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IDENTIFICATION OF HUMAN AND RAT NOVEL RIBOFLAVIN TRANSPORTER RFT1 BASED ON THE IN SILICO GENE EXPRESSION DATABASE

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About 400 transporters have been identified using expression cloning or PCR cloning. However, many transporters remain to be identified yet. We searched for novel transporters using our rat kidney mRNA expression database, which consists of high expression genes in the kidney [Horiba N, et al. Kidney Int 66: 29-45, 2004], and SOSUI program, which can predict the number of transmembrane domains from amino acid sequence. We found a predicted 10-transmembrane protein, Gpr172b, in this database. Gpr172b does not show the similarity to ABC and SLC transporters. Real-time PCR revealed that human GPR172B mRNA was expressed strongly in the placenta and small intestine, and was detected in all tissues examined. GPR172B was also expressed in HEK293 and Caco-2 cells. HEK293 cells transfected with green fluorescent protein-tagged GPR172B exhibited a fluorescent signal in the plasma membrane. By overexpression system with HEK293 cells, screening of 26 compounds were carried out. However, we could not identify the substrate of Gpr172b. Small interfering RNA targeting GPR172B significantly decreased the uptake of [3H]riboflavin, but not other compounds. Transfection of rat Gpr172b in siRNA-transfected HEK293 cells induced a increase in [3H]riboflavin uptake. Based on these results, we renamed GPR172B as riboflavin transporter 1, RFT1. Furthermore, functional analysis was carried out using HEK293 cells natively expressing RFT1. Riboflavin uptake by HEK293 cells was independent of Na+, membrane potential and pH-gradient. Kinetic analysis demonstrated that the Michaelis-Menten constant for [3H]riboflavin uptake by HEK293 cells was 28.1 nM. In conclusion, we successfully identified novel riboflavin transporter RFT1 in combination of informatics and functional characteristics [Yonezawa A, et al. Am J Physiol Cell Physiol in press].

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STRUCTURE-ACTIVITY RELATIONSHIPS OF THE INHIBITORY EFFECTS OF RESVERATROL AND CONPOUNDS WITH SIMILAR STRUCTURES ON P-GLYCOPROTEIN-MEDIATED TRANSPORT IN KB-C2 CELLS

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It has been revealed that flavonoids and other polyphenols modulate P-gp activity. We studied the effects of stilbene derivatives, resveratrol, and piceatannol as well as nordihydroguaiaretic acid, phenethyl caffeate, rosmarinic acid, and probucol whose chemical structures resemble with those of the stilbene derivatives on P-gp function in multidrug-resistant P-gp overexpressing KB-C2 cells. Piceatannol, nordihydroguaiaretic acid, and phenethyl caffeate significantly increased the accumulation of rhodamine-123 and daunorubicin dependent on their chemical structure. Intracellular accumulation of resveratrol and piceatannol were similar in both KB-C2 cells and the drug sensitive KB-3-1 cells. From this, resveratrol and piceatannol did not seem to be P-gp substrates. Piceatannol penetrated into the cells promptly in a concentration-dependent manner and reached equilibrium within 30 minutes. Piceatannol has one more hydroxyl group as a catechol ring and hydrophobicity of resveratrol is larger than that of piceatannol. The effect of piceatannol on the accumulation of P-gp substrates were larger than that of resveratrol. The effects of polyphenols tested on the accumulation of daunorubicin in KB-C2 cells were in the order of nordihydroguaiaretic acid > piceatannol, phenethyl caffeate > resveratrol, probucol. The effects on the accumulation of rhodamine-123 were in the order of phenethyl caffeate > nordihydroguaiaretic acid > piceatannol > resveratrol, probucol. Caffeic acid had no inhibitory effect. From the findings the number of hydroxyl groups as well as hydrophobicity seemed to determine the inhibitory effect.