Metastatic growth is a pivotal, often lethal step in cancer progression in which cancer cells leave the primary tumor to travel to distal sites where they form new (i.e., secondary) tumors (Langley and Fidler, 2011). While the shedding of cancer cells from primary tumors is common, the formation of secondary tumors at distant sites is, fortuitously for the patient, usually highly inefficient. Only <0.01% of circulating cancer cells successfully establish secondary growth (Fidler, 1970). This lack of success is likely due to anoikis (literally meaning “without home”) that occurs when cancer cells leave the tissue microenvironment, also called tumor stroma (literally meaning “bed”), provided by the primary tumor. Loss of stroma means loss of attachment of the cancer cells to the extracellular matrix (ECM) in the stromal tumor microenvironment.

Loss of attachment deprives the cancer cells of essential growth and survival signals. Therefore, isolated cancer cells are often much less tumorigenic than those embedded in stroma, and immune destruction of cancer stroma substantially reduces the success of cancer cells to implant and cause tumors (Singh et al., 1992).

As a prominent exception to this rule, high-grade serous ovarian cancer (HGSOC) has found an efficient way to overcome this fundamental hurdle. For reasons that had remained mostly obscure, cancer cells leaving the primary ovarian tumor establish metastases highly successfully in the abdominal or pleural cavities. This property makes HGS OC the most lethal female malignancy. In this issue of JEM, Gao et al. report the reasons for the metastatic success of HGSOC cells: the formation of heterotypic spheroids of HGSOC cells with fibroblasts. This discovery was made possible by using intrapatient-paired tumor sample sets, three for each patient: primary tumor, ascites, and metastasis. These precious resources have been prominently missing from most previous studies (Bowtell et al., 2015), and their availability allowed Gao et al. (2019) to overcome the problems with interpatient variability. Thus, Gao et al. (2019) compared matched primary, ascites, and metastatic cancer samples from individual HGSOC patients with the three types of matched samples from patients with low-grade serous ovarian cancer (LGSOC), a cancer that also produces ascitic cancer cells but has a more favorable prognosis. The authors observed that HGSOC ascites contained large cellular aggregates while LGSOC ascitic cells were finely dispersed as single cells. The largest qualitative difference was the remarkably increased percentage of fibroblasts in HGS OC ascites. Histologically, the HGSOC aggregates showed a prominent inner core of fibroblasts that was surrounded by the cancer cells, and Gao et al. (2019) termed these heterotypic aggregates “metastatic units.” Metastatic units adhered more avidly to fibronectin and mesothelium-covered surfaces and were significantly more invasive in vitro. Also, ascitic tumor cells from a HGS OC patient established metastases in mice more efficiently than cancer cells derived from the primary tumor or a metastasis of the same HGSOC patient. No significant differences in these assays were found comparing cancer cells from the three types of matched samples from LGSOC patients.

HGSOC ascitic cancer cells specifically reduced expression of E-cadherin mRNA while up-regulating ITGA5 expression encoding integrin subunit α5. No integrin was dominantly up-regulated in LGSOC ascitic tumor cells. Deleting ITGA5 by CRISPR/Cas technology severely reduced adhesion and metastatic success of HGSOC cells. All evidence is consistent with the notion that integrin α5β3 provides ascitic HGSOC cells with the essential capability of attaching to an initial primitive fibronectin/collagen matrix for pro-survival signaling to escape death due to anoikis while traveling in the peritoneal cavity. Previous work already demonstrated that the loss of E-cadherin causes epithelial–mesenchymal transition (EMT) to allow the cancer cells to leave the primary tumor and metastasize by up-regulating integrin α5β1 (Sawada et al., 2008). This allowed the cancer cells to attach to the primitive fibronectin matrix to receive key mitogenic signals. Also, there is an inverse correlation between ITGA5 levels and ovarian cancer patient survival (Sawada et al., 2008).

Fibroblasts not only provide the primitive matrix for attachment of the HGSOC cells but are also an essential part of the bidi-

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Heterotypic aggregates are responsible for the unusual metastatic success of HGSOC, the most lethal female malignancy. When the tumor cells leave the primary ovarian cancer to enter the abdominal cavity, they do not travel alone. Instead, they rapidly form small aggregates by surrounding an inner tissue core of fibroblasts, macrophages, and ECM. This inner core provides the HGSOC cells with attachment and reciprocal signals to escape death by anoikis. The signals also help the aggregates attaching to mesothelium-covered surfaces and establish metastatic growth. Neutrophils are essential in the recruitment of progenitors of macrophages from the BM and in the recruitment of fibroblasts from local perivascular reservoirs. All HGSOCs harbor numerous patient-specific mutations that may be recognized by T cells as cross-presented antigens on fibroblasts and/or macrophages in tumor stroma. This would provide a truly cancer-specific, truly personalized approach to stromal targeting in cancer therapy.

The realization that tumor-associated fibroblasts play a decisive role in the aggressiveness of ovarian cancer calls for a reevaluation of realistic possibilities to target the stroma of cancers specifically and effectively. The authors used the Abelson-kinase inhibitor imatinib to inhibit CAFs or liposome-clodronate to destroy TAMs. However, both drugs are toxic and inhibited metastatic spread only transiently. Already two generations ago, outstanding immunologists showed that the most effective targets for cancer rejection were the so-called unique tumor-specific rejection antigens recognized by T cells. In 1995, these unique antigens were shown to be caused by nonsynonymous single nucleotide variants (nsSNVs) and shown to be effective targets for mutation-specific adoptive T cell transfer (Monach et al., 1995). These nsSNVs are now also simply referred to as mutant neoantigens, and it appears that these random, mutant, truly cancer-specific antigens represent the relevant targets of most successful human immunotherapies (see references in Deniger et al., 2018). HGSOC has an average of 46 nsSNVs; if only half of them were expressed at sufficient levels, the set of usually 12 different antigen-presenting HLA molecules could create >250 cancer-specific targets. At least some of these nsSNVs will be cross-presented by BM-derived as well as non-BM-derived stromal cells, both of which must be targeted to prevent cancer escape in experimental models (Zhang et al., 2007). HGSOC diagnosed with ascites is presently almost invariably lethal, but most of these patients can be effectively treated by chemotherapy, which results in a relapse-free interval often lasting ≥1 yr. Thus HGSOC is an ideal starting point for mutation-specific T cell therapy because this interval could be used to generate a set of autologous T cell receptors for specifically targeting the patient’s neoantigens. Indeed, recent results showed that autologous, truly cancer-specific T cells to mutant antigens could be induced in five of seven HGSOC patients and that responses are not limited by a relatively low mutational burden (Deniger et al., 2018). Such autologous TCRs transduced into autologous peripheral T cells and adaptively transferred into the patient during remission may well prevent relapse of HGSOC and would represent a truly personalized, truly cancer-specific therapy. The research by Gao et al. (2019) is an important guide to focus on those mutant neoantigens that are...
highly expressed to become effective cancer-specific targets, not only for the cancer cell but also for the tumor stroma.

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