RESEARCH HIGHLIGHT

Promoting T and NK cell attack: preserving tumor MICA/B by vaccines

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The activating receptor NKG2D, first discovered in the late 1990's, participates in the immune surveillance of cytotoxic lymphocytes through recognition of the stress-induced ligands MICA/B on the surface of malignant cells. Recently, a study by Badrinath et al. in *Nature* described a vaccine targeting approach that prevents proteolytic cleavage of MICA/B, leading to enhanced immune infiltration and anti-tumor responses highlighting the importance of NKG2D interactions on both natural killer cells and T cells.

In both mice and humans, ligands for the stimulatory receptor NKG2D are upregulated in response to activation of the DNA damage checkpoint pathway. NKG2D ligands in mice (Rae1, MULT1, and H60) and humans (ULBPs, MICA, and MICB) serve to alert the immune system to potentially dangerous cells that have experienced genotoxic stress.[1] Engagement of NKG2D on natural killer (NK) cells and $CD8^+$ T cells provides co-stimulatory signaling that triggers direct cell-mediated cytotoxicity.[2] However, human tumors have the capability to avoid NKG2D recognition through shedding of MICA/B from the cell surface.[3] Shedding occurs via metalloproteinase-mediated cleavage within the membrane-proximal α3 domains of these molecules such that only the α3 domain remains on the tumor and the α1/2 domains are shed.[4] When this occurs, soluble MICA/B can bind NKG2D on cytotoxic lymphocytes, causing endocytosis and degradation of NKG2D to further promote tumor immune evasion through decreased NK cell activity.[5]

Previously, the Wucherpfennig group hypothesized that MICA/B shedding could be blocked with antibodies binding to α3 domains such that the Fc segment of these antibodies could enhance immunotherapy by engaging Fc receptors on immune cells, including the low-affinity Fc receptor CD16 on NK cells to trigger antibody-mediated cellular cytotoxicity. To test this hypothesis, they immunized mice with the recombinant MICA α3 domain and identified several α3 domain-specific antibodies. The monoclonal antibody that was most effective at stabilizing MICA and MICB on the surface of tumor cells, 7C6, was tested further. 7C6 enabled robust NK cell-mediated cytotoxicity against human tumor cells and triggered the production of high amounts of interferon (IFN)-γ. The authors took advantage of the fact that human MICA is recognized by murine NKG2D to establish an immunocompetent mouse model where B16F10 melanoma cells were transduced to overexpress human MICA and injected subcutaneously into syngeneic mice. 7C6 treatment resulted in increased MICA levels on the surface of tumor cells in vivo and significantly delayed tumor growth. Antibody-mediated depletion experiments demonstrated that NK cells, but not $CD8^+$ T cells, were essential for the antitumor activity of 7C6 against lung metastases. To test this therapeutic approach with human cancer cells and NK cells, the authors injected human NK cells into immunodeficient mice followed by injection of human A2058 melanoma cells and treatment with 7C6. In these studies, 7C6 reduced tumor metastasis and extended survival, albeit to a more modest degree relative to syngeneic mouse experiments.[6]

The current study by Badrinath et al.[7] is a logical extension of the previous work demonstrating antibody-mediated stabilization of MICA.[6] The authors developed a vaccine consisting of the α3 domain of MICA or MICB fused to the N-terminus of ferritin from *Heliobacter pylori* for multivalent antigen display. A biodegradable scaffold incorporating granulocyte-macrophage colony-stimulating factor (GM-CSF) for dendritic cell (DC) recruitment and CpG ODN1826 (an adjuvant) was used for vaccine delivery. A MICB α3 domain version of the vaccine (MICB-vax) induced high-titer antibodies in mice that strongly bound to B16F10 melanoma cells expressing human MICB and prevented ligand shedding. The vaccine demonstrated considerable efficacy at limiting tumor growth and extending the survival of mice with subcutaneous B16F10 cells expressing MICB. Importantly, the authors also demonstrated immunological memory in this model by re-challenging mice that remained tumor free 4 months after immunization with another dose of B16F10 (MICB) cells. These mice remained fully protected. The authors also found that mice treated with the MICB-vax were protected in metastatic melanoma and triple-negative breast cancer models of disease recurrence after primary tumor removal.[7]

To enable a comparison of the tumor microenvironment between mice given a control vaccine and mice given the MICB-vax, the authors used B16F10 melanoma cells expressing a doxycycline-inducible MICB. After mice had been vaccinated and tumors had been established, MICB was induced by doxycycline treatment. The authors observed substantial enrichments of $CD4^+$ T cells, $CD8^+$ T cells, and NK cells in the tumors of these mice and reduced percentages of $FoxP3^+$ regulatory T cells. Furthermore, single-cell RNA-seq analysis revealed that the effector lymphocyte populations had altered chemokine receptor expression profiles suggestive of enhanced homing to the tumor sites. Cell depletion experiments in this model showed that $CD4^+$ T cells, $CD8^+$ T cells, and NK cells all substantially contributed to the efficacy of the vaccine. Additionally, both $CD4^+$ T cells and NK cells were

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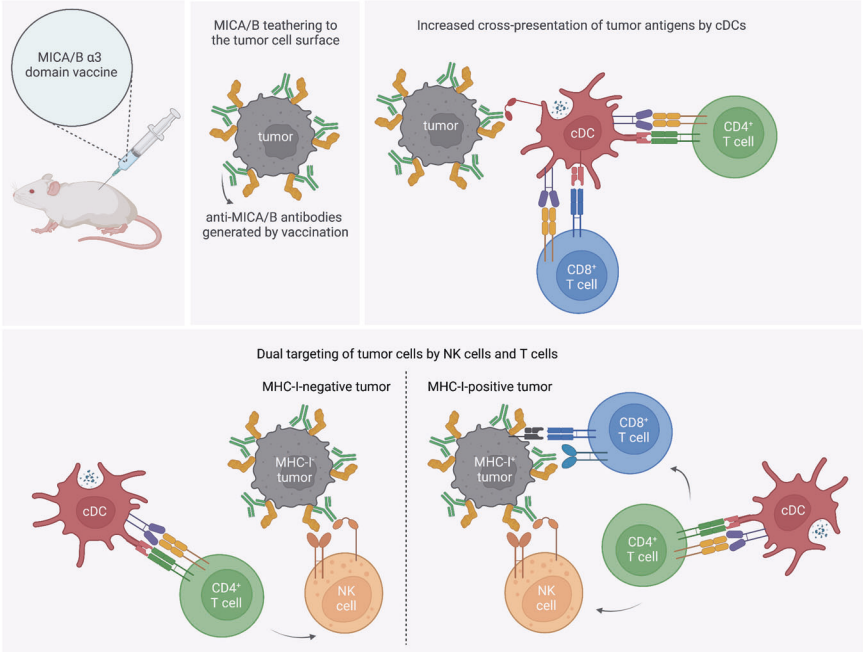

Fig. 1 Mechanisms of action for antibodies induced by MICA/B-vax. Mice injected with the MICA/B-vax generate antibodies that prevent MICA/B cleavage, stabilizing the ligands on the surface of tumor cells. DCs that recognize antibody-bound tumor cells become activated and cross-present antigens to $CD8^+$ and $CD4^+$ T cells. Tumors that downregulate or eliminate MHC-I to avoid $CD8^+$ T cell detection can still be targeted by NK cells with the help of activated $CD4^+$ T cells. Tumor cells that maintain MHC-I can be targeted by both NK cells and $CD8^+$ T cells with the help of activated $CD4^+$ T cells. This figure was modified from Badrinath et al.[7]

important for effective control of tumors with mutations resulting in loss of MHC-I, MHC-II, or IFN-γ receptor.

While exploring mechanisms by which $CD4^+$ T cells could promote NK cell recruitment to tumors, the authors observed a significant increase in the numbers of migratory conventional DCs (cDCs) in the tumor-draining lymph nodes from mice treated with MICB-vax. $CD4^+$ T cell depletion abrogated cDC migration, and cDC ablation significantly decreased the numbers of $CD4^+$ T cells, $CD8^+$ T cells, and NK cells within tumors. Finally, the authors used *Fcer1g*-knockout DCs and mice to demonstrate the importance of the interactions between antibodies triggered by MICB-vax and Fc receptors on DCs to promote $CD8^+$ T cell proliferation in vitro and control tumor growth in vivo.[7] A model describing how MICB-vax bridges innate and adaptive immunity to target $CD8^+$ T cell-resistant and -sensitive tumors is shown in Fig. 1.

This important study provides compelling proof-of-principle data that supports the translation of a MICA/B α3 domain vaccine in planned clinical trials to treat patients with cancer. A limitation of this study, which is acknowledged by the authors, is the use of mouse tumor cells expressing human MICA/B. Whether the same compelling antitumor responses seen in this study will translate in human disease with more complex endogenous regulation of MICA/B expression in heterogeneous tumor cells and potential immunosuppressive conditions that limit B cell function and antibody production remains to be determined. Regardless of the outcome of these planned trials, this and previous studies highlight how MICA/B can be targeted on tumors by either injection of an α3 domain-specific antibody or administration of a vaccine to induce the generation of an antibody targeting the α3 domain to keep the natural NKG2D–ligand complex on the surface. These findings have broad implications across multiple immune lineages with the potential for enhanced antitumor efficacy.

REFERENCES

1. Gasser, S., Orsulic, S., Brown, E. J. & Raulet, D. H. *Nature* **436**, 1186–1190 (2005).
2. Bauer, S. et al. *Science* **285**, 727–729 (1999).
3. Kaieser, B. K. *Nature* **447**, 482–486 (2007).
4. Wang, X. et al. *Biochem. Biophys. Res. Commun.* **387**, 476–481 (2009).
5. Groh, V., Wu, J., Yee, C. & Spies, T. *Nature* **419**, 734–738 (2002).
6. Ferrari de Andrade, L. et al. *Science* **359**, 1537–1542 (2018).
7. Badrinath, S. et al. *Nature* https://doi.org/10.1038/s41586-022-04772-4 (2022).

ADDITIONAL INFORMATION

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