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Identification and functional characterization of a novel UGT2A1 splice variant: Potential importance in tobacco-related cancer susceptibility

Supplemental Materials and Methods

Generation of a UGT2A1_i2 over-expressing cell line and UGT2A1_i2 cell homogenate. UGT2A1exonΔ3 was cloned from pooled lung RNA using *Pfu* Polymerase and the UGT2A1_S1 and UGT2A1_AS1 primers. Following gel extraction and sequencing of the PCR product of the appropriate size, the verified UGT2A1exonΔ3 cDNA was cloned into the pcDNA 3.1/V5-His-TOPO vector using standard protocols and grown in One Shot TOP10 competent E.Coli. After direct dideoxy sequencing for sequence confirmation and a large-scale plasmid preparation, electroporation (200 V, 1000 μF) with 10 μg of the pcDNA 3.1/V5-His-TOPO_UGT2A1exonΔ3 vector was used to generate the HEK293 cell line over-expressing UGT2A1_i2. Cells were grown in DMEM supplemented with 10% FBS, 1% penicillin-streptomycin, and 400 μg/mL G418 to 75% confluence. Cell homogenates were prepared essentially as previously described in 1X Tris-buffered saline (25 mM Tris base, 138 mM NaCl and 2.7 mM KCl; pH 7.4) (Dellinger et al., 2006; Sun et al., 2006). Total RNA was extracted using the RNeasy Mini kit using the manufacturer’s protocols. Homogenate protein concentrations were determined using the BCA protein assay.

Determination of UGT2A1_i1 homo-oligomerization. An inducible co-expression system, similar to that used to investigate UGT2A1_1:UGT2A1_i2 hetero-oligomerization, was used to examine potential UGT2A1_i1 homo-oligomerization.

Creation of the pcDNA 6.2/V5/GW/D-TOPO_wtUGT2A1 vector was described previously in the Materials and Methods. Wild-type UGT2A1 was cloned into the FLAG tagged, hygromycin resistance containing pEGSH vector, using UGT2A1_S3 and UGT2A1_AS3 primers as described previously. A HEK293 cell line stably expressing the pcDNA6.2/V5/GW/D-TOPO_wtUGT2A1, pEGSH_wtUGT2A1, and pERV vectors was created as described in the Materials and Methods. UGT2A1_i1_FLAG expression was induced by treating HEK293 cells at 50% confluence with 10 μM of PonA (in ethanol) for 12 h. Vehicle (0.01% ethanol) was added to HEK293 cells as a negative control. Determination of UGT2A1_i1_V5 and UGT2A1_i1_FLAG expression levels using the anti-V5 and anti-FLAG antibodies, the use of the anti-UGT2A1 antibody to confirm UGT2A1_i1_V5 and UGT2A1_i1_FLAG levels, and co-IP experiments were completed using identical conditions to that described for UGT2A1_i1_V5 and UGT2A1_i2_FLAG hetero-oligomerization.