Cation-Selective Transporters are Central to AMPK-Mediated Anti-proliferative Efficacy of Metformin in Endometrial Cancer Cells
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Purpose
Metformin is the first-line treatment for Type 2 diabetes mellitus, but it has also been shown to have anti-cancer effects against multiple types of cancer, including endometrial cancer (EC). Retrospective studies have shown that metformin treatment may improve overall survival and enhance recurrence free survival rates in diabetic EC patients compared with patients not taking the medicine. Metformin is believed to exert direct anti-neoplastic effects via activation of its intracellular target, adenosine monophosphate kinase (AMPK). Since metformin is positively charged (pKa 12.4) at all physiological conditions and hydrophilic (logD -6.13 at pH=7.0), it cannot passively traverse cellular membranes and needs cation-selective transporters to mediate its cellular uptake. Our laboratory has previously shown that the multidrug and toxin extrusion (MATE)1 and MATE2 transporters are the two predominant cation-selective transporters in the human EC cancer cell lines, ECC-1 and Ishikawa, followed by the plasma membrane monoamine transporter (PMAT). The goal of this study is to demonstrate the importance of MATE1 and 2 in metformin-mediated anti-proliferative activity and AMPK activation in EC cell lines, and to compare cation-selective transporter expression between EC cell lines and human EC tissue specimens.

Methods
ECC-1 and Ishikawa cells were maintained in RPMI 1640 and DMEM media supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic. Cells were treated with varying concentrations (0.1 mM-100 mM) of metformin for 72 hours, and cell viability was assessed by an MTT assay. The 50% inhibitory concentration (IC50) and 90% effective inhibition concentration (EC90) of metformin for ECC-1 and Ishikawa cells were calculated using GraphPad. Cellular uptake (5 min) of [14C] metformin at the IC50 concentrations was measured in both cell types in the presence or absence of pyrimethamine (50 μM and 500 μM), a selective inhibitor of MATE1 and 2. AMPK phosphorylation was evaluated by Western blot analysis following treatment with 1 mM metformin for 48 hours in the presence or absence of 50 μM pyrimethamine in culture media containing 1% FBS, using antibodies that are specific for p-AMPK and total AMPK. GAPDH was used as a loading control. Protein band intensities were quantified and compared by densitometric analysis. Fifty six human EC tissue samples were analyzed for MATE1, MATE2 and PMAT gene expression by real-time polymerase chain reaction (RT-PCR). Statistical significance was determined by a student’s t-test.

Results
Metformin exhibited dose-dependent anti-proliferative activity against ECC-1 and Ishikawa cell lines, with an IC50 of 8.6 mM and 5.8 mM, and EC90 values of 12.2 mM and 20.5 mM for ECC-1 and Ishikawa cells, respectively. Metformin (5.8 mM) uptake in Ishikawa cells was significantly inhibited by 500 μM pyrimethamine (4208.0 vs. 1684.4 pmol/mL/min, p<0.05). A trend towards an inhibition of metformin (8.6 mM) uptake in ECC-1 cells was observed in the presence of 500 μM pyrimethamine (3030.3 vs 1841.4 pmol/mL/min), although the inhibition was not statistically significant. Western blot and densitometric analyses revealed that 50 μM pyrimethamine significantly decreases metformin-mediated phosphorylation of AMPK, relative to total AMPK, with a 51% and 45% decrease in AMPK phosphorylation in ECC-1 and Ishikawa cells, respectively (p<0.05). RT-PCR results showed that approximately 30% of the EC specimens analyzed expressed one or more of the three cation-selective transporters (MATE1, MATE2 and PMAT) at varying levels, of which MATE1 was the predominant transporter.

Conclusion
The results show that MATE1 and MATE2 play a role in the uptake and anti-proliferative efficacy of metformin in EC cell lines, and in metformin-mediated AMPK activation. Our results showing that MATE1 is the predominant cation-selective transporter in human EC specimens suggest that MATE1 may play a role in metformin uptake in EC tissues in humans, and that the highly variable cation-selective transporter expression profiles in human EC could result in variable outcomes to metformin treatment for EC in the clinic. Preclinical and clinical studies need to be conducted to confirm the role of cation-selective transporters in the anti-neoplastic activity of metformin in EC.