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INSIGHTS

When tumor contact reshapes CAR-T cells

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Repeated tumor contact leaves more behind than simple CAR-T exhaustion. This study by Gu et al. (https://doi.org/10.1084/jem.20252564) shows that chronic antigen exposure impairs a Rab5-dependent endocytic program, allowing trogocytosed antigen to accumulate, functional CAR to decline, and fratricide to increase.

CAR-T cells targeting solid tumors often fail for many reasons, including poor infiltration, antigen heterogeneity, and other barriers within the tumor microenvironment (Du et al., 2025). Gu and colleagues focus instead on a more specific consequence of repeated tumor contact (Gu et al., 2026). They show that chronic tumor exposure progressively disables endocytic activity in CAR-T cells, turning repeated target engagement into a liability. In this framework, chronic antigen exposure is not only a problem of exhaustion or signaling (Delgoffe et al., 2021; Good et al., 2021). It is also a problem of membrane handling.

CAR-T dysfunction has centered on exhaustion, tonic signaling, suppressive cytokines, and poor persistence, all of which are real and important barriers in solid tumors. But repeated contact between cells leaves something behind. Much like wet paint brushing onto a sleeve, repeated immune synapses can leave tumor-derived antigen on the CAR-T cell surface even after the two cells part. Prior work established that CAR-T cells can trogocytose target antigen from tumor cells, lowering antigen density on the tumor while coating CAR-T cells with the very antigen they were engineered to recognize. That transferred antigen can then drive fratricide and deepen dysfunction (Hamieh et al., 2019). More recently, tumor cells themselves were shown to strip CAR molecules from CAR-T cells, creating a second layer of damage by depleting usable receptor from the effector cell surface (Zhai et al., 2023).

Gu and colleagues add a mechanistic bridge between chronic exposure and the consequences of trogocytosis (Gu et al., 2026). Using a continuous antigen exposure (CAE) model (Good et al., 2021), they found that repeatedly stimulated CAR-T cells downregulated endocytosis-associated genes and showed impaired uptake in functional endocytosis assays. Unlike Rab4 or Rab11, which are more closely linked to recycling or exocytosis (Ward et al., 2005; Longatti et al., 2012), overexpression of Rab5 restored endocytic activity and prolonged tumor control during repeated antigen stimulus, whereas a dominant-negative Rab5 mutant (S35N) had the opposite effect; together, these data demonstrate that Rab5 is not only a marker of dysfunction but also functions as a trafficking regulator.

Mechanistically, Rab5 does not stop CAR-T cells from engaging tumor cells. Instead, it helps them clear what remains after repeated contact. Rab5 overexpression did not block early antigen acquisition, but it did promote removal of trogocytosed antigen from the CAR-T cell surface. By limiting persistent surface display of acquired antigen, Rab5 reduced fratricide and helped preserve CAR-T cell fitness under chronic stimulation. This suggests that CAR-T dysfunction may arise in part from a failure to properly handle the material accumulated during repeated tumor encounters.

The authors then asked whether Rab5 influences not only antigen clearance but also the availability of functional CAR on the T cell surface (Zerial and McBride, 2001).

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Here, Rab5 promoted internalization of antigen-bound CAR, facilitated dissociation of CAR from target, recycled unbound CAR back to the plasma membrane, and reduced tumor-mediated capture of CAR molecules. The net effect was to preserve a functional surface pool of unengaged CARs. This shifts the story from antigen clearance alone to the broader question of how CAR-T cells maintain receptor availability under chronic tumor exposure. In this sense, Rab5 helps CAR-T cells clear what repeated tumor contacts leave behind while keeping their receptors available for the next target.

This study builds on prior work on trogocytosis but shifts attention to what happens after the tumor antigen is transferred. Hamieh and colleagues showed that CAR-mediated trogocytosis can drive antigen loss, fratricide, and exhaustion (Hamieh et al., 2019). Zhai and colleagues extended the story by showing that tumor cells can acquire CAR molecules from CAR-T cells, contributing to antigen masking and receptor depletion (Zhai et al., 2023). Olson and colleagues

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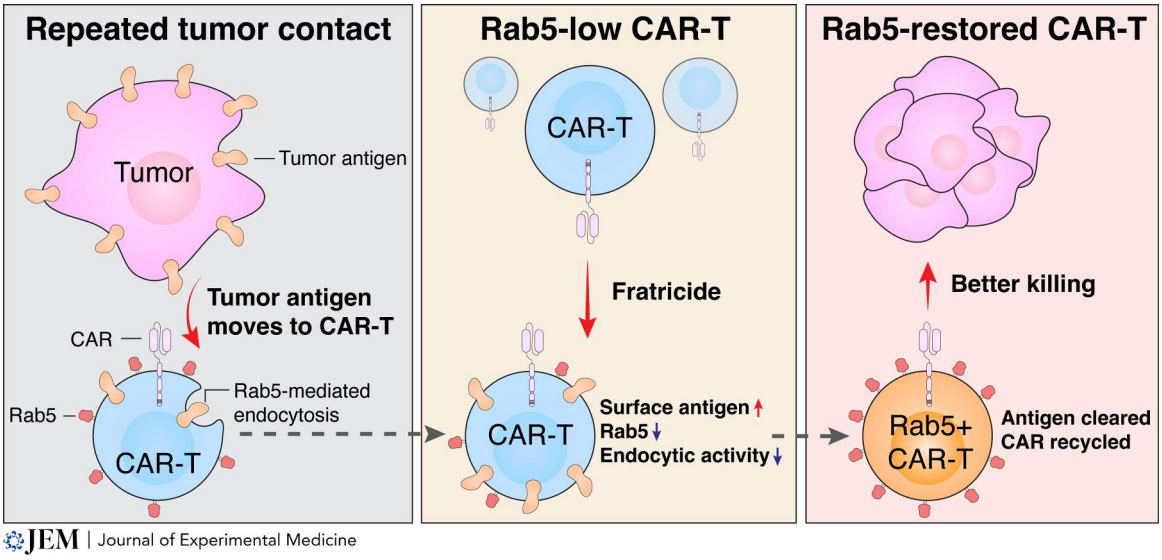

Rab5 preserves CAR-T function after repeated tumor contact. Repeated tumor contact leaves molecular residue on CAR-T cells through bidirectional trogocytosis. Tumor antigens are transferred onto the CAR-T cell surface, while CAR molecules can also be captured by tumor cells. When Rab5 is low, CAR-T cells fail to adequately clear this acquired antigen via Rab5-mediated endocytosis, leading to accumulation of surface antigen, loss of available CAR, and increased fratricide. By contrast, restoration of Rab5 improves handling of these postcontact consequences by promoting clearance of trogocytosed antigen and recycling of functional CAR back to the cell surface. In this way, Rab5 helps maintain a usable receptor landscape on CAR-T cells and supports more sustained tumor killing after repeated tumor encounters.

reported that lower-affinity CARs can reduce trogocytosis while preserving antitumor activity, suggesting that less aggressive binding may improve CAR-T cell expansion and persistence (Olson et al., 2022). Gu and colleagues approach this problem from a different angle. Rather than focusing on how to reduce the initial transfer event, they examine how CAR-T cells handle transferred antigen and receptor complexes after repeated tumor contact. This shows that Rab5-mediated endocytosis can be a potential complement to affinity tuning in limiting trogocytosis-associated dysfunction.

The authors then examined whether Rab5 could improve CAR-T function in clinically relevant models and patient-derived samples. Rab5 improved function not only in model CD19 CAR-T cells but also in BCMA CAR-T cells from patient bone marrow aspirates after infusion, where transient Rab5 expression enhanced CAR availability and improved tumor control upon rechallenge (Garfall et al., 2023). The same principle extended into solid tumor models, including mesothelin-directed CAR-T cells, where Rab5 expression improved tumor control, persistence, and surface CAR maintenance *in vivo*. Together, these findings suggest that preserving endocytic function may be a practical way to improve CAR-T durability. This may be especially relevant in solid tumors, where repeated antigen exposure steadily wears down receptor availability and sustained killing.

These findings suggest that CAE may damage CAR-T cells in a way that exhaustion alone does not fully explain. A CAR-T cell that cannot clear acquired antigen, recycle occupied receptors, or protect itself from receptor theft is not simply exhausted. It is gradually losing the ability to maintain a functional cell surface during repeated tumor contact. In this setting, Rab5 appears to help preserve that surface organization. This may be especially important in solid tumors, where repeated contact is hard to avoid and loss of receptor availability can steadily undermine continued killing. Future studies should define how chronic tumor exposure suppresses Rab5 and related endocytic pathways and how this process can be restored in ways that are useful therapeutically.

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References

Delgoffe, G.M., et al. 2021. *Cancer Cell*. https://doi.org/10.1016/j.ccell.2021.06.012
Du, B., et al. 2025. *Cancer Cell*. https://doi.org/10.1016/j.ccell.2025.03.019
Garfall, A.L., et al. 2023. *Blood Cancer Discov*. https://doi.org/10.1158/2643-3230.BCD-22-0074
Good, C.R., et al. 2021. *Cell*. https://doi.org/10.1016/j.cell.2021.11.016
Gu, M., et al. 2026. *J. Exp. Med*. https://doi.org/10.1084/jem.20252564
Hamieh, M., et al. 2019. *Nature*. https://doi.org/10.1038/s41586-019-1054-1
Longatti, A., et al. 2012. *J. Cell Biol*. https://doi.org/10.1083/jcb.201111079
Olson, M.L., et al. 2022. *Leukemia*. https://doi.org/10.1038/s41375-022-01585-2
Ward, E.S., et al. 2005. *Mol. Biol. Cell*. https://doi.org/10.1091/mbc.e04-08-0735
Zerial, M., and H. McBride. 2001. *Nat. Rev. Mol. Cell Biol*. https://doi.org/10.1038/35052055
Zhai, Y., et al. 2023. *Signal Transduct. Target. Ther*. https://doi.org/10.1038/s41392-023-01708-w