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Differing expression of enzymes of the glyoxalase system in superficial and invasive bladder carcinomas

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Abstract

This work aimed to study the activities of the glyoxalase system enzymes (glyoxalase I (GI) and glyoxalase II (GII) and their gene expression in human bladder carcinomas compared with the corresponding normal mucosa. Samples of these tissues were collected from 26 patients with superficial (SBC) or invasive bladder cancer (IBC) and used to evaluate enzyme activity and gene expression by northern blot analysis. In keeping with the electrophoretic pattern and the expression level of the respective genes, GI activity significantly increased in SBC samples, while it remained unchanged in IBC samples compared with the normal mucosa. In contrast, GII showed a higher activity in the tumour (either SBC or IBC samples) versus normal tissues. These results confirm the role of the glyoxalases in detoxifying cytotoxic methylglyoxal (MG) in bladder cancer. The differing levels of GI activity level and gene expression of *GI* between the SBC and IBC samples could help in their differential diagnosis.

Keywords: Methylglyoxal; Glyoxalase system; Gene expression; Bladder cancer

1. Introduction

The glyoxalase system consists of two enzymes, glyoxalase I (GI) and glyoxalase II (GII), that convert methylglyoxal (MG) to D-lactate, through the intermediate S-D-lactoylglutathione. GI (EC 4.4.1.5) catalyses the formation of S-D-lactoylglutathione from MG with reduced glutathione (GSH) acting as a cofactor. GII (EC 3.1.2.6) catalyses the hydrolysis of S-D-lactoylglutathione to D-lactate and regenerates GSH [1,2]. MG formation in mammalian cells mainly arises as a product of glycolysis by the non-enzymatic degradation of triose phosphates (dihydroxyacetone phosphate and glyceraldehyde-3-phosphate) [3] and, to a lesser extent, from ketone body metabolism [4], threonine catabolism [5] and the degradation of glycated proteins [6].

Even if the exact biological significance of the glyoxalase system is so far uncertain, the ubiquitous presence of GI and GII in plants and microorganisms [1,7] suggests they have important functions in cell metabolism. Such an assumption is also strengthened by the extensive sequence homology observed between the genes in humans and those of Procaryota and plants [7,8].

MG shows a strong cytotoxicity in both normal and tumour cells, binding to DNA, RNA and proteins, inhibiting their synthesis, stopping cell growth and inducing apoptosis [9,10]. Several studies suggest GI expression is increased in malignant cells and tissues, most likely to effectively detoxify cells by reducing MG levels. This is required since the formation of MG from triose phosphates is increased in malignant tissues due to their high glycolytic activity [11–13]. However, high GI activity has also been detected in proliferating tissues such as embryonic cells [14] and regenerating liver [15].

A number of recent reports show a detectable GII activity in tumour cells and tissues, although this is far lower than that observed for GI in the same samples [16–18].

Our recent study, concerning the expression of GI and GII in normal and breast cancer tissues [19], showed a far higher activity of GI and GII in tumour compared with pair-matched normal tissues. Such increased activ-

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ities likely result from an enhanced enzyme synthesis due to an increased expression of the respective genes in the tumour tissues.

The numerous papers reporting glyoxalase activity measurements display a wide range of levels in different human tumour cell lines [12,16,20] and tumour tissues [11,12]. The level of gene expression, so far restricted almost exclusively to GI, is also varied.

A previous report [21] showed large individual variations in GI and GII activities in human tumour and non-tumour urogenital tissues. Moreover, a seemingly random increase or decrease in activity of either enzyme in the tumour compared with the normal tissue of pair-matched samples was seen. In particular, no significant difference was found in the mean activity values of both GI and GII between tumour (transitional cell carcinoma) and normal samples of human bladder.

The present study aimed to evaluate GI and GII activity and, in addition, the expression of the genes in samples of human bladder carcinoma at different stages of progression (superficial or invasive) compared with the corresponding normal adjacent tissue.

2. Materials and methods

2.1. Sample collection

Bladder tissue samples were collected from May to July 2000 from two groups of 13 patients, who underwent surgery either for superficial (11 men, 2 women; mean age 68.4 years, range 49–80 years) or invasive (11 men, 2 women; mean age 65.5 years, range 48–76 years) bladder carcinoma, respectively.

All tumours, measuring a mean diameter of 0.5 cm, were histologically classified as:

1. Superficial bladder carcinoma (SBC)
   - Stage: 8 Ta–5 T1
   - Grade: 7 G1–2 G1-G2–3 G2–1 G2-G3
2. Invasive bladder carcinoma
   - Stage: 8 T2–5 T3
   - Grade: 2 G2–3 G2-G3–8 G3

Routinely, a sample of approximately 0.2–0.3 g was excised from each freshly removed tumour, split in two, quickly placed into liquid nitrogen and stored at −80 °C until subsequent use. At the same time as the surgical ablation of the tumour, a sample of approximately 0.2–0.3 g was also drawn from the adjacent normal tissue (bladder mucosa) and handled in the same way as described above.

2.2. Preparation of tissue extracts

A fraction of the excised tumour or normal tissue from each patient was thawed and homogenised (1/10 w/v) at 5 °C in 20 mM Tris–HCl buffer, pH 7.4, containing 0.1 mM phenylmethylsulphonyl fluoride (PMSF), using an Ultraturrax blender. The resulting homogenate was centrifuged at 100 000 *g* for 60 min at 5 °C in a Beckman L60 ultracentrifuge (SW 41 Ti rotor). The supernatant thus obtained was used for GI and GII activity measurements, as well as for polyacrylamide gel electrophoresis (PAGE).

2.3. Glyoxalase I and II assay

The activity of either GI or GII was assayed spectrophotometrically as in Ref. [19]. In particular, one enzyme unit (IU) was defined as the amount of enzyme catalysing the formation (GI) or the hydrolysis (GII) of 1 μmol of S-D-lactoylglutathione per minute at the saturating substrate concentration.

Protein concentrations were measured by the method of Lowry and colleagues [22] using bovine serum albumin (BSA) as a standard.

2.4. PAGE

20-μl samples of tumour (either SBC or IBC) or normal tissue extracts were electrophoresed under non-denaturing conditions on a vertical slab-gel (8×7.3×0.1 cm). To stain for GI, a starch-polyacrylamide gel was used for the electrophoresis and the subsequent staining carried out as described by McLellan and Thornalley in Ref. [23]. For GII, samples were electrophoresed on a 7% acrylamide gel using a 20-mA current and enzyme activity was stained according to the method of Uotila described in Ref. [24].

2.5. RNA extraction and northern analysis

Total cellular RNA was extracted by a single-step method described in Ref. [25] using frozen samples of bladder tissue (approximately 0.2 g) from each patient, consisting of SBC or IBC as well as control normal tissue. As regards the subsequent northern analysis, RNA electrophoresis, prehybridisation and detection of mRNAs specific for *GI* and *GII* were carried out following procedures that we reported in Ref. [19]. In particular, the same cDNA probes from human colon (*GI*) and liver (*GII*) were used for the northern blots. Normalisation and autoradiography were also performed as previously described.

3. Results

3.1. Glyoxalase I and II activity

Table 1 shows the values for GI and GII activity in SBC or IBC, as well as in the normal adjacent tissues,

Table 1
Specific activities of glyoxalase I (GI) and glyoxalase II (GII) in extracts of superficial (SBC) or invasive bladder carcinoma (IBC) and normal adjacent bladder tissue (N)

Tissue	GI			GII		
	Specific activity (mIU/mg)	Comparison C⇒N	C/N ratio	Specific activity (mIU/mg)	Comparison C⇒N	C/N ratio
Superficial carcinoma (SBC)	178.4±123.1	$P<0.01$	1.93	19.7±8.2	$P<0.05$	1.66
Normal tissue (N)	92.5±55.0			11.9±3.9		
Invasive carcinoma (IBC)	110.1±66.6	NS	1.08	19.9±13.3	$P<0.05$	1.73
Normal tissue (N)	102.0±52.9			11.5±5.4		

NS, non-significant; S.D., standard deviation.

Activity values are given as mean ±S.D. of 13 determinations. Statistical comparisons were carried out by a variance analysis, keeping the effect of individual variability separated from the experimental error.

observed in the two groups of 13 patients. GI shows a significantly higher activity (nearly double) in the superficial tumour. In contrast, GI activity in the invasive carcinoma does not differ significantly from that in the normal tissue. As for GII, enzyme activity in both the superficial and invasive carcinoma appears to be significantly higher than in the normal control tissues.

3.2. PAGE

Fig. 1 shows a representative result of the PAGE of tissue extracts from either SBC or IBC and from the normal bladder tissue (NT) of the same patients, stained for GI and GII activity. Both enzymes display single activity bands migrating towards the anode. As regards GI, a slight increase in the staining intensity appeared in the superficial carcinoma sample compared with the normal tissue extract; in contrast, the invasive carcinoma sample showed a similar staining to its normal tissue counterpart. Clear differences in the staining intensity were also detectable for GII activity bands from extracts of both SBC or IBC compared with the normal adjacent tissue. These results were reproducible using extracts from other patients to those shown in Fig. 1.

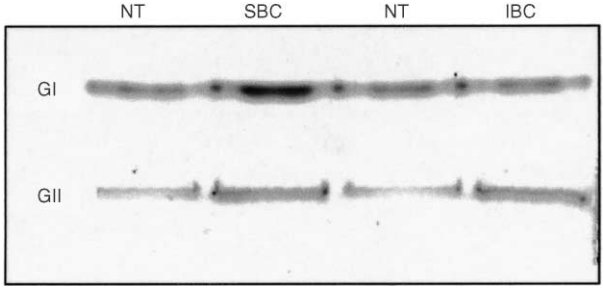

Fig. 1. Non-denaturing polyacrylamide gel electrophoresis (PAGE) of extracts from superficial (SBC) or invasive (IBC) bladder carcinoma and normal bladder mucosa tissue (NT) with subsequent staining for glyoxalase I (GI) or glyoxalase II (GII) activity.

3.3. Northern analysis of RNA

Fig. 2 shows a representative northern analysis with RNA extracted from the other half of the samples of SBC or IBC and corresponding normal tissue (NT) employed for the PAGE analysis. Hybridisation of total RNA with ^{32}P-labelled human *GI* cDNA and subsequent autoradiography gave a visibly more intense transcript band for the SBC sample than for normal tissue. In contrast, no clear difference in the intensity of the transcript bands was detectable between the IBC sample and its pair-matched normal tissue. Experiments carried out using the same total RNA hybridised with the ^{32}P-labelled human *GII* cDNA showed an elevation of *GII* transcript in both the SBC and IBC samples compared with the normal control tissues. Results using

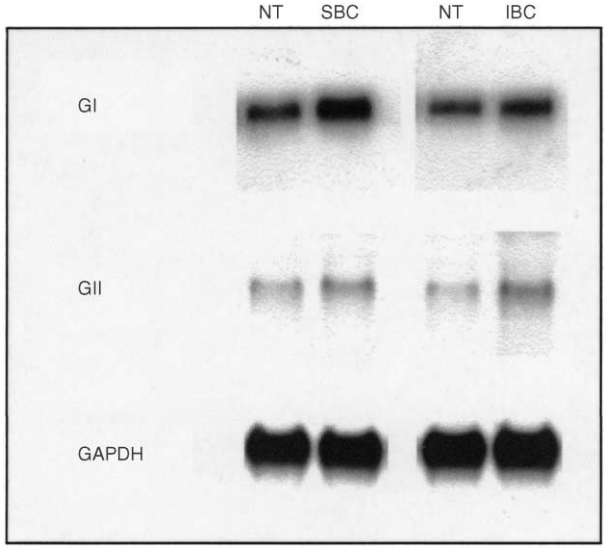

Fig. 2. Northern analysis of the total cellular RNA from superficial (SBC) or invasive (IBC) bladder carcinoma and normal bladder mucosa tissue (NT). Total RNA (20 μl) was electrophoresed through an agarose/formaldehyde gel, transferred to a nylon filter and hybridised with ^{32}P-labelled cDNA probes for human glyoxalase I (*GI*) and glyoxalase II (*GII*) or rat glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as a control.

Table 2
Densitometric quantitation of northern analyses using mRNA for glyoxalase I (*GI*) and glyoxalase II (*GII*) from superficial (SBC) or invasive (IBC) bladder carcinoma and normal adjacent bladder tissue (N)

Tissue	GI			GII		
	DV	Comparison C⇒N	C/N ratio	DV	Comparison C⇒N	C/N ratio
Superficial carcinoma (SBC)	1.80±0.41	$P<0.01$	1.8	0.15±0.02	$P<0.01$	1.5
Normal tissue (N)	1.02±0.25			0.10±0.02		
Invasive carcinoma (IBC)	1.01±0.19	NS	1.0	0.18±0.03	$P<0.01$	1.8
Normal tissue (N)	1.03±0.14			0.10±0.02		

NS, non significant; S.D., standard deviation.

Densitometric values (DV, arbitrary units) are given as mean ±S.D. of 13 determinations. Statistical comparisons were performed by a variance analysis, keeping the effect of individual variability separated from the experimental error.

material from the other patients showed comparable results. Table 2 gives the densitometric data for these northern analyses allowing a quantitation of transcription levels for either gene. Thus, SBC displayed a significantly higher concentration of both *GI* and *GII* transcripts in comparison with the normal tissue, whilst IBC showed a significant elevation of the *GII* transcript alone over the normal counterpart.

4. Discussion

The results of the present study showed a significant increase (nearly double) in GI activity in the tissue samples of SBC when compared with the adjacent normal tissues. These findings are in keeping with the results from both the electrophoresis and northern analyses that showed a higher level of GI in the tumour tissue. In particular, the rise in GI activity as a result of enhanced expression of the *GI* gene is also supported by the marked similarly in the ratios seen in Tables 1 and 2 representing specific activity and gene expression, respectively. All these results suggest that the *GI* gene is overexpressed in the tumour resulting in the conversion of toxic MG into S-D-lactoylglutathione that is present at enhanced levels in actively proliferating tissues [11,14,15].

In contrast, the GI activity and *GI* expression were not significantly different between IBC samples and their normal counterparts. Clearly, further and more extensive investigation is required to explain these differences.

It is well known that the human GI protein consists of two subunits that are expressed by two allelic genes. Therefore, GI may exist as three phenotypes, $GI_{(1\text{-}1)}$, $GI_{(1\text{-}2)}$ and $GI_{(2\text{-}2)}$, which represent the homozygous and heterozygous association of the two alleles, $GI_{(1)}$ and $GI_{(2)}$ [12,26]. Although the allelic combination in our patients was unknown, it is possible that the differences discussed above may be due to different phenotypic expression of the above-mentioned isoenzymes in the tumours that we analysed.

In addition, the increased MG level in the IBC, that is typical of tumour tissues, in the absence of an enhanced GI activity, might be removed throughout metabolic pathways other than the glyoxalase system, such as mechanisms involving aldehyde reductase (EC 1.1.1.2) and aldose reductase (EC 1.1.1.21) [12,27]. In support of this hypothesis, Vander Jagt and colleagues [27] believe it is likely that protection from MG toxicity is a normal function of aldose reductase, since MG is a preferred substrate for this enzyme.

Several studies are presently in progress in our laboratory to clarify the phenotypic expression of the *GI* gene in human SBC and IBC, as well as the possible involvement of enzymes other than those of glyoxalase system in the metabolism of MG in IBC tissues. However, the results obtained in this study may represent useful parameters for the differential diagnosis of SBC and IBC.

As for GII activity, our results showed significant increases in both SBC and IBC samples compared with the corresponding tissues. These findings were strengthened by the significantly enhanced expression of the relative genes by northern analysis. The rise of GII activity in IBC without a parallel increase in that of GI may seem to be in contrast with a well-balanced method of action of both enzymes in removing MG and GSH regeneration within the glyoxalase pathway [1,10]. However, it has been shown that S-D-lactoylglutathione effects a growth arrest and increased toxicity in cultured HL60 promyelocytic leukaemia cells [12,28]; hence, a quick removal of this intermediate product of glyoxalase activity could be required in certain solid tumours as well. It is also well known that GII shows, as observed in several normal or tumour tissues, a specific activity approximately 10-fold lower than GI [19,29,30]. Therefore, an increased GII expression and level of GII activity, being the rate-limiting enzyme of glyoxalase pathway, might be a common adaptive feature of metabolism used in some tumour tissues.

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