RECOMBINANT CD30 NANOBODIES AS ROBUST AND COST-EFFECTIVE ALTERNATIVES TO CONVENTIONAL ANTIBODIES FOR DIAGNOSTICS OF HEMATOLOGIC MALIGNANCIES

Urša Štrancar [1], Ario de Marco [1], Tim Božič [2], Živa Pišljar [2], Urša Lampreht Tratar [2,3], Maja Čemažar [2,4]

[1] *Laboratory for Environmental and Life sciences, University of Nova Gorica, Nova Gorica, Slovenia*
[2] *Department of Experimental Oncology, Oncology Institute Ljubljana, Ljubljana, Slovenia*
[3] *Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia*
[4] *University of Primorska, Faculty of Health Sciences, Izola, Slovenia*

Elektronski naslov: ursa.strancar@ung.si

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Human CD30 (TNFRSF8) is a transmembrane glycoprotein receptor of the tumor necrosis factor receptor superfamily, usually highly expressed on activated T and B lymphocytes. It serves as a positive regulator of apoptosis that limits the proliferative potential of autoreactive CD8 effector T cells, thereby protecting against autoimmunity. CD30 activation leads to diverse cellular responses including proliferation, survival, cytokine secretion, and cell death. CD30 serves as a selective biomarker in several cancers, including classical Hodgkin lymphoma, anaplastic large cell lymphoma (ALCL), embryonal carcinoma, diffuse large B-cell lymphoma (DLBCL) and T-cell lymphoma.[1] Detection of CD30 is based on the combination of conventional immunohistochemistry (IHC) and flow cytometry (FC). While IHC allows the detection of large and fragile Hodgkin and Reed-Sternberg cells, flow cytometry offers higher sensitivity and accuracy particularly for cells showing low CD30 expression levels. Therefore, the use of both methods is recommended for reliable assessment. Standardization of FC analysis has been successfully established for anaplastic large cell lymphomas, DLBCL and other rare lymphomas[2] using commercial IgG antibodies. Our data show that alternative detection reagents such as nanobodies may offer comparable specificity with respect to traditional antibodies but provide advantages in terms of stability and production costs. Nanobodies (VHHs) are small (~15 kDa), stable, single-domain antibody fragments derived from camelids characterized by low immunogenicity.[3] VHHs achieve selective target recognition through their unique single variable domain. CD30-specific nanobody-derived CAR-T cells have been developed for treating lymphomas, showcasing as well the therapeutic potential of these agents.[4] In our laboratory, nanobodies targeting CD30 have been isolated by panning a phage display naive library against the purified extracellular domain of CD30 used as the antigen. Their sequences were recovered and used for subcloning into expression vector and production in *E. coli* BL21 SOX. After purification, their binding characteristics and half maximal effective concentration were determined by ELISA on recombinant CD30 and by flow cytometry using human cell line expressing CD30. Our results demonstrate that the developed CD30-specific nanobodies are reliable reagents that allow obtaining results comparable to those recovered using conventional antibodies. However, their production in bacteria makes VHHs cheaper and their recombinant nature ensures excellent batch-to-batch consistency, reducing variability and enhancing reproducibility in both research and clinical settings. Additionally, they can be produced as Fc-fusions into IgG-like formats fully compatible with standardized protocols developed for commercial antibodies.

Literatura

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