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Galectin-3 inhibition suppresses drug resistance, motility, invasion and angiogenic potential in ovarian cancer

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HIGHLIGHTS

- We blocked Galectin-3 in ovarian cancer, by using Galectin-3C, a dominant-negative inhibitor of Galectin-3.
- Galectin-3C, alone or with Paclitaxel, reduces ovarian cancer growth and drug resistance and interferes with angiogenesis.
- We provide evidence of the relevance of Galectin-3 in ovarian cancer and the activity of Galectin-3C in this disease.

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ABSTRACT

Objective. Ovarian cancer is the most deadly gynecologic malignancy worldwide. Since the pathogenesis of ovarian cancer is incompletely understood, and there are no available screening techniques for early detection, most patients are diagnosed with advanced, incurable disease. In an effort to develop innovative and effective therapies for ovarian cancer, we tested the effectiveness of Galecti-3C *in vitro*. This is a truncated, dominant negative form of Galectin-3, which is thought to act by blocking endogenous Galectin-3.

Methods. We produced a truncated, dominant-negative form of Galectin-3, namely Galetic-3C. Ovarian cancer cell lines and primary cells from ovarian cancer patients were treated with Galectin-3C, and growth, drug sensitivity, and angiogenesis were tested.

Result. We show, for the first time, that Galectin-3C significantly reduces the growth, motility, invasion, and angiogenic potential of cultured OC cell lines and primary cells established from OC patients.

Conclusions. Our findings indicate that Galectin-3C is a promising new compound for the treatment of ovarian cancer.

Introduction

Ovarian cancer (OC) is the leading cause of mortality from gynecologic malignancies in the United States, with more than 15,000 deaths expected in 2012 [1]. Despite extensive research in the field, the origin and molecular pathogenesis of OC are still poorly understood. This has significantly limited the development of new, OC-tailored therapeutic interventions for this disease [2]. Due to the lack of effective screening, over 70% of OC patients present with advanced stage disease, and more than 60% ultimately succumb to progressive disease. The recommended primary treatment for OC is based on stage and involves a combination of cytoreductive surgery followed by platinum and taxane-based combination chemotherapy. More recently, the addition of bevacizumab, an anti-angiogenesis agent, to chemotherapy has been reported to improve patient progression-free survival. Despite the observed improvement in survival rates with the use of adjuvant chemotherapy following surgery, the vast majority of patients with advance OC will ultimately recur and die of disease progression [3,4]. Moreover, the development of drug resistance and cumulative toxicity from cytotoxic therapies further limit the efficacy of the currently available treatment options for progressive OC [5]. It is for these reasons that novel therapeutic interventions are desperately needed to improve patient outcomes.

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The development of drug resistance is tightly associated to the acquisition of an invasive and motile phenotype during tumor progression [6,7] that results in tumor spread, induction of angiogenesis and poor prognosis [8,9]. Tumor-induced angiogenesis is one of the main causes of increased tumor burden in OC [10–12]. Therefore, targeting angiogenesis represents an attractive way of interfering with OC progression and several studies testing this strategy in the clinical setting have produced promising results.

Galectins are S-type lectins which bind β-galactose-containing glycoconjugates and have been implicated in the regulation of key cellular processes, including cell adhesion, growth, neoplastic transformation and apoptosis [13]. Since the discovery of the first galectin in animal cells in 1975, fifteen mammalian galectins have been isolated and accumulating evidence suggests a role for certain members of the galectin family in the pathogenesis of OC [13,14]. Galectin-3 (Gal-3) is unique in that it displays both a carboxyl-terminal carbohydrate recognition domain (CR), which binds to β-galactosides, and the N-terminal domain, critical for Gal-3 multivalent behavior and cross-linking activity [23]. Gal-3 is of special interest since it is involved in many features of tumor progression, such as cellular adhesion, proliferation, motility and metastasis [15,16]. Intracellularly, Galectin-3 functions through activation of the Akt/NF-kB axis [17–19]. Although Galectin-3 can be internalized in the cytoplasm of tumoral and normal cells, its most relevant functions affect the interaction of tumor cells with the microenvironment. Galectin-3 functions as an extracellular protein are better elucidated, and it is due to its ability to cross-link and cluster integrins and to stabilize focal adhesions [23,16,19].

These observations support the importance of Gal-3 as a potential therapeutic target in OC.

We have previously shown that Gal-3C reduced chemotaxis and angiogenesis, as well as enhanced the effects of bortezomib, in multiple myeloma [21]. In this study, we examined the effects of Gal-3 inhibition in OC cell lines and primary cells derived from OC patients, by using Galectin-3C (Gal-3C), a truncated, dominant-negative inhibitor of Gal-3. Gal-3C consists of the last 143 carboxyl-terminal amino acid residues of human Gal-3 and lacks its N-terminal domain, in this way preserving the molecule's carbohydrate binding ability, while blocking its cooperative binding properties and molecular cross-linking ability. Thus, Gal-3C functions as a dominant negative inhibitor of Galectin-3 [20,21].

In this study we show that Gal-3C, alone or in combination with paclitaxel, reduces growth, invasion, migration, and drug resistance of OC cells *in vitro*, and interferes with OC cell angiogenic potential. Our results provide evidence that Gal-3C can effectively block Gal-3-mediated actions in OC cells, supporting the role of Gal-3 as a promising therapeutic target in this disease.

Materials and methods

Reagents and drugs

Paclitaxel was purchased from Ben Venue Labs (Bedford, OH, USA). Gal-3C was prepared as previously described [21]. Briefly, truncated recombinant Galectin-3 (N-terminal 6xHis-tagged) was produced in *Escherichia coli* DH5α, containing the pQ30 plasmid, then purified by the Ni-NTA Fast Start Kit (QIAGEN). Purified Gal-3C was analyzed by SDS-PAGE (purity was about 98%, and was assayed to be endotoxin-free, data not shown). In all the experiments, control cells were cells exposed to equal volumes of Paclitaxel and Gal-3C vehicles (PBS and purified water, respectively).

Cell cultures

The OC cell lines used in this study were SKOV-3 and ID8 (American Type Culture Collection, Manassas, VA, USA), while primary OC cells from patients (Pt1 and Pt2) were provided by Professor Martin J Cannon (University of Arkansas for Medical Sciences, Little Rock, AR, USA).

All OC cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS, Thermo Scientific, Rockford, IL, USA) in 5% CO_2 atmosphere at 37 °C. Human Umbilical Vein Endothelial Cells (HUVEC, American Type Culture Collection) were maintained in EGM-2 medium supplemented with endothelial cells growth factors (Lonza, Houston, TX, USA) and were used within 10 passages.

Flow cytometry analysis

The expression of Galectin-3 was analyzed by flow cytometry. Briefly, OC single-cell suspensions were distributed into 12 × 75 mm flow cytometry tubes (1×10^5 cells/tube). Cells were incubated with 1 μg/mL mouse monoclonal anti-human/mouse Galectin-3 IgG1 (LifeSpan Biosciences, Inc., Seattle, WA, USA, clone B2C10) in 20 μL PBS (pH = 7.4) for 1 h on ice. 1 μg/mL mouse IgG1 was used as isotype matched control (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Then, cells were washed three times with ice-cold PBS [0.2 mL], and incubated with 0.2 μg/mL FITC-conjugated rat anti-mouse Ig (BD Biosciences, San Jose, CA, USA) in 20 μL PBS for 1 h on ice in the dark. Cells were analyzed with a FACScan flow-cytometer (BD Biosciences) after washing two times with 0.3 mL ice-cold PBS.

Viability assay

Cell proliferation was assessed with a ViaLight Plus Cell Proliferation and Cytotoxicity BioAssay Kit (Lonza, Walkersville, MD, USA) according to the directions of the manufacturer. In brief, OC cells were seeded in 100 μL RPMI-1640 with 10% heat-inactivated FBS in 96-well plates (8×10^3/well). Each drug/drug combination was tested in triplicate. Luminescence was measured with a 1-s integrated setting in a Berthold luminometer.

Apoptosis assay

Apoptotic rates were measured by the use of the Apoptosis Detection Kit (based on Annexin-V staining, BD Biosciences) and flow-cytometry analysis, following the manufacturer's instructions.

Migration assays

4×10^5 cells were plated in 100 μL serum-free culture medium (supplemented with drugs or vehicle) in the top chambers of 24-well Transwell™ polycarbonate inserts (Corning Costar, NY, USA) with 8-μm pores. Complete culture medium (600 μL) was added to the bottom chamber. After incubation for 4 h, cells on lower side of the filter were fixed, stained and counted as described [21]. For HUVEC, the assay was run using 5×10^4 cells/well, and the lower chamber was filled with 600 μL EGM-2 medium supplemented with 20% *V/V* conditioned medium from OC cells (obtained after treating of OC cells with 10 μg/mL Gal-3C, 8 nM paclitaxel, or combined drugs). The experiments were run in triplicate and the results are expressed as the mean number of migrated cells in the presence of different stimuli.

Invasion assay

OC invasion potential was measured as described [21], using Transwell™ polycarbonate inserts (5-μm pore size).

Tubule formation assay

A capillary tubule formation assay was performed as previously described [21]. Briefly, growth factor-reduced Matrigel™ (50 μL/well; Becton Dickinson) was added with 30 ng/mL recombinant basic fibroblastic growth factor (bFGF; R&D Systems) and incubated for 1 h at