Cation-Selective Transporters are Critical Determinants of the Anti-proliferative Effects of Metformin in Breast Cancer Cells
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Purpose
Antidiabetic agent, metformin exerts anticancer effects against several cancers, including breast cancer. Studies suggest that intracellular adenosine monophosphate-activated protein kinase (AMPK) mediates metformin anticancer effect. Because metformin is highly hydrophilic and positively charged at physiological pHs, it requires transporters for entry into cells. Therefore, we hypothesize that cation-selective transporters are central to metformin anti-proliferative effects in breast cancer cells. This hypothesis is tested by measuring anti-proliferative effect of metformin in genetically modified BT20 breast cancer cell line expressing organic cation transporter 3 (OCT3) (OCT3-BT20) as a representative cation-selective transporter, and comparing it with wild-type BT20 cells devoid of organic cation transporters.

Methods
BT20 human breast cancer cells expressing OCT3 were generated by electroporation. OCT3 expression was confirmed by determining gene and protein expression, and functional activity of OCT3 was confirmed by measuring metformin uptake in the presence/absence of OCT3-specific and pan cation transporter inhibitors. Metformin anti-proliferative effect was evaluated by Alamar blue assay, and IC50 values were determined.

Results
OCT3-BT20 cells showed >13-fold increase in metformin uptake at 5min compared to BT20 cells (108.38 vs 8.09 pmol/mg protein/min). OCT3 inhibition by famotidine and quinidine decreased uptake by 88% and 96%, respectively. Metformin-mediated inhibition of cell proliferation in OCT3-BT20 cells was significantly higher (p<0.001) compared to BT20 cells, with an IC50 of 1.91 mM in OCT3-BT20 cells vs 7.46 mM in BT20 cells.

Conclusion
These data support our hypothesis, and highlight the role of cation-selective transporters in cellular uptake and anti-proliferative effects of metformin in breast cancer cells. BT20 and OCT3-BT20 cells will be used to generate xenograft mice to evaluate the role of metformin transporters in the in vivo efficacy of metformin against breast cancer.