

The Two Faces of NKG2D

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One of the defining "hallmarks" of cancer is metastasis, i.e. the ability of tumor cells to advance from their site of origin, invade surrounding tissue, enter the circulation, and establish new tumor growth at distant body parts. In order to do that, certain traits need to be developed in a process called cancer cell plasticity, which is regulated by the epithelial mesenchymal transition (EMT) and the interrelated acquisition of certain cancer stem cell-like features.

In 2011, a team of Fred Hutch researchers from the Spies Lab in the Clinical Research Division published unexpected findings regarding the stimulatory lymphocyte receptor NKG2D, a well-known mediator of immune surveillance of malignant cells. Signal-triggering molecules that bind to this receptor, NKG2D ligands, are generally not expressed on the surface of normal cells but are induced in most types of cancers, making them a target for cancer-fighting immune cells. The observation that surprised the investigators was that certain cancer cells also express the NKG2D receptor, possibly corrupting this key player into stimulating their own tumor growth (Caballero-Benitez et al. 2011). NKG2D signaling was demonstrated in breast, ovarian, prostate and colon cancer cells, and additional support for the hypothesis was provided through analysis of clinical specimens, positively correlating NKG2D expression with tumor size and spread.

Intrigued by these discoveries, Dr. Xin Cai and colleagues in the Spies group undertook further studies to shed light on the mechanisms through which tumor-associated NKG2D promotes metastasis. They first examined whether EMT could be induced in an *in vitro* model using epithelial tumor lines that were modified to express the NKG2D receptor in complex with its activating protein DAP10 (NKG2D-DAP10). Phase contrast microscopy demonstrated that modified cells indeed altered their structure in comparison with control cells, displaying features that facilitate invasion and migration. When tested for motility, i.e. ability to move spontaneously and actively, the NKG2D-DAP10-expressing cells migrated 6 to 12 times more than the control cells through various membranes *in vitro*. The findings were confirmed in a variety of tumor lines and premalignant mammary epithelial cells, which normally lack NKG2D; after NKG2D-DAP10-modification all cell lines exhibited significantly increased migratory and invasive activities. Flow cytometry analysis showed that this transition required cell contact and was thus ligand-dependent. Further experiments identified PI3K-AKT as the crucial signaling axis downstream of NKG2D-DAP10.

Importantly, these *in vitro* results were confirmed in actual tumor specimens from patients with primary invasive breast cancer. It was shown that NKG2D was associated with expression of various EMT-like protein signatures, providing evidence for the importance of NKG2D as an EMT activator. Findings in other cell populations and *in vivo* xenografts in mice added to the support of this theory.

The researchers also showed that expression of NKG2D-DAP10 induced cancer stem cell-like attributes that are known to overlap with progression through EMT, and therefore are vital for cancer cell plasticity. The mechanisms underlying the regulatory synergies of EMT/stem cell reprogramming are only beginning to emerge, but it is known that they can involve cooperation between various DNA-binding proteins called transcription factors. Dr. Cai and colleagues found that in comparison to control cells, NKG2D-DAP10-modified cells expressed significantly more of the transcription factor Sox9. This is notable given that Sox9, in combination with the EMT transcription factor Snail2, is crucial for breast cancer stem cell-reprogramming. Most importantly, the Fred Hutch researchers demonstrated an association between NKG2D expression and expression of Sox9 and Snail2 also in patient samples of breast cancer tumors. This body of evidence supports the important role of receptor NKG2D as a promoter of cancer cell plasticity. Cancer cell NKG2D expression occurs not only in breast but also in other types of cancers, suggesting that the dual NKG2D role is broadly applicable.

"The discovery is a paradigm changer, and first example of its kind," said Dr. Veronika Groh, adding that preliminary data indicate that more is to be found. The ability of cancer cells to hijack an important part of our immune system adds a "provocative twist" to our current knowledge. "NKG2D and its ligands are considered central innate immune regulators of successful and/or failed tumor immune surveillance. Now we show that cancer cells co-opt NKG2D to complement presence of its ligands for self-stimulation of high-malignancy traits."

The "misuse" of NKG2D makes cancer cells even more "evil", according to Dr. Groh, and its presence on human cancer cells could prove crucial for clinical outcomes. By uncovering the Janus-faced nature of NKG2D, the poor scenarios that are associated with expression of its ligands on tumors may finally have gotten an explanation.

[Cai X, Dai Z, Reeves RS, Caballero-Benitez A, Duran KL, Delrow JJ, Porter PL, Spies T, Groh V.](#) 2014. Autonomous stimulation of cancer cell plasticity by the human NKG2D lymphocyte receptor coexpressed with its ligands on cancer cells. *PLoS One*. 9(10), e108942. PMID: PMC4188595.

See also: [Caballero-Benitez A, Dai Z, Mann HH, Reeves RS, Margineantu DH, Gooley TA, Groh V, Spies T.](#) 2011. Expression, signaling proficiency, and stimulatory function of the NKG2D lymphocyte receptor in human cancer cells. *PNAS*. 108 (10): 4081-4086.

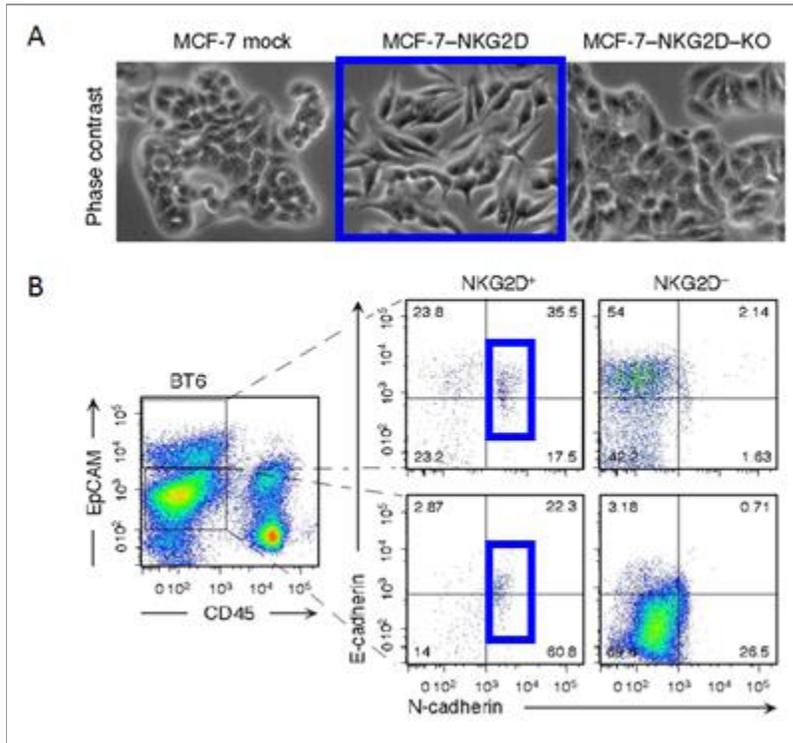


Image provided by Dr. Xin Cai

Association of NKG2D with mesenchymal cell traits, highlighted by blue squares, in a breast cancer cell line (A) and in ex vivo breast cancer cells (B).