In 2010, more than 21,000 women were diagnosed with ovarian cancer in the United States. The majority of patients present with advanced-stage disease, after tumors have metastasized throughout the abdominal cavity. Despite aggressive surgery and chemotherapy, less than 30% of patients with widely spread intra-abdominal ovarian cancer attain long-term progression-free survival (1). The biology of ovarian cancer differs from hematogenously spreading tumors, such as breast and colon, in that the cells detach from the primary ovarian tumor and disseminate throughout the peritoneal cavity by the clockwise flow of peritoneal fluid (2). Successful implantation is characterized by the adhesion, migration, proliferation, and invasion of the tumor cells into the peritoneal surface or the omentum. The peritoneal cavity is also a site of metastasis for other epithelial cancers, including gastric, colon, appendiceal, and pancreatic cancer, which have metastatic patterns that parallel ovarian cancer dissemination. Patients diagnosed with these cancers generally present at a late stage, thus micrometastases are rarely evident. These circumstances create an eminent challenge to investigate the initial interaction between cancer cells and the peritoneal surface. We are “in the dark” regarding our knowledge of the first steps of metastasis in the abdominal cavity.

The peritoneal cavity is lined by a continuous single layer of mesothelial cells (Fig. 1A), a unique cell type that covers only peritoneal, pleural, and pericardial serosal surfaces (3). However, mesothelial cells are absent under the peritoneal tumor mass in widespread disease (Fig. 1B). An explanation for the absence of mesothelial cells in advanced disease is uncertain. It has been suggested that the fragile mesothelial cells easily detach from the peritoneal surface, making the collection of and observation of their early interaction with tumor cells even more difficult.

Contributing to the mystery surrounding tumor and mesothelial cell interaction is the general lack of adequate in vivo model systems and intraperitoneal imaging techniques that allow investigation of these early events. Mesothelial cells are thought to be the first line of defense against all abdominally metastasizing tumors. The initial effects of mesothelial cells on ovarian cancer cell adhesion and invasion have been explored in vitro. In 1985, Niedbala and colleagues (4) presented an in vitro model system co-culturing primary human mesothelial cells grown on extracellular matrix-coated culture dishes with primary ovarian cancer tumor cells from ascites. This study found that ovarian cancer cells attach more efficiently to extracellular matrix as compared to mesothelial cells. A subsequent study used primary human peritoneal mesothelial cells and fibroblasts with extracellular matrix, and discovered that mesothelial cells inhibit ovarian cancer cell adhesion and invasion, whereas fibroblasts had the opposite effect (5). The inhibitory effect of mesothelial cells on ovarian cancer cell adhesion dissipated with the senescence of mesothelial cells (6). These studies established a role for mesothelial cells as the “first line of defense” against intra-abdominal cancer cell metastasis. However, the mechanism by which cancer cells clear mesothelial cells to gain access to the basement membrane remained elusive.

Iwanicki and colleagues (7) developed and employed a live, real-time image-based in vitro model to gain spatial and temporal resolution of the initial interaction between ovarian cancer tumor spheroids and mesothelial cells. Through the use of cutting-edge time-lapse fluorescent microscopy, they monitor and measure the interaction of ovarian cancer cells and green fluorescent protein-labeled mesothelial cells, including mesothelial cell clearance. Their elegant study appears in the current issue of Cancer Discovery and provides novel insights into the initial interaction between cancer cells and mesothelial cells. The key mechanism elucidated by the authors is ovarian cancer spheroids use myosin-generated force to “breach” and remove the mesothelial cell monolayer, termed “mesothelial cell clearance” (illustrated in Fig. 7 of ref. 7).

Cells exert force on their environment through association of myosin and integrin networks using recruitment of talin I to adhesion sites (8). The authors establish that attenuation of myosin II, using a combination of myosin IIα short hairpin RNA (shRNA) and myosin IIβ short interfering RNA (siRNA) treatment, inhibits the ability of the ovarian cancer cells to clear mesothelial cells. However, decreasing myosin II has no effect on mesothelial cell apoptosis as measured...
by immunoblot and immunofluorescent analysis of cleaved-caspase 3. Iwanicki and colleagues (7) discover that blocking talin I expression in cancer spheroids by treatment with talin I shRNA inhibits ovarian cancer–induced mesothelial cell clearance. The authors then report that talin I recruitment is required for myosin-generated mesothelial cell clearance. Blocking α5β1-integrin in ovarian cancer cells expressing high levels of α5β1-integrin decreases mesothelial cell clearance, while overexpressing α5β1-integrin increases mesothelial cell clearance. Taken together, these data reveal that the ovarian cancer spheroid clears the mesothelial cells from its path in an α5β1-integrin- and talin-dependent manner.

Alternative mechanisms of mesothelial clearance have been previously explored in vitro. Scanning electron microscopy showed that ovarian cancer cells disrupted intracellular junctions, leading to the retraction of mesothelial cells and the exposure of the underlying extracellular matrix (4). Further, cancer cells may clear mesothelial cells by inducing apoptosis in the mesothelial cells. Treatment of a colonic cancer cell line with an inhibitory Fas-ligand antibody reduced mesothelial cell apoptosis, suggesting that tumor-induced mesothelial cell apoptosis is mediated, in part, by a Fas-dependent mechanism (9). Similarly, Iwanicki and colleagues (7) found that ovarian cancer spheroids clear mesothelial cells at the site of contact; however, the ovarian cancer cells have no effect on mesothelial cell apoptosis.

Supporting the findings by Iwanicki and colleagues (7) is a study, using Fourier transform traction microscopy, that described a role for the fibronectin receptor, α5β1-integrin, in myosin-generated force (10). Cancer cell lines from various organs expressing high levels of α5β1-integrin showed increased invasion through dense 3D collagen fiber matrices and were able to generate very strong contractile forces when compared to cells expressing low levels of α5β1-integrin (10). Accordingly, treatment of ovarian cancer cells with α5β1-integrin–specific antibody or siRNA significantly inhibited in vivo attachment, metastasis, and even survival of mice injected intraperitoneally with ovarian cancer cell lines (11, 12). Taken together, these studies suggest that the α5β1-integrin–driven “mechanical force” of cancer cells is instrumental in the clearance of mesothelial cells and the invasion of the extracellular matrix (7, 10, 11).

The tumor spheroid-induced integrin-talin-myosin force that was able to clear mesothelial cells was studied by the authors using a benign immortalized mesothelial cell line (LP-9) and later passages of primary human mesothelial cells. A caveat to this approach is the recent finding that the morphology and senescent state of early passages of primary human peritoneal cells is different than late-passage primary mesothelial cells (6). The authors reported a difference in the tumor-induced migratory response of mesothelial cells from the pleural and peritoneal cavities; thus the source of

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**Figure 1.** Ovarian cancer cell metastasis. Hematoxylin and eosin-stained human peritoneal surface from a cancer-free patient (A) and a microscopic tumor implant on the omental surface from a patient with metastatic serous ovarian carcinoma (B). Mesothelial cells (arrows) line the abdominal surface but are not detected under the proliferating ovarian cancer cell implant (asterisk).
mesothelial cells likely has an effect on the interaction of tumor and mesothelial cells. Last but not least, the peritoneal microenvironment is not composed exclusively of mesothelial cells but is a complex microenvironment of mesothelial cells, fibroblasts, inflammatory cells, and extracellular matrices (i.e., fibronectin, vitronectin, and collagen type I) affecting the interaction of tumor and mesothelial cells (2). Additional investigations using this new imaging technique will allow us to further elucidate early abdominal metastasis and test the effects of different therapies that will be useful for adjuvant therapy after complete tumor removal.

In summary, Iwanicki and colleagues (7) provide the first evidence that ovarian cancer spheroids “force” the mesothelial cells out of their way, leading to mesothelial clearance. This data, together with that from other studies (4, 5), suggest that the cancer cells “plow” through the protective layer of mesothelial cells. Once the cancer cells reach the extracellular matrix, they have access to underlying stromal cells, which can be recruited to support their survival and rapid growth.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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The First Line of Intra-abdominal Metastatic Attack: Breaching the Mesothelial Cell Layer


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