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Pharmacogenomic Application for Gene Targeting and Molecular Characterization of a Nucleoside Transporter hCNT2 in Human Intestine

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We have cloned and functionally expressed a sodium dependent human nucleoside transporter, hCNT2, from a CNS cancer cell line U251. Our cDNA clone of hCNT2 had the same predicted amino acid sequence as the previously cloned hCNT2 transporter. Of the several cell lines studied, the best hCNT2 transport function was obtained when transiently expressed in U251 cells. Na+-dependent uptake of [3H]-inosine in U251 cells transiently expressing hCNT2 was 50 fold greater than that in non-transfected cells, and uptake in Na+-containing medium was approximately 30 fold higher than at Na+-free condition. The hCNT2 displayed saturable uptake of [3H]-inosine with Km of 12.8 M and Vmax of 6.66 pmol/mg protein/5 min. Uptake of [3H]-inosine was significantly inhibited by the purine nucleoside drugs dideoxyinosine and cladribine, but not by acyclic nucleosides including acyclovir, ganciclovir, and their prodrugs valacyclovir and valganciclovir. This indicates that the closed ribose ring is important for binding of nucleoside drugs to hCNT2. Among several pyrimidine nucleosides, hCNT2 favorably interacted with the uridine analog floxuridine. Interestingly, we found that benzimidazole analogs including maribavir, 5,6-dichloro-2-bromo-1-β-D-ribofuranosylbenzimidazole (BDCRB), and 5,6-dichloro-1-β-D-ribofuranosyl-benzimidazole (DRB) were strong inhibitors of inosine transport, even though they have a significantly different heterocycle structure compared to a typical purine ring. As measured by GeneChip® arrays, mRNA expression of hCNT2 in human duodenum was 15-fold greater than that of hCNT1 or hENT2. Further, the rCNT2 expression in rat duodenum was 20-fold higher than rCNT1, rENT1 or rENT2. This suggests that hCNT2 (and rCNT2) may have a significant role in uptake of nucleoside drugs from the intestine and is a potential transporter target for the development of nucleoside and nucleoside-mimetic drugs. In conclusion, (1) we found

predominant expression of hCNT2 mRNA in human intestinal tissues, (2) we have successfully cloned hCNT2 from human duodenum, (3) U251/hCNT2 expression system was very effective for functional characterization of hCNT2, (4) and intact sugar ring structure appears to be important for binding and (5) we found benzimidazole riboside analogs as strong inhibitors of hCNT2 transport activity.

Keyword : Pharmacogenomics, Gene targeting, Transporter rCNT2, Human intestine