Biochemical and Biophysical Research Communications 414 (2011) 694–699

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Relevance of the OCT1 transporter to the antineoplastic effect of biguanides

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ARTICLE INFO

Article history:
Received 24 September 2011
Available online 2 October 2011

Keywords:
Cancer
Biguanides
Metformin
Phenformin
Organic cation transporter 1

ABSTRACT

Epidemiologic and laboratory data suggesting that metformin has antineoplastic activity have led to ongoing clinical trials. However, pharmacokinetic issues that may influence metformin activity have not been studied in detail. The organic cation transporter 1 (OCT1) is known to play an important role in cellular uptake of metformin in the liver. We show that siRNA knockdown of OCT1 reduced sensitivity of epithelial ovarian cancer cells to metformin, but interestingly not to another biguanide, phenformin, with respect to both activation of AMP kinase and inhibition of proliferation. We observed that there is heterogeneity between primary human tumors with respect to OCT1 expression. These results suggest that there may be settings where drug uptake limits direct action of metformin on neoplastic cells, raising the possibility that metformin may not be the optimal biguanide for clinical investigation.

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1. Introduction

Metformin is an orally active biguanide that lowers systemic glucose and insulin and is commonly used for the treatment of type II diabetes. Retrospective studies suggest that metformin may have a protective role against cancer, possibly by reducing elevated systemic insulin levels and/or by directly inhibiting cellular proliferation via AMPK pathway activation within neoplastic cells [1]. Studies have demonstrated induction of apoptosis by metformin in pancreatic [2], prostate, and colon cancer [3]. Several publications reported a potential therapeutic effect of metformin in epithelial ovarian cancer (EOC) [4–6]. We previously demonstrated that metformin enhances ovarian cancer cell cytotoxicity in a dose- and time-dependent manner, an effect potentiated by cisplatin [5]. Metformin induces apoptosis through the modulation of the Bcl-2 family of proteins in some experimental systems [7], but other mechanisms including AMPK-dependent inhibition of mTOR may also play a role [8,9].

Phenformin is another biguanide with anti-diabetic activity [10]. It was withdrawn from the market in the late 1970s due to a small risk of lactic acidosis in patients treated for diabetes [11]. Phenformin has been shown to have anti-neoplastic activity, including p21 cell-cycle inhibition leading to apoptosis. Moreover, phenformin was shown to reduce tumor growth in several animal models [12–16]. While risk/benefit considerations clearly favor use of metformin over phenformin for treatment of diabetes, the risk of phenformin-associated lactic acidosis is low enough that this agent certainly would not be contraindicated for cancer treatment if it had demonstrated superior antineoplastic activity.

The organic cation transporter 1 (OCT1) is responsible for organic cation uptake into hepatic cells via facilitated diffusion as well as active transport [17]. It is well known that OCT1 is highly expressed particularly in hepatic cells, where many drugs such as metformin and phenformin act [17,18]. OCT1 has previously been reported to have several polymorphisms that can influence the cellular uptake of metformin [19]. The most common mutation, OCT1-420del, occurs with an allelic frequency of 16% [20] and was found to be present in 20% of Caucasian Americans displaying a reduced response to metformin [19]. While germ-line OCT1 polymorphisms may have modest but detectable effects on metformin efficacy in diabetes, we conducted *in vitro* studies to investigate whether differences in OCT1 expression amongst neoplastic tissues may be a more important consideration for potential applications in oncology.

2. Materials and methods

2.1. Cell culture

The ovarian cancer cell lines OVCAR-3 and SKOV-3 (American Tissue Culture Collection, Manassas, VA, USA) were grown in RPMI-1640 medium (Wisent Bioproducts, Saint-Bruno, QC, Canada) supplemented with 10% fetal bovine serum (FBS), 2 mM

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doi:10.1016/j.bbrc.2011.09.134

glutamine, and 10 μg/ml gentamicin. Each cell line was passaged every 5–7 days and maintained at 37 °C in a 5% CO_2, 95% air atmosphere incubator.

2.2. Chemicals and antibodies

Metformin (catalog# D150959), Phenformin (catalog# P7045) and Anti-OCT1 (catalog# AV41516) were purchased from Sigma–Aldrich (Oakville, ON, Canada) OCT1 siRNA (sc-42552) was obtained from Santa Cruz Biotechnologies (Santa Cruz, CA, USA). Anti-phospho-AMPK (Thr^{172}), and anti-β-actin antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA).

2.3. Cytotoxicity assays

Cells were treated separately with increasing doses of metformin and phenformin in the presence or absence of OCT1 siRNA, and then incubated for 72 h. AlamarBlue colorimetric assay was performed in medium containing 1% FBS in triplicates as described earlier [21].

2.4. siRNA transfection

Cells were seeded in 6- and 96-well flat-bottom cell culture plates (Corning Incorporated, NY, USA). Lipofectamine for siRNA treatment was obtained from Invitrogen (Burlington, Ontario, Canada). Lipofectamine (1:1) was mixed with negative siRNA and OCT1 siRNA separately in RPMI-1640 with no FBS. Following 30 min of incubation at room temperature, both negative and OCT1 siRNA were added to their respective wells. The cells were incubated at 37 °C for 5 h and medium was changed containing increasing doses of phenformin and metformin in 1% FBS, respectively.

2.5. Protein assay

Total protein content was determined by the Lowry method [22] using a colorimetric assay (Bio-Rad, Mississauga, ON, Canada).

2.6. Protein extraction and Western blot analysis

Cells were lysed in radioimmunoprecipitation assay (RIPA) buffer (25 mM Tris·HCl pH 7.6, 150 mM NaCl, 1% NP-40, 0.25% sodium deoxycholate, 0.1% SDS) supplemented with protease inhibitor cocktail tablets (Roche Diagnostics, Mannheim, Germany). Protein lysates (110 μg) were resolved electrophoretically on 10% denaturing SDS-polyacrylamide gels and transferred to nitrocellulose membranes. Membranes were blocked in 5% milk and probed with antibodies specific for anti-OCT1 anti-phospho-AMPK (Thr^{172}) and anti-β-actin. Following incubation with horseradish peroxidise (HRP)-conjugated secondary antibodies, immunoblotted proteins were visualized by enhanced chemoluminescence (ECL). Relative levels of negative and positive siRNA for OCT1 were quantified by scanning densitometry and normalized by β-actin. The level of positive siRNA for OCT1 was significantly different from the level under control conditions $P < 0.05$.

2.7. Tissue microarray

TMA was kindly provided by the Terry Fox Research Institute and contains 105 patients with serous papillary epithelial ovarian

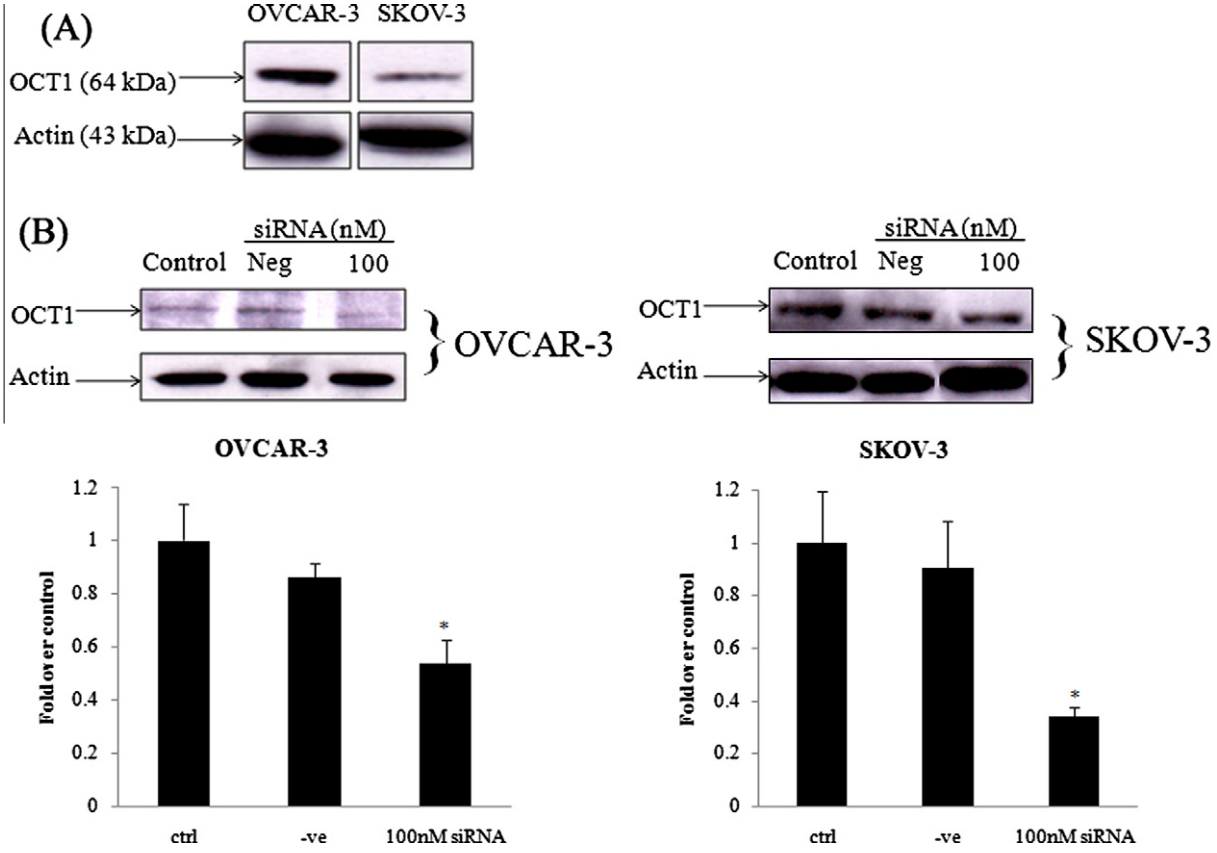

Fig. 1. OCT1 expression in human EOC cell lines. (A) Cell lysates from OVCAR-3 and SKOV-3 cell lines were subjected to western blot for OCT1 and actin. One representative experiment out of three is shown. (B) Inhibition of OCT1 protein expression with increasing doses of siRNA in OVCAR-3 and SKOV-3 cell lines. Cells were incubated with nonspecific and OCT1 siRNA for 72 h in RPMI without FBS. Densitometric analysis is shown. *$P < 0.05$ when compared to positive and negative controls.

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