Acta Biochimica Polonica
Vol. 40 No. 1 1993

Glutathione-*S*-transferase isoenzymes in Wilms' tumor and normal kidney tissue

Joanna Gajewska, Magdalena Szczypka and Teresa Laskowska-Klita

Department of Biochemistry, National Research Institute of Mother and Child, M. Kasprzaka 17a, 00-211 Warsaw, Poland

Glutathione-*S*-transferases (GSTs) form a family of enzymes catalyzing the conjugation of glutathione with a large variety of xenobiotics as well as with endogenous substrates. According to their structural, kinetic and immunological properties, GSTs[1] isolated from mammalian tissues can be conveniently grouped into main three classes: alpha, mu and pi [1]. Several reports indicate that GSTs, in particular GST class pi, play an important role in the cellular inactivation of anti-cancer drugs, and, in some cases, the acquisition of enhanced resistance of cancer cells to these drugs has been related to the GST expression [2].

Wilms' tumor (nephroblastoma), an embryonal malignancy of the kidney, is one of the commonest solid tumors of childhood in Poland. As so far described, with the therapy currently used the prognosis for children with Wilms' tumor especially for the patients with "unfavourable histology", is not good. The latter is often associated with a high rate of relapse and death [3]. Although GST pi and GST alpha have been found in Wilms' tumor [4], nevertheless the information on characteristics of distribution of either isoenzyme in this tumor is rather scarce. In the present study we identify the pattern of GST alpha and pi expression in Wilms' tumor in comparison with the neighbouring normal kidney tissue.

Neoplastic and normal kidney tissues were obtained at operation from 16 patients with Wilms' tumor. All patients had received prior chemotherapy (actinomycin D, vincristine). The tumor and kidney samples were immediately transferred to a cold saline solution, washed exhaustively and homogenized in 4 vol. of 0.25 M sucrose. The homogenates were centrifuged at 3000 *g* for 15 min and then at 16000 *g* for 30 min. We have investigated the distribution of GST isoenzymes by high-performance analytical isoelectric focusing according to the modified method of Shea *et al.* [5]. Isoelectric focusing was carried out at 1800 V for 2 h on 0.5 mm thick, 5% polyacrylamide gels containing 6% (v/v) ampholytes, pH 3-10. The 16000 *g* supernatant (20 µl) of 20% (w/v) homogenate of tumors or kidney tissues and marker proteins were applied in adjacent lanes of the gel. After focusing the gels were divided into 5 mm wide segments along the pH gradient. Gel slices were incubated with 1.0 cm^3 of 0.1 M potassium phosphate buffer (pH 6.5) at 4°C for 4 h, and then GST activity was measured. The isoelectric points of the GST isoenzymes were determined by comparison to the position of marker proteins. Total GST activity and GST isoenzymes activities were assayed with 1-chloro-2,4-dinitrobenzene as a substrate according to the method of Habig *et al.* [6]. Protein concentrations were measured by the method of Lowry *et al.* [7].

Our data demonstrate existence of the differences in the pattern of GST isoenzymes between Wilms' tumor and normal kidney tissue (Fig. 1, 2). In Wilms' tumor, as compared with normal kidney tissues, we observed a significant decrease in total GST activity (Table 1). This decrease usually was due to the lower activity of GST alpha in comparison to the nor-

[1]Abbreviation: GST, glutathione-*S*-transferase

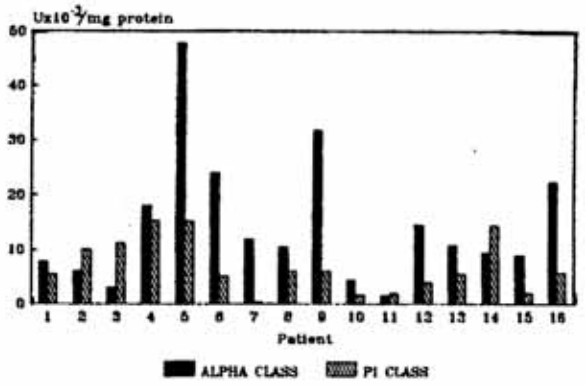

Fig. 1. Activity of GST isoenzymes in unaffected kidney tissue

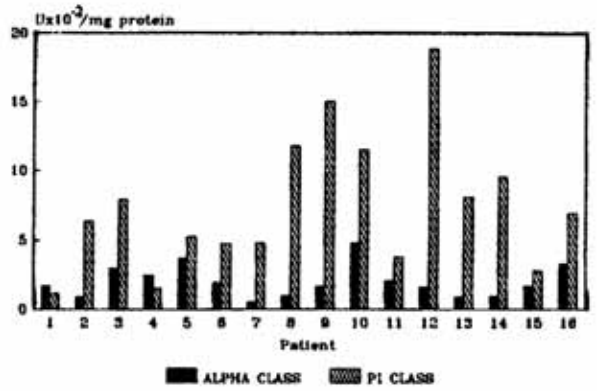

Fig. 2. Activity of GST isoenzymes in Wilms' tumor

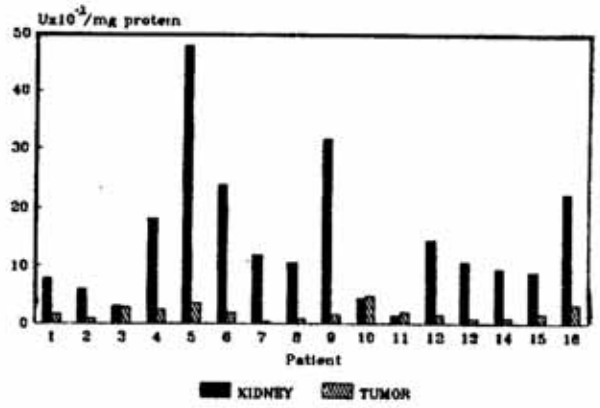

Fig. 3. Activity of GST alpha in kidney tissue and Wilms' tumor

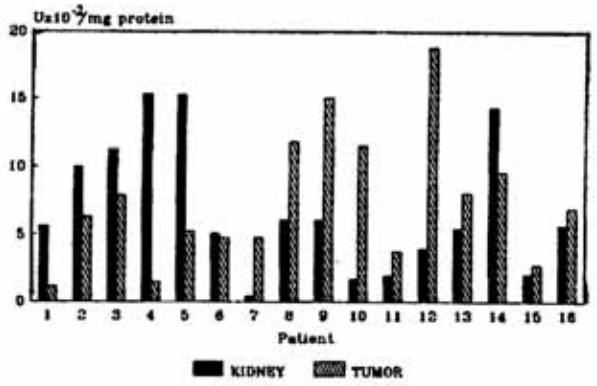

Fig. 4. Activity of GST pi in kidney tissue and Wilms' tumor

mal kidney tissue (Fig. 3). The predominant isoenzyme of GSTs in Wilms' tumor, unlike in kidney tissue, was GST pi. This isoenzyme has been found to be overexpressed also in a number of human tumor tissues [5] except liver tumor [8]. Therefore recent studies have suggested the possibility of GST pi being used as a tumor marker for human extrahepatic tumors [9].

Other studies have shown that GST could also serve as a predictive marker of responsiveness of tumor to chemotherapy [10] and could be related to the tumor stage [11]. We have observed that Wilms' tumors of I stage and "favourable histology" show lower activity of GST pi, but tumors with "unfavourable histology" or at higher stages (irrespective of histology) show higher activity of this isoenzyme in com-

Table 1

Total GST activity in Wilms' tumor and non-tumor human kidney tissue

Patient	Stage	Histology*	GST activity (U/mg protein)	
			Non-tumor	Tumor
1	I	FH	0.61	0.17
2	I	FH	1.67	0.51
3	I	FH	0.78	0.49
4	I	FH	1.12	0.49
5	I	FH	1.95	0.95
6	I	FH	1.86	0.95
7	II	FH	0.79	0.61
8	II	FH	1.32	0.63
9	II	FH	1.35	0.98
10	III	FH	0.49	0.76
11	I	FH/UH	0.34	0.42
12	I	UH	1.02	1.41
13	II	UH	1.10	0.74
14	II	UH	1.24	0.99
15	II/IV	UH	0.81	0.53
16	IV	UH	1.59	0.97
			1.13 ± 0.48	0.73 ± 0.30**

*FH, "favourable histology"; UH, "unfavourable histology"

** Values are expressed as mean ± S.D. Statistical significance ($P < 0.002$) was estimated with Student's paired *t*-test

parison to the normal kidney tissue (Fig. 4). The studies on the relation between GST pi activity and the patient's history might be useful in designing new strategies of tumors therapy.

REFERENCES

1. Mannervik, B., Alin, P., Gurhenberg, C., Jensson, H., Tahir, M.K., Warholm, M. & Jornvall, H. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7202 - 7206.
2. Black, S.M. & Wolf, C.R. (1991) *Pharmac. Ther.* **51**, 139 - 154.
3. Beckwith, J.B. & Palmer, N.F. (1978) *Cancer* **41**, 1937 - 1948.
4. Harrison, D.J., Hallam, L. & Lauder, J. (1990) *Br. J. Cancer* **61**, 836 - 840.
5. Shea, T.C., Kelley, S.L. & Henner, W.D. (1988) *Cancer Res.* **48**, 527 - 533.
6. Habig, W.H., Pabst, M.J. & Jakoby, W.B. (1974) *J. Biol. Chem.* **249**, 7130 - 7139.
7. Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951) *J. Biol. Chem.* **193**, 265 - 275.
8. El Monehli, M., Didolkar, M., Elias, E.G., Guengerich, F.P. & Kauffman, F.C. (1987) *Cancer Res.* **47**, 460 - 466.
9. Sato, K. (1989) *Adv. Cancer Res.* **52**, 205 - 255.
10. Batist, G., Tulpule, A., Sinha, B.K., Katki, A.G., Myers, C.E. & Cowan, K.H. (1986) *J. Biol. Chem.* **261**, 15544 - 15549.
11. Clapper, M.G., Hoffman, S.J., Carp, N., Watts, P., Seestaller, L.M., Weese, J.L. & Tew, K.D. (1991) *Carcinogenesis* **12**, 1957 - 1961.