Production and purification of anti-VEGFR2 single chain fragment variable antibody

Shirafkan Kordi[a,b], Mehdi Asghari Vostakolaei[c],Mohammad Rahmati Yamchi[d], Jalal Abdolalizadeh[b, e,*]

Authors' Affiliations:
[a] Department of Medical Biotechnology, Faculty of Advanced Medical Science, Tabriz, Iran;
[b]Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran;
[c]Pharmaceutical biotechnology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran;
[d] Department of Clinical Biochemistry, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran;
[e]Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran;

Abstract Presenter:
ShirafkanKordi; PhD; Department of Medical Biotechnology, Faculty of Advanced Medical Science, Tabriz, Iran;
E-mail: shirafkankordi@yahoo.com

****Correspondence:***
Jalal Abdolalizadeh; PhD; Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran;;
E-mail:jabdolalizadeh@gmail.com:

Introduction:
The antibody display technology (ADT) such as phage display (PD) has substantially improved the production of monoclonal antibodies (mAbs) and Ab fragments through bypassing several limitations associated with the traditional approach of hybridoma technology.
In the current study, we capitalized on the PD technology to produce high affinity single chain variable fragment (scFv) against Vascular Endothelial Growth Factor Receptor 2 (VEGFR2). VEGFR and its receptor (VEGFR2) play an important role in angiogenesis associated with tumor growth and metastasis.
Methods and Results:
To pursue production of scFv antibody fragments against human VEGFR2, we performed four rounds of biopanning using stepwise decreased amount of VEGFR2 peptide (1 to 0.1 µg), a semi-synthetic phage antibody library (Tomlinson I + J) and TG1 cells.
Antibody clones were isolated and selected through enzyme-linked immunosorbent assay (ELISA) screening. The selected scFv antibody fragments were further characterized by means of ELISA, PCR and Western blot analyses as well as fluorescence microscopy and Wound healing assay. Based upon binding affinity to VEGFR2, 5 clones were selected out of 30 positive clones enriched from PD in vitro selection. The selected scFvs displayed high specificity and binding affinity with Kd values at nm range to human VEGFR2. The immunofluorescence analysis revealed significant binding of the selected scFv antibody fragments to the HUVEC cell line. The effectiveness of the selected scFv fragments was further validated by Western blot analyses and Wound healing assay.
Conclusions:
Based on these findings, we propose the selected fully human anti- VEGFR2 scFv antibody fragments as potential immunotherapy agents that may be translated into preclinical/clinical applications.

Key words: Monoclonal Antibody, scFv, Human VEGFR2, Phage display.

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