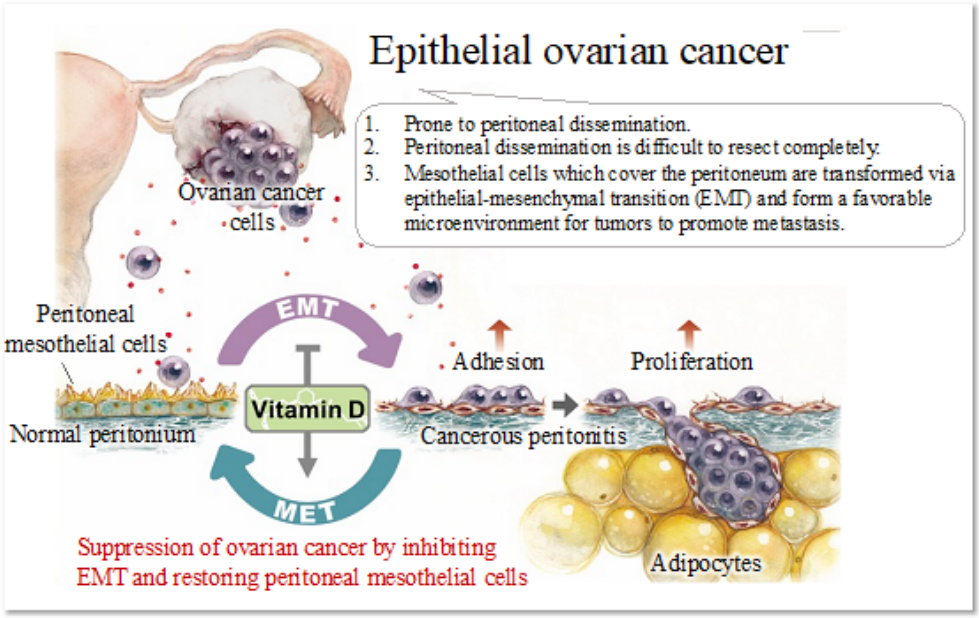

Title

Peritoneal restoration by repurposing vitamin D inhibits ovarian cancer dissemination via blockade of the TGF-β1/thrombospondin-1 axis

Key points

- Ovarian cancer is associated with the highest mortality rate, as most patients are diagnosed at an advanced stage with peritoneal dissemination.
- During the peritoneal dissemination, mesothelial cells (MCs) are transformed to a mesenchymal state via a process called epithelial-mesenchymal transition (EMT) induced by TGF-β1 secreted from cancer cells.
- Mesenchymal-changed MCs promote ovarian cancer cell adhesion, proliferation and invasion by secreting various chemokine or extracellular matrix (ECM).
- Vitamin D inhibits EMT in MCs, furthermore, vitamin D induces MET and normalized ECM expression in fixed mesenchymal MCs, thereby preventing peritoneal dissemination.
- These findings suggest that restoration of the peritoneal environment can be used for preventing OvCa peritoneal dissemination

Summary

Ovarian cancer (OvCa), a lethal gynecological malignancy, disseminates to the peritoneum. Mesothelial cells (MCs) act as barriers in the abdominal cavity, preventing the adhesion of cancer cells. However, in patients with OvCa, they are transformed into

cancer-associated mesothelial cells (CAMs) via mesenchymal transition and form a favorable microenvironment for tumors to promote metastasis. However, attempts for restoring CAMs to their original state have been limited. Here, we investigated whether inhibition of mesenchymal transition and restoration of MCs by vitamin D suppressed the OvCa dissemination *in vitro* and *in vivo*. The effect of vitamin D on the mutual association of MCs and OvCa cells was evaluated using *in vitro* coculture models and *in vivo* using a xenograft model. Vitamin D restored the CAMs, and thrombospondin-1 (component of the extracellular matrix that is clinically associated with poor prognosis and is highly expressed in peritoneally metastasized OvCa) was found to promote OvCa cell adhesion and proliferation. Mechanistically, TGF-β1 secreted from OvCa cells enhanced thrombospondin-1 expression in CAMs via Smad-dependent TGF-β signaling. Vitamin D inhibited mesenchymal transition in MCs and suppressed thrombospondin-1 expression via vitamin D receptor/Smad3 competition, contributing to the marked reduction in peritoneal dissemination *in vivo*. Importantly, vitamin D restored CAMs from a stabilized mesenchymal state to the epithelial state and normalized thrombospondin-1 expression in preclinical models that mimic cancerous peritonitis in vivo. MCs are key players in OvCa dissemination and peritoneal restoration and normalization of thrombospondin-1 expression by vitamin D may be a novel strategy for preventing OvCa dissemination.

Background

Among gynecological cancers, ovarian cancer (OvCa) is associated with the highest mortality rate, as most patients are diagnosed at an advanced stage with peritoneal dissemination. OvCa is more likely to cause peritoneal dissemination and rarely metastasizes hematogenously, as the ovary is directly exposed to the abdominal cavity. Complete resection of peritoneal dissemination is difficult, as it forms numerous metastases in the peritoneum, omentum, mesentery, and diaphragm. Therefore, for achieving complete remission, physicians rely on chemotherapy, mainly using anticancer drugs (e.g., cisplatin and paclitaxel). However, even though recently developed novel agents such as molecular targeted drugs prolong progression-free survival, they do not prolong overall survival, and effective treatment for peritoneal dissemination of OvCa has not yet been established.

It is believed that controlling peritoneal dissemination may play an important role in improving the refractory nature of OvCa. However, the mechanism underlying dissemination and adhesion of metastasized cancer cells must be understood to control peritoneal dissemination. Soon after OvCa cells detach from the primary site, they are

carried by ascites to secondary implantation sites, including the peritoneal wall, diaphragm, mesentery, and omentum, which are the most common sites of OvCa metastasis. These sites are covered with mesothelial cells (MCs) in a monolayer, which protect internal organs from pathogens and cancer cells. Many studies, including ours, have suggested that MCs play cancer-promoting functions in the tumor microenvironment.

During the peritoneal dissemination, MCs are transformed to a mesenchymal state via a process called epithelial-mesenchymal transition (EMT) induced by TGF-β1 secreted by cancer cells. Thus, EMT-induced MCs are called cancer-associated mesothelial cells (CAMs), and are an important cellular component of the tumor microenvironment that facilitates OvCa progression.

We have shown that CAMs promote OvCa cell adhesion and proliferation by decreasing the expression of the miR200 family, enhancing angiogenesis by secreting VEGF, and migration and invasion of OvCa cells by secreting CCL2, IL-8, and CXCL5. In addition, CAMs contribute to resistance to platinum-based chemotherapies, key drugs used for treating OvCa, by increasing fibronectin secretion. Other groups have also reported that CAMs play a tumor-promoting role in gastric, pancreatic, and colorectal cancers, by modifying the normal stroma to tumor-promoting stroma.

Vitamin D is a hormone synthesized in the skin through exposure to ultraviolet (UV) radiation in sunlight and plays an important role in calcium and bone metabolism. Indeed, vitamin D has been used clinically for osteoporosis for many years and does not have any serious side effects. Recently, there have been many reports that vitamin D is effective against cancer. For example, vitamin D replacement therapy reduces cancer-related mortality. Functionally, vitamin D inhibits cell cycle, proliferation, and angiogenesis. Vitamin D also enhances the therapeutic effect of pancreatic cancer by reprogramming the stroma. Then, we hypothesized that restoration and reprogramming of cancer-associated MCs (CAMs) by administering vitamin D may be a new strategy for treating peritoneal dissemination that can simultaneously target cancer cells and tumor stroma, including MCs.

Research results

EMT was induced in MCs derived from omentum by TGF-β1 to transform them into CAMs. CAMs lost their microvilli of cell surface that MCs possess and showed enhanced adhesion to OvCa cells. Furthermore, when CAMs and OvCa cells were co-cultured, proliferation of OvCa cells was enhanced. On the other hand, MCs in which EMT was suppressed by vitamin D maintained their microvilli and suppressed cancer cell adhesion

and proliferation. Transcriptome analysis (RNA sequencing analysis) of MCs revealed that thrombospondin-1, an extracellular matrix involved in cell adhesion and proliferation, is upregulated during EMT, whereas it is downregulated by vitamin D.

Next, we confirmed the involvement of thrombospondin-1 by suppressing its expression in CAMs and by adding recombinant protein to MCs and found that thrombospondin-1 plays an important role in the adhesion between MCs and OvCa cells. The molecular mechanism is that the vitamin D receptor competes with the transcription factor Smad2/3 downstream of TGF-β pathway and inhibits its binding to DNA, thereby suppressing thrombospondin-1 expression. The high level of thrombospondin-1 expression in OvCa tissue is a poor prognostic factor, and its expression is higher in peritoneal dissemination sites than in primary OvCa sites. When mice models were tested under conditions that mimic cancerous peritonitis of OvCa, vitamin D suppressed thrombospondin-1 expression in MCs and inhibited formation of peritoneal dissemination. Using a tumor-derived organoid model and mice model, we also confirmed that vitamin D induced mesenchymal-epithelial transition (MET) in CAMs and normalized thrombospondin-1 expression.

Research Summary and Future Perspective

This study suggests that peritoneal restoration and normalization of thrombospondin-1 expression by repurposing vitamin D may be a novel strategy for OvCa dissemination. The combination of vitamin D and chemotherapy may enhance the therapeutic efficacy of OvCa. Vitamin D has proven safe drugs in clinical use, and clinical trials are expected to plan and verify its clinical efficacy for advanced OvCa.

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