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Effects of ovarian cancer cells manipulating mesothelial cells that line the peritoneal cavity

Ovarian epithelial cancers frequently spread within the peritoneal cavity, resulting in cell implantation and metastasis at many secondary sites. The peritoneal cavity and associated organs are lined by a single layer of mesothelial cells that it has been suggested not only provides a physical barrier to prevent implantation and invasion but also plays a more complex interactive role in regulating cancer spread. The authors investigated the hypothesis that ovarian cancer cells use exosomes—small membrane vesicles that enable cell-to-cell transfer of mRNA, microRNA, or proteins—to directly communicate with mesothelial cells and manipulate them to provide a favorable environment for cancer cell implantation and growth. They tested their hypothesis using cell culture. The authors isolated exosomes from ovarian carcinoma cell lines, evaluated their physical properties by electron microscopy, and characterized their protein and RNA content by Western blotting and capillary electrophoresis, respectively. To study the effects of these exosomes on cancer cell invasion, they created an invasion assay where a monolayer of human peritoneal mesothelial cells was grown on top of Matrigel, a gelatinous protein mixture used to create three-dimensional cell cultures. The authors then pretreated the mesothelial cells with exosomes isolated from ovarian carcinoma cells or exosomes isolated from control immortalized ovarian surface cells. After pretreatment, they added ovarian carcinoma cells and assessed their invasion into the Matrigel. Significantly more invasion was noted in the presence of exosomes isolated from ovarian carcinoma cells. A control assay without a mesothelial monolayer showed no difference in ovarian cancer cell invasion, demonstrating that the exosomes acted on the mesothelial cells and not the carcinoma cells. In examining the biological mechanism, the authors found that exosomes appeared to reduce the expression of E-cadherin in mesothelial cells, resulting in a mesenchymal-like, spindled appearance. The exosomes, which were enriched for CD44, were also internalized by the mesothelial cells and resulted in increased CD44 expression. This increase in CD44 expression caused the mesothelial cells to secrete matrix metalloprotease 9, resulting in degradation of the extracellular matrix and enhanced invasion by the ovarian carcinoma cells. Therefore, cancer cell-derived exosomes appeared to integrate with the mesothelial cells, modifying their appearance and function to promote cancer cell invasion. The authors confirmed this mechanism in three elegant experiments. In the first experiment, they showed that blockade of exosome formation using a sphingomyelinase inhibitor reduced invasion of the carcinoma cells in a dose-dependent manner and blocked transition to a mesenchymal phenotype in the mesothelial cells. In the second, they demonstrated that exosomes derived from carcinoma cells that had reduced CD44 expression through siRNA knockdown had minimal effect. In the third, they directly transfected mesothelial cells with a CD44 expression vector, which resulted in downregulation of E-cadherin, transition to spindle cell morphology, and increased invasion by carcinoma cells that mimicked the effects of CD44-containing exosomes from cancer cells. The authors validated their findings in human ovarian cancer specimens. Whereas mesothelial cells from the omentum in noncancerous regions were negative for CD44, mesothelial cells adjacent to ovarian carcinoma implants showed strong CD44 expression in 60 percent of cases, using immunohistochemistry. The authors concluded that their study demonstrates that exosomes from carcinoma cells may reprogram mesothelial cells to affect the tumor microenvironment to promote implantation and invasion. Therefore, targeting exosome formation or interaction with mesothelial cells could have therapeutic implications.

Nakamura K, Sawada K, Kinose Y, et al. Exosomes promote ovarian cancer cell invasion through transfer of CD44 to peritoneal mesothelial cells. *Mol Cancer Res.* 2017;15(1):78–92.

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Heterogeneity in DNA copy number in colon cancer

Cancer is often biologically heterogeneous, with molecular genetic diversity seen temporally and spatially. The heterogeneity seen at the DNA level not only stems from different point mutations in various oncogenes and tumor suppressor genes but also from copy number variation. Colorectal cancer is widely studied at the molecular level, and mutations in different genes determine eligibility for various treatment strategies. However, tumors often become refractory due to the development of additional molecular alterations. The authors of this study used a targeted DNA sequencing panel to analyze tumor heterogeneity in colon cancer. They collected tumors from 27 patients with colon carcinoma, each with a primary tumor and at least one lymph node or distant metastasis. They used targeted sequencing at a mean depth of 1,500 reads to sequence a panel of 100 cancer-related genes. From all the tumors examined, they found 88 distinct mutations in 20 genes, including mutations in *TP53* (in 78 percent of patients), *APC* (70 percent), and *KRAS* (44 percent). Of the 27 patients, only four (15 percent) exhibited single nucleotide variant heterogeneity between their tumors. However, using an algorithm specifically designed to estimate copy number frequency, the authors found that copy number was highly discordant between tumors from the same patient. They found that the most frequent copy number gain was seen in *CDX2* (82 percent of samples) and *WFDC2* (56 percent), while copy number loss was most frequently seen in the tumor suppressor gene *SMAD4* (52 percent). To confirm some of the most commonly seen copy number variant findings, the authors verified the results using FISH. Experiments using centromeric FISH probes demonstrated that some of the genes, including *CDX2* and, occasionally, *EGFR*, were due to aneuploidy, while others, such as *MMP9*, showed only local gene amplification. The authors then determined the spatial heterogeneity within a single tumor. They subdivided a primary colon carcinoma into 68 samples representing a variety of proximal, distal, left, right, luminal, and deeply invasive areas, with a separate block of uninvolved colon tissue serving as the control. Each of the 68 samples was again sequenced, using the gene panel, at a mean coverage of 1,800 reads. All parts of the tumor exhibited the same *APC* (c.C4099T, p.Q1367*) and *TP53* (c.G524A, p.R175H) point mutations. No variants were seen in any of the other genes in the panel. When examining copy number variants, they found alterations characteristic of colon carcinoma, including loss of 17p and gain of 20q, but once again, the copy number variants often differed in the 68 regions of the single tumor. The authors created a three-dimensional reconstruction to better visualize patterns of copy number variation. They performed hierarchical clustering, identifying two clusters—one representing the proximal portion of the tumor and the other the distal portion. The authors concluded that this study shows that even though point mutations, and likely driver mutations, were consistent across the tumor, there was wide variability in copy number variation among tumors within the same patient, and even within a single tumor. Many of the copy number variants may be due to aneuploidy; there is often chromosomal instability in cancer. However, such molecular diversity has been shown to be associated with resistance to therapy, especially in colon carcinoma.

Mamlouk S, Childs LH, Aust D, et al. DNA copy number changes define spatial patterns of heterogeneity in colorectal cancer. *Nat Commun.* 2017;8:14093. doi:10.1038/ncomms14093.

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