IN VITRO DEVELOPMENT OF FOLATE TRANSPORT AND ENDOCYTOSIS BY PCFT, RFC, and FRα, IN A TRANSWELL SYSTEM

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Abstract

Folate, aka vitamin B9, including the dietary folate and synthetic folic acid, plays an important role in numerous vital cellular processes. Its major circulating active form 5-methyltetrahydrofolate (5-MTHF), folate is used as a center for one carbon fragments in DNA synthesis, repair and methylation, as well as biosynthesis of the essential amino acid methionine. Folate is especially important during rapid cell proliferation. Therefore, a number of folic acid derivatives and folates, have been developed as anticancer drugs. Methotrexate has been widely used by and approved in cancer chemotherapy in non-ontological neoplastic diseases and NY-ESO-1-expressing tumor. Permethrex is a unique enzyme targeting multiple folate metabolic enzymes.

Folate is a hydrophilic nutrient and an ion in physiological pH. Its permeability across epithelial cell membranes is low. 5-MTHF transport rates on both in the small intestine and colon. Proton-coupled folate transport (PCFT, SLCO6A1) is mainly localized in the upper [acidic] part of the intestine including the duodenum and proximal jejunum. As a proton-folate symporter that couples the uptake of folates to the flow of protons down an electrochemical gradient, the activity of PCFT is high in low pH. The reduced folate carrier (RFC, SLC19A1) is expressed ubiquitously in mammalian tissues, including intestinal and cutaneous epithelia. However, it functions optimally at around neutral pH and favors reduced folates, as its name stands for. RFC transports folates and their conjugates, being responsible for folate intestinal absorption.

In this study, we aimed to develop PCFT, RFC uptake assays and FRα endocytosis assay in MDCK cell lines by using transwell transfection and transfection in the intestinal space via endocytosis. FRα is normally expressed in secretory epithelial cells, and it is not FRβ, is overexpressed on the basolateral surface of tumor cells. Some ABC transporters in the group of MRP and SLCO transporters in the families of SLCT and 22 have been reported to be involved in folic acid absorption and secretion. However, the mechanisms and their contributions remain largely unclear compared to PCFT, RFC, and FRα.

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Methods

MDCK cells were maintained in DMEM with low glucose and 10% FBS. Cells were seeded in Millicell inserts (PCFT) or in polycarbonate filter (RFC) and filter cell receptor (FRα) and allowed to adhere in DMEM plus 5% FBS. After 24 hours, folate or folic acid was added to the transwell plate. A 10:1 volume ratio was made to measure the intracellular accumulation of the radiolabeled substrate by autoradiography.

Results

PCFT transport of folic acid

RF transport of methotrexate

RFC transport of methotrexate

RFC transport did NOT transport folic acid

Conclusions & Discussion

MDCK cells include PCFT and RFC transport assays and FRα endocytosis assay have been successfully developed and characterized. Both PCFT (SLCO6A1) and RFC (SLC19A1) expression are not only applied to conventional transport assays, but also good for receptor endocytosis, and potentially transcytosis.

PCFT is expressed on the apical side of MDCK cells and is optimal at acidic pH (pH 5.5) for transport of both folic acid and methotrexate. FRα is robust with 31.7X and 122X assay windows for folate acid and methotrexate, respectively.

RFC is predominantly expressed on the basolateral side of MDCK cells and performs better at neutral pH. There exist abundant endogenous RFC or other methotrexate transporting transporters, rendering a more 268X assay window for methotrexate transport. However, the assay window is wide enough to generate meaningful IC50 inhibition curves for BSP and pemetrexed.

FRα seems equally expressed on the apical and basolateral side of MDCK cells. Folic acid endocytosis is linear within 3 hours tested, and cellular accumulation of folate acid is inhibited by BSP and pemetrexed, respectively.

Using the validated assays, a number of integrase inhibitors were assessed for potentials on folate pathways, and clinical xenotransplant predicted no concerns of decreases in maternal and fetal folate levels [6].

References