Published in final edited form as:

Histochem Cell Biol. 2015 May ; 143(5): 505–515. doi:10.1007/s00418-014-1295-x.

Immunolabeling of Gamma-glutamyl transferase 5 in Normal Human Tissues Reveals Expression and Localization Differs from Gamma-glutamyl transferase 1

Marie H. Hanigan^{1,3}, Elizabeth M. Gillies², Stephanie Wickham¹, Nancy Wakeham¹, and Celeste R. Wirsig-Wiechmann¹

¹Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK

²Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK

Abstract

Gamma-glutamyl transferase (GGT5) was discovered due to its ability to convert leukotriene C₄ (LTC₄, a glutathione *S*-conjugate) to LTD₄ and may have an important role in the immune system. However, it was not known which cells express the enzyme in humans. We have developed a sensitive and specific antibody that can be used to detect human GGT5 on western blots and in fixed tissue sections. We localized GGT5 expression in normal human tissues. We observed GGT5 expressed by macrophages present in many tissues, including tissue-fixed macrophages such as Kupffer cells in the liver and dust cells in the lung. GGT5 was expressed in some of the same tissues that have been shown to express gamma-glutamyl transferase (GGT1), the only other enzymatically active protein in this family. But, the two enzymes were often expressed by different cell types within the tissue. For example, GGT5 was expressed by the interstitial cells of the kidney; whereas, GGT1 is expressed on the apical surface of the renal proximal tubules. Other tissues with GGT5-positive cells included: adrenal gland, salivary gland, pituitary, thymus, spleen, liver, bone marrow, small intestine, stomach, testis, prostate and placenta. GGT5 and GGT1 are cell surface enzymes. The different pattern of expression results in their access to different extracellular fluids and therefore different substrates. GGT5 has access to substrates in blood and intercellular fluids, while GGT1 has access primarily to fluids in ducts and glands throughout the body. These data provide new insights into the different functions of these two related enzymes.

Keywords

Gamma-glutamyl transferase; gamma-glutamyl transpeptidase; Leukotriene C₄; glutathione; glycoproteins

³Correspondence to: Dr. Marie H. Hanigan, Stanton L. Young Biomedical Research Center Room 264, 975 N.E. 10th Street, Oklahoma City, OK 73104. marie-hanigan@ouhsc.edu Phone: 405-271-3832, FAX: 405-271-3785.

CONFLICT OF INTEREST

None

STATEMENT OF HUMAN RIGHTS

This manuscript does not contain clinical studies or patient data.

INTRODUCTION

Gamma-glutamyl transferase (GGT5) is a cell surface protein that hydrolyzes the gamma-glutamyl bond of glutathione and glutathione *S*-conjugates (Wickham et al. 2011; Heisterkamp et al. 1991). Its substrates are extracellular and include a large number of molecules critical to redox regulation, drug metabolism, immune function and other processes within the body (Wickham et al. 2011). For many years the cell surface protein gamma-glutamyl transferase (GGT1, a.k.a. gamma-glutamyl transpeptidase) was thought to be the only enzyme that could cleave this gamma-glutamyl bond. However, in 1991 Heisterkamp and colleagues reported the discovery of a human enzyme with 39.5% homology to human GGT1 that could cleave glutathione and metabolize the glutathione-conjugate leukotriene C₄ (LTC₄) to leukotriene D₄ (LTD₄) (Heisterkamp et al. 1991). This new enzyme was unable to cleave the gamma-glutamyl bond of gamma-glutamyl-p-nitroanalide (GpNA), which is the commonly used substrate for assaying GGT1 activity, and therefore had not been detected in routine assays for GGT activity. They named this GGT-related enzyme GGT-rel (Heisterkamp et al. 1991). In 1997 Carter and colleagues identified the mouse homolog calling it gamma-glutamyl leukotrienase (Carter et al. 1997). Human GGT-rel (P36269) and murine gamma-glutamyl leukotrienase (Q9Z2A9) show 76% identity and 89% similarity. In 2008, the enzyme name was standardized to GGT5 (Heisterkamp et al. 2008).

Both GGT1 and GGT5 are type II membrane glycoproteins, with their catalytic activity on the surface of the cell (Hanigan and Ricketts 1993; West et al. 2010; Heisterkamp et al. 2008). GGT1 is present in bacteria and throughout the plant and animal kingdoms while GGT5 is present only in vertebrates (Law et al. 2012; Okada et al. 2006; Bello and Epstein 2013). Phylogenetic analyses indicate that GGT5 arose from a duplication of the GGT1 gene in the vertebrate lineage after divergence from Branchiostoma but before the divergence of amphibians and teleost fish (Law et al. 2012). We have expressed GGT5 and conducted kinetic analysis which has shown that human GGT5 (hGGT5) cleaves glutathione, LTC₄ and other glutathione conjugates but at a much slower rate than human GGT1 (hGGT1), with second order rate kinetics approximately 30-fold lower for hGGT5 compared to hGGT1 (Wickham et al. 2011). Despite the reduced activity, differential localization of GGT5 and GGT1 may contribute to unique functions of each enzyme within the body. The phenotypes differ between GGT1 and GGT5 knockout mice. GGT1 knockout mice are unable to catabolize glutathione in the glomerular filtrate as it passes through the proximal tubules resulting in the excretion of glutathione in the urine. The enhanced excretion of glutathione, 250-fold more than normal, results in the loss of its three constituent amino acids, glutamate, cysteine and glycine and the development of a cysteine deficiency that is fatal by 10 weeks of age. In contrast, GGT5 knockout mice do not have any phenotypic abnormalities until they are put under stress (Carter et al. 1997). During zymosan-A-induced peritonitis, GGT5-deficient mice cannot metabolize LTC₄ in peritoneal fluid resulting in attenuated neutrophil infiltration into the peritoneum (Shi et al. 2001). In *Aspergillus fumigatus* induced asthma, GGT5 knockout mice have increased airway hyper-responsiveness (Han et al. 2002). The double GGT1/GGT5 knockout mice reveal that there

is some functional complementarity as the double knockouts have a similar but more severe phenotype than the GGT1 knockouts, dying by 4 weeks of age (Shi et al. 2001).

Little is known about expression of GGT5 in humans. Data from microarrays and western blot analysis of normal human tissues indicates that GGT5 is expressed in several organs, but the data are inconsistent. Further, it is unclear which cell types within the organs express GGT5. We have developed an anti-hGGT5 antibody and have evaluated GGT5 expression in normal human tissues. These results combined with our data on substrate-specificity provides new insight into the role of GGT5 in normal human physiology and disease.

MATERIALS AND METHODS

Production of GGT5-Ab797 antibody

An affinity-purified antibody to a peptide corresponding to amino acids 371–387 of hGGT5 isoform b was prepared in rabbits and purified under contract by Pacific Immunology (Ramona, CA.) using their standard protocols.

Protein Samples

hGGT1 was expressed in yeast and purified as previously described (King et al. 2009). The N-glycans were removed from an aliquot of hGGT1 with EndoH (New England Biolabs, Ipswich, MA) as previously described (West et al. 2013). Whole cell lysates and membrane were prepared from NIH3T3 control cells and from NIH3T3 cells stably transfected with hGGT5 (3T3/GGT5 cells), a generous gift from Dr. Nora Heisterkamp, Children's Hospital of Los Angeles, Los Angeles, CA (Heisterkamp et al. 1991). For whole cell lysates, the cells were lysed in PBS, 0.5% Chaps, 110.4 KIU Aprotinin and 1 μ M Leupeptin at 4°C. To prepare membrane fractions, the cells were resuspended in PBS and sonicated on ice. The membranes were pelleted by centrifugation at $14,000 \times g$ for 15 min, 4°C. The membrane pellet was washed twice in PBS, then resuspended in PBS, 0.5% Chaps, 110.4 KIU Aprotinin and 1 μ M Leupeptin and mixed gently for 1 h at 4°C. GGT1 and GGT5 activity were assayed with the Glutamate Release Assay (Wickham et al. 2011). One unit of activity is defined as the amount of enzyme that releases 1 μ M L-glutamate per min at 37°C in the 140 μ L assay. The detergent solubilized membranes from the 3T3/GGT5 cells contained 0.04 Units of GGT activity/mg protein.

Human kidney microsomes were prepared from normal human kidneys obtained from the National Disease Research Interchange (NDRI, Philadelphia, PA) and stored frozen at -80°C (West et al. 2010). Tissue from the kidney cortex was minced then homogenized in 25 mM Tris, pH 7.5, 0.33 M sucrose, 0.2 mM EDTA, 1.4 μ g/ml Aprotinin and 1 μ M Leupeptin. The homogenate was centrifuged at $500 \times g$ for 15 min, 4°C. The supernatant was centrifuged at $9,000 \times g$ for 15 min, 4°C to pellet organelles. The supernatant was centrifuged at $100,000 \times g$ for 30 min, 4°C to pellet the microsomal fraction. The pellets were homogenized in 25 mM Tris-HCl, pH 7.35, 0.5% Triton X-100, incubated for 30 min 4°C, then centrifuged at $100,000 \times g$ for 30 min. The supernatant contained detergent extracted microsomes. N-glycans were removed from aliquots of the NIH3T3 membranes

and the detergent solubilized kidney microsomes with PNGase F (New England Biolabs, Ipswich, MA) as previously described (West et al. 2010).

SDS-PAGE and Western Analysis

The protein concentration of all samples was determined by the BCA protein assay (Pierce Biotech., Rockford, IL). hGGT1 (150ng), NIH3T3 cells (5µg) and detergent extracted kidney microsomes (20µg) per lane were resolved on a 10% SDS-polyacrylamide gel, then electroblotted onto Protran BA-83 0.2 µm nitrocellulose membrane (Whatman, Dassel, Germany) and incubated sequentially with GGT5-AB797 and HRP-conjugated donkey anti-rabbit secondary antibody (LNA934V, GE Healthcare, UK). Protein bands were visualized by luminol chemiluminescence detection.

For immuno-detection of GGT5 in normal human tissues, a Human Normal Tissue Blot was purchased from ProSci (Poway, CA). The blot contained 15 µg protein per lane from each tissue homogenate. The proteins were resolved on a 4–20% gradient SDS-PAGE gel and blotted onto a polyvinylidene difluoride (PVDF) membrane. The primary antibody was peptide affinity-purified rabbit anti-GGT5-Ab797 diluted 1:1,500 in TBST containing 0.16% Tween-20 and 1.0% BSA.20). The secondary antibody, HRP-conjugated goat anti-rabbit antibody (SC-2004, Santa Cruz Biotech, Dallas, TX), was diluted 1:100,000 with TBST. The protein bands were visualized by luminol chemiluminescence detection.

Immunohistochemistry

Three tissue arrays of normal human tissue (FDA999b, BN117, BN00011) were purchased from The Tissue Array Network, Rockville, MD. The arrays contained 5 µm sections of formalin-fixed, paraffin-embedded normal human tissues. Any normal tissue that was adjacent to tumor was not included in our scoring. One section from each array was immunolabeled for GGT5, the second section served as a control and was processed in the absence of the primary antibody. The slides were immunolabeled and counter-stained by the Pathology Laboratory at OU Medical Center using a Ventana BenchMark XT with an Immunohistochemistry Staining Module (Ventana Medical Systems, Inc. Oro Valley, AZ). The paraffin was removed with the Ventana EZ Prep solutions. The sections were pretreated with a Tris-based buffer for 60 min at 95°C for antigen retrieval. The affinity-purified GGT5-Ab797 was diluted 1:100 and incubated on the tissue for 32 min at 37°C. The antibody was visualized with a Ventana *ultraView* Universal DAB Detection System which included H₂O₂ pretreatment and *ultraView* Universal HRP Multimer HRP labelled goat anti-rabbit antibody. All slides were counterstained with hematoxylin. Tissue sections were processed in the absence of primary antibody as negative controls. With each set of tissues immunocytochemically labeled, sections of normal human kidney and adrenal gland, obtained from the Pathology Department, were included as positive controls. Immunolabeling of tissues for GGT1 followed the same protocol as that for GGT5. The GGT129 antibody was used as the primary antibody (Hanigan and Frierson 1996). The affinity-purified GGT129 antibody was diluted 1:500, which is 1:6700 relative to the starting serum concentration.

Statement of Animal Rights

The antibody GGT5-Ab797 was produced in rabbits by Pacific Immunology (Ramona, CA.) which is fully NIH compliant (NIH Animal Welfare Assurance Number: A4182-01).

RESULTS

Specificity of anti-human GGT5-Ab797 antibody

The specificity of the GGT5-Ab797 was evaluated with western blots (Fig. 1A). The antibody, which was developed against a peptide sequence in the large subunit of hGGT5, did not detect hGGT1 (Fig. 1A, Lanes 1,2). Analysis of whole cell lysates from control NIH/3T3 cells and cells transfected with hGGT5 showed a single band at approximately 54.5 kDa in the transfected cells Fig. 1A, Lanes 3,4). A single band of the same molecular mass was observed in membranes isolated from human kidney (Fig. 1A, Lane 7). Removal of the N-glycans from the proteins in the cell lysate by PNGaseF resulted in a decrease in the apparent molecular mass of the immunolabeled band to approximately 41.1 kDa, consistent with the predicted molecular mass of 41.5 kDa for the unmodified heavy subunit of GGT5 (Fig. 1A, Lanes 6,8). There are 4 potential N-glycosylation sites on the large subunit of hGGT5. The decrease in the apparent molecular mass of the heavy subunit following deglycosylation of both NIH/3T3 expressed GGT5 and GGT5 from human kidney indicates that N-glycans may account for almost one-fourth of the molecular mass of the heavy subunit.

GGT5 expression in normal human tissues

A blot of whole human tissue homogenates provided insight into the relative amount of GGT5 protein expressed in various tissues (Fig. 1B). Each lane contained 15 µg of protein. When immunoblotted with GGT5-Ab797, the highest levels of GGT5 among the six tissues analyzed were in the kidney and the lung (Fig. 1B, Lanes 4,6). Colon and heart tissue contained lower levels of GGT5 (Fig. 1B, Lanes 2,3). Expression in the liver was very low (Fig. 1B, Lane 5). None was detected in the brain (Fig. 1B, Lane 1).

Immunohistochemical localization of GGT5 and GGT1 in human kidney and liver

Immunolabeling of human kidney with GGT5-Ab797 showed that the interstitial cells between the renal tubules were strongly GGT5-positive (Fig. 2A). These results correspond with the immunoblot (Fig. 1B) which showed strong expression of GGT5 in human kidney. GGT1 was also highly expressed in human kidney (Fig. 2B). However, GGT1 was expressed exclusively by the proximal tubules and localized to the apical surface of these epithelial cells. Analysis of GGT5 and GGT1 expression in the liver also revealed differences in expression and localization. GGT5 was strongly expressed by the Kupffer cells, the tissue-fixed macrophages in the liver and weakly expressed in hepatocytes (Fig. 2C). The immunoblot (Fig. 1B) indicated a lower level of GGT5 expression in human liver than was observed by immunohistochemistry. The liver tissue on the blot shows several light bands, indicating that there may have been some degradation of the GGT5 in that sample. GGT1 was expressed by hepatocytes and localized to the bile canalicular surface (Fig. 2D). It is interesting to note that both GGT1 and GGT5 were expressed by hepatocytes,

but GGT1 was localized to one surface in these polarized cells while GGT5 was evenly distributed.

Immunohistochemical localization of GGT5 in normal human tissues

We analyzed 156 sections of normal human tissues. The cells that immunostained positive for hGGT5 are summarized in Table 1.

Lymphoid Tissues—Sections of thymus from seven individuals ranging in age from 1 month to 42 years of age were labeled for GGT5 expression. The epithelial cells within Hassall's corpuscles were GGT5-positive in sections from adults (Fig. 3A). However, Hassall's corpuscles were just beginning to form in the 1 month old infant and the epithelial cells within them showed only very light GGT5 labelling. Macrophages in all sections were positive for GGT5. The macrophages in the section from the infant were more lightly labeled than those from the adults. All lymphocytes in lymph nodes were negative for GGT5 expression. Lymphocytes in tonsils were GGT5-negative, while the macrophages were GGT5-positive. In the spleen, some germinal centers were negative for GGT5 while others contained GGT5-positive cells, which appeared to be dendritic cells (Fig. 3B). Macrophages within the spleen were strongly GGT5-positive.

Endocrine Glands—There was variability in the GGT5 immunolabeling among the adrenal glands, which may reflect the physiological state of the individual particularly with regard to the stress level. In most sections the interstitial cells of the adrenal cortex were GGT5-positive (Fig. 3C). In each of three adrenal glands from infants less than 6 months of age there was very, light labeling of the glandular cells throughout the cortex. Among the adrenal glands from adults the labeling of the glandular cells ranged from negative (62 year old male) to intense labeling of all the glandular cells (21 year old female, Fig. 3D). In addition, there was sometimes a gradation of labeling between cortical zones in the same tissue sections. Within each section of thyroid tissue there were both GGT5-positive and GGT5-negative follicles (Fig. 3E). The colloid within the follicles was either GGT5-positive or negative corresponding to the labeling of the follicular cells. The GGT5-positive follicular cells were more cuboidal suggesting that they may be actively secreting thyroglobulin, while the GGT5-negative follicular cells were more flattened. Differences in the content of the colloid among different follicles within the same gland has been observed by other investigators (Carcangia 2007). The pituitary contained both GGT5-positive and GGT5-negative glandular cells. The acidophils and chromophobes were GGT5-positive while the basophils failed to label for GGT5 (Fig. 3F).

Digestive Tract—The intercalated ducts, striated ducts and excretory ducts in the salivary glands were GGT5-positive, but the acini were negative (Fig. 4A). Esophageal tissue from nine different donors was examined. Keratinocytes in the mid and superficial spinosum layer of the stratified squamous epithelium generally showed weak GGT5-positive immunolabel (Fig. 4 C). In a limited region of one section, keratinocytes in the mid and superficial regions were very darkly labeled which may have been a response to irritation or damage of the tissue. In the cardiac region of the stomach, surface mucous cells were GGT5-positive. However, in the fundic region, surface mucous cells were unlabeled. In the fundic region,

the chief cells were GGT5-positive although the intensity of the labeling varied among donors (Fig. 4B). Parietal cells and mucous neck cells also varied in intensity of labeling ranging from moderate to intense. In sections of small intestine the absorptive epithelial cells, the enterocytes, were GGT5-positive (Fig. 4D). Lymphocytes within the sections were GGT5-negative, but macrophages were positive. Among tissue sections of colon there was very light labeling of the crypt cells in some sections with no labeling in others. GGT5-positive macrophages were present in all sections. Tissue sections of rectum showed labeling patterns similar to colon with very lightly labeled crypt cells in some sections and no labeling of the crypt cells in others. Macrophages were GGT5-positive. GGT5 staining in the liver is discussed above.

The secretory acini of the pancreas were GGT5-positive (Fig. 4E). The acini from some individuals stained more lightly than others. The pancreatic ducts were GGT5-negative. Only one Islet of Langerhans was observed in the immunolabeled sections from three pancreases. The cells in that islet were more intensely GGT5-labeled than the surrounding exocrine pancreas.

Bone Marrow—Within the bone marrow megakaryocytes expressed GGT5 (Fig. 4F). Other cells within the bone marrow were also GGT5-positive but we were unable to definitively classify these cells.

Respiratory System—The dust cells, the resident macrophages of the lung, expressed GGT5 (Fig. 5A). Dust cells scavenge particulates in the lung and therefore often appear dark in tissue sections. However, the lack of brown staining in the control section (Fig. 5B) demonstrates that the macrophages are GGT5-positive. The intense labeling of lung tissue on the immunoblot (Fig. 1B) suggests that the lung used for the immunoblot contained a large number of dust cells.

Reproductive System—The interstitial Leydig cells in the testis were GGT5-positive although not all cells labeled with the same intensity. Primary oocytes were GGT5-positive (Fig. 5C). The intensity of the labeling varied among oocytes within the same tissue section. The glands of the endometrium were negative, although macrophages within the tissue labeled GGT5-positive. In the placenta, the syncytial trophoblasts were GGT5 positive (Fig. 5D).

Muscle—Each of three sections of skeletal muscle was negative for GGT5 expression. In sections of cardiac muscle there was no labeling of the muscle fibers, but there was a small area of dense punctate labeling adjacent to the cell nuclei. The localization of GGT5 in the sections of cardiac muscle was distinct from all other tissues and was localized to the perinuclear region where lipofuscin was observed in the control sections. Expression of GGT5 in cardiac muscle was confirmed by western blot analysis. In a homogenate of human heart tissue, a single band at 54.5 kD labeled positive for GGT5 (Fig. 1B).

Skin—There was no specific labelling for GGT5 within sections of the skin.

Nervous System—The peripheral nerves including the axon, and myelin sheath were GGT5-negative. Macrophages in the same tissue sections labeled positive for GGT5. In the brain, the pyramidal cells in the cerebral cortex were GGT5-positive (Fig. 5E). Oligodendrocytes in the white matter deep to layer VI were negative for GGT5 just as observed for the GGT5-negative Schwann cells in the peripheral nervous system. Sections of the cerebellum showed positive labelling of Purkinje cells (Fig. 5F). The immunoblot that was labeled with GGT5-Ab797 (Fig. 1B) did not show any expression of GGT in the brain. The blot was purchased from a commercial vendor and no information was provided as to the area of the brain used to prepare the homogenate.

DISCUSSION

We have developed an anti-peptide antibody that is sensitive and specific for human GGT5. The antibody can be used to detect the large subunit of GGT5 on western blots and to immunolabel GGT5 in formalin-fixed, paraffin-embedded tissues. Analysis of the protein on western blots reveals that the large subunit is *N*-glycosylated both in primary human tissues and in human tissues grown in culture.

In this study, GGT5 expression was observed in macrophages present in many tissues. GGT5-positive macrophages were observed in the thymus, spleen, small intestine, endometrium, liver (Kupffer cells) and lung (dust cells). A study of mRNA expression in human monocytes and macrophages found that GGT5 mRNA was present at a similar level throughout differentiation of monocytes to macrophages and there was a slight but consistent increase in GGT5 mRNA in macrophages activated by either interferon-gamma (classical activation) or IL-4 [National Center for Biotechnology Information GEO dataset GDS2429 (Martinez et al. 2006)]. In the lung, the dust cells were the only cells that were GGT5-positive. Comparison of microarray datasets in which multiple normal human tissues were assessed for expression of GGT5 mRNA reveals considerable variability in the tissues that are found to express the highest level of GGT5 mRNA (National Center for Biotechnology Information GEO datasets GDS442, GDS596, GDS3113, and GDS3834). This variability may be due to differing levels of macrophage infiltration in the tissue among the samples analyzed. These data highlight the need for immunohistochemical studies to identify the cell type within a tissue that is expressing the protein of interest. In earlier studies of GGT1 expression in normal human tissues, the only organs in which macrophages were immunolabeled for GGT1 were lymph nodes and appendix, with weak labelling of the alveolar macrophages (dust cells) of the lung (Hanigan and Frierson 1996).

Little is known regarding the regulation of GGT5. Stimulation of human fibroblasts in cell culture with a sonic hedgehog homolog resulted in a 27-fold induction of GGT5 mRNA (National Center for Biotechnology Information GEO dataset GDS4512). GGT5 expression in Hassall's corpuscles in the thymus varied according to the age of the tissue donor. There was little to no expression in infants and young children where the corpuscles were just beginning to form, but in adults high expression of GGT5 was observed in some corpuscles suggesting that it may be induced in cells that were dying.

In many human tissues that express both GGT1 and GGT5 the enzymes are expressed by different cell types and exposed to different extracellular fluids. The kidney is one such tissue. The cell surface expression of GGT5 by the interstitial cells in the kidney results in direct contact of the enzyme with the interstitial fluid. The renal cortical interstitium has been shown to contain two cell types: fibroblasts and dendritic cells (Kaissling and Le Hir 2008). The latter are part of the mononuclear phagocyte system which includes macrophages. We were not able to ascertain which cell type within the interstitium of the renal cortex was GGT5-positive. However, it is likely that these cells were dendritic cells since we commonly observed GGT5-positive macrophages but not GGT5-positive fibroblasts in the tissues examined. In contrast, GGT1 is expressed on the apical surface of the epithelial cells lining the proximal tubules. GGT1 is in contact with the glomerular filtrate, where it serves an essential role by hydrolyzing the gamma-glutamyl bond of glutathione preventing the excretion of glutathione into the urine.

In the brain, both pyramidal cells and Purkinje cells expressed GGT5. Neither of these types of neurons express GGT1 (Hanigan and Frierson 1996). In the brain, extracellular glutathione and other gamma-glutamyl compounds are substrates for GGT5 and one of the products of the reaction is glutamate, which is a neurotransmitter. While pyramidal cells are known to release glutamate, Purkinje cells release gamma-amino butyric acid, which is synthesized from glutamate. In previous studies of GGT1 expression, the only cells within the human brain that were GGT1-positive were the capillary endothelium (Hanigan and Frierson 1996; Tsuji et al. 1987). Studies in cell culture have shown that GGT1 is expressed in murine endothelial cells isolated from brain capillaries only when the endothelial cells are in direct contact with glial cells suggesting that GGT1 is a component of the blood-brain barrier (DeBault and Cancilla 1980).

Hepatocytes are one of the few human cell types that express both GGT1 and GGT5. It is interesting to note that these two enzymes are localized differently by the hepatocyte. GGT1 is localized to the bile canaliculi surface where it would be in contact with the bile being secreted by the hepatocytes. Likewise, GGT5 is localized to the apical surface of many epithelial cells including the proximal tubules of the kidney, ductal epithelium of the salivary gland, the glandular surface of the prostate, cervical glands and ducts in the breast (Hanigan and Frierson 1996). In contrast, GGT5 was not localized to a specific region of the cell surface in any of the GGT5-positive polarized cells examined in this study.

Information on the expression of GGT5 in other vertebrates is derived primarily from mRNA studies in mice, rats and fish (Carter et al. 1998; Potdar et al. 1997; Law et al. 2012). The most striking difference in GGT5 expression between mouse and human tissues was that in the mouse, macrophages lacked GGT5 expression, while in humans GGT5-positive macrophages were observed in many tissues (Carter et al. 1998). In addition, Carter and colleagues measured LTC₄ hydrolysis activity in organs from GGT1 knockout mice and reported the highest levels of GGT5 activity in uterus, spleen, small intestine, kidney, pancreas, liver and lung (Carter et al. 1997; Shi et al. 2001).

GGT5 was originally discovered as a leukotrienease (Heisterkamp et al. 1991). Subsequent studies have shown that it also cleaves glutathione and other glutathione-conjugates

(Wickham et al. 2011). GGT1 can cleave these same substrates more efficiently. As shown in this study, GGT1 and GGT5 are expressed by different cell types within the body, which may result in unique roles for GGT1 and GGT5. Much remains to be learned about the role of GGT5 in both physiological processes and in disease. This study provides new information regarding the expression and localization of the enzyme in human tissues. The strong expression of GGT5 by macrophages throughout the body suggests it has an important role in the immune system.

Acknowledgments

We would like to thank the National Disease Research Interchange (NDRI, Philadelphia, PA) for procurement of the kidney samples used in this study and Ms. Jeanne Frazier, Medical Technologist, OU Anatomic Pathology Laboratory for immunolabeling of the tissues. Research reported in this publication was supported by The Oklahoma Center for the Advancement of Science and Technology (OCAST) grant number HR11-085 (M.H.H.), and by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM1103640.

References

- Bello MH, Epstein L. Clades of gamma-glutamyltransferases (GGTs) in the ascomycota and heterologous expression of *Colletotrichum graminicola* CgGGT1, a member of the peizizomycotina-only GGT clade. *J Microbiol.* 2013; 51(1):88–99.10.1007/s12275-013-2434-0 [PubMed: 23456716]
- Carcangia, ML. Thyroid. In: Mills, SE., editor. *Histology for Pathologists*. 3. Lippincott Williams and Wilkins; Philadelphia, PA: 2007. p. 1130-1148.
- Carter BZ, Shi ZZ, Barrios R, Lieberman MW. gamma-glutamyl leukotrienase, a gamma-glutamyl transpeptidase gene family member, is expressed primarily in spleen. *J Biol Chem.* 1998; 273 (43): 28277–28285. [PubMed: 9774450]
- Carter BZ, Wiseman AL, Orkiszewski R, Ballard KD, Ou CN, Lieberman MW. Metabolism of leukotriene C4 in gamma-glutamyl transpeptidase-deficient mice. *J Biol Chem.* 1997; 272 (19): 12305–12310. [PubMed: 9139674]
- DeBault LE, Cancilla PA. gamma-Glutamyl transpeptidase in isolated brain endothelial cells: induction by glial cells in vitro. *Science.* 1980; 207 (4431):653–655. [PubMed: 6101511]
- Han B, Luo G, Shi ZZ, Barrios R, Atwood D, Liu W, Habib GM, Sifers RN, Corry DB, Lieberman MW. Gamma-glutamyl leukotrienase, a novel endothelial membrane protein, is specifically responsible for leukotriene D(4) formation in vivo. *Am J Pathol.* 2002; 161 (2):481–490. [PubMed: 12163373]
- Hanigan MH, Frierson HF Jr. Immunohistochemical detection of gamma-glutamyl transpeptidase in normal human tissue. *J Histochem Cytochem.* 1996; 44(10):1101–1108. [PubMed: 8813074]
- Hanigan MH, Ricketts WA. Extracellular glutathione is a source of cysteine for cells that express gamma-glutamyl transpeptidase. *Biochemistry.* 1993; 32 (24):6302–6306. [PubMed: 8099811]
- Heisterkamp N, Groffen J, Warburton D, Sneddon TP. The human gamma-glutamyltransferase gene family. *Hum Genet.* 2008; 123 (4):321–332.10.1007/s00439-008-0487-7 [PubMed: 18357469]
- Heisterkamp N, Rajpert-De Meyts E, Uribe L, Forman HJ, Groffen J. Identification of a human gamma-glutamyl cleaving enzyme related to, but distinct from, gamma-glutamyl transpeptidase. *Proc Natl Acad Sci U S A.* 1991; 88 (14):6303–6307. [PubMed: 1676842]
- Kaissling B, Le Hir M. The renal cortical interstitium: morphological and functional aspects. *Histochem Cell Biol.* 2008; 130(2):247–262.10.1007/s00418-008-0452-5 [PubMed: 18575881]
- King JB, West MB, Cook PF, Hanigan MH. A novel, species-specific class of uncompetitive inhibitors of gamma-glutamyl transpeptidase. *J Biol Chem.* 2009; 284 (14):9059–9065. doi:M809608200. [PubMed: 19203993]
- Law SH, Redelings BD, Kullman SW. Comparative genomics of duplicate gamma-glutamyl transferase genes in teleosts: medaka (*Oryzias latipes*), stickleback (*Gasterosteus aculeatus*), green

- spotted pufferfish (*Tetraodon nigroviridis*), fugu (*Takifugu rubripes*), and zebrafish (*Danio rerio*). *J Exp Zool B Mol Dev Evol.* 2012; 318 (1):35–49.10.1002/jez.b.21439 [PubMed: 21898790]
- Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. *J Immunol.* 2006; 177(10):7303–7311. [PubMed: 17082649]
- Okada T, Suzuki H, Wada K, Kumagai H, Fukuyama K. Crystal structures of gamma-glutamyltranspeptidase from *Escherichia coli*, a key enzyme in glutathione metabolism, and its reaction intermediate. *Proc Natl Acad Sci U S A.* 2006; 103 (17):6471–6476. [PubMed: 16618936]
- Potdar PD, Andrews KL, Nettesheim P, Ostrowski LE. Expression and regulation of gamma-glutamyl transpeptidase-related enzyme in tracheal cells. *Am J Physiol.* 1997; 273 (5 Pt 1):L1082–1089. [PubMed: 9374738]
- Shi ZZ, Han B, Habib GM, Matzuk MM, Lieberman MW. Disruption of gamma-glutamyl leukotrienase results in disruption of leukotriene D(4) synthesis in vivo and attenuation of the acute inflammatory response. *Mol Cell Biol.* 2001; 21 (16):5389–5395.10.1128/MCB.21.16.5389-5395.2001 [PubMed: 11463821]
- Tsuji T, Mimori Y, Nakamura S, Kameyama M. A micromethod for the isolation of large and small microvessels from frozen autopsied human brain. *J Neurochem.* 1987; 49 (6):1796–1800. [PubMed: 2890713]
- West MB, Chen Y, Wickham S, Heroux A, Cahill K, Hanigan MH, Mooers BH. Novel insights into eukaryotic gamma-glutamyl transpeptidase 1 from the crystal structure of the glutamate-bound human enzyme. *J Biol Chem.* 2013; 288 (44):31902–31913. doi:M113.498139. [PubMed: 24047895]
- West MB, Segu ZM, Feasley CL, Kang P, Klouckova I, Li C, Novotny MV, West CM, Mechref Y, Hanigan MH. Analysis of site-specific glycosylation of renal and hepatic gamma-glutamyl transpeptidase from normal human tissue. *J Biol Chem.* 2010; 285 (38):29511–29524. doi:M110.145938. [PubMed: 20622017]
- Wickham S, West MB, Cook PF, Hanigan MH. Gamma-glutamyl compounds: Substrate specificity of gamma-glutamyl transpeptidase enzymes. *Anal Biochem.* 2011; 414:208–214. doi:S0003-2697(11)00197-7. [PubMed: 21447318]

Fig. 1.

GGT5-Ab797 specifically recognizes the large subunit of human GGT5. Western immunoblot analysis (A) of purified hGGT1 expressed in yeast (Lane 1), hGGT1 deglycosylated with EndoH (Lane 2), whole cell lysate of NIH3T3 cells (Lane 3) or NIH3T3 cells expressing hGGT5 (Lane 4), membranes from NIH3T3 cells expressing hGGT5 intact or deglycosylated with PNGaseF (Lanes 5 and 6) and detergent extracts of human kidney microsomes intact or deglycosylated with PNGaseF (Lanes 7 and 8). GGT5-Ab797 detected a single band at 54.5 kD in samples containing hGGT5 and at 41 kD in samples containing deglycosylated hGGT5. The line between lanes 3 and 4 indicates lanes with samples not relevant to this publication were omitted from this image. Western immunoblot analysis (B) of tissue homogenates from human brain (Lane 1), colon (Lane 2), heart (Lane 3), kidney (Lane 4), liver (Lane 5) and lung (Lane 6).

Fig. 2. Immunolabeling of GGT5 and GGT1 in normal human kidney and liver. In kidney (A, B) GGT5 antibodies labeled (brown) interstitial cells (A, arrow) while GGT1 antibodies labeled proximal tubule cells (B). In liver (C, D) GGT5 antibodies labeled Kupffer cells (C, arrow) and to a lesser extent hepatocytes while GGT1 antibodies labeled hepatocyte bile canalicular membrane (D, arrow). Sections are counterstained with hematoxylin (blue). Bar = 50 μ m

Fig. 3.

Human lymphatic and endocrine tissues immunolabeled for GGT5. Immunolabeled cells (brown) were observed in Hassall's corpuscles in the thymus (A), and in germinal center cells in the spleen (B). Endothelial cells lining the sinusoids of adrenal cortex were consistently GGT5-positive (C,D) while immunolabeling of glandular cells varied. Shown are unlabeled (C, zona fasciculata) or labeled (D, zona reticularis) glandular cells. The thyroid contained both GGT5-positive and GGT5-negative follicular cells and colloid (E). Acidophils and chromophobes in the pituitary were GGT-5 positive (F). Sections are counterstained with hematoxylin (blue). Bar = 50 μ m

Fig. 4. GGT5 immunolabeling in organs of the human digestive tract and hematopoietic tissues. Ducts of salivary gland labeled with GGT5 antibodies (A, brown stain). Stomach glandular cells were labeled as exemplified by chief cells at the base of fundic glands (B). In the esophagus in GGT5 labeling was observed in mid to superficial regions while cells near the basal layer (arrow) were only very lightly labeled (C). Enterocytes of the small intestine (D), and exocrine pancreatic cells (E) showed moderate labeling. In the bone marrow (F) many cells were labeled as exemplified by the large megakaryocyte (arrow). Some of the white and red blood cell precursors were also labeled. Sections are counterstained with hematoxylin (blue). Bar = 50 μ m

Fig. 5. GGT5 in human lung, reproductive organs and brain. The dust cells in the lung (A, arrow) were strongly GGT5-positive (brown). Control lung tissue (B), obtained from the same specimen as in A and processed in the absence of the primary antibody, shows macrophages (arrow) containing black particulate matter that is different than the brown immunolabeling. Primary oocytes in the ovary were GGT5-positive (C), and trophoblasts (arrow) of the placenta immunolabeled for GGT5 (D). In sections of the brain, expression of GGT5 was observed in pyramidal cells of cerebral cortex (E) and Purkinje cells in the cerebellum (F). Sections are counterstained with hematoxylin (blue). Bar = 50 μ m

Table 1

Immunohistochemical detection of GGT5 in normal human tissues

Tissue	Immuno-positive cells
Immune System	
Thymus (7) ^a	Hassall's corpuscles, macrophages
Lymph Nodes (4)	Macrophages
Spleen (16)	Germinal centers, macrophages
Endocrine Glands	
Adrenal (12)	interstitial cells in the cortex, glandular cells
Thyroid (11)	Some follicular cells and colloid
Pituitary (2)	Acidophils and chromophobes
Digestive Tract	
Salivary gland (2)	Intercalated, striated and excretory ducts
Esophagus (9)	Squamous epithelium (weak)
Stomach (9)	Mucous glands (cardiac stomach), fundic glands
Small Intestine (3)	Enterocytes, macrophages
Colon (6)	Crypt cells (very weak), macrophages
Rectum (6)	Crypt cells (very weak), macrophages
Liver (10)	Endothelial cells lining the sinusoids, Kupffer cells, Hepatocytes (weak)
Pancreas (3)	Secretory acini
Bone Marrow (3)	Megakaryocytes and other cells types
Urinary System	
Kidney (10)	Interstitial cells
Respiratory System	
Lung (9)	Dust Cells
Reproductive system	
Testis (3)	Leydig cells and granular labeling within the cells of the seminiferous tubule
Prostate (3)	Epithelial cells (granular labeling)
Ovary (3)	Primary oocytes
Placenta (8)	Syncytial trophoblasts
Brain	
Cerebral Cortex (6)	Pyramidal cells
Cerebellum (3)	Purkinje cells.

^aNumber of sections analyzed. Each section was from a different donor.