Gene splicing found to reduce effectiveness of CD20-targeting monoclonal antibodies for blood cancers and disorders

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Immunotherapies that target the CD20 antigen have revolutionized how patients with a variety of blood cancers and hematologic disorders have been treated. However, many patients develop resistance to these treatments due to a loss of the antigen that's being targeted.

Now, a new study from researchers at Children's Hospital of Philadelphia (CHOP) and the Perelman School of Medicine at the University of Pennsylvania (Penn) has found that gene splicing occurring within these cells can cause significant changes in CD20 protein levels that render the therapies ineffective.

The findings, published today by the journal *Blood*, also demonstrate that patients with very low levels of CD20 may still be responsive to CAR-T therapy, something that was previously thought not possible. These findings may lead to more appropriate choices for therapy that maximize benefits to patients affected by a variety of blood cancers.

CD20 is a cell-surface protein involved in the fine-tuning of B cell responses to foreign agents like viruses. It is expressed exclusively on the surface of normal and malignant B cells and is not expressed by other cell types in the body. This makes CD20 an attractive target for monoclonal antibody therapies which have been used to treat a variety of B-cell lymphomas, including follicular lymphoma, Burkitt lymphoma, diffuse large B cell lymphomas and high-grade B-cell lymphomas.

Despite anti-CD20 therapies revolutionizing the way these conditions are treated, some patients with these conditions do not respond to antibodies

targeting CD20, while others initially respond before eventually developing resistance.

Prior research had shown that a loss of CD20 reduced the effectiveness of these immunotherapies, since it would remove their intended target. However, the mechanism by which CD20 levels can be reduced were poorly understood. Researchers at CHOP suspected that CD20 messenger RNA was not being properly translated into the CD20 protein expressed on the surfaces of cells.

"It does not matter how much mRNA is being made, it's how effectively it is translated that matters," said senior study author Andrei Thomas-Tikhonenko, Ph.D., chief of the Division of Cancer Pathobiology and a professor with the Department of Pathology and Laboratory Medicine at CHOP and Penn. "In this study, we found that certain isoforms of the mRNA responsible for producing CD20 were impacted by splicing in a way that the proteins were not being made at the levels necessary for these immunotherapies to do their job."

In the study, researchers focused on the MS4A1 gene, which encodes for CD20. The gene undergoes splicing, or stitching together of its building blocks called exons, to produce several mRNA isoforms, which may encode the identical amino acid sequence, but differ in the efficiency with which the protein is made.

The researchers identified four variants in total among normal and malignant cells. Of the four variants, V1 and V3 were by far the most abundant, yet only V3 is efficiently translated into CD20. In contrast, variant V1 had trouble recruiting ribosomes responsible for making proteins, making it difficult for monoclonal antibodies to target affected cells.

Surprisingly, CHOP researchers and their Penn collaborators, including

Drs. Steven Schuster and Marco Ruella, also found that CAR T-cells were still able to effectively kill both V3- and V1-expressing cells. CAR T-cell therapy modifies patient's own immune cells to kill cancer cells.

Historically, CAR T-cell therapy presented challenges and was thought not to be effective in these malignancies, but the study showed that it was more effective in targeting both variants compared with the monoclonal antibody mosunetuzumab, which was only effective against V3-expressing cells.

"If a patient has relapsed because CD20 levels are downregulated, CAR T-cell therapy may still be an option, as it requires a lower threshold of the protein in order to be effective," said first study author Zhiwei Ang, Ph.D., Research Associate Scientist in the Thomas-Tikhonenko lab. "These findings may help clinical staff offer more precise options when treating these hematologic malignancies."

More information: Zhiwei Ang et al, Alternative splicing of its 5' UTR limits CD20 mRNA translation and enables resistance to CD20-directed immunotherapies, *Blood* (2023). DOI: 10.1182/blood.2023020400

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