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METABOLIC ACTIVATION/INACTIVATION

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Glutathione transferase activities in renal carcinomas and adjacent normal renal tissues: factors influencing renal carcinogenesis induced by xenobiotics

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Abstract In general, the biological activation of nephrocarcinogenic chlorinated hydrocarbons proceeds via conjugation with glutathione. It has mostly been assumed that the main site of initial conjugation is the liver, followed by a mandatory transfer of intermediates to the kidney. It was therefore of interest to study the enzyme activities of subgroups of glutathione transferases (GSTs) in renal cancers and the surrounding normal renal tissues of the same individuals ($n = 21$). For genotyping the individuals with respect to known polymorphic GST isozymes the following substrates with differential specificity were used: 1-chloro-2,4-dinitrobenzene for overall GST activity (except GST θ); 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole for GST α; 1,2-dichloro-4-nitro-benzene for GST μ; ethacrynic acid and 4-vinylpyridine for GST π; and methyl chloride for GST θ. In general, the normal tissues were able to metabolize the test substrates. A general decrease in individual GST enzyme activities was apparent in the course of cancerization, and in some (exceptional) cases individual activities, expressed in the normal renal tissue, were lost in the tumour tissue. The GST enzyme activities in tumours were independent of tumour stage, or the age and gender of the patients. There was little influence of known polymorphisms of GSTM1, GSTM3 and GSTP1 upon the activities towards the test substrates, whereas the influence of GSTT1 polymorphism on the activity towads methyl chloride was straightforward. In general, the present findings support the concept that the initial GST-dependent bioactivation step of nephrocarcinogenic chlorinated hydrocarbons may take place in the kidney itself. This should be a consideration in toxicokinetic modelling.

Key words Glutathione transferases · Kidney tissue · Renal carcinomas · Phenotyping · Genotyping

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Introduction

The much-debated toxicological issue of human carcinogenicity of trichloroethylene (for recent reviews, see Brüning and Bolt 2000, Goeptar et al. 1995, and Wartenberg et al. 2000) has focused new interest on the mechanisms of human renal carcinogenicity of chlorinated hydrocarbons (Lash et al. 2000a, 2000b). Besides trichloroethylene, renal carcinogenicity is suspected for dichloroacetylene (DFG 1995; IARC 1986), hexachloro-1,3-butadiene (DFG 1983), 1,3-dichloropropene (DFG 1985) and tetrachloroethylene (DFG 1997).

The mechanism of biological activation of nephrocarcinogenic chlorinated hydrocarbons has been elucidated. It proceeds via conjugation with glutathione and subsequent formation of reactive metabolites by β-lyase (Birner et al. 1993; Dekant et al. 1993). To extrapolate from animal experiments to the risk prevailing in humans, toxicokinetic (PBPK) modelling is a very useful tool (Fisher 2000). However, a difficulty with this

approach, as outlined very recently (Lash et al. 2000b), is that expression of glutathione transferase (GST) isozymes in humans is different from that in the rat which is the main experimental species. It is generally assumed that the main site for the initial GSH conjugation of halogenated hydrocarbons is the liver, followed by a mandatory transfer of the gluthathione or cysteine conjugates to the kidney (Green et al. 1997).

As the question of human variability and individual susceptibility is much debated (Pastino et al. 2000), it was of importance to determine whether the activities of GST θ (i.e. polymorphic hGSTT1-1) are expressed in human renal tissue (Thier et al. 1998a, 1998b). Moreover, it is well established that GST α and GST π are constitutive enzymes in the proximal and the distal renal tubule, respectively. The urinary excretion of both isoforms has been taken as indicative of specific toxic damage to these sites of the nephron (Sundberg et al. 1993, 1994). This has been a specific cornerstone in the discussion of nephrocarcinogenicity of trichloroethylene (Brüning et al. 1999).

There is an ongoing discussion about possible changes in expression of GST π (i.e. hGSTP1-1) in the course of cancerization of human renal tissue (see Discussion). This is relevant to the problem of nephrocarcinogenicity of chlorinated hydrocarbons, as chemical carcinogenesis is a multistage process, and further genotoxic hits might also be relevant in later stages of carcinogenesis. In the case of trichloroethylene, it has been shown that multiple mutations of the von Hippel-Lindau tumour suppressor gene are not uncommon (Brauch et al. 1999; Brüning et al. 1997). Therefore, it would be of interest to know about possible differential changes in the renal expression of individual subforms of GST in renal cancer development. Moreover, a number of recently discovered human polymorphisms of individual GST isozymes (Eaton and Bammler 1999) must be considered in this context.

This was the reason for studying the phenotypes of different subgroups of GSTs in renal cancers and the surrounding normal renal tissues of the same individuals using an array of substrates of differential specificity, in relation to the genotype of the individuals with respect to known polymorphic GST isozymes.

Materials and methods

Specimens of normal kidney tissue and the corresponding tumour tissue were obtained from 21 renal cell cancer patients, as listed in Table 1, which also shows the histopathological diagnoses, classifications and gradings.

Genotypings of polymorpic GST

Extraction of DNA from normal kidney tissues was performed using a commercial DNA isolation kit (Qiagen, Hilden, Germany). The multiplex-PCR method of Kempkes et al. (1996) was used to determine the presence of a functional *hGSTT1* or *hGSTM1* allele (see also Thier et al. 1999). The hGSTM1 polymorphism of GSTM1-positive genotypes was obtained by a semiquantitative PCR using allele-specific primers (Fryer et al. 1993). Determination of the GSTM3 polymorphism was carried out as described by Inskip et al. (1995). The *hGSTP* polymorphism at codon 105 was determined by PCR-RFLP by the method of Harries et al. (1997) and of the codon 114 by the method of Harris et al. (1998).

The nomenclature of allelic variants of GST followed the proposals of Ali-Osman et al. (1997) and Watson et al. (1998), as summarized by Eaton and Bammler (1999).

Enzyme assays

The preparation of kidney cytosols from the tissue specimens followed the procedure described by Thier et al. (1998a). Protein contents were determined with a commercial BCA Protein Assay Reagent Kit (Pierce). The GST enzyme activities were determined using the methods described in the following sections.

Table 1 Composition of the study group and morphological characterization of the kidney tumours

No.	Gender (m, f)	Age (years)	pT/Grading	Morphology
1	m	67	pT2/G2	Clear cell cancer
2	m	47	pT2/G2	Clear cell cancer
3	f	65	pT3b/G2	Clear cell cancer
4	m	64	ptTb/G2	Clear cell cancer
5	f	63	pT3B/G2	Clear cell cancer
6	m	64	pT3b/G2	Clear cell cancer
7	m	62	pT2/G2	Clear cell cancer
8	f	68	pT3a/G2	Clear cell cancer
9	m	61	pT3a/G2	Clear cell cancer
10	m	55	pT2/G2	Clear cell cancer
11	f	63	pT3a/G2	Clear cell cancer
12	f	67	pT2/G2	Clear cell cancer
13	m	72	pT3b/G2	Clear cell cancer
14	m	52	pT3a/G2	Clear cell cancer
15	m	63	pT3a/G2	Chromophilic cancer
16	f	76	pT3a/G2	Chromophilic cancer
17	m	61	pT3a/G2	Chromophobic cancer
18	m	65	pT2/G2	Chromophobic cancer
19	f	74	pT2/G2	Chromophobic cancer
20	m	69	pT3a/G2	Chromophobic cancer
21	m	67	pT1/G2	Cancer of ductus Bellini

Overall GST activity (except GST θ)

Conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) was determined spectrophotometrically as described by Habig and Jacoby (1981).

GST α

Activity towards 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) was measured spectrophotometrically according to the method of Ricci et al. (1994).

GST μ

The activity towards 1,2-dichloro-4-nitrobenzene (DCNB) was determined spectrophotometrically according to the method of Habig et al. (1974).

GST π

The activities were spectrophotometrically quantitated towards ethacrynic acid (EA) and 4-vinyl-pyridine (VP), the methods of Habig and Jacoby (1981) and Berhane et al. (1994), respectively.

GST θ

Activity towards methyl chloride was measured by gas chromatographic monitoring of methyl chloride in a closed vial, according to the method of Peter et al. (1989).

Results

The results are presented in Figs. 1, 2, 3, 4, 5 and 6. All enzyme assays were performed in triplicate, and the results are expressed as the means ± SD of these triplicates. The figures show the individual data of the normal and cancer tissues from the 21 renal cell cancer patients, as well as the results of the individual genotypings of polymorphic GST isozymes. The designations of the polymorphisms followed the proposals of Eaton and Bammler (1999).

Figure 1 refers to the activities towards CDNB which is considered nonspecific for individual GST classes (except class θ, which does not transform CDNB; Schröder et al. 1996), Figs. 2, 3, 4, 5 and 6 display the results using individual GST substrates which are considered specific for distinct classes of GST. The results of genotypings for polymorphic GSTs, i.e. hGSTM1, hGSTM3 (Fig. 3), hGSTP1 (Figs. 4 and 5), hGSTT1 (Fig. 6) are also shown. In general, a decrease in individual GST enzyme activities was apparent in the course of cancerization, and in some (exceptional) cases individual activities which were expressed in the normal renal tussue were practically absent in the tumour tissue. However, there were also cases in which an enzyme activity was (slightly) greater in the tumour. The mean percentage decreases were 46% for CDNB (Fig. 1; $P < 0.0001$), 69% for NBD-Cl (Fig. 2; $P < 0.0001$), 56% for DCNB (Fig. 3; $P < 0.0001$), 45% for EA (Fig. 4; $P < 0.0001$), 14% for VP (Fig. 5; $P < 0.194$, n.s.), and 69% for MC (Fig. 6; $P < 0.0001$). All GST enzyme activities in tumours were independent of the tumour stage, or the age and gender of the patient.

In general, it can be stated that, using an array of standard GST substrates, activities towards these substrates could be demonstrated in all samples of normal (non-cancer) renal tissues examined. There was a considerable individual variation of activities, and obviously it must be considered that the substrates used had overlapping affinities towards the different GST subtypes, although they are considered specific in the literature. In the course of cancer development there is a clear tendency towards a decrease in all the GST sub-

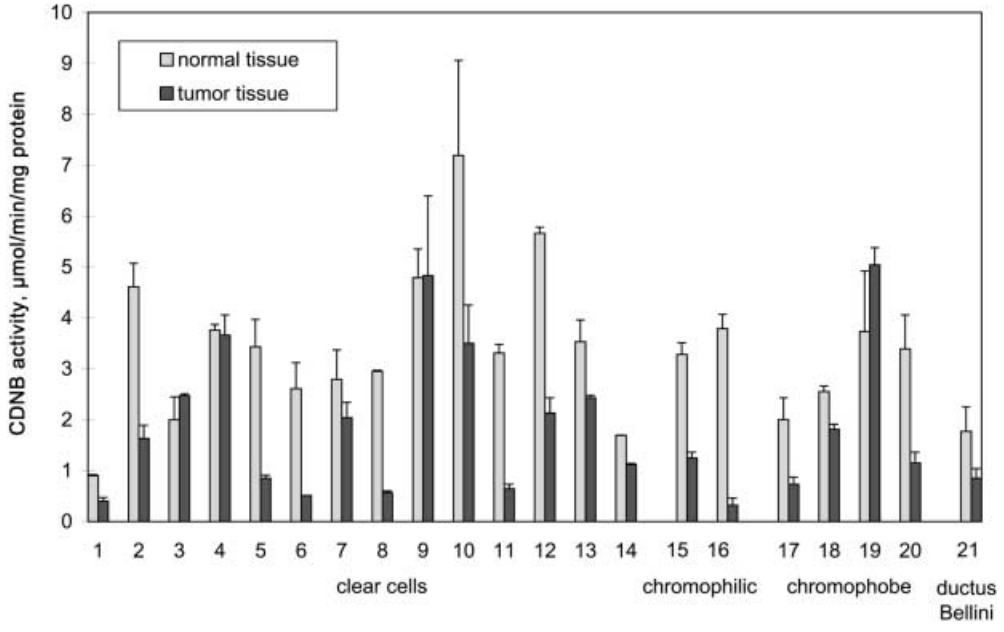

Fig. 1 GST activities in normal and tumour renal tissues determined by the substrate 1-chloro-2,4-dinitrobenzene (CDNB). This substrate is considered nonspecific, being transformed by all GST forms except GST θ

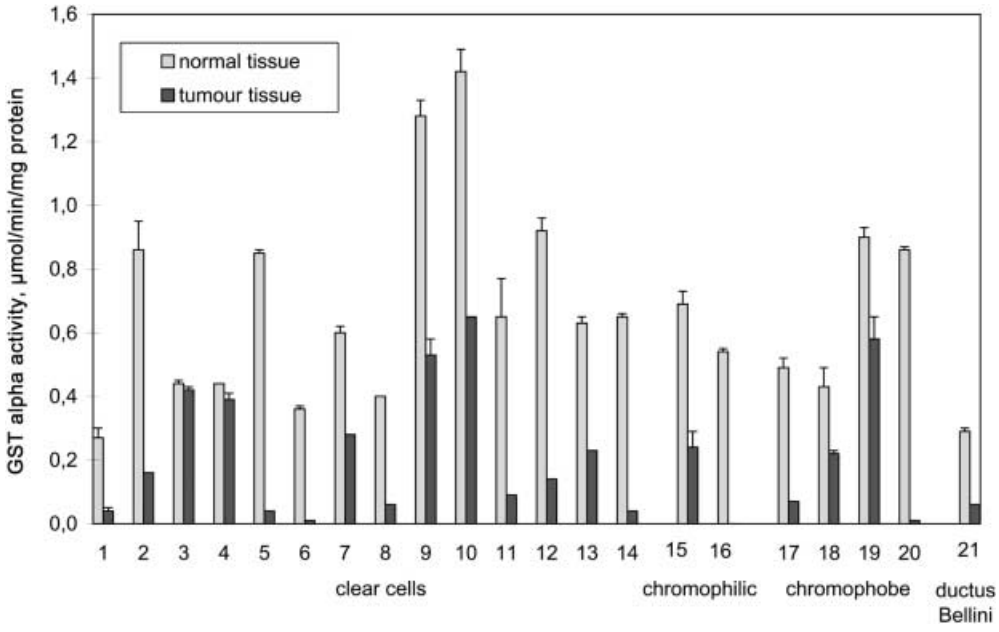

Fig. 2 GST activities in normal and tumour renal tissues determined by the substrate 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl), considered specific for GST α

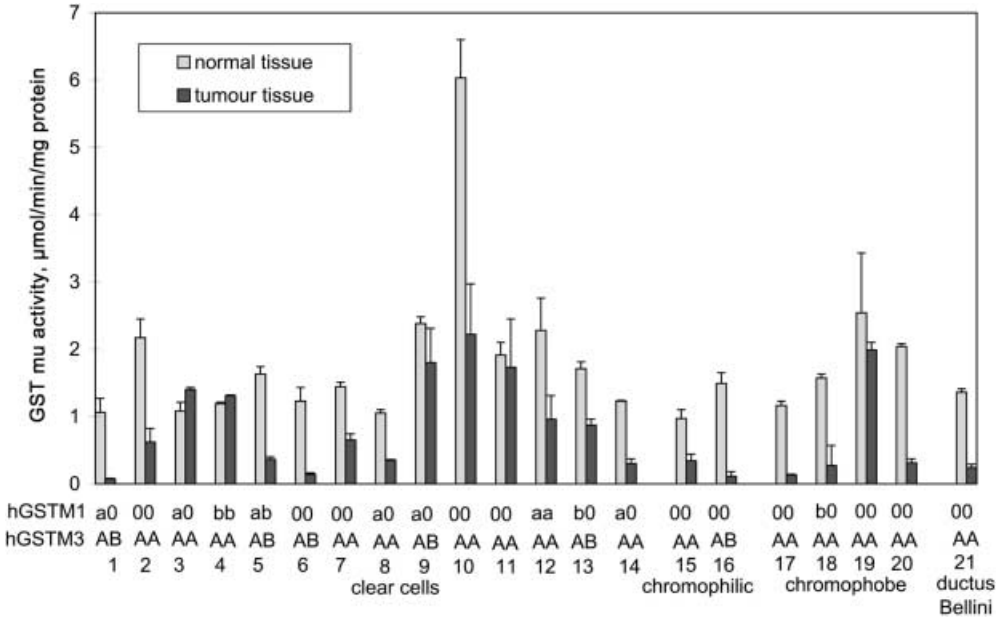

Fig. 3 GST activities in normal and tumour renal tissues determined by the substrate 1,2-dichloro-4-nitrobenzene (DCNB), considered specific for GST μ. The results of individual genotypings for the polymorphic GST μ enzymes hGSTM1 and hGSTM3 are also shown

types, although in single cases increases or even the absence of single GST subtypes can also be observed. Except for the known clear coherence between the hGSTT1 genotype and the phenotype (using methyl chloride as substrate; Pemble et al. 1994), no straightforward connection between polymorphisms of individual GST subtypes and activities towards the standard substrates has been observed.

Discussion

The presence of subtypes of GSTs in human tumour tissues has been a matter of research in conjunction with questions of chemoprotection and drug resistance (Hayes and Pulford 1995). In particular, the influence of cancerization upon the expression of human renal GST π (hGSTP1-1) has been much debated since Di Ilio et al. (1987) proposed the use of this isoenzyme as a biomarker of human renal cell carcinomas. Since that time, reported results on expression levels of hGSTP1-1 in renal cell cancer tissues have been contradictory. Whilst Di Ilio et al. (1987) and Toffoli et al. (1992) have reported an overexpression of the isozyme in tumours, Howie et al. (1990) and Rodilla et al. (1998) have reported an unchanged expression, and Klöne et al. (1990) a decrease in GST π in tumours down to about half the level of the surrounding normal renal

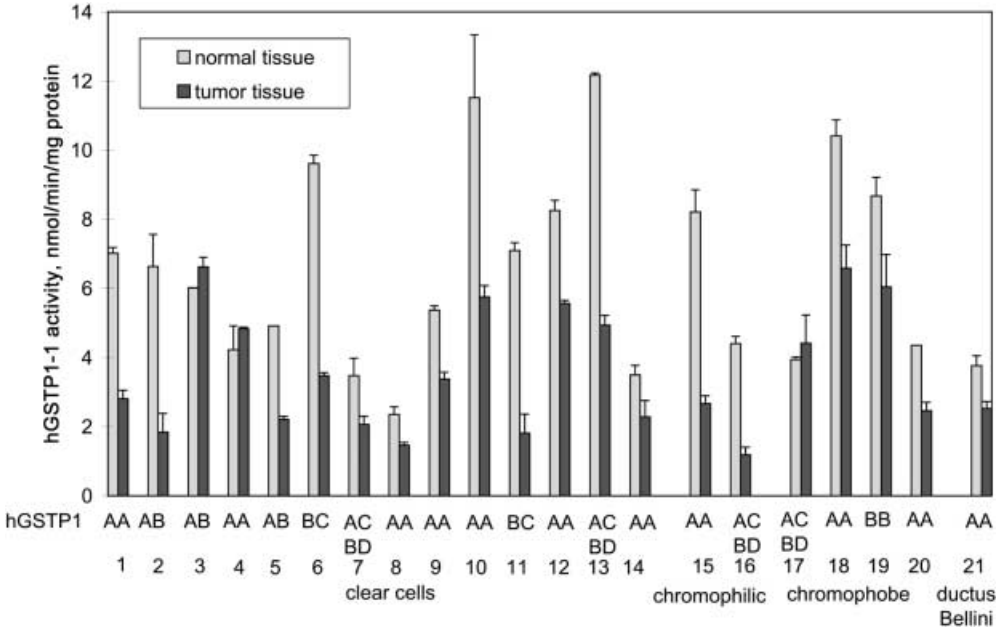

Fig. 4 GST activities in normal and tumour renal tissues determined by the substrate ethacrynic acid (EA), considered specific for GST π. The results of individual genotypings for the polymorphisms of hGSTP1 are also shown

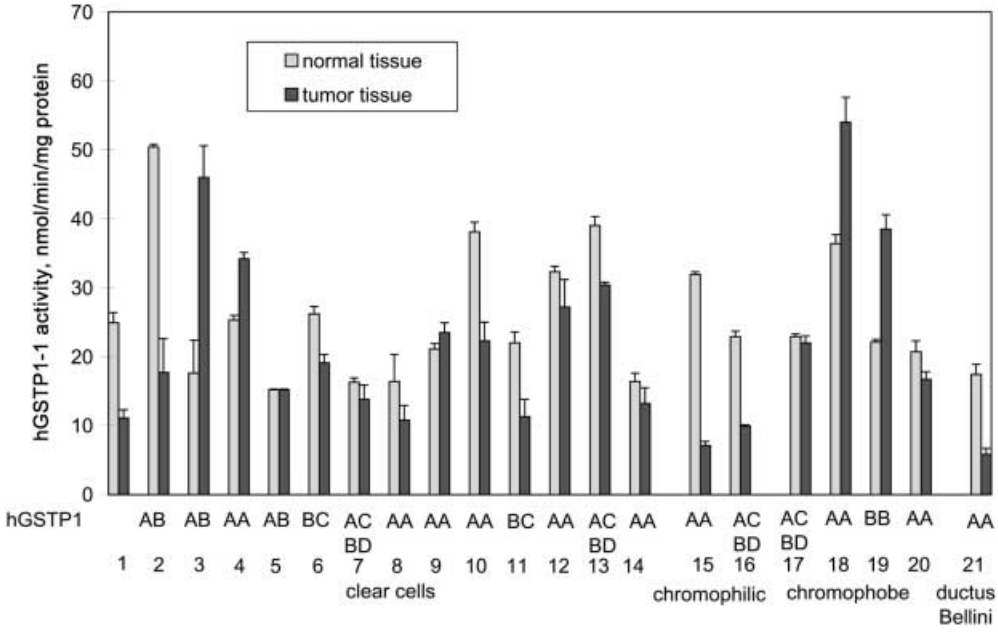

Fig. 5 GST activities in normal and tumour renal tissues determined by the substrate 4-vinylpyridine (VP), considered specific for GST π. The results of individual genotypings for the polymorphisms of hGSTP1 are also shown

tissue. Moreover, Klöne et al. (1990) reported a decrease in GST α protein, which is highly expressed in the normal proximal tubule, down to levels of practically zero in renal carcinomas. The present data showed a clear general tendency towards decreased activities of GSTs in renal tumours, compared to the surrounding non-cancer tissues, but there was high individual variation. This variability obviously accounts for the discrepancies between the results of previous studies which were mostly based on very low sample numbers (with the exception of the study of Klöne et al. (1990) with 30 cases in total).

Against the background of the present debate on the human carcinogenicity of trichloroethylene (see Introduction), it has been suggested (Lash et al. 2000a) that work is currently needed to better define the isoenzyme composition of renal GSTs in various species, including humans. The primary object of the present study was to determine the enzyme activity levels because of the possibility of bioactivation of a whole group of proven or suspected nephrocarcinogens by renal GSTs in situ. As a result, it can clearly be stated that the human kidney does indeed express the potentially relevant GST isozymes, and that at least some GST enzyme activities are generally retained in the process of cancerization,

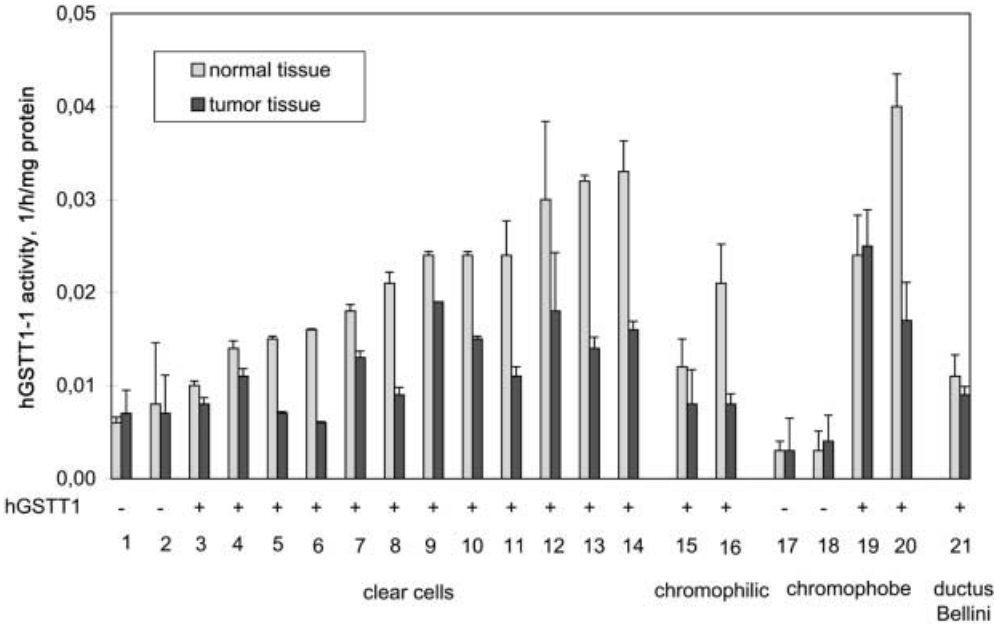

Fig. 6 GST activities in normal and tumour renal tissues determined by the substrate methyl chloride (MC), considered specific for GST θ. The results of individual genotypings for the polymorphisms of hGSTT1 are also shown

although they tend to decrease to about half of the original level. Importantly, there is considerable variation in the activities of renal GST isozymes between individuals, and considerable variation in individual activity changes during the cancerization process. However, it was also found that, with the exeption of GST θ (GSTT1-1, Fig. 6), single known polymorphisms of GSTs have little influence on the GST activities towards standard substrates.

In general, the present data are consistent with, and supportive of, the concept that the initial GST-dependent bioactivation step of nephrocarcinogenic chlorinated hydrocarbons can take place in the kidney itself (Lash 2000a). Previous toxicokinetic models, in which GSH conjugation is assumed to take place exclusively in the liver followed by a distribution (and dilution) of the glutathione conjugate as the initial carcinogen within the entire organism before the ultimate carcinogen is formed by renal β-lyase (Green et al. 1997), should therefore be modified.

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