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Resveratrol inhibits ovarian cancer cell adhesion to peritoneal mesothelium in vitro by modulating the production of α5β1 integrins and hyaluronic acid

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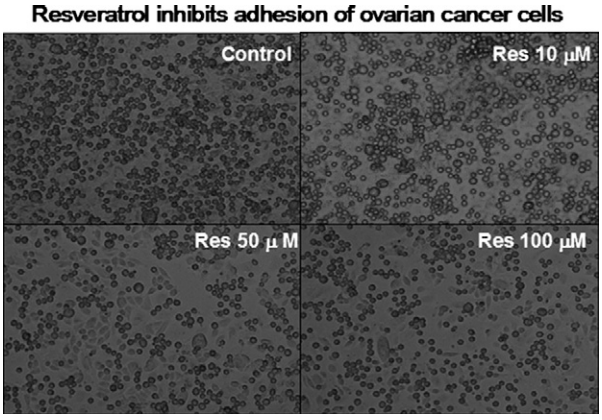
HIGHLIGHTS

- Resveratrol (Res) inhibits ovarian cancer cell adhesion to peritoneal mesothelial cells (HPMCs).
- Res-dependent decrease in cancer cell adhesion is related to decreased α5β1 integrin production and increased hyaluronic acid release.

GRAPHICAL ABSTRACT



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ABSTRACT

Objective. Resveratrol (Res) is known to inhibit adhesion of numerous malignancies though its effect on an adherence of ovarian cancer cells to peritoneal mesothelium remains undefined.

Methods. To address this issue, ovarian cancer cells (A2780, OVCAR-3, SKOV-3) were subjected to Res (10, 50, 100 μM), and then their adhesion to omentum-derived human peritoneal mesothelial cells (HPMCs) was assayed.

Results. The study showed that Res inhibits adhesion of all ovarian cancer cell lines investigated. More importantly, this effect was evident either when cancer cells were directly treated with Res (cell-dependent activity) or when intact cancer cells were pretreated with conditioned medium (CM) generated by their counterparts subjected to Res (medium-dependent activity). Cell-dependent activity of Res has been recognized to be linked with decreased level of cellular α5β1 integrins which decreased functionality corresponds with reduced efficiency of cancer cell adhesion. Medium-related effects have been, in turn, associated with up-regulated secretion of soluble HA to environment (CM). The experiments with exogenous HA revealed the inverse relation between HA concentration in CM and cancer cell adhesion. When the CM from cells subjected with Res (with elevated HA) was supplemented with hyaluronidase, the restoration of cell adhesive capabilities occurred.

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Conclusions. Our studies evidenced that Res affects ovarian cancer cell adhesion to HPMCs by decreasing cellular α5β1 integrin level and by increasing the secretion of HA to environment.

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Introduction

Peritoneal cavity is the preferential place for ovarian cancer metastasis. In the 3rd and 4th stages of the disease, malignant cells disseminate by ovarian surface shedding, then are carried with peritoneal fluid, to finally sediment on the surfaces of the peritoneal cavity, especially the omentum [1]. It is believed that the effective colonization of the peritoneum by cancer cells largely depends on the success of the very initial stage of metastasis, that is the solid attachment to the peritoneal mesothelium [2]. The body of evidence suggests that the reciprocal interplay between ligand–receptor pairs, in particular α5β1 integrins—fibronectin (FN), αVβ3 integrins—vitronectin (VN), and CD44—hyaluronic acid (HA) present on the surface of both cancer and normal cells, plays the prominent role in this process [2–5].

Resveratrol (3,4′,5-trihydroxy-*trans*-stilbene; Res) is a natural phytoestrogen widely acknowledged for its anti-cancer activity [6]. It has been found that Res is capable of restricting the progression of numerous malignancies, including lymphoid and myeloid cancers, melanoma, breast, cervical, colon, gastric, head and neck, liver, lung, pancreas, prostate, and thyroid tumors. The anti-cancer effects attributed to the activity of Res are related to an interference of this compound with various cellular processes that are crucial for cancer cell expansion, such as proliferation, differentiation, apoptosis, inflammation, angiogenesis, and redox homeostasis. This, in turn, encompasses a suppression by Res of several signaling pathways, of which AP-1, COX-2, Egr-1, MAPK, NF-κB, and PKC seem to be the most important (see [7] for extensive review).

In recent years, much attention has also been paid to the effect of Res of ovarian tumors. It has been found that this stilbene may effectively inhibit ovarian cancer cell proliferation [8] and invasion [9] as well as to induce their apoptotic [10] and autophagic death [11]. Moreover, it has been recognized to modulate cell signaling related to angiogenesis [12] and drug-resistance [13], and to sensitize ovarian cancer cells to chemotherapeutic agents [14]. At the same time, the effect of this compound on the ovarian cancer cell adhesion to peritoneal mesothelium remains undefined. Hence, in order to fulfill this niche we designed the in vitro experiments in which three lines of ovarian cancer cells, A2780, OVCAR-3, and SKOV-3 were subjected to Res, and then their adhesion to the omentum-derived human peritoneal mesothelial cells (HPMCs) was assayed. To address this issue mechanistically, the expression of major mediators of ovarian cancer cell adhesion to HPMCs, including α5β1 and αVβ3 integrins, FN, VN, CD44, and HA upon exposure to Res was evaluated.

Methods

Chemicals

Unless otherwise stated, all reagents were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). The tissue culture plastics were from Nunc (Roskilde, Denmark). Approximately 99% pure RVT was obtained from Sigma-Aldrich Corp. A stock solution was prepared in dimethyl sulfoxide (DMSO) and diluted in a culture medium to desired final concentration. The concentration of DMSO was always 0.05% (v/v). During the experiments, the ovarian cancer cells were exposed to medium supplemented with Res (10, 50 and 100 μM) for three days. A chemical stability of Res in culture media within three-day period was confirmed using high performance liquid chromatography (HPLC), as described in [15].

Cell cultures

The ovarian cancer cell lines A2780 and SKOV-3 were obtained from the European Collection of Cell Cultures (Porton Down, UK) and propagated in McCoy's 5a and RPMI 1640 media, respectively, both supplemented with L-glutamine (2 mmol/l), penicillin (100 U/ml), streptomycin (100 g/ml), and 10% fetal bovine serum (FBS). The ovarian cancer cell line OVCAR-3 was purchased from the American Type Culture Collection (Rockville, MD) and maintained in RPMI 1640 medium supplemented with L-glutamine (2 mmol/l), HEPES (10 mmol/l), sodium pyruvate (1 mmol/l), glucose (4500 mg/l), and 10% FBS.

Human peritoneal mesothelial cells (HPMCs) were isolated from the pieces of omentum, as described elsewhere [16]. The tissues were obtained from patients undergoing abdominal surgery. The study was approved by the institutional ethics committee. The cells were cultured in medium M199 enriched in L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 μg/ml), hydrocortisone (0.4 μg/ml), and 10% FBS. The experiments were performed on HPMCs from the 2nd and 3rd passages.

Cell adhesion assay

Adhesion of ovarian cancer cells to HPMCs was measured as described by Alkhamesi et al. [17] with minor modifications. In brief, HPMCs were seeded in 96-well plates (1×10^5 cells/well) and left to settle overnight. Ovarian cancer cells were trypsinized, washed with phosphate buffered saline, and probed with 5 μM calcein-AM (Molecular Probes, Eugene, OR, USA) for 20 min at 37 °C. The labeled cells were washed with M199 medium to remove the free dye and added (3×10^4 cells/well) on top of mesothelial cells. After 60 min of incubation at 37 °C, total fluorescence was recorded in a spectrofluorimeter using 485 nm and 535 nm wavelengths for excitation and emission, respectively. Then, the non-adherent cells were removed by washing and the measurement of fluorescence was repeated. Recorded values were compared to total fluorescence to calculate the percent of cells bound.

In some experiments, the efficiency of cancer cell adhesion to HPMCs was evaluated after cell or cell-derived conditioned medium pre-incubation with exogenous HA (R&D Systems Inc., Minneapolis, MN, USA), hyaluronidase type 1 (Sigma) (80 U/ml [2]), or exogenous recombinant CD44 (5–20 μg/ml [18]) (Abcam, Cambridge, UK), for 6 h at 37 °C with shaking.

Immunoblotting

Cancer cells were lysed in a buffer containing 20 mM Tris–HCl at pH 7.3, 200 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1 mM PMSF, and protease inhibitor cocktail (Sigma), and homogenized by sonication. Samples corresponding to 40 μg protein were subjected to SDS–PAGE. The proteins were transferred to Immobilon-P polyvinylidene difluoride membranes (Sigma) and immunoblotted with specific antibodies for α5β1 (Biorbyt, Cambridge, UK) and αVβ3 integrins (Novus Biologicals, Littleton, CO, USA), fibronectin, vitronectin, CD44, GAPDH (all from Millipore) and hyaluronic acid (Cloud-Clone Corp, Houston, TX, USA). Bound antibodies were visualized following incubation with the peroxidase-labeled secondary antibodies (Dako, Glostrup, Denmark) and the exposure to chemiluminescence reagent (ECL; Amersham Pharmacia Biotech, Castle Hill, Australia).

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