Fung et al., *MDR1* Synonymous Polymorphisms Alter Transporter Specificity and Protein Stability in a Stable Epithelial Monolayer

Supplementary Materials and Methods

Preparation of pcDNA-MDR1 constructs

The pcDNA-MDR1 constructs were subcloned from the pTM-MDR1 constructs used by Kimchi-Sarfaty et al (1). 3 vectors were constructed as follows 1) pcDNA-MDR1(1236C-2677G-3435C), 2) pcDNA-MDR1(1236T-2677T-3435T), and 3) pcDNA-MDR1(1236T-2677T-3435A). The *MDR1* coding sequences were confirmed using sequencing primers as follows.

(MDR1-537-reverse) 5'-GACATCATCTGTAAGTCGGGTG-3';

(MDR1-407) 5'-GGTGCCTGGCAGCTGGAAGAC-3';

(MDR1-1078) 5'-GCAAATGCAAGAGGAGCAGCTTATG-3';

(MDR1-1400) 5'-GGAAATCATTGGTGTGGTGA-3';

(MDR1-1885) 5'-CAGACAGCAGGAAATGAAGT-3';

(MDR1-2355) 5'-CAAGCGGCTCCGATACATGG-3';

(MDR1-3120) 5'-TGTATTCAACTATCCCACCC-3';

(MDR1-3475) 5'-GAGTCACGCCTAATA-3'.

Sequence alignment with the reference human *MDR1* gene sequences (NM_000927.4) reveals that all the pTM- and pcDNA-*MDR*1 DNA sequences contain a synonymous mutation at position 2134 (C>T). The names of the primers indicate the first nucleotide where the primer anneals to the *MDR1* sequence.

Generation of LLC-MDR1 cell lines

LLC-PK1#7, a subclone from the parental LLC-PK1 cell line, was isolated by clonal selection. Using LLCPK1#7, four stable cell lines (LLC-vector, LLC-MDR1-WT, LLC-MDR1-3H, LLC-MDR1-3HA) were generated by lipofectamine 2000™-mediated transfection (Invitrogen). After transfection, cells were incubated for 48 hrs in complete medium before adding 500 μg/mL Geneticin. A minimum of 30 clones were isolated for clone selection.

Reference

1. Kimchi-Sarfaty C, Oh JM, Kim IW, Sauna ZE, Calcagno AM, Ambudkar SV, et al. A "silent" polymorphism in the MDR1 gene changes substrate specificity. Science. 2007;315:525-8.