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CANCER IMMUNOTHERAPY

Natural killers join the fight against cancer

An antibody overcomes cancer cell immune evasion and activates natural killer cells

By **Adelheid Cerwenka**[1] *and* **Lewis L. Lanier**[2]

Immunotherapy represents one of the major breakthroughs in the treatment of cancer patients. Current therapies focus on harnessing the adaptive immune system, with great success achieved by interfering with immune checkpoints to unleash antitumor CD8$^+$ T cell responses. There is emerging evidence that cancers develop multiple strategies to escape CD8$^+$ T cell recognition. These tumors, however, can be preferentially attacked by natural killer (NK) cells. NK cells are innate lymphocytes that express activating receptors, including the NK group 2D (NKG2D) receptor, which recognize ligands displayed on the surface of tumor cells and pathogen-infected cells. On page 1537 of this issue, Ferrari de Andrade *et al.* (*1*) present an elegant approach to improve NK cell recognition of tumor cells, extending the range of immunotherapies beyond T cells.

The NKG2D–NKG2D ligand axis represents a major activating pathway for human NK cell–mediated recognition of tumor cells and virus-infected cells (*2*–*4*). Several strategies targeting NKG2D ligands (*5*, *6*) expressed on cancer cells or the NKG2D receptor (*7*) on NK cells and certain other immune cells (including CD8$^+$ T cells) have been reported and are now under evaluation in preclinical studies. These approaches are challenging because tumors have evolved mechanisms to escape NK cell surveillance. One major mechanism of tumor escape is the shedding of the NKG2D ligands, major histocompatibility complex (MHC) class I polypeptide–related sequence A (MICA) and MICB, from the tumor cell surface by matrix metalloproteinases (MMPs) and ADAMs (a disintegrin and metalloproteinases), thus impairing NK cell recognition (*8*–*10*). Moreover, shed MICA and MICB that might block NKG2D receptor interaction with its cellular ligands are found in the sera of cancer patients, which frequently correlates with poor prognosis and impaired NK cell function.

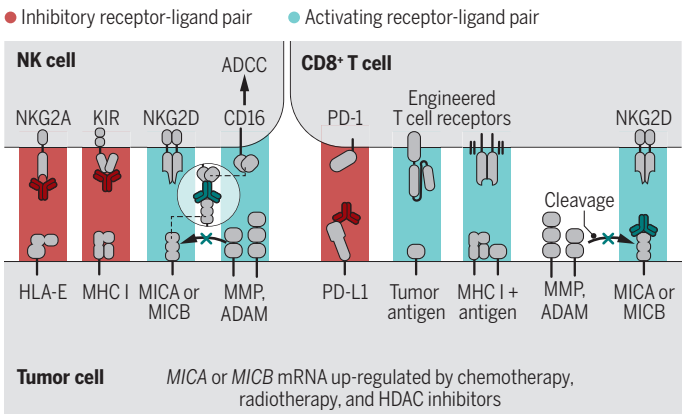
To circumvent tumor immune escape and to efficiently target NK cells to tumors, Ferrari de Andrade *et al.* generated a monoclonal antibody (mAb) against MICA and MICB that masks the region of their extracellular domains that is cleaved by MMPs and ADAMs. The use of a mAb that binds to the cleavage site, but does not interfere with the sites for NKG2D receptor binding, preserves the expression of MICA and MICB on tumor cells and allows NKG2D-dependent activation of NK cell effectors. Additionally, the Fc portion of this mAb can mediate antibody-dependent cellular cytotoxicity (ADCC), triggering target-cell killing by NK cells. The application of this mAb greatly reduced the growth of subcutaneous tumors and metastases in an immune-competent mouse model and in an immunocompromised xenograft mouse model in which human NK cells were administered. The study introduces an exciting concept for a therapeutic mAb with the potential to improve NK cell–based cancer immunotherapy.

The biology of NKG2D ligands is complex (*4*, *10*). The *MICA* and *MICB* genes are highly polymorphic, and different alleles of these ligands vary in expression and their affinity for the NKG2D receptor. The mAb generated in this study recognizes the most common MICA and MICB variants, which are expressed in many tumors but rarely by healthy cells. Expression of MICA and MICB can be enhanced at the transcriptional level by radiotherapy, histone deacetylase (HDAC) inhibitors, or chemotherapy. The present approach, however, is more specific. Moreover, tumor cells often evolve strategies to down-regulate NKG2D on NK cells or T effector cells in the host. In this respect, tumor cell–produced transforming growth factor β (TGF-β) and kynurenine were shown to transcriptionally down-regulate NKG2D expression on NK cells (*11*). These effects are transient, and NKG2D expression can be restored by activation of NK cells with cytokines, for example, interleukin-2 (IL-2) and IL-15. Thus, a combination of the MICA-MICB mAb with cytokines that are now being used in the clinic might further increase the therapeutic efficiency of this approach. Additional combinatorial strategies could involve the blockade of inhibitory NK cell pathways such as killer cell inhibitory receptors (KIRs) or NKG2A, which are now in clinical trials in different types of cancer (*12*) (see the figure).

In humans, NKG2D is not only expressed by NK cells but also by all CD8$^+$ T cells in which a costimulatory function of the NKG2D–NKG2D ligand axis has been reported (*13*). Ferrari de Andrade *et al.* used a poorly immunogenic metastatic melanoma model to show that after application of the MICA-MICB mAb, tumor growth control was mainly mediated by NK cells and not by CD8$^+$ T cells. It will be important to assess whether blocking MICA and MICB shedding in more immunogenic tumor models will also elicit an antigen-specific CD8$^+$ T cell response. Accordingly, it is feasible to combine the MICA-MICB mAb with immune checkpoint inhibitors such

Combinatorial strategies with MICA-MICB mAb

The MICA-MICB mAb stabilizes these NKG2D ligands on tumor cells, inducing tumor cell killing by NK and CD8$^+$ T cells. Combinations with immune checkpoint inhibitors (anti–PD-1 or anti–PD-L1), engineered immune cells, or antibodies blocking NKG2A or KIR could amplify antitumor activity. HLA-E, human leukocyte antigen E.



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as anti–programmed cell death protein 1 (PD-1), anti–PD-1 ligand 1 (PD-L1), or other T cell–based therapies, including the adoptive transfer of engineered T cells to further enhance T cell activation. These combinations would not only enhance both $CD8^+$ T cell and NK cell activation against cancer cells but, in addition, broaden the spectrum of tumor cells that can be attacked.

MICA and MICB expression has been reported in healthy individuals in barrier tissues such as the gut. It is unknown whether these cells also shed NKG2D ligands. Expression and stabilization of MICA and MICB in these tissues could cause excessive inflammation resulting from aberrant immune cell activation and might lead to serious side effects. Additionally, circulating monocytes and tumor-infiltrating myeloid cells in some cancer patients express MICB. Activated T cells can express NKG2D ligands as well (*14*). Moreover, under homeostatic conditions, NKG2D ligands were detected on mouse endothelial cells and might modulate NK cell function (*15*). Whether NKG2D ligands on myeloid cells, T cells, and endothelial cells are also stabilized by the MICA-MICB mAb, potentially promoting inflammation, has not been addressed. Undoubtedly, future studies are needed to provide a comprehensive analysis of MICA and MICB expression in homeostatic conditions and during disease.

Bispecific mAbs targeting additional antitumor effector cells, such as $CD3^+$ T cells (which infiltrate solid tumors at higher numbers than NK cells), to MICA- and MICB-bearing tumors could be generated. Moreover, the MICA-MICB mAb could also be engineered into T cells or NK cells for adoptive cell transfer, potentially resulting in efficient tumor cell targeting, provided there is no toxic off-target cell killing. Ferrari de Andrade *et al.* reveal an innovative approach to counteract a major mechanism of cancer immune escape from NK cell recognition that, if safe in patients, harbors high potential and versatility for future clinical application. ■

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NEUROSCIENCE

Making room for new memories

Clearing neuronal networks from transient memory engrams during sleep consolidates memories

By **Andreas Draguhn**

What are our memories made of? Plato suggested imagining a block of wax in our soul, where perceptions and thoughts leave impressions that we can remember as long as they have not been erased. This historic metaphor captures the transience of some memories and the stability of others, and it illustrates the brain's plasticity. The mechanisms of memory formation and retention remain a key question in neuroscience. Groundbreaking work on the rodent hippocampus (a network in the temporal lobe) revealed that certain neurons form transiently stable representations of places (*1*). Hence, this brain region has become an important focus for studying spatial memory (or engram) formation. It also serves as an experimentally accessible proxy for declarative (knowledge) and episodic (experience) memory in humans, which involves the same brain structures and mechanisms. However, how can we remember an almost infinite number of items with the limited storage capacity of the hippocampus? There is good evidence that relevant representations are transferred to neocortical networks before forming long-lasting engrams. The hippocampus is then reset for acquisition of new memories. Studies in animals (*2*) and humans (*3*) show that neuronal activity during sleep plays a major role in these processes. The underlying mechanisms, however, have remained mostly enigmatic. On page 1524 of this issue, Norimoto *et al.* (*4*) show how sleep-associated activity patterns induce "negative" neuronal plasticity in the hippocampus, erasing remote memories. A previous, related paper by Khodagholy *et al.* (*5*) reveals similar activity patterns in the neocortex, which, hence, may mediate long-term consolidation of transient engrams at their final location.

> ***"...how can we remember an almost infinite number of items with the limited storage capacity of the hippocampus?...neuronal activity during sleep plays a major role..."***

Norimoto *et al.* show that excitatory synapses between hippocampal neurons are weakened by sharp wave–ripple (SWR) complexes, patterns of coordinated network activity that typically occur during sleep (*6*) (see the figure). Surprisingly, neurons contributing to recently acquired engrams are excluded from this weakening and remain stably active. Behavioral tests suggest that this mechanism supports the formation of new memories, in line with the idea that the hippocampal memory system must be regularly cleared. This requires, however, that "old" memories (if relevant) must be stored elsewhere, fostering the idea of engram transfer from the hippocampus to the neocortex (*7*).

The representation of spatial contexts in hippocampal networks involves three major mechanisms. First, special neurons called "place cells" are selectively activated when the animal is in a certain spot of its environment (*1*). Second, exploring an environment strengthens the coupling of sequentially activated place cells, which then form neuronal ensembles representing the spatial experience (*8*). Third, coherent membrane potential oscillations of all local neurons provide a common time frame for coordinating the activation of coupled neurons (*9*). The resulting spatiotemporal activity patterns form transiently stable representations of spatial experience. A key observation from multineuronal recordings in rats links such coactive neuronal ensembles to memory consolidation: Sequences of place cell activity that were formed during spatial exploration are replayed in the same order during phases of immobility or slow-wave sleep (*2*). This sleep state, better known as deep or non-REM (rapid eye movement) sleep, is exactly the phase where humans stabilize recently formed memories (*3*). Compared to memory acquisition, however, replay of

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