

## عنوان مقاله:

Developing and characterizing a single-domain antibody (nanobody) against human cytotoxic T-lymphocyte-associated protein ۴ (hCTLA-۴)

## محل انتشار:

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## خلاصه مقاله:

Objective(s): Cytotoxic T-lymphocyte-associated protein-۴ (CTLA-۴) is the most important human immune checkpoint that modulates T cells activity and brings about immune-homeostasis. Accordingly, checkpoint inhibitor cancer therapy has been approved as a growing method to block over-expressed immune checkpoints, such as CTLA-۴ receptors. Considering the competitive characteristics of single-domain antibodies with monoclonal antibodies, we tried to develop a camelid Nanobody against human CTLA-۴. Materials and Methods: We have constructed the VHH gene library by using immunized-camel peripheral blood mononuclear cells and carrying out the Nested-PCR technique. VHH-library was screened by phage display technique and specific nanobodies against CTLA-۴ protein were selected and amplified with bio-panning steps. Stronger binders were screened by Periplasmic Extract-ELISA, followed by estimating the complexity of the library. Specific anti-CTLA-۴ Nanobody and ۳hCTL۵۵, with longer CDR۳ and a higher binding rate, were selected for more assays. Results: Results revealed the existence of two different clones in the library with ۱۰۸ binders. In comparison with seven different antigens, using the ELISA technique confirmed the specificity of Nanobody ۳hCTL۵۵ against human CTLA-۴ antigen. We calculated Nanobody ۳hCTL۵۵ affinity for human CTLA-۴ antigen at  $۵ \times 10^{-9}$  M, approximately. Performing western blot and Flow-cytometry techniques showed that Nanobody ۳hCTL۵۵ was able to specifically detect and attach both commercial human CTLA-۴ protein and human CTLA-۴ antigen on the cell surface and in the cell lysate. Conclusion: Taken together, this developed camelid-specific anti-CTLA-۴ Nanobody ۳hCTL۵۵, selected from a high-quality immune library by phage display technique, may be effective for further study about cancer diagnosis and cancer-therapy purposes.

## کلمات کلیدی:

CTLA-۴ antigen, Immune checkpoint- proteins, Immunotherapy, Nanobody, Single-domain antibodies

