Anoikis

Major Finding: Sustained blebbing confers resistance to anoikis by inducing survival signals in melanoma cells.

Concept: The membrane curvature of blebs recruits septin scaffolds that assemble NRAS and downstream effectors.

Impact: These results reveal bleb signaling as a potential therapeutic target, including in RAS-driven tumors.

PLASMA MEMBRANE BLEBS INDUCE PROSURVIVAL ONCOGENIC SIGNALING SCAFFOLDS

Most cell types within multicellular organisms require anchorage and undergo programmed cell death via anoikis in the absence of cell adhesion–mediated prosurvival signals, whereas cancer cells must acquire resistance to anoikis to maintain the anchorage-independent growth required for cancer progression. After detaching, normal cells typically form transient, small plasma membrane protrusions known as blebs, while cancer cells in low-adhesion environments can sustain blebbing indefinitely. To examine whether persistent blebbing may help cancer cells overcome anoikis, Weems and colleagues cultured human melanoma cell lines in nonadherent or adherent conditions and treated cells with increasing concentrations of wheat germ agglutinin to reduce plasma membrane deformability, revealing that bleb inhibition specifically induced death in detached cells. Blebbing can induce pronounced plasma membrane contours, which have been shown in other contexts to recruit septin proteins. Indeed, live-cell 3D imaging of detached melanoma cells demonstrated that sustained, bleb-generated membrane curvature was required for the recruitment of septins to the plasma membrane, and the subsequent polymerization of septin oligomers was necessary for the generation of stable septin scaffolds. Moreover, these scaffolds were important for bleb-dependent resistance to anoikis, as sustained blebbing in the context of pharmacologic septin inhibition was not sufficient for the survival of detached melanoma cells. Proximity labeling analysis revealed prominent interactions between SEPT6 and NRAS, which prompted an exploration of septin-mediated signaling through downstream NRAS effectors. Septin inhibition in detached melanoma cells reduced the spatial organization of NRAS in signaling hotspots as well as MAPK and PI3K activity, supporting the role of septins in promoting NRAS-mediated prosurvival signaling. The phenomenon of bleb- and septin-mediated resistance to anoikis appeared to be highly conserved, since sustained blebbing was sufficient to prevent anoikis upon detachment in nonmalignant mouse embryonic fibroblasts. Overall, these findings uncover a mechanism by which cancer cells endure low-adhesion environments through bleb-mediated formation of oncogenic scaffolds that maintain survival signaling and suggest that targeting bleb signaling through antiseptin drugs could improve therapeutic outcomes, including in RAS-driven tumors. ■

Weems AD, Welf ES, Driscoll MK, Zhou FY, Mazloom-Farsibaf H, Chang BJ, et al. Blebs promote cell survival by assembling oncogenic signalling hubs. Nature 2023;615:517–25.

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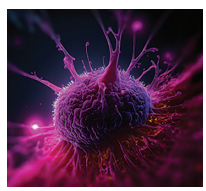
Immunology

Major Finding: Lymphatic vasculature regulates CD8$^+$ T-cell function and retention in the tumor microenvironment.

Concept: Lymphatic vessels express CXCL12 to drive the egress of tumor-reactive, CXCR4-expressing T cells.

Impact: Treatments that prevent CD8$^+$ T-cell egression from tumors may enhance the response to immunotherapy.

LYMPHATIC VESSELS SUPPRESS T-CELL ACCUMULATION IN MELANOMA TUMORS



Though the efficacy of immunotherapy depends on antigen-specific CD8$^+$ T-cell accumulation in tumors, little is known about T-cell transit. In this study, Steele and colleagues investigated the role of lymphatic vessels in limiting CD8$^+$ T-cell control of tumors by facilitating their egress from the tumor microenvironment (TME) and showed in a mouse model of melanoma that removal of the peritumoral lymphatic vessels led to significantly more CD8$^+$ T-cell accumulation in the tumors, suggesting that lymphatic vessels reduce intratumoral T-cell retention. Indeed, high lymphatic vessel density was observed in areas of T-cell exclusion in melanoma patient samples, while in mouse models, tumor-specific CD8$^+$ T cells were found to egress from implanted tumors into the draining lymph nodes, with these egressed T cells expressing low levels of exhaustion markers and retained effector function. Mechanistically, egressed T cells were found to express high levels of CXCR4, the chemokine receptor for CXCL12, and CXCR4 deletion reduced T-cell egress from tumors. Antigen encounter reduced surface CXCR4 expression proportional to the strength of T-cell receptor signaling as well as upregulated the CXCL12 decoy receptor ACKR3, which led to decreased T-cell migration toward CXCL12 gradients. High CXCL12 levels were observed in lymphatic endothelial cells (LEC) at the tumor periphery and found to attract CD8$^+$ T cells to these regions, and LEC-specific deletion of *Cxcl12* as well as pharmacologic inhibition of CXCR4 increased tumor infiltration by CD8$^+$ T cells. Moroever, while treatment with anti–PD-L1 alone was insufficient to control the growth of immunogenic tumors, the addition of a CXCR4 inhibitor contributed to significant suppression of tumor growth. Additionally, adoptive transfer of CD8$^+$ T cells lacking CXCR4 expression or lymphatic deletion of CXCL12 also enabled tumor control by CD8$^+$ T cells. Altogether, this study showed that chemokine expression by LECs regulates the activity and retention of CD8$^+$ T cells in the TME and suggests that targeting this axis could improve intratumoral T-cell quality and quantity as well as immunotherapy efficacy. ■

Steele MM, Jaiswal A, Delclaux I, Dryg ID, Murugan D, Femel J, et al. T cell egress via lymphatic vessels is tuned by antigen encounter and limits tumor control. Nat Immunol 2023;24:664–75.

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