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Research Article

Metabolic changes during ovarian cancer progression as targets for sphingosine treatment

Angela S. Anderson[a], *Paul C. Roberts*[b], *Madlyn I. Frisard*[a], *Ryan P. McMillan*[a], *Timothy J. Brown*[a], *Michael H. Lawless*[a], *Matthew W. Hulver*[a,*], *Eva M. Schmelz*[a,**]

[a]*Department of Human Nutrition, Foods and Exercise, Virginia Tech, Blacksburg, VA 24060, United States*
[b]*Biomedical Science and Pathobiology, Virginia Tech, Blacksburg, VA, United States*

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ABSTRACT

Tumor cells often exhibit an altered metabolic phenotype. However, it is unclear as to when this switch takes place in ovarian cancer, and the potential for these changes to serve as therapeutic targets in clinical prevention and intervention trials. We used our recently developed and characterized mouse ovarian surface epithelial (MOSE) cancer progression model to study metabolic changes in distinct disease stages. As ovarian cancer progresses, complete oxidation of glucose and fatty acids were significantly decreased, concurrent with increases in lactate excretion and ^{3}H-deoxyglucose uptake by the late-stage cancer cells, shifting the cells towards a more glycolytic phenotype. These changes were accompanied by decreases in TCA flux but an increase in citrate synthase activity, providing substrates for *de novo* fatty acid and cholesterol synthesis. Also, uncoupled maximal respiration rates in mitochondria decreased as cancer progressed. Treatment of the MOSE cells with 1.5 μM sphingosine, a bioactive sphingolipid metabolite, decreased citrate synthase activity, increased TCA flux, decreased cholesterol synthesis and glycolysis. Together, our data confirm metabolic changes during ovarian cancer progression, indicate a stage specificity of these changes, and suggest that multiple events in cellular metabolism are targeted by exogenous sphingosine which may be critical for future prevention trials.

Introduction

Ovarian cancer is the leading cause of gynecological cancer deaths and the fifth leading cause of cancer deaths in women. While the origins of ovarian cancer are still debated, most ovarian cancers are thought to originate in the layer of epithelial cells surrounding the ovary or the fimbriae of the fallopian tubes. While early diagnosis leads to a 92% rate of survival [1], the cancer is most commonly diagnosed when it has progressed to stage III or IV, drastically reducing the chance of survival. Understanding the etiology of ovarian cancer and identifying early events in ovarian cancer for diagnostic and prevention purposes are critical to increase the survival rates of women afflicted with this disease.

The observation that cancer cells have an altered metabolic phenotype, allowing them to adapt to and survive dynamic

Abbreviations: So, sphingosine; TCA, tricarboxylic acid cycle; FASN, fatty acid synthase; β-HAD, β-hydroxyl-CoA dehydrogenase; PDH, pyruvate dehydrogenase; OCR, oxygen consumption rate; ECAR, extracellular acidification rate.

*Corresponding author
**Corresponding author. Fax: +001 540 231 5522.
E-mail addresses: eschmelz@vt.edu (E.M. Schmelz), hulvermw@vt.edu (M.W. Hulver).

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microenvironmental conditions, was first made by Otto Warburg and has been subsequently been labeled the "Warburg Effect" [2,3]. Warburg observed a higher uptake of glucose and elevated lactate production and secretion, and concluded that cancer cells primarily use aerobic glycolysis rather than oxidative phosphorylation as the primary source for ATP synthesis, due to impaired mitochondria. Although this glycolytic shift takes place under normoxic conditions, anaerobic metabolism is also critical for tumor development where the hypoxic core of growing tumors limits access to oxygen. To avoid acid-induced apoptosis, monocarboxylate transporters must export excess lactic acid [4,5]. The resulting acidic microenvironment generated by the secreted lactate, carbon dioxide production, bicarbonate depletion and other mechanisms has been shown to promote progression and metastasis [6,7].

Since Warburg's first communication of the glycolytic nature of cancer cell metabolism, there has been a debate as to whether this hypothesis encompasses all changes pertaining to cancer cell metabolism. It is now clear that mitochondrial dysfunction is not apparent in all cancer cells [8], and that the aberrant metabolic phenotype is the consequence of mutations or epigenetic silencing modulating the expression of metabolic enzymes or their regulators such as *p53*, *MYC*, *AMP-activated kinase* (*AMPK*) or *Hypoxia-inducible factor 1 alpha* (*HIF1α*) [9] rather than their cause. The glycolytic shift now is understood as a survival mechanism to produce the macromolecules and reducing equivalents required to meet the cancer cells need of proteins, nucleic acids and lipids to support rapid cell growth. This confers a distinct growth advantage even in normoxic conditions with functional mitochondria [10]. Since these glycolytic cells mostly have a more aggressive and metastatic phenotype [11], it has been suggested that aerobic glycolysis is a hallmark for invasive cancers [12]. Therefore, chemopreventive and chemotherapeutic strategies that modify these metabolic pathways could successfully remove the cancer cells' growth advantage and prevent cancer growth.

Sphingolipids are a class of structurally diverse bioactive lipids involved in a variety of cellular processes including the regulation of growth, apoptosis, autophagy, motility and many more in a metabolite-specific manner [13,14]. Sphingolipid metabolites are also involved in cellular metabolism, inhibiting (sphingosine, So) or activating (sphingosine-1-posphate, S1P) glucose uptake, inhibiting (So) or activating (S1P) insulin signaling pathways and mediating AKT (protein kinase B) inhibition (So) or activation (S1P). The endogenous sphingolipid metabolites S1P and ceramide have been implicated in reduced pancreatic -cell function, insulin resistance, atherosclerotic plaque formation, and other factors associated with obesity and metabolic syndrome (see recent comprehensive review [15]). In contrast, our previous studies have shown that the administration of complex sphingolipids via the diet suppressed carcinogen-induced [16–19], mutant *Adenomatous Polyposis Coli*-mediated [20,21] or inflammation-driven colon cancer [22], and suppressed breast xenograft progression [23] in mice without any apparent side effects. The sphingolipid bases that are generated from the dietary complex sphingolipids are likely the effective metabolites in the observed suppression of tumor growth and progression [20,24]. The effects of exogenous sphingolipid metabolites on the altered metabolism in ovarian cancer cells and their impact on tumor growth and progression are not known.

We have recently developed and characterized a mouse ovarian surface epithelial (MOSE) cell model of progressive ovarian cancer [25,26] to identify and delineate events involved in ovarian cancer progression. Serial passaging of primary MOSE cells induced spontaneous immortalization and transformation and allowed for the capturing of genetically and phenotypically distinct early (benign), intermediate and late (highly aggressive and invasive) stages of ovarian cancer. This model is unique in its ability to compare syngeneic stages of cancer progression, a model that does not exist for the human disease. As cells progress, they increase their growth rate, acquire the ability to grow as spheroids, invade collagen and form tumors *in vivo* [25]. The progression of MOSE cells is accompanied by changes in their gene expression levels and a successive dysregulation of their cellular architecture also reported in the human disease [26]. In the present studies, this ovarian cancer model was used to determine when during cancer progression metabolic changes are taking place and identify potential targets for exogenous So in the prevention of ovarian cancer. We investigated shifts in cellular metabolism and substrate utilization during ovarian cancer cell's progression, determined changes in mitochondrial function, and investigated how exogenous So affects the observed changes. Together, our results suggest that alterations in the metabolic phenotype of the progressive MOSE cells are targets for a cancer prevention regimen with exogenous So.

Methods and materials

Cell culture.

MOSE cells were cultured in DMEM (Sigma) supplemented with 4% FBS (Atlanta Biologicals) and 100 ug/ml each of penicillin and streptomycin (Gibco). Classification into early-benign (MOSE-E), intermediate (MOSE-I), and late-aggressive (MOSE-L) phenotypes was established as previously described [25]. So-treated cells were cultured in medium containing 1.5 μmol/L So (Avanti Lipids) as a BSA complex (60 μM fatty acid-free fraction V, Calbiochem) for at least three passages to mimic long-term exposure to diet-derived sphingolipid metabolites. This treatment regimen has been shown to be necessary to induce significant changes in gene and protein expression level and cytoskeleton organization (data not shown). This concentration was not toxic for any cell line. Cells were cultured at 37 °C in a humidified incubator with 5% CO_2.

Real-time PCR (qPCR).

Cells were seeded in 100 mm culture dishes and harvested 24 h later. Total RNA was extracted using the RNeasy Mini Kit (Qiagen). cDNA synthesis was performed on 500 ng of RNA using the ImProm-II Reverse Transcription System (Promega). qPCR was performed using 50 ng of cDNA with SensiMix Plus SYBR Mastermix (Quantace) in the ABI 7900HT (Applied Biosystems) with the following parameters: 42 cycles at 95 °C for 10 min, 95 °C for 15 s, 58 °C for 30 s and 72 °C for 15 s, followed by a dissociation curve segment. Data was quantified using the ΔΔCT method and expressed relative to RPL19 as the housekeeping gene [26]. Primer pairs were designed with the Beacon Design software and were as followed: pyruvate dehydrogenase (PDHb) Fwd: CAT TCG GCA