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Detection of Human Taurine Transporter and Production of Monoclonal Antibody

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Taurine (2-ethaneaminosulfonic acid) is one of the major intracellular β-amino acids in mammals and is required for a number of biological processes including membrane stabilization, osmoregulation, antioxidation, detoxification, modulation of calcium flux and neuromodulation. The taurine transporter (TAUT) which contains 12 hydrophobic membrane-spanning domains has been cloned from dog kidney, rat brain, mouse brain, human thyroid, placenta and retina. In this study, The TAUT cDNA from the human intestinal epithelial cell, HT-29 was cloned and sequenced. Reverse-transcription polymerase chain reaction (RT-PCR) was performed to amplify partial cDNA encoding human intestinal TAUT. The coding region of the PCR product was 732 bp long. The primers were designed to encode highly conserved amino acid sequences near the transmembrane domains III (IPYFIFLF) and VI (KYKYNSYR) both in human and mouse. The TAUT cDNA amplified was ligated into the pGEX 4T-1 expression vector. The resulting sequence of human intestinal TAUT cDNA (Accession number of NCBI Genebank is AF346763) was identical to the sequences of the TAUTs previously determined in the human placenta and retina except 3 base pairs from that of the reported human thyroid. TAUT specific antibodies were generated to use them as biological tools in the studies of the biological role of TAUT. Peptides of 149-162 amino acid residue (14 amino acids) of the TAUT were synthesized. The synthetic peptide used in this study was LFQSFQKELPWAHC. This region was chosen not only to avoid putative glycosylation sites but also to exclude regions of known homology with GABA transporters in the extracellular hydrophilic domains. The synthetic peptide, TAUT-1 was conjugated with carrier protein, kehole lympet hemocyanin (KLH) to use as an antigen. When used for immunization on a rabbit to produce polyclonal antiserum, the conjugates elicited high-titered specific anti-TAUT-1 antibodies, which reacted well with the ovalbumin (OVA) conjugated peptides in ELISA. The KLH-conjugated peptide was also used as immunizing antigen in BALB/c mice to produce TAUT specific monoclonal antibodies. From the culture supernatant of the hybridoma, the specificity of anti-TAUT-1 monoclonal antibodies was confirmed by ELISA. Further applications of more tools in TAUT expression analysis will be performed such as western blotting and flow cytometry. *Supported from the Interdiciplinary Research Program of KOSEF (1999-2-209-013-5).*