted in the whole array field of the sections of 3000 μm diameter to estimate the apoptotic activity.

Ki67 immunostaining

For Ki67 immunostaining the antigen retrieval was performed by heating the sections in a microwave oven for 2x7 min in 10 mM Tris/10 mM EDTA (pH 9). The monoclonal antibody MIB-1 (IgG1, Immuno-tech S. A: Marseille, France) was used with a dilution of 1:110. Counter staining was done using 0.4% ethyl green in acetate buffer. The staining was evaluated using a computer-assisted image analysis system (CAS-200 Software, Becton Dickinson & Co., USA). The MIB-1 index was defined as the percentage of cells with immunopositivity in nuclei.

Western blotting experiments

50 μg of protein was run under denaturating and reducing conditions (BioRad, Hercules, CA), transferred to nitrocellulose membranes, and treated with antibodies to MnSOD, ECSOD, Trx, TrxR, GLCL-c and GLCL-r antibodies with a dilution of 1:20000, 1:10000, 1:4000, 1:10000, 1:20000, and 1:10000, respectively. Beta-actin was used as a marker for protein loading, for this purpose mouse monoclonal anti-beta-actin antibody (Sigma, St Louis, MO) at 1:20000 dilution was used. Proteins were detected by enhanced chemoluminence system (ECL; Amersham), and the luminol excitation was imaged on X-ray film.

Statistical analysis

SPSS for Windows (Chicago, IL) was used for statistical analysis. The significance of associations were determined using Fisher's exact probability test, correlation analysis and two tailed t-test. Survival was analysed with the Kaplan-Meier curve, and significance of associations with log rank, Breslow and Tarone-Ware tests. Cox regression multivariate model (stepwise forward) was used in multivariate analysis. Probability values ≤ 0.05 were considered significant.

Results

In non-neoplastic kidney variable expression of all AOEs was seen in kidney tubular cells except for

Table 1. Expression of AOEs and nitrotyrosine in non-neoplastic kidney.

LOCATION	MnSOD	ECSOD	Trx	TrxR	GLCLc	GLCLr	CAT	NITROTYROSINE
Glomerulus	+	-	-	+	+	-	+	+
Proximal tubules	+	-	++	+	+	+	++	+
Loop of henle	++	-	+	+	+	+	+++	+++
Distal tubules	++	-	+	+	+	+	++	++
Collecting duct	+	-	+	+	+	+	+	++

ECSOD (Fig. 1a, Table 1). Positivity in a minor population of glomerular cells was detected for MnSOD, TrxR, CAT and GLCLc. In kidney tumors, the positivity was variable (Fig. 1b, c, d). The highest frequency of positivity was seen for MnSOD while ECSOD showed least immunoreactivity (Fig. 2). The percentage of positivity was 83.7%, 28%, 58.7%, 73.9%, 54.4%, 50.4%, and 50.4% for MnSOD, ECSOD, Trx, TrxR, GLCLc, GLCLr and CAT, respectively. The expression in different histological subtypes is presented in Table 2.

None of the AOEs associated with size of the tumors when they were divided into two groups (T1-2/T3-4). Similarly, no association was found with tumor diameter. Stage 1-2 tumors had significantly more often high (++, +++) MnSOD expression than stage 3-4 tumors (p=0.024). Tumors of male patients expressed significantly more often ECSOD than those of female patients (p=0.0045). Similarly, TrxR (p=0.002), GLCLc (p=0.005) and GLCLr (p=0.008) expression was more often found in tumors of men. In contrast, CAT expression was more often seen in tumors of women (p=0.03 and p=0.013, respectively). Patients with tumors showing Trx positivity were older (65.8±10.9 years v. 60.0±10.4 years, p=0.002). Also, RCCs showing Trx expression had significantly lower apoptotic activity than other cases (2.38±3.35 v. 3.89±5.26/ 3000 μm diameter field, p=0.05), whereas those showing ECSOD expression had a higher extent of apoptosis than negative cases (2.39±3.72 versus 4.90±5.35/ 3000 μm diameter field, p=0.004). GLCLr positive tumors also tended to

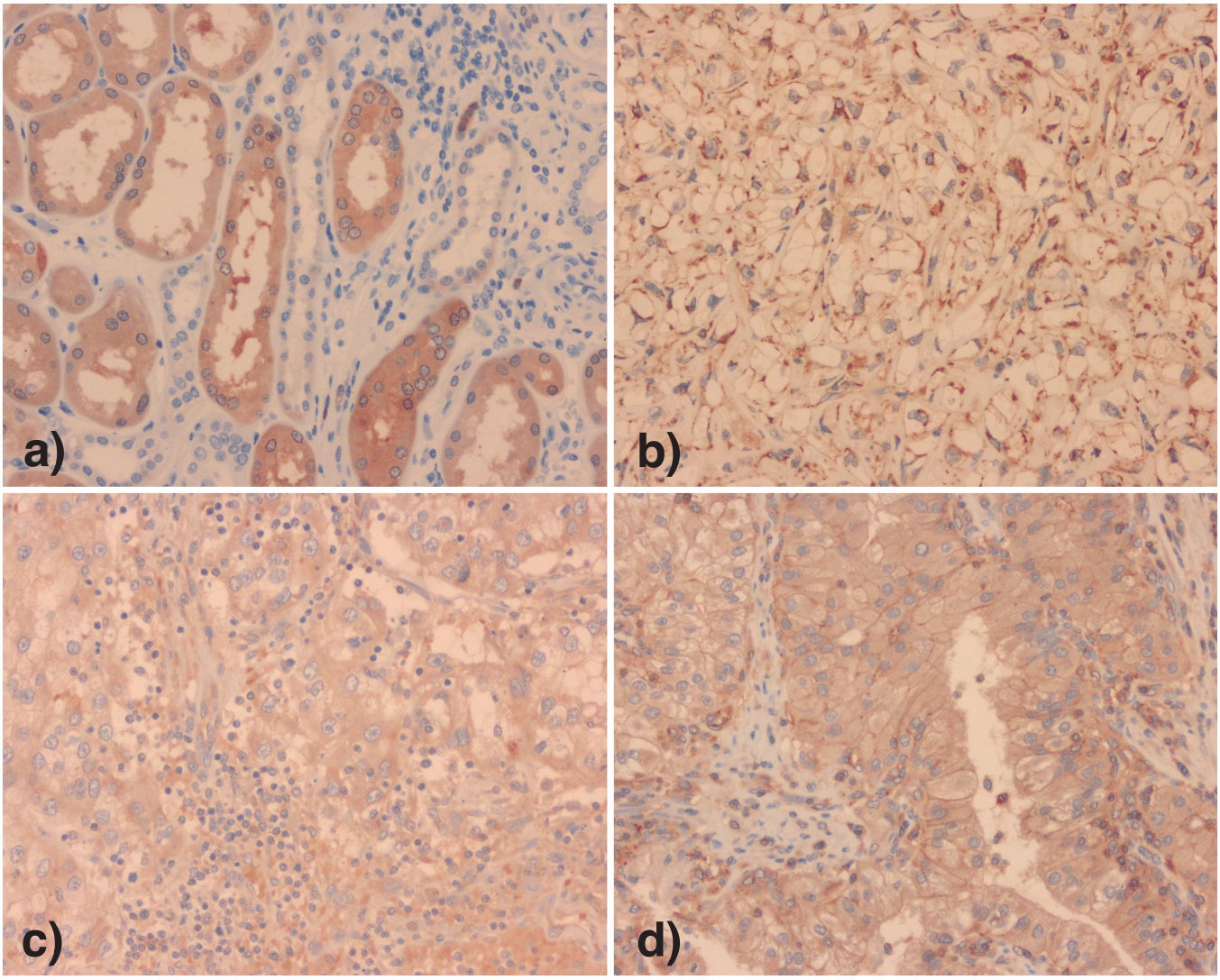

Fig. 1. Examples of positive immunostaining in different renal cell carcinomas and in non-neoplastic kidney. Positivity for Trx can be seen in kidney tubular cells **(a)**. Renal adenocarcinomas show cytoplasmic positivity for MnSOD **(b)**, ECSOD **(c)** and GLCLc **(d)**.

have more apoptosis but the association did not reach statistical significance (p=0.08). However, expression of GLCLr was associated with increased proferative acticity (p=0.001). GLCLr-positive cases showed a MIB index of 8.56±10.17% compared with 3.85±5.12% in low or negative ones. The mean value for apoptosis in the material was 3.03±4.32/ 3000 μm field and for the MIB index 6.15±8.28%/ 3000 μm diameter field. Expression of the AOEs and nitrotyrosine in RCCs in relation to apoptosis and proliferation, tumor stage and Fuhrman grade is presented in Table 3.

Nitrotyrosine expression was seen in 77.6% of the cases (fig. 2), mainly in tumor cells, but sometimes also stroma showed positivity, which was also seen in tubular cells of non-neoplastic renal tissue. Nitrotyrosine expression was significantly associated with a high Fuhrman grade and also with WHO grade III tumors (p=0.001 and p=0.004, respectively). There was an association between nitrotyrosine expression and TrxR and GLCLc (both p>0.001), and a nearly significant association with Trx (p=0.057).

In western blotting experiments, presence of all antioxidant enzymes studied were also seen in western blot experiments performed on the tumor samples (Fig. 3a,b).

Discussion

We investigated the expression of AOEs and related proteins MnSOD, ECSOD Trx, TrxR, GLCLc, GLCLr, and CAT in a large set of RCCs. These enzymes are expressed in RCC to a similar extent as in other tumors (Janssen et al., 2000; Kahlos et al., 2000, 2001a,b; Soini et al., 2001a-c; Järvinen et al., 2002; Haapasalo et al., 2003; Nozoe et al., 2003). AOEs and redox modulatory proteins have been shown to influence the progression of other tumors, such as gastrointestinal malignancies, brain tumors and mesotheliomas, where the expression of MnSOD, Trx and/or CAT has prognostic significance (Haapasalo et al., 2003; Kahlos et al., 2001a, b; Nozoe et al., 2003; Janssen et al., 2000).

AOEs have a potential role in the regulation of cellular redox state and, therefore, they not only affect the oxidant resistance but may also take part in the regulation of cell growth and apoptosis (Zelko et al., 2002). In non-small cell lung carcinomas, Trx and TrxR positivity was inversely associated with apoptosis (Soini et al., 2001a). Similar to this, RCCs showing Trx expression had a significantly lowered apoptosis. Support for the observation of an antiapoptotic function of Trx comes from in vitro studies, where adenoviral transfection of the Trx gene to hepatocytes leads to abrogation of apoptosis (Tsutsui et al., 2003). Inversely, absence of Trx in mouse embryonic development leads to massive apoptosis (Nonn et al., 2003). In non-small cell lung carcinomas also, GLCL is associated with apoptosis, GLCL positive tumors having a higher apoptotic index (Soini et al., 2001c). However, in RCC we could not find any significant association between apoptosis and GLCLr or GLCLc expression. In our recent study, we found an association between proliferation and GLCLc in astrocytomas and between GLCLr and proliferation in pilocytic astrocytomas (Haapasalo et al., 2003), but no such trend could be seen in mesothelioma even though they had high GLCL expression (Järvinen et al., 2002). Overall it appears that Trx is a very potent modulator of tumor apoptosis and proliferation not only in vitro but also in several human

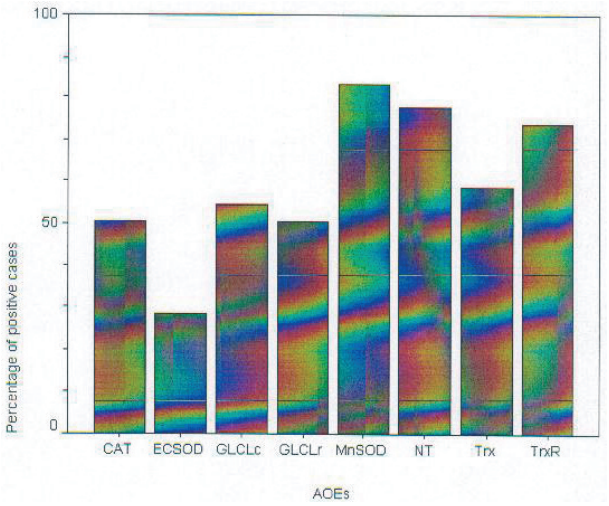

Fig. 2. Frequency of AOE positivity in renal cell carcinomas. Expression of CAT, ECSOD, GLCLc, GLCLr, MnSOD, Trx, TrxR and NT is shown. The most frequent expression is for MnSOD and the least for ECSOD.

Table 2. Expression of AOEs in different histological subtypes of RCC. The number of negative cases is shown on left and positive on fight.

	MnSOD Negative/ positive	ECSOD Negative/ positive	Trx Negative/ positive	TrxR Negative/ positive	GLCLc Negative/ positive	GLCLr Negative/ positive	CAT Negative/ positive	Nitrotyrosine Negative/ positive
Clear cell	22/101	88/34	50/73	32/91	52/70	64/59	63/61	56/66
Papillary	1/5	3/3	3/3	2/4	4/2	1/5	1/5	4/2
Chromophobe	0/2	1/1	2/0	1/1	1/1	1/1	1/2	1/1
Collecting duct	1/1	2/0	2/0	1/1	1/1	1/1	2/0	2/0
Others	0/2	2/0	1/1	0/2	0/2	0/2	1/1	0/2

malignancies in vivo.

Only 28% of RCCs showed ECSOD immunoreactivity. This is in accordance with a study which also showed hardly detectable positivity of ECSOD in lung carcinoma (Svensk et al., 2004). ECSOD is, however, widely expressed in healthy tissues (Marklund, 1984; Oury et al., 1994). Being extracellular, low or absent ECSOD in the tumors may also modulate the extracellular redox milieu with consequent effects on tumor growth or invasion. In renal carcinomas cases showing high ECSOD activity had a high extent of apoptosis. Expression of MnSOD, on the other hand, can

Table 3. Expression of AOEs and nitrotyrosine in relation to apoptosis, proliferation, tumor stage and Fuhrman grade in RCC.

	EXPRESSION*	APOPTOSIS%	PROLIFERATION%	RCC LOW STAGE	RCC HIGH STAGE	FUHRMAN GRADEI-II	FUHRMAN GRADEIII-IV
MnSOD	low	1.83±1.59	5.4±8.1	28	38	27	39
	high	2.60±4.59	6.4±9.0	42	27	37	32
EcSOD	low	2.39±3.72	5.4±7.6	52	44	43	53
	high	4.90±5.36	8.3±9.9	17	21	20	18
Trx	low	3.89±5.26	5.5±7.8	29	28	24	33
	high	2.38±3.35	6.6±8.8	41	36	39	38
TrxR	low	2.59±3.62	5.7±8.9	20	16	17	19
	high	3.22±4.60	6.3±8.2	50	49	47	52
GLCLc	low	3.41±4.84	4.7±7.2	32	26	27	31
	high	2.77±3.88	7.3±9.1	37	38	35	40
GLCLr	low	2.39±4.08	3.8±5.1	36	31	32	35
	high	3.76±4.53	8.5±10.2	34	34	32	36
CAT	low	2.42±3.66	7.2±9,4	40	28	34	34
	high	3.55±4.77	5.1±7.0	32	37	31	38
Nitrotyrosine	low	3.60±4.43	6.4±8.9	30	33	39	24
	high	2.32±4.06	5.9±8.0	39	32	25	46

*for MnSOD low=-, + and high=++, +++; for others low =- and high= +,++,+++

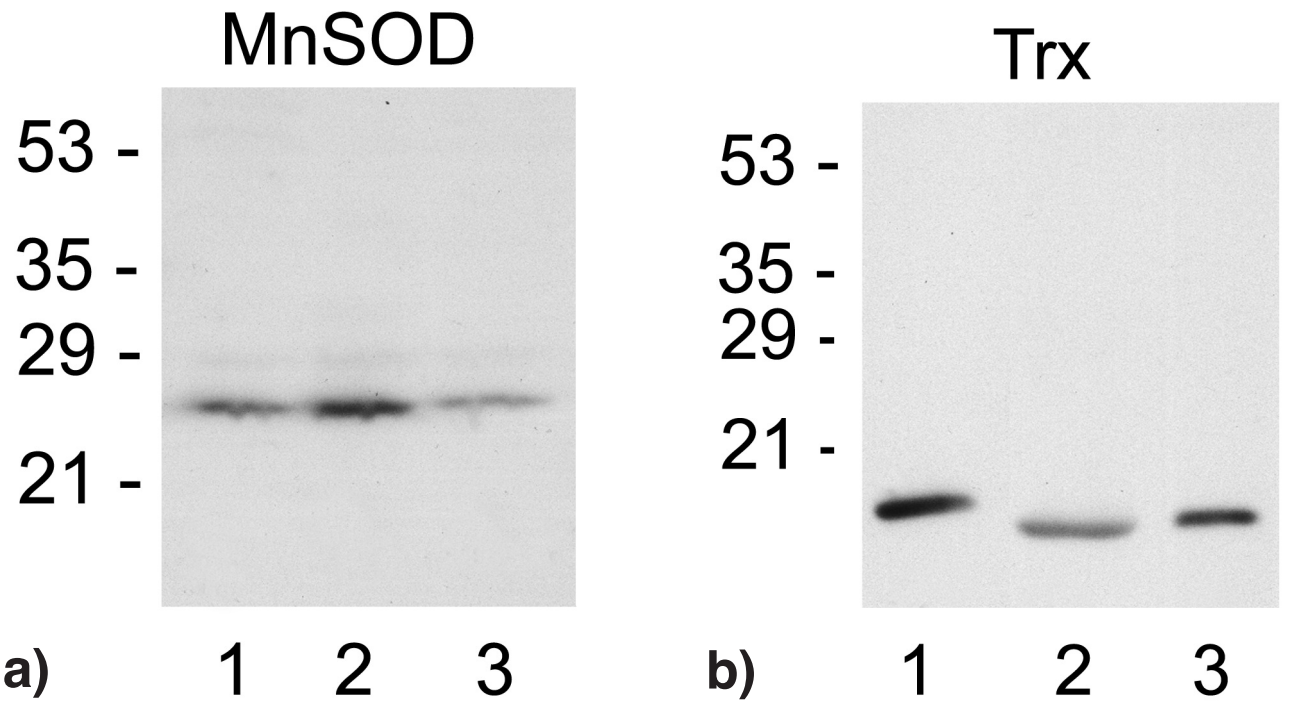

Fig. 3. Some immunoblotting experiments performed on fresh tissue samples on renal cell carcinomas. With antibodies to MnSOD **(a)**, and Trx **(b)** bands corresponding to the proteins could be observed in all samples.

prevent apoptosis (Epperly et al., 2003) but, similar to studies on breast carcinoma and mesothelioma (Kahlos et al., 2001a; Soini et al., 2001b), no such effect could be seen in renal tumors. Overexpression of MnSOD has previously been shown in RCC which is in line with our results (Shi et al., 2004). In the studies where the MnSOD gene has been transfected to a number of cells and also in mesotheliomas, MnSOD has been shown to be inversely correlated with cell proliferation (Kahlos et al., 2001a; Oberley et al., 1995a) , but this trait could not be observed in renal tumors.

In non-neoplastic kidney, expression of AOEs was seen mainly in kidney tubular epithelial cells. This is in line with previous studies showing AOEs in hamster renal tubular cells already before birth (Oberley et al., 1995b). Since renal cell carcinomas originate from tubular cells, expression of these AOEs in renal tumors could be regarded as a trait of differentiation of neoplastically transformed tubular epithelial cells. Interestingly, expression of ECSOD was lowest in renal tumors. This AOEs was also the only one which was not detected in tubular cells of non-neoplastic kidney.

There was a close association between the expression of different AOEs in the renal tumors. This is in line with previous studies on brain tumors and bronchial dysplasia (Haapasalo et al., 2003; Soini et al., 2003) where similar trends and/or high expression of several AOEs have been detected. Such a phenomenon most likely speaks for the fact that the oxidative load and formation of ROS in RCCs simultaneously leads to induction of many AOEs and related proteins. An indirect evidence of this is the close association of nitrotyrosine expression and some of the AOEs such as TrxR and GLCLc.

Expression of MnSOD was higher in tumors of lower stage. In fact, in vitro studies with transfected MnSOD and high MnSOD expression have suggested that MnSOD functions as a tumor suppressor gene (Li et al., 1995). On the other hand, oxidant milieu of these cells is very different compared to situation in vivo where tumors have been shown to have increased oxidant burden and induction of several AOEs. Moreover MnSOD has been shown to be highly overexpressed in a number of human malignancies when assessed by mRNA, protein and/or activity (Kinnula and Crapo 2004) .

Interestingly also, the expression of nitrotyrosine was associated with higher grade tumors. This could indicate that in their development tumors of higher grade have been susceptible to oxidative damage, induction of some AOEs but due to the disturbance in their redox balance accumulated more genetic damage leading to higher anaplasia of the tumor cells. Increased oxidative damage in tumors has also been shown in a number of previous studies (Kinnula and Crapo, 2004), but our study clearly shows that the damage can be associated not only with the expression of several defense mechanisms but also with tumor behavior.

RCCs with ECSOD, TrxR, GLCLC or GLCLR expression were more likely found in men. The association of expression of these AOEs with male gender is obscure. It has, however, been found that oxidative damage in skeletal muscle increases with age and is higher in elderly males than females (Fano et al., 2001). A higher expression of AOEs in renal tumors might simply indicate a higher ROS load also in neoplastic tissue of male patients. In contrast, CAT was elevated in tumors of female patients. The putative reason for the association of the AOE expression with gender in RCC might be more complex and associated also with hormonal metabolism. MnSOD, ECSOD and Trx expression, for example, has been shown to be induced by estrogen while catalase was not affected (Chiueh et al., 2003; Strehlow et al., 2003). Estrogen receptors are, however, only rarely found in RCC (Brown et al., 1998), even though some responses to medroxy progesterone acetate have been reported in metastatic RCC (Naglieri et al., 2002).

In conclusion, our results show that AOEs and related proteins are expressed in RCC to a variable degree. Their presence can also be shown in normal kidney where site and cell specific difference in tubular and glomerular compartments can be observed. Expression of nitrotyrosine was present in renal cell tumors as evidence of oxidative/nitrosative damage and was associated with the expression of several enzymes. The expression of some of these enzymes might influence histological aggressivity of tumors by protecting tumor cells from oxidative damage and consequent increased genetic instability.

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