The Proton-Coupled Folate Transporter: Impact on Pemetrexed Transport and on Antifolates Activities Compared with the Reduced Folate Carrier

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ABSTRACT

The reduced folate carrier (RFC) and the proton-coupled folate transporter (PCFT) are ubiquitously expressed in normal and malignant mammalian tissues and in human solid tumor cell lines. This article addresses the extent to which PCFT contributes to transport of pemetrexed and to the activities of this and other antifolates relative to RFC at physiological pH. Either RFC or PCFT cDNA was stably transfected into a transporter-null HeLa cell variant to achieve activities similar to their endogenous function in wild-type HeLa cells. PCFT and RFC produced comparable increases in pemetrexed activity in growth medium with 5-formyltetrahydrofolate. However, PCFT had little or no effect on the activities of methotrexate, *N*-(5-[*N*-(3,4-dihydro-2-methyl-4-oxyquinazolin-6-ylmethyl)-*N*-methyl-amino]-2-thenoyl)-L-glutamic acid (raltitrexed, Tomudex; ZD1694), or N^{α}-(4-amino-4-deoxypteroyl)-N^{δ}-hemiphthaloyl-L-ornithine (PT523) in comparison with RFC irrespective of the folate growth source. PCFT, expressed at high levels in *Xenopus laevis* oocytes and in transporter-competent HepG2 cells, exhibited a high affinity for pemetrexed, with an influx K_m value of 0.2 to 0.8 μM at pH 5.5. PCFT increased the growth inhibitory activity of pemetrexed, but not that of the other antifolates in HepG2 cells grown with 5-formyltetrahydrofolate at physiological pH. These findings illustrate the unique role that PCFT plays in the transport and pharmacological activity of pemetrexed. Because of the ubiquitous expression of PCFT in human tumors, and the ability of PCFT to sustain pemetrexed activity even in the absence of RFC, tumor cells are unlikely to become resistant to pemetrexed as a result of impaired transport because of the redundancy of these genetically distinct routes.

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CF FC .

Materials and Methods

Chemicals. 3,5,7,9-^{3}

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(., 2004 ; ., 2006). ,
2(□), -
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CF -4, , 1-11- ,
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5.5, CF ,
1-11- CF -4 (F . 1B).

Impact of Expression of RFC or PCFT on Cellular Folate Growth Requirement. C - 5- F . - A . B , - . A F . 2, , 50% (EC$_{50}$) 1-11-

25 5- I F (F . 2,). A
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1-11- -1 1/14 1/17 I
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3 5- I F
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11. I , CF -
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A Comparison of he Impac of RFC or PCFT on An ifola e Ac i i ies. A -
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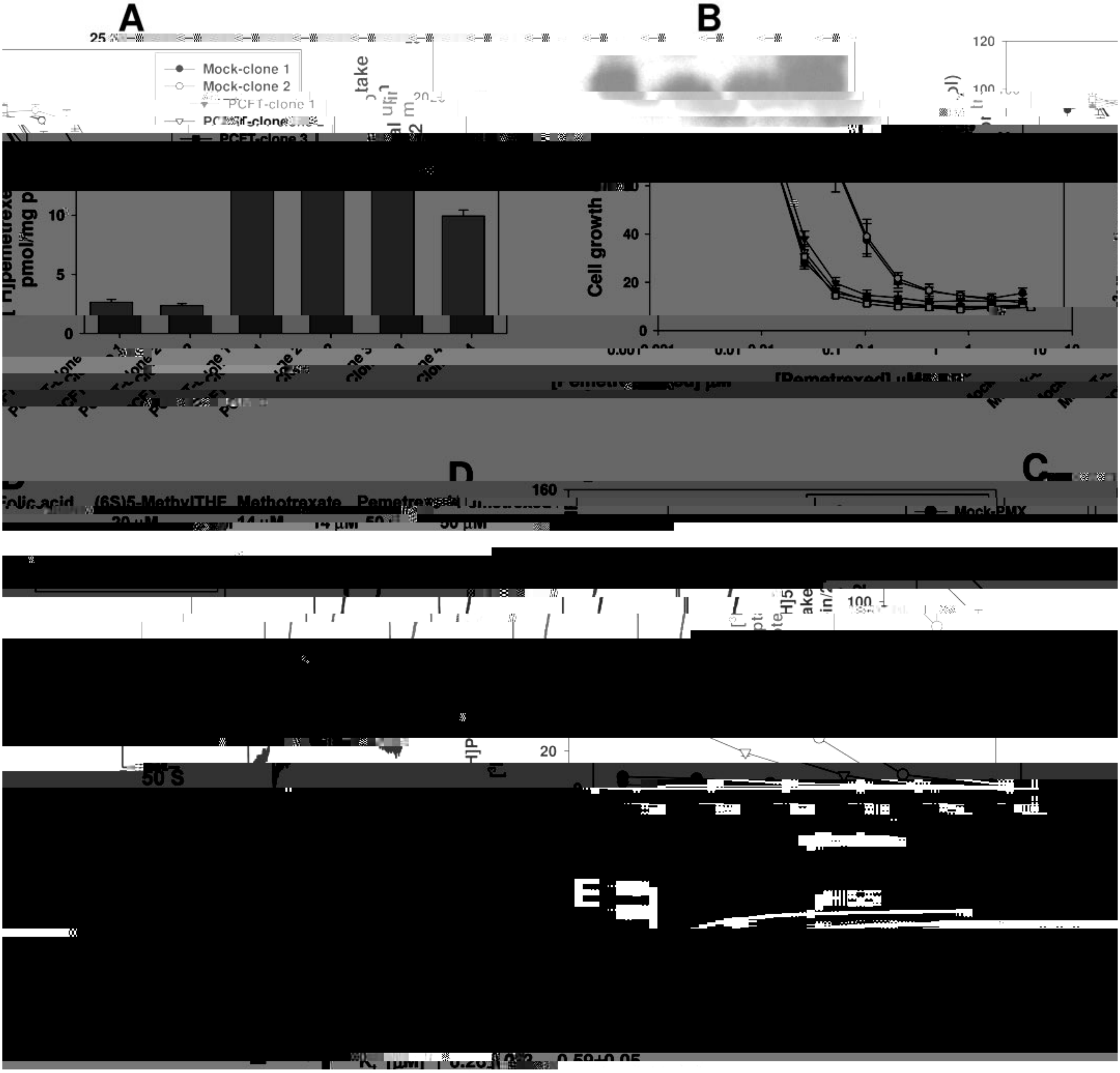

Fig. 5. A, 0.5 μ ³I - CF - I 2 I 7.0 2 . D .E. . , CF . B, - CF - I 2 . C 25 (6*R*,)5- I F. D .E. . . *C*, 0.5 μ ³I ³I (6)5- I F - CF -I 2 (CF - 2 - -1) I 2 . .E. . . D, 20 μ , 14 μ (6)5- I F, 50 μ , 7 μ , 20 25 *K* I 5.5 CF . E, CF I 5.5 6.5, *I*/*I* . *K* .E. .

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5.5 □ 1.2 ; P □ 0.33), (19 □ 2 16 □ 2
; P □ 0.42), 523 (3.6 □ 0.7 4.5 □ 0.4 ; P □
0.26) - CF - , ,
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(P □ 0.014) -
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(F . 4).

Discussion

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2 , K 0.26
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K . ,
7.4 K 13 μ -
K 130 μ ,
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FC -
C -15 ;
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632 (523) (., 2004 ; C -
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CF FC
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(., 1995; ., 2004).
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FC -
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3397(,)3334( )-3( )-3(7(8( )-3( )
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767(767 (-10.032 D153 637 09/ 1 11 1.271)0

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