News Release

Mesothelial cells promote peritoneal invasion and metastasis of ascites-derived ovarian cancer cells through spheroid formation

Key Points

- Epithelial ovarian cancer (EOC) is a fatal gynecological cancer and more than 75% of patients with EOC are diagnosed at an advanced stage due to rapidly induce metastasis in abdominal cavity using abdominal fluid (ascites).

- We found that **EOC cells compose spheroids with mesothelial cells** and **alter their characteristics through spheroid formation to acquire invasion capabilities** into the mesothelial layer.

- EOC cells can induce peritoneal metastasis without direct dynamic RNA expression changes. **EOC cells then followed the route created by the mesothelial cells.**

- These findings represent a unique characteristic of ovarian cancer in that it **rapidly induces numerous peritoneal metastases within a few months of acquiring malignant characteristics,** and it is difficult to detect early-stage EOC. This model also explains that EOC cells control the unique tumor microenvironment in ascites to rapidly induce abdominal dissemination (Fig. 1).

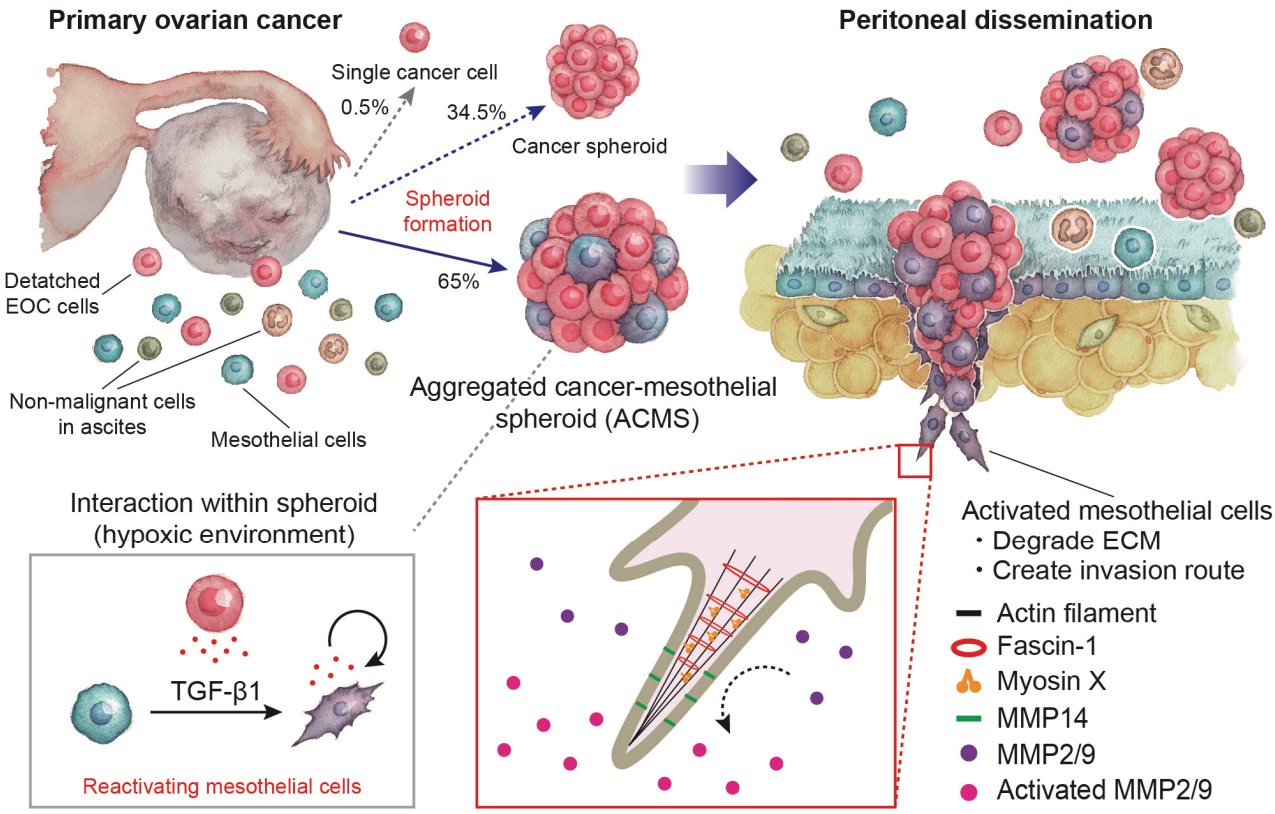

Fig. 1. A model of mechanisms by which ovarian cancer cells survive in non-adherent ascites and rapidly form broad peritoneal metastases in the abdominal cavity.

Almost all the EOC cells identified in the ascites were in a spheroids formation and 65% were accompanied by mesothelial cells, referred to as aggregated cancer-mesothelial spheroids (ACMS). The formation of ACMS enabled EOC cells to alter the RNA expression profiles of mesothelial cells via TGF-β1 related pathway. These alternations increased the expression of fascin-1 in this pathway, which caused invadopodia formations in mesothelial cells to mature, and this degraded collagen with MMP14. Mesothelial cells interacted with EOC cells, which aggressively invaded the collagen and mesothelial layer. These results show that EOC cells can induce peritoneal metastasis without direct dynamic RNA expression changes. EOC cells then followed the route created by the mesothelial cells. This model explains that EOC cells control the unique tumor microenvironment in ascites to rapidly induce abdominal dissemination. ACMS, aggregated cancer-mesothelial spheroid; EOC, epithelial ovarian cancer.

Summary

Patients with epithelial ovarian cancer (EOC) are often diagnosed with peritoneal metastasis and ascites, the accumulation of intraperitoneal fluid containing non-malignant cells. However, the interactions between EOC and non-malignant cells before peritoneal metastasis remain

unclear. To investigate this, whole EOC spheroids were observed using a multiphoton microscope, and their invasion ability was assessed. Mesothelial cells were identified as significant components of ascites through morphological assessment, immunohistochemical and immunofluorescent staining, and single-cell RNA-sequencing analyses. Almost all EOC cells were spheroids, with 60% containing mesothelial cells. EOC cells quickly generate aggregated spheroids with mesothelial cells, and these aggregated cancer-mesothelial spheroids (ACMS) invade collagen or mesothelial layers. Mesothelial cells forming ACMS initiated the invasion. RNA-sequencing analysis revealed dramatic RNA expression changes in mesothelial cells, whereas the changes in EOC cells were minor. TGF-β1-stimulated mesothelial cells showed increased invadopodia formation along with fascin-1 upregulation. These findings suggest that EOC cells alter mesothelial cells through ACMS, thereby elucidating the rapid spread of EOC in the abdominal cavity.

Research Background

Epithelial ovarian cancer (EOC) is a fatal gynecological cancer. More than 75% of patients with EOC are diagnosed at an advanced stage because there is currently no effective screening process and there are no specific symptoms in the initial stages. Early detection primarily fails because EOC can almost immediately metastasize to other organs inside the abdominal cavity once it develops in the ovary or fallopian tube. Induction of metastasis via ascites (abdominal fluid) is known as trans-coelomic dissemination, which is a unique characteristic of EOC. However, the mechanism by which EOC cells survive in ascites and the induction of peritoneal disseminations through interaction with the tumor microenvironment in ascites have not yet been fully elucidated.

The surface of the abdominal cavity is covered with a single layer of mesothelial cells. The interior of the cavity is filled with ascites fluid, which acts as a lubricant for the internal organs. The fluid comprises various cell types, including macrophages, mesothelial cells, lymphocytes, and neutrophils, creating a unique microenvironment. Although mesothelial cells may play an important role in EOC progression, few studies have assessed mesothelial cells in relation to peritoneal disseminations.

When EOC cells metastasize to other organs, they are initially transported by the ascites and must attach to the mesothelial cell layer to reach the basement membranes below. However, the mechanism by

which cancer cells modify mesothelial cells, which are considered protective barriers of the intraperitoneal cavity, into invasive entities capable of metastasizing to other internal organs remains unclear.

We suspect that direct contact between cancerous and mesothelial cells in ascites is necessary for mesothelial cell transformation. In this study, we hypothesized that EOC cells interact with surrounding non-malignant cells in the ascites before inducing peritoneal metastasis and that EOC cells can survive and induce peritoneal dissemination via the formation of hetero-cellular spheroids with unique cellular components in the ascites.

Research Results

In this research, we have clarified below 5 points with latest research techniques.

1: Ovarian cancer cells are present as spheroids.
2: Mesothelial cells are a major component of ascites.
3: Mesothelial cells are present in ovarian cancer spheroids, forming ACMS.
4: ACMS are highly invasive into collagen and mesothelial layers, and in mouse model
5: EOC cells alter the RNA expression profile of mesothelial cells through ACMS formation and use them as invasive leader cells.

We will describe each point in detail below.

1: Ovarian cancer cells are present as spheroids.

Through observing clinical ascites samples, we showed that almost all (99.5%) of the EOC cells identified in this study were cellular aggregates (spheroids), although previous studies have mentioned that EOC cells occur as “single cells,”. The sizes and constituents of the spheroids varied among patients (Fig. 2).

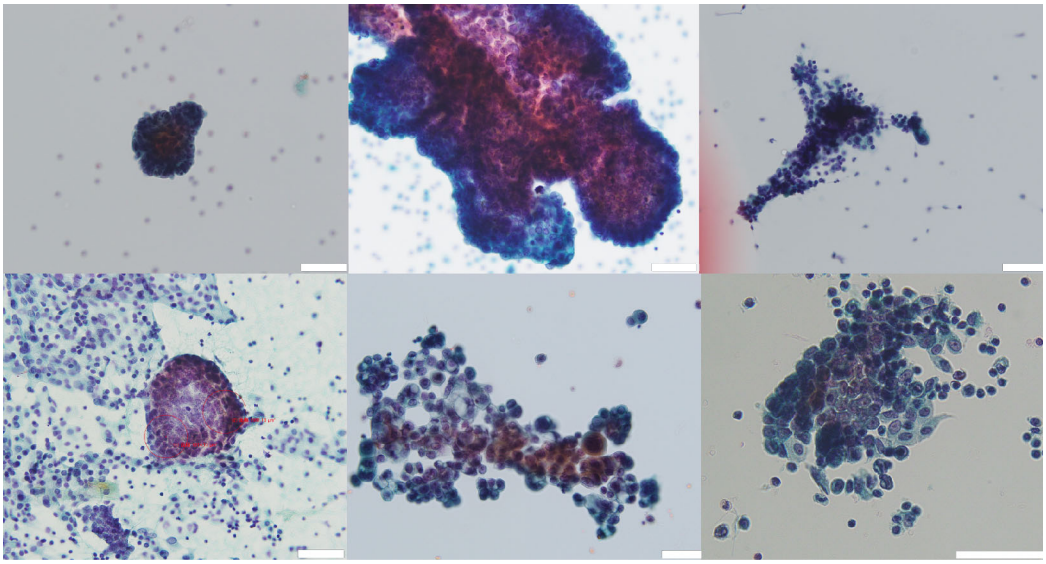

Fig. 2. Ovarian cancer cells are present as spheroids.
Papanicolaou staining results from different patients. The size and shapes of spheroids were different in each patient, but almost all EOC cells in ascites were present as spheroids. Bar = 50 μm.

2: Mesothelial cells are a major component of ascites.

Although mesothelial cells are the primary and essential components of ascites, several previous studies did not consider them in their studies using single-cell RNA-sequencing (scRNA-seq) analysis. To address this issue, we reanalyzed three public scRNA-seq data and revealed that their fibroblast clusters expressed not only fibroblast markers but also mesothelial markers (KRT8 andKRT18), which are normally negative in fibroblasts. Stromal cells in ascites are strongly positive for various mesothelial cell markers (Fig. 3).

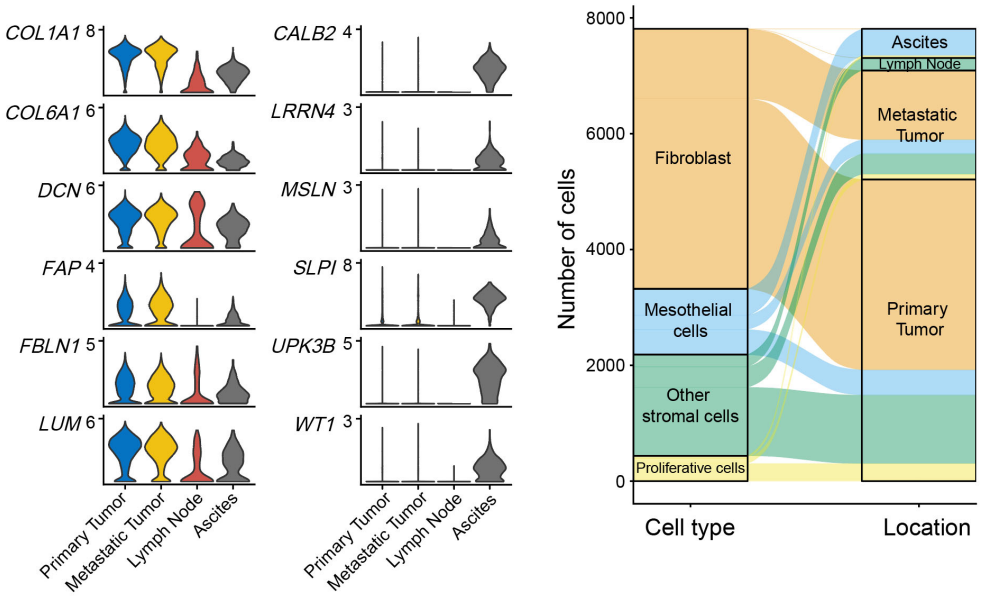

Fig. 3. Mesothelial cells are major components of ascites.
Violin plots showing the expression of fibroblast (left) and mesothelial markers (middle) in stromal cells depending on tumor location. Alluvial plot illustrating the number of cells and proportion of stromal cell components depending on tumor location (right).

3: Mesothelial cells are present in ovarian cancer spheroids, forming ACMS.

From observational analysis, we hypothesized that EOC spheroids were composed of EOC and mesothelial cells and that these hetero-cellular spheroids have survival advantages in ascites and induced peritoneal metastasis. A multiphoton microscope was used to observe whole spheroid structures because its observations are deeper than those of an ordinary laser confocal microscope. The multiphoton microscope could observe whole spheroids and HBME1 clearly distinguished mesothelial cells within EOC spheroids in the 3D images (Fig. 4). Overall, 383 EOC spheroids were observed in 28 patients with HGSOC, of whom 13 had received chemotherapy before surgery and 15 were chemotherapy naïve. The results revealed that >60% were composed of mesothelial cells. Notably, when EOC spheroids were compared before and after chemotherapy, the proportion of HBME1-positive mesothelial cells was significantly higher in patients who received chemotherapy before surgery than in those who did not (Fig. 5). EOC cells can form compact spheroids with surrounding mesothelial cells, which are termed as aggregated cancer-mesothelial spheroids (ACMS), in ascites immediately after detachment from the primary site.

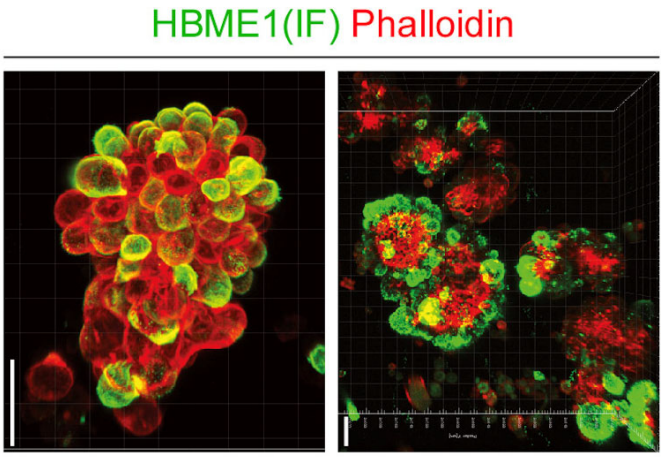

Fig. 4. Mesothelial cells are present in ovarian cancer spheroids, forming ACMS.
Representative images of spheroids with HBME1 staining for mesothelial cells (green) and phalloidin for structure of cancer spheroids (red) using a multiphoton microscopy. Bar = 50 μm.

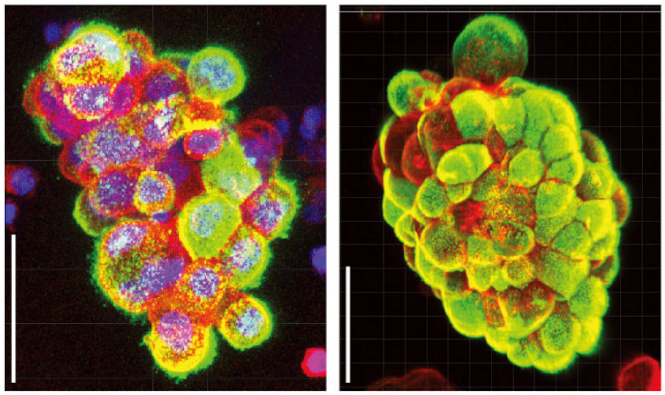

Fig. 5. Proportion of HBME1-positive mesothelial cells was significantly higher in patients who received chemotherapy before surgery.
Representative images of spheroids with HBME1 (green) after chemotherapy. Bar = 50 μm.

4: ACMS are highly invasive into collagen and mesothelial layers, and in mouse model.

We compared the invasion ability of spheroids containing only EOC cells and ACMS. When spheroids were composed of only EOC cells, a few EOC cells invaded the collagen layer. In contrast, the red-stained mesothelial cells from ACMS aggressively invaded the collagen layer. Red-stained mesothelial cells from ACMS aggressively invaded the mesothelial layer. Then, green-EOC cells started to invade from the spheroids and move freely into the mesothelial layer (Fig. 6).

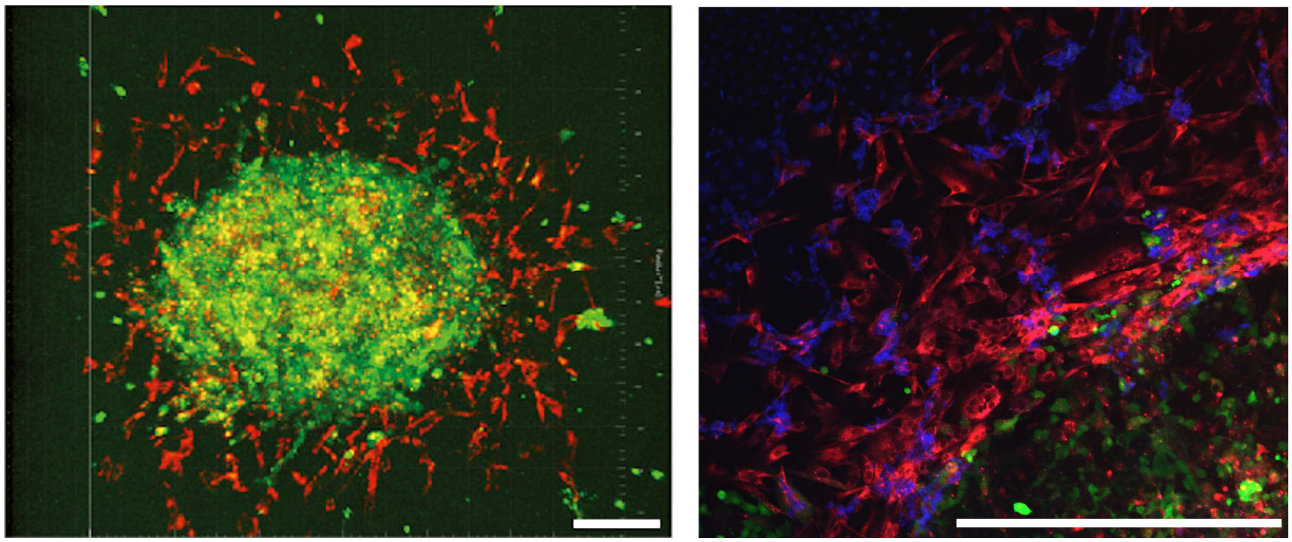

Fig. 6. ACMS has an aggressive invasion ability and mesothelial cells inside ACMS. Representative images of spheroids collagen invasion (Left). Red-stained mesothelial cells invaded the collagen first, followed by green-stained EOC cells, which used the same route. Bar = 200 μm. Representative images of the invasion front from ACMS into the mesothelial layer (blue) (right). Bar = 200 μm.

5: EOC cells alter the RNA expression profile of mesothelial cells through ACMS formation and use them as invasive leader cells.

We analyzed RNA expression changes in both EOC and mesothelial cells. Gene expression changes were relatively minor in EOC cells by the interaction with mesothelial cells. In contrast, RNA expression in mesothelial cells was drastically affected by EOC spheroid formation (Fig. 7). These results show that ACMS formation drastically alters RNA expression in mesothelial cells, whereas the RNA expression profile of EOC cells is similar. TGF-β1-stimulated mesothelial cells increased the number of invadopodia, which is an essential structure for the degradation of the ECM.

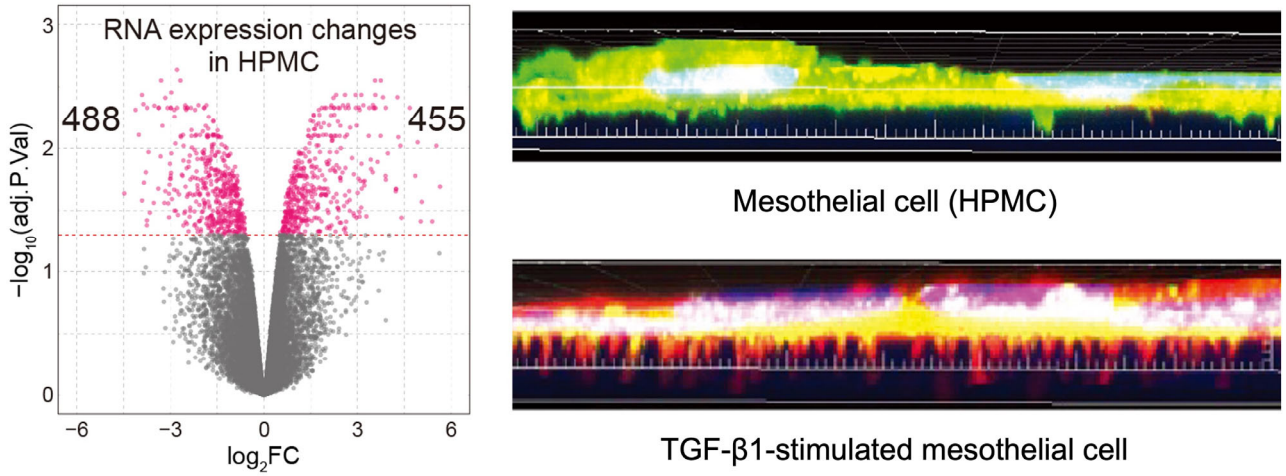

Fig. 7. EOC cells alter the RNA expression profile and morphology of mesothelial cells through ACMS formation.

Volcano plot of dramatical RNA expression changes in mesothelial cells interacted with EOC cells (right). Morphological changes of TGF-β1 in mesothelial cells. They increased number of invadopodia which is essential structure of invasion.

Research Summary and Future Perspective

This study reveals a unique characteristic of ovarian cancer in that it rapidly induces numerous peritoneal metastases within a few months of acquiring malignant characteristics, and it is difficult to detect early-stage EOC. The results also indicate that EOC cells can control the tumor microenvironment in ascites fluid. This indicates that targeting ACMS may be a suitable treatment strategy to reduce EOC cell survival in ascites, invasion of the peritoneal cavity, and resistance to chemotherapy.

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