Great Lakes Drug Metabolism & Disposition Group

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Immunohistochemical Localization of Glutathione S-Transferases in Normal and Diseased Humans using Tissue Microarray Technology and a Panel of Isoform-Specific Antibodies

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INTRODUCTION

Cytosolic glutathione S-transferases (GSTs) comprise a large family of proteins (18 gene products divided into eight major Classes (designated as A (alpha), M (mu), P (pi), T (theta), K (kappa), Z (zeta), O (omega) and S (sigma). The GSTs play prominent roles in biotransformations of many endogenous compounds and in the detoxification of xenobiotics. Studies to date indicate that GSTs exhibit distinct yet overlapping, tissue expression patterns. For example, in humans, GST A1-1 is the predominant GST in liver, but other GST Class A isoforms have been detected in nearly every tissue examined.

It is well documented that GSTs are released by cells upon damage due to toxin exposure. Hence, given relatively recent discovery of many additional isoforms, GSTs have enormous untapped potential to serve as organ-specific toxicity biomarkers. Therefore, we have developed and characterized a comprehensive panel of GST isoform-specific antibodies and herein report their use to map the expression of GST isoforms in a broad range of human tissues.

ABSTRACT

Differential expression of GST A, M & P classes in mammalian tissues has long been recognized, and class-specific antibodies have been successfully employed to differentiate mechanism(s) of action of toxins. The discovery of multiple A and M class isoforms, as well as additional classes of cytosolic GSTs may allow for their broader applications for organ-specific toxicity. Several investigators have examined the expression of specific GST isoforms in a number of human and rodent tissues. However, localization has been rigorously confirmed for only some selected GSTs and only in a limited number of tissues.

We have developed a panel comprised of 12 monoclonal and polyclonal antibodies to recombinant human cytosolic GSTs, and have rigorously confirmed the absolute specificity of these antibodies for individual GST isoforms. Using Tissue MicroArray (TMA) technology, we have determined the localization of most cytosolic GST isoforms in specimens obtained from 25 normal human organs as well as over 20 different tissues with distinct pathologies. In addition to visual inspection of the expression of GST isoforms in all of the tissues examined, quantitative IHC analysis was performed using Defineins® technology. The resulting expanded knowledge base for the localization of GST isoforms in human tissues should enrich their applications for drug development and toxicological analyses. Also, some of the observed differences in the expression of individual GST isoforms between normal and diseased specimens may be exploited as an aid to the diagnosis and management of specific diseases. One intriguing example involves observed differences in GST isoform expression among normal prostate specimens vs. prostatic hyperplasia and adenocarcinoma of the prostate.

MATERIALS AND METHODS

Expression and purification of recombinant human GST isoforms: Full-length cDNAs for human GSTs were cloned into expression vectors along with a sequence encoding an amino-terminal 6X histidine epitope tag to facilitate purification. Sequence-verified expression constructs were then introduced into an appropriate bacterial expression host. Expressed GSTs from bacterial lysates were purified using a Cobalt-agarose resin to purify individual GST isoforms with purities consistently exceeding 98%.

Antibody Production: Purified recombinant human GST proteins were used as immunogens for the production of polyclonal antibodies in goats (A1) and rabbit (M1 and P1) or mouse monoclonal antibodies M1, M3, K1, O1, S1, T1 and Z1. After fusions and hybridoma subcloning, antibodies were screened against all recombinant human GST isoforms by ELISA and clones that exhibited the proper GST specificity were be expanded and purified.

Human Tissue Microarrays: Tissue microarrays were prepared by the William Beaumont Hospital BioBank from 1.0mm core samples obtained from paraffin-embedded blocks of normal tissues and tissues with specific pathologies following review by a board-certified pathologist. Normal tissue cores were arrayed in a 7 x 8 pattern, and disease specimens in a separate 5 x 9 array. Each block was cut into 4 µm sections and mounted on multiple microscope slides. *Immunohistochemistry:* Separate slides were incubated with each of the 1:100 dilutions of one of the anti-GST antibodies described for 3 hours, rinsed with PBS-Tween 20, followed by incubation in peroxidase blocking solution for 10 minutes at room temperature. After rinsing in PBS-Tween 20, the slides were incubated with biotinylated secondary antibody in PBS for 30 minutes at room temperature, rinsed in PBS-Tween 20 for 3x2min, then incubated in streptavidin-HRP in PBS for 30 minutes at room temperature, rinsed and incubated in DAB solution for 3 minutes. Counterstaining was with H&E.

Analysis of Stained Tissue Sections: A total of 12 anti-GST antibodies were evaluated on each TMA array [45 sections for diseased tissues and 56 sections for normal tissues]. These were analyzed both visually by a trained histo-technician, and also scanned into an Aperio® e-slide manager and quantitative digital analysis of the entire section performed using Definiens® Tissue Studio 4.0™ software.

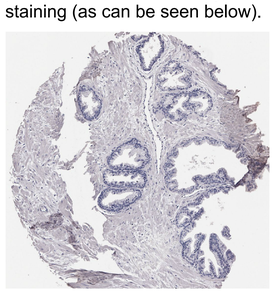
Tabulation of Results: In order to present the results from this very large dataset in a manageable fashion, the visual and Definiens analyses have been combined into the results presented in tablular format. A key is provided.

IMMUNOLOCALIZATION OF HUMAN GST ISOFORMS IN NORMAL AND DISEASED TISSUES

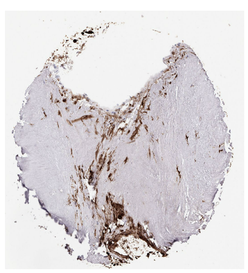
Anti-GST Antibodies	OBR Product Code	GS41e	GS12	GS15	GS25	GS17c	GS67	GS19	GS26	GS46e	GS27	GS20	GS21
	Antibody Type	Polyclonal	Monoclonal	Monoclonal	Monoclonal	Monoclonal	Polyclonal	Monoclonal	Monoclonal	Polyclonal	Monoclonal	Monoclonal	Monoclonal
	Immunogen	rec.protein	Peptide	Peptide	rec.protein	rec.protein	rec.protein	rec.protein	rec.protein	rec.protein	rec.protein	rec.protein	rec.protein
	Isoform specificity	A1	A3	A4	K1	M1	M1	M3	O1	P1	S1	T1	Z1
Tissue Type	**Pathology**												
Adrenal	Normal	H(N/C)	H(N/C)	-	L(N/C)	V(N/C)	-	-	H(N/C)	H(N/C)	-	-	H(N/C)
	adrenal gland renal cell carcinoma	H(N/C)	H(N/C)	-	H(N/C)	V(N/C)	-	-	H(N/C)	H(N/C/M)	-	-	H(N/C)
Aortic valve	Normal	-	H(N)	-	-	L,V(N/C)	-	-	L,V(N)	H(N/C)	L(C)	-	H(N)
	aortic stenosis	-	L(N)	-	-	L,V(I)	L,V(N)	L,V(C/M)	V(N)	H(N)	V(C)	-	H(N/C)
Appendix	Normal	-	V(N,C)	-	-	-	L,V(N,C)	-	H(N)	H(N)	-	-	H(N/C)
Bladder	Normal	-	-	-	-	L(N,C)	L(N,C)	-	-	H(N)	-	-	L(N,C)
	carcinoma bladder papillary	-	-	-	-	-	H(N)	-	H(N)	H(N)	-	-	-
	high grade paillary urothelial	-	-	-	-	V(C)	L,V(N,C)	-	-	-	-	-	V(C)
Breast	Normal		L,V(N)										
	carcinoma breast	-	H(N)	-	-	-	H,V(N)	-	H(N)	H(N)	-	-	H(N/C)
	Carcinoma breast	-	-	-	-	-	H,V(N)	-	L(N)	H(N)	-	-	H,V(N/C)
Colon	Normal	L,V(N)	L,V(N)		L,V(N,C)		L(C)		-	L(N,C)			H(N/C)
	adenocarcinoma of cecum	-	H,V(N,C)	-	L(C)	L(C)	L(C,N)	-	L(N,C)	H(N)	-	-	H(N/C)
	moderately differentiated adenocarcinoma	-	L,V(N,C)	-	-	-	L(N,C)	-	H(N)	H(N,c)	-	-	H(N/c)
Esophagus	Normal		H(N)			H(N,c)	H(N,c)			H(N,c)			H(N/C)
	adenocarcinoma of stomach	-	H(N,c)	-	-	-	H(N,c)	-	H(N,c)	H(N,c)	-	-	H(N/C)
	Invasive squamous cell carcinoma								H,V(N)	H,V(N)			H,V(N,c)
GBM	GBM Control Block	-	H(N,C)	-	-	H(C)	H,V(N,C)	-	H(N)	H,V(N,C)	-	-	H(N/c)
Kidney	Normal	H(N,C)	H(N,C)		L,V(N,C)	L(C)	L,V(C)		H(N,c)	H(N,c)			H(N/C)
	renal cell carcinoma kidney		H,V(N)										H,V(N)
	renal cell carcinoma papillary	-	-	-	L(C)	-	-	-	H(N,c)	H(N,C)	-	-	H(N/c)
Liver	Normal	H,V(N,C)	H(N,C)		L,V,C)	V(N,C)			H(N)				H(N,C)
Lung	Normal		V(N,C)			H,V(N,C)	H,V(N,C)		H(N,c)	H(N,c)			H(N/C)
	adenocarcinoma metastatic breast	-	H(N,c)	-	-	-	H(N,C)	-	H(N)	-	-	-	H(N/C)
	invasive adenocarcinoma	H,V(N)	H,V(N)						H,V(N)	H,V(N)			H,V(N,C)
Lymph Node	Normal		V(N)							V(N,C)			V(C)
	Lymphoma L. Node Control Block	-	V(N,C)	-	-	-	-	-	H(N,C)	H(N,c)	-	-	V(N,c)
Muscle	Normal	V(N,C)								V(N,c)	L(C)		H,V(N/C)
Ovary	Normal	H,V(N,C)	H(N,c)			H,V(N,c)			H(N)	H(N)			H(N/C)
	carinoma ovary	-	H(N,c)	-	-	-	V(N,C)	-	H(N,c)	H,V(N,C)	-	-	H,V(N,C)
	cystadenocarcinoma ovary	H,V(N,c)	H,V(N,C)	-	-	-	-	L,V(C)	H(N,c)	H(N,C)	-	-	H(N/c)
Pancreas	Normal	H(N,C)	H(N,C)				L,V(N,C)		H(N)	H(N,c)			H(N/C)
	adenocarcinoma pancreas	-	-	-	-	-	H,V(N,C)	V(N)	H(N,c)	H(N,c)	-	-	V(N,C)
	Pancreatic neurendocrine	H(N,C)	H(N,C)	-	H(C)	H,V(C)	L(C)	-	H(N,c)	L,V(n,c)	-	-	H(N/C)
Parathyroid	Normal	L,V(n,C)	H(N,C)		H,V(N,C)		L,V(n,c)		H(N,C)	H(N,C)	-	-	H(N/C)
	adenoma parathyroid	-	H(N,C)	-	L,V(C)	-	L,V(C,N)	L(C)	H(N,C)	H(N,C)	-	-	H(N/C)
	adenoma parathyroid	-	H(N,C)	-	H(N,C)	-	L,V(C)	H(C)	H(N,C)	H(N,C)	-	-	H(N/C)
Placenta	Normal	-	H(N,c)							H(N,c)			H(N/C)
Prostate	Normal		H(N,c)				H(N,C)		H(N)	H(N,c)			H(N,c)
	hypertrophy of prostate	-	H,V(N,C)		-	L,V(C)		-	-	V(N,C)	-	-	L,V(N,C)
	Adenocarcinoma prostate	-	-	H(N,C)	L(N,C)	-		-	-	H(N,C)	-	-	-
Skin	Normal		L,V(N,C)	L,V(N)			H(N,C)			H,V(N)			
	Melanoma Control Block	-	H(N,C)	-	L(C)	H(C)	-	-	H,V(N,c)	H,V(N,c)	-	-	H(N,C)
Small Intestine	Normal	V(N,C)	H(N,c)			L,V(C)			H,V(N)	H(N,c)			H(N,c)
	adenoma	H(N,C)	H(N,C)	-	H(C)	L(C)	-	-	H(N,C)	H(N,C)	-	-	H(N,C)
	adenoma duodenum	H(N,C)	H(N,C)	-	L(C)	L(C)	L,V,C)	-	H(N,c)	H(N,C)	-	-	H(N,C)
Spinal Cord	Normal					L(N,C)	L,V(C)						H(N)
Spleen	Normal		H(N,C)						H(N,c)	H(N,c)			H(C)
Stomach	Normal						L(N,C)			H(N)			H(N,c)
Sarcoma	Synovial sarcoma	L,V(C)	H(N,C)	-	-	-	H(C)	-	H(N)	H(N)	-	-	H(N,C)
Testicle	Normal	H(N,C)	H(N,C)			H(N,c)	H(N,c)	H(N,c)		H(N)			H(N,c)
Thyroid	Normal									H,V(N)			
	carcinoma thyroid papillary	-	H(N,C)	-	H(C)	H(C)	L(C)	H(C)	H(N,C)	H(N,c)	-	-	H(N,C)
	carcinoma thyroid papillary	-	L,V(C)	-	-	-	-	-	L,V(N,C)	H(N,c)	-	-	-
Trachea										H(N)			
Uterus	Normal								H(N)	H(N,c)			H(N,c)
	carcinoma uterus	-	H(N,C)	-	-	L(C)	L(C)	L(C)	H(N)	H,V(N)	-	-	H(N,C)
	Endometrial carcinoma	-	H(N,C)	-	L(C)	L(C)	L(C)	L(N)	H(N,c)	H(N,c)	-	-	H(N,C)

EXPRESSION OF GST-M3 IN HUMAN PROSTATE

Upon visual assessment and using the Definiens software to score every core and every stain, the antibody specific for GST P1 shows the best uptake and distribution. From the statistical analysis, anti GST A4 as well as the polyclonal specific to GST M1 show a unique specificity profile for Prostate. Visually, normal and diseased prostate tissues showed areas of differential staining (as can be seen below).



Entire TMA normal human prostate core specimen stained using Anti-GST M3



Entire TMA core specimens from patients with pathologies of the prostate stained using Anti-GST M3

KEY

- ☐ = Blank = not readable (e.g. TMA section did not adhere to slide)
- \- = No staining detected, or very weak staining by visual and – by Definiens
- L = Low level of staining by Definiens, +/- to 2+ staining by visual
- H = High level of staining by Definiens, 3+ to 5+ staining by visual
- V = Variability between Definiens and visual analysis OR between different specimens
- N = Nuclear staining
- C = Cytoplasmic staining
- n/C= Staining of nucleus observed but weaker than that observed for cytoplasm
- N/c= Staining of cytoplasm observed but weaker than that observed for nucleus

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