Identification of transporters associated with etoposide sensitivity of stomach cancer cell lines and methotrexate sensitivity of breast cancer cell lines by quantitative targeted absolute proteomics

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Molecular Pharmacology

Supplementary Table 1 Peptide probe sequences and selected ions for absolute quantification of membrane proteins and metabolic enzymes

Gene name	Alias	Probe sequence	SRM/MRM transition (m/z)				
			Q1	Q3-1	Q3-2	Q3-3	Q3-4
ABCA9	ABCA9	QHISDAK	399.7	420.2	533.3	581.3	652.3
		QHISD**A***K	401.7	424.2	537.3	581.3	656.3
ABCB5	ABCB5	SADLIVTLK	480.3	686.5	801.5	573.4	460.3
		SADLIVT**L***K	483.8	693.5	808.5	580.4	467.3
SLC16A3	MCT4	LLDLSVFR	482.4	849.5	736.4	621.4	508.3
		LLDLSV**F***R	487.4	859.5	746.4	631.4	518.3
SLC22A9	UST3	DTLTLEILK	523.4	716.4	829.5	502.3	615.4
		DTLTLEI**L***K	526.9	723.4	836.5	509.3	622.4
SLC22A14	OCTL2	DQPLSESLNHSSQIR	570.9	590.3	727.4	503.3	841.4
		DQPLSESLNHSSQ**I***R	573.2	597.3	734.4	510.3	848.4
SLC47A2	MATE2	YLQNQGWLK	575.3	873.5	745.4	631.4	986.5
		YLQNQGW**L***K	578.8	880.5	752.4	638.4	993.5
SLC47A2	MATE2k	TPEEAHALSAPTSR	733.9	939.5	802.4	731.4	618.3
		TPEEAHALSA**P***TSR	736.9	945.5	808.4	737.4	624.3
DHFR	DHFR	NGDLPWPPLR	582.8	765.4	482.3	668.4	385.3
		NGDLPWPP**L***R	586.3	772.4	489.3	675.4	392.3
FPGS	FPGS	DPIFQPPSPPK	611.8	622.4	341.2	897.5	750.4
		DPIFQPPSP**P***K	614.8	628.4	347.2	903.5	756.4
GGH	GGH	YYIAASYVK	539.3	638.4	567.3	751.4	496.3
		YYIAASY**V***K	542.3	644.4	573.3	757.4	502.3

Typically, doubly charged precursor ions (singly charged for some peptides) were selected (Q1). Four transitions per peptide (Q3-1, -2, -3 and -4), corresponding to high-intensity fragment ions, were selected. Bold letters with asterisks indicate amino acid residues labeled with stable isotope (^{13}C and ^{15}N). Other peptides were listed in previous reports (Ohtsuki et al., 2011; Uchida et al., 2011).

Ohtsuki S, Uchida Y, Kubo Y and Terasaki T (2011) Quantitative targeted absolute proteomics-based ADME research as a new path to drug discovery and development: methodology, advantages, strategy, and prospects. *J Pharm Sci* **100**(9):3547-3559.

Uchida Y, Ohtsuki S, Katsukura Y, Ikeda C, Suzuki T, Kamiie J and Terasaki T (2011) Quantitative targeted absolute proteomics of human blood-brain barrier transporters and receptors. *J Neurochem* **117**(2):333-345.

Supplementary Table 2 Conditions of LC-MS/MS analysis in uptake studies

Molecule	SRM/MRM transition (m/z)		DP (V)	CE (V)
	Q1	Q3		
Etoposide	589.2	229.2	76	23
Gemcitabine	264.1	112.1	66	25
Methotrexate (MTX)	455.4	308.2	151	27
MTX-Glu_2	584.6	308.2	156	37
MTX-Glu_3	713.7	308.2	181	47
MTX-Glu_4	842.8	308.2	150	51
MTX-Glu_5	971.9	308.2	150	63
MTX-Glu_6	1101.0	308.2	150	78
MTX-Glu_7	1230.1	308.2	150	89

Supplementary Information 1. Derivation of Eq. 1 and 2

Based on the model shown in Fig S1A, medium-to-cell influx rate and cell-to-medium efflux rate are described as:

Medium-to-cell influx rate = PS_{inf} x C_{medium} Supp Eq. 1

Cell-to-medium efflux rate = ($PS_{eff,MK\ insensitive}$ + $PS_{eff,MK\ sensitive}$) x C_{cell} Supp Eq. 2

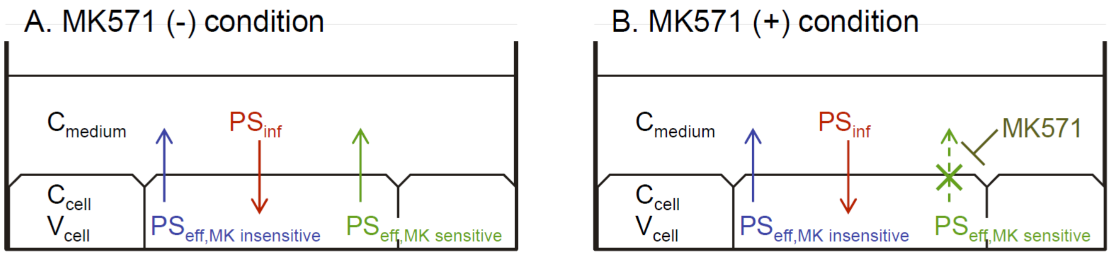

Fig S1. Schematic diagram illustrating the PS products for the accumulation of substrates in human stomach cancer cells.

PS_{inf}, $PS_{eff,MK\ insensitive}$ and $PS_{eff,MK\ sensitive}$ represent influx clearance, MK571-insensitive efflux clearance and MK571-sensitive efflux clearance, respectively.

C_{medium} and C_{cell} represent drug concentration in medium (nM) and cell (nmol/μg ceullar protein), respectively. V_{cell} represents volume of cells (μL).

Cell-to-medium (C/M) ratio represents the apparent distribution volume of the substrate in the cell generated by the polarized transport rate across the plasma membrane, and is defined as (C_{cell} x V_{cell}) / C_{medium}. Considering the steady-state condition, medium-to-cell influx rate is equal to cell-to-medium efflux rate. Therefore, steady-state C/M_{ss} ratio can be transformed to Eq.1 in the text using Supp Eq. 1 and 2.

PS_{inf} x $C_{mediium}$ = ($PS_{eff,MK\ insensitive}$ + $PS_{eff,MK\ sensitive}$) x C_{cell}

C/M_{ss} ratio = (C_{cell} x V_{cell}) / C_{medium} = PS_{inf} x V_{cell}/ ($PS_{eff,MK\ insensitive}$ + $PS_{eff,MK\ sensitive}$)

Eq. 1 in the text

In the presence of MK571, $PS_{eff,MK\ sensitive}$ becomes zero, as shown in Fig S1B. Therefore, steady-state C/M_{ss} ratio,MK571 can be described as:

C/M_{ss} ratio,MK571 = PS_{inf} x V_{cell} / $PS_{eff,MK\ insensitive}$

Then, Eq. 2 in the text was derived as follows:

$1/(C/M_{ss}\ \text{ratio}) - 1/(C/M_{ss}\ \text{ratio,MK571})$
$= (PS_{eff,MK\ insensitive} + PS_{eff,MK\ sensitive}) / (PS_{inf} \times V_{cell}) - PS_{eff,MK\ insensitive} / (PS_{inf} \times V_{cell})$
$= PS_{eff,MK\ sensitive} / (PS_{inf} \times V_{cell})$

Eq. 2 in the text