SPOT LIGHT

A revelation in cancer cell behavior: The NK cell story

WENDY FANTL, PHD

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On the right path to better ovarian cancer diagnosis and treatment

Wendy Fantl, PhD, and her team at Stanford University have discovered a major mechanism in tumor growth and progression. Inspired to help cancer patients, they are finding ways to investigate unanswered questions about tumor heterogeneity and immune response.

Fantl and her lab work primarily with multiparametric single-cell technology platforms like mass cytometry (cytometry by time-of-flight, used in CyTOF® systems) to identify disease-causing cell subpopulations and understand their signaling networks. She notes that with CyTOF, the ability to analyze so many parameters simultaneously in single cells "is just unbelievably powerful." She can then translate these discoveries focusing on areas of unmet clinical need, including early detection of disease, monitoring of disease progression and precision medicine.

Most recently, her team chose a new approach to investigate high-grade serous ovarian cancer (HGSOC), the most common form of ovarian cancer. Typically, the standard of care for women with ovarian cancer is surgery combined with carboplatin-based chemotherapy. While many patients initially respond, most will relapse. New treatments are therefore urgently needed to prolong survival and cure this deadly disease. The team's approach used CyTOF to analyze single intact cells that make up the ovarian tumor. Initial exploration of newly diagnosed ovarian tumors revealed a tumor cell type, present at low frequency, that correlated with poor patient prognosis and probability of relapse within a year after chemotherapy.

The team then focused on immune cells within the same tumors, namely the tumor immune infiltrate. "Tumors contain a variety of both tumor cells and immune cells, and it is important to learn how they interact and direct each other's behavior," says Fantl. Determining tumor-immune cell interactions could provide a basis for new therapeutic targets to destroy the tumor. Little did the team realize how much they would discover.

"Ovarian cancer is immunogenic. When there are more CD8 T immune cells infiltrating the tumor, prognosis is better, as this is an indication of the patient's immune system mounting a response to tumor antigens," Fantl explains.

"However, many ovarian tumors are infiltrated with T cells expressing the PD-1 immune checkpoint protein, a marker of exhaustion due to chronic antigen stimulation. To override immune exhaustion, immune checkpoint blockade treatments have entered the clinic, resulting in a dramatic paradigm shift in managing cancer patients. This treatment modality is effective in patients with melanoma and kidney cancer, for example, but is just not working for women with HGSOC." Fantl and her team

hypothesized that an overriding mechanism of HGSOC must be preventing immune checkpoint blockade clinical efficacy.

Single-cell analysis of the tumor immune infiltrate

The diversity of immune cells within the tumor immune infiltrate poses a challenge to any type of single-cell analysis. Fantl was confident that with the analytical power of CyTOF successfully applied to HGSOC tumor cells, she and her team could repeat their success with the tumor-infiltrating immune cells.

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She notes that single-cell technologies are essential to illuminating critical information about true tumor heterogeneity, especially about minority populations involved in tumor initiation, relapse or therapeutic resistance. This information is largely lost in bulk-processed tumors.

“CyTOF technology characterizes intact single cells in suspension based on protein co-expression patterns—about 60 parameters per cell—and measures hundreds of thousands to millions of cells. Its ability to work with intact single cells empowers us to discover previously unrecognized cell populations in multiparameter space,” Fantl comments.

The team first correlated the frequencies of tumor cells with those of intratumoral T cells and natural killer (NK) cells. While no correlations were noted when examining exhausted PD-1-expressing T cells, the team discovered an NK cell subpopulation that expressed CD9, correlated positively with overall tumor mass and also correlated with tumor cells co-expressing E-cadherin and vimentin. For the latter, this co-expression pattern indicates tumor cells that are likely undergoing epithelial-to-mesenchymal transition, a process contributing to tumor progression and metastasis.

NK cells are part of the innate immune system, directly killing tumor cells through the release of cytotoxic granules containing perforin and granzyme and antitumor cytokines. The specific intratumoral NK cells that Fantl’s lab identified were noteworthy for their expression of CD9. In normal physiology, CD9 delineates decidual NK (dNK) cells that constitute about 70% of lymphocytes in the first trimester of pregnancy. These dNK cells are extremely immunosuppressive and are responsible for maintaining the fetal-maternal barrier, one of the strongest correlations in biology for a mother-to-be to tolerate her fetus.

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Tumor cells can modulate NK cell function

Fantl reasoned that ovarian tumors co-opted the powerful immunosuppressive properties of dNK cells to promote and maintain their survival. To accomplish this, tumor cells would likely need to recruit more than one mechanism to endow intratumoral NK cells [in this study named decidual-like (dl)] with immunosuppressive properties.

The first mechanism the team evaluated was expression levels of NK receptor ligands present on ovarian tumor cells. These ligands represent a complex network of proteins that bind to an equally complex network of activating or inhibitory receptors present on NK cells. The killing response by NK cells is triggered when signals emanating from NK-activating receptors are at levels greater than signals emanating from NK-inhibitory receptors. Moreover, in order to exploit the killing activity of NK cells, the tumor needs to express ligands for NK-activating receptors to unleash NK cytotoxic granules to directly kill tumor cells.

CyTOF measurements revealed that the NK receptor ligands were expressed in different combinations within three distinct ovarian tumor cell types, each representing different states of tumor progression within the same tumor sample. These three cell types represent tumor cells within a primary tumor mass where cells all adhere to one another, cells transitioning to a metastatic state and cells that have transitioned to the metastatic state. The most immunosuppressive combination of ligands was expressed by the metastatic cell types. These data provide insight into how tumor cells can differentially regulate NK cell function to bypass killing activity and permit tumor expansion.

Multiple studies have shown that a variety of chemotherapeutic agents increase expression of ligands for NK cell activating receptors and decrease expression of ligands for NK cell inhibitory receptors. However, when Fantl's lab measured NK receptor ligands on ovarian tumor cells after in vitro treatment with carboplatin, cells took on a more inhibitory NK receptor ligand phenotype. Furthermore, in a small pilot of ovarian tumor specimens removed from patients before and after neoadjuvant chemotherapy, there was a significant increase in expression of the inhibitory NK receptor ligand nectin-4 in 4 out of 10 matched ovarian tumor specimens.

The significance of these findings is that if a woman with ovarian cancer has failed several rounds of platinum-based chemotherapy, she may be invited to take part in a clinical trial for new treatments such as NK cell-based immunotherapy. The data from ovarian cancer cell lines and tumors strongly recommend measuring NK receptor ligand expression in a biopsied tumor before a patient receives NK immunotherapy, not

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only to spare health care costs but most importantly to spare a patient false hope and intense disappointment.

CD9 is key

It is notable that, aside from decidual NK cells, other NK cells are devoid of CD9. Yet Fantl's team demonstrated that when cultured with ovarian tumor cells, NK cells began expressing CD9 within 15 minutes of coculture. This result generated two key questions: How do NK cells acquire CD9? Could CD9 have a role in conferring immunosuppressive properties to NK cells?

The Fantl Lab demonstrated that in primary ovarian tumors, not only was CD9 expressed by dl-NK cells but it was abundantly expressed on ovarian tumor cells themselves. Using reliable in vitro models of ovarian tumors, the team cocultured ovarian tumor cells representing the three stages of tumor progression with the NK-92 cell line, chosen because it is used in the clinic in several engineered forms.

When grown in monoculture, NK-92 cells do not express CD9. However, upon coculture with ovarian tumor cell lines, they acquired CD9 by a process called trogocytosis (from the Greek, trogo—to gnaw) whereby a recipient cell extracts a membrane fragment from a neighboring cell. In this case, the NK-92 cells acquired an ovarian tumor cell plasma membrane fragment in which CD9 was embedded.

The Fantl Lab demonstrated that the functional consequences of CD9 acquisition were reduced levels of antitumor cytokine production and suppressed killing activity toward ovarian tumor cells. The team was excited to observe a significant increase in tumor cell killing with either a CD9-blocking antibody or a CD9 CRISPR knockout in ovarian tumor cells prior to coculture.

While the findings in this study provide new information about the ovarian tumor microenvironment, Fantl always returns to the overarching goal of her lab: How will this information help patients? The data have spawned many avenues for investigation but evaluating the mechanism of how CD9 suppresses the benefits of NK cell-based immunotherapy is of the highest priority. NK cells are in clinical trials in a variety of formats, in many cases to exploit their cytotoxic function. Fantl hypothesizes that to have any efficacy in HGSOC, acquisition and/or function of CD9 by NK cells must be suppressed.

Priming patients with a CD9-blocking antibody before NK immunotherapy or even administering a CD9-blocking antibody as a monotherapy to patients could awaken endogenous NK cells, allowing them to destroy the tumor. Fantl has a published patent for CD9 utility in NK immunotherapy.

"I think this is an intriguing mechanism that tumor cells have evolved to suppress host immune function. I think we've revealed

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another major mechanism, where CD9 is a target on a par with PD1 and/or CTLA4," she says. Additionally, the group found a T cell subset that expresses CD9, and like the dl-NK cells, correlates positively with tumor growth.

Decidual-like NK cells have also been discovered in other cancers, such as colorectal, lung and prostate cancer (Albini and Noonan, 2021). Thus, the Fantl Lab findings have important implications in treatment of other tumors.

"CyTOF is very powerful in leading you toward undiscovered secrets and mechanisms that you wouldn't have seen otherwise," Fantl says. "It opens new areas for investigation. How would we have ever come to NK cells mimicking the immunosuppressive properties of decidual NK cells so essential for successful pregnancy, or to trogocytosis and CD9?"

References

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Read the associated article on Stanford's blog: How ovarian cancers evade the immune system.

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