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SOFIA UNIVERSITY MARKING MOMENTUM FOR INNOVATION AND TECHNOLOGICAL TRANSFER

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MARKING MOMENTUM
FOR INNOVATION AND TECHNOLOGICAL TRANSFER

National Recovery and Resilience Plan
OF THE REPUBLIC OF BULGARIA

Selection of high affinity recombinant scFv antibodies against human complement proteins and human Galectin-3

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INTRODUCTION

The recombinant antibody fragments in single-chain fragment variable format (scFv) are characterized with a promising therapeutic application to treat viral, inflammatory and cancer diseases. One of the main research topics in the laboratory of molecular immunology (Faculty of Biology – Sofia University) is the structural and functional properties of scFv antibodies as a newly developed class of therapeutic and diagnostic agents. The scFv antibodies could be applied as a powerful tool to study the autoantigenicity of a human molecules which are a binding target from autoantibodies in many autoimmune diseases.

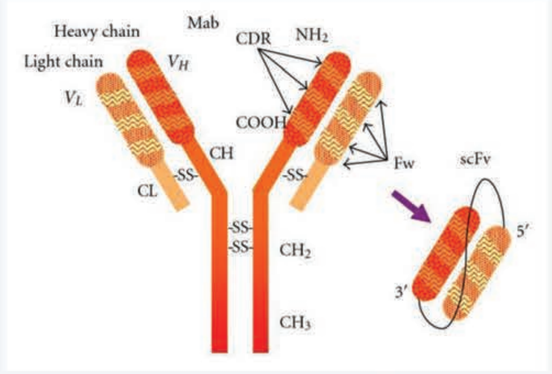

Figure 1. Structure of scFv antibody (*Ahmad et al., 2012*).

In our research we focused to study the molecular aspects of autoantigenicity of the human complement molecules C3 and Factor H (FH), as well as human Galectin-3 (Gal-3). We aimed to select a high affinity scFv antibodies against human C3, FH and Gal-3 from the "Griffin 1" phage display library expressing human scFv antibodies.

METHODOLOGY

- Screening the "Griffin-1" phage library expressing human scFv antibodies
- Selection of monoclonal anti-C3, anti-FH and anti-Gal-3 scFv using a gradual decrease of the amount of the antigen during the rounds of selection.
- Transfer of selected scFv antibodies into a non-suppressor *E.coli* HB2151
- Induction and expression of soluble monoclonal scFv antibodies
- Quantitative analysis by ELISA to determine the affinity of selected scFv clones to human C3
- Dot Blot assay with selected scFv antibodies to determine the affinity of selected scFv clones to human C3 and human Gal-3
- Two induction approaches – IPTG induction and autoinduction, for expression of soluble scFv antibodies.

RESULTS

Rounds of phage selection	Titer of eluted phages (anti-C3 scFv)
Round II	1.26 x 10^5 PFU/ml
Round III	2.74 x 10^4 PFU/ml

Figure 2. Amount of eluted phages with anti-C3 scFv antibodies after III round and IV round of selection from Griffin-1 phage library.

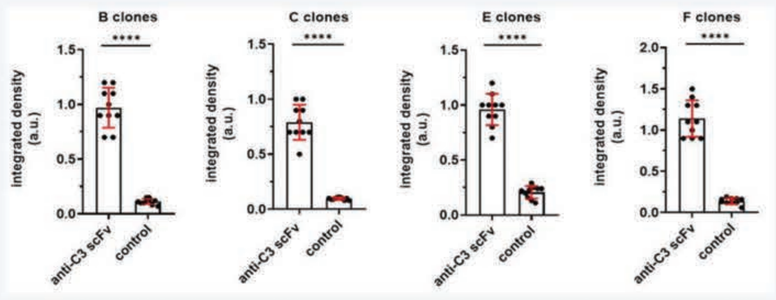

Figure 3. Dot Blot assay. Measurement of integrated density of positive anti-C3 scFv antibodies from selected in round III (B clones – C clones) and anti-C3 scFv antibody clones selected in round IV (E clones – F clones). 1 dot = mean value of duplicate measurement of integrated density of a sample normalized to a control. Statistical analysis was done with paired student t test, error bars: mean±SD, **** ($p \leq 0.0001$).

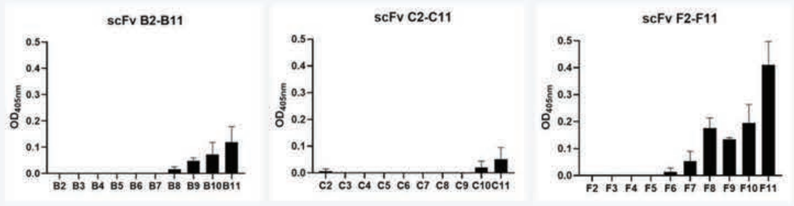

Figure 4. Comparative ELISA analysis of soluble monoclonal anti-C3 scFv antibodies from clones B2-B11, clones C2-C11 (selection round III) and clones F2-F11 (selection round IV).

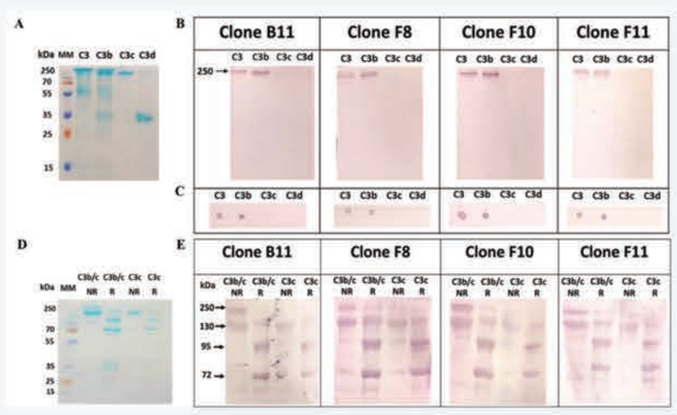

Figure 5. Immunoblotting of the selected high affinity scFv clones for the recognition of the intact C3 and its smaller fragments C3b, C3c, C3d. (A) NC with blotted proteins after non-reducing 15% SDS PAGE. Western Blot (B) and Dot Blot (C) of antigens with selected anti-C3 scFv clones. (D) NC with blotted proteins after reducing 15% SDS PAGE. (E) Western Blot of reduced antigens with selected anti-C3 scFv clones.

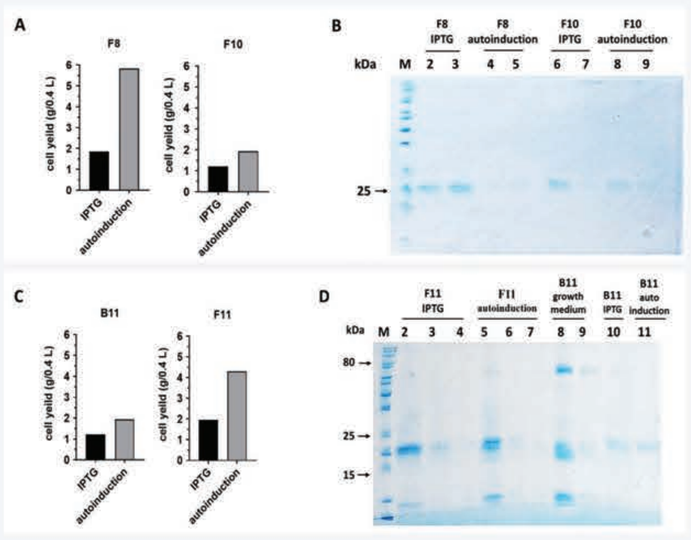

Figure 6. *E. coli* cell yield obtained after IPTG-induction and autoinduction of (A) clones F8 and F10 and (C) clones B11 and F11; (B) non-reducing 15% SDS-PAGE of anti-C3 scFv eluates of clones F8 and F10. Lanes: M, molecular marker; 2 and 3: clone F8 eluates from IPTG induction; 4 and 5: clone F8 eluates from autoinduction; 6 and 7: clone F10 eluates from IPTG induction; 8 and 9: clone F10 eluates from autoinduction; (D). non-reducing 15% SDS-PAGE of anti-C3 scFv eluates from clones B11 and F11. Lanes: M, molecular marker; 2, 3, 4: clone F11 eluates from IPTG induction; 5, 6, 7: clone F11 eluates from autoinduction; 8 and 9: eluates from culture growth medium of clone B11; 10 - cell lysate from IPTG induction of clone B11; 11 - cell lysate from auto-induction of clone B11. The relative protein molecular weight (kDa) was estimated with ImageJ software.

Figure 7. The three major families of human galectins (*Souchak et.al, 2024*).

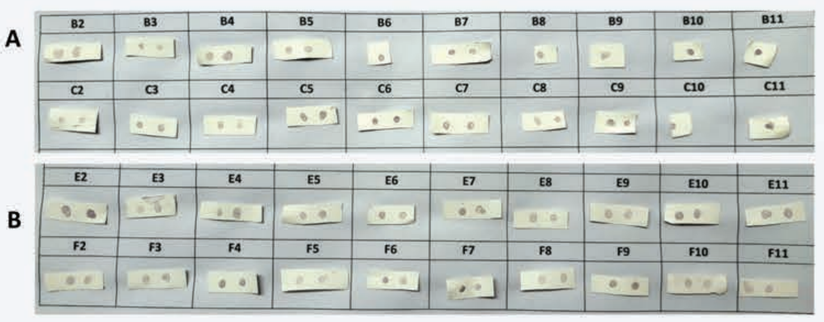

Figure 8. (A) Dot blot assay for detection of positive anti-Gal-3 scFv antibody clones selected in round III (clones B2 - C11) and (B) anti- Gal-3 scFv antibody clones selected in round IV (clones E2 - F11).

Rounds of phage selection	Titer of eluted phages (anti-Gal-3)
Round II	8 x 10^3 PFU/ml
Round III	1.6 x 10^3 PFU/ml

Figure 9. Amount of eluted phages with anti-Gal-3 scFv antibodies after II round and III round of selection from Griffin-1 phage library.

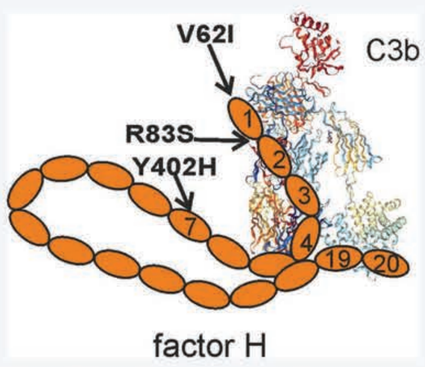

Figure 10. Factor H domain structure *(Meri S and Haapasalo K., 2020).*

Rounds of selection	Titer of eluted phages (anti-FH)
Round II	1 x 10^4 PFU/ml
Round III	1.3 x 10^6 PFU/ml

Figure 11. Amount of eluted phages with anti-FH scFv antibodies after II round and III round of selection from Griffin-1 phage library.

CONCLUSION

- The gradual decrease of the amount of antigen the successive rounds resulted in the selection of high-affinity antibodies to human C3, FH and Gal-3.
- Comparative quantitative ELISA analysis highlighted 4 monoclonal anti-C3 scFv antibodies suitable for further experimental work - B11, F8, F10 and F11
- The anti-C3 scFv antibodies B11, F8, F10 and F11 recognized C3, C3b, but not C3c and C3d.

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