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Striking a Balance: scFv affinity modulates expression and activation in CAR T cells

By Carson Gray

Chimeric-antigen receptor (CAR) T cells represent a substantial advance in anti-tumoral therapies due, in large part, to their cell-specific toxicity and minimal off-target effects. These therapeutics are produced by transduction of a CAR construct containing, at least, an extracellular antigen-binding domain and a T cell activation domain in the form of CD3ζ into autologous T Cells from the patient.[1] Most commonly, the CAR will also include costimulatory motifs such as the signaling region of CD28.[2] Together, these allow the cell to bind targets in an antigen-dependent manner and mount a highly specific cytotoxic response. The antigen-binding domain of most CARs is a modified antibody variable fragment (Fv) known as a single-chain variable fragment (sFv).[1] This approach has yielded significant clinical successes, including some complete remissions in patients who otherwise had exhausted their therapeutic options.[3]

Since their inception in the 1980s, improvements have enhanced the durability and efficacy of CAR-based therapies. One area of insight has been in the avidity of CAR constructs. Avidity of a CAR is a combination of two important attributes: surface expression and antigen binding.[4] While it is clear that CARs must have a robust avidity to mount a response, excess signaling can also be problematic. Specifically, excessively potent CAR-mediated activation can result in T cell exhaustion, off-target tissue damage, and cytokine toxicity. An important advancement in this area was the use of CRISPR platforms to target CAR to the endogenous T cell receptor (TCR) promoter.[5] This placed CAR surface expression under the same regulation as the endogenous TCR, which is dynamically expressed in the presence of antigen. This has been beneficial in optimizing surface expression and avidity, but research into modulating antigen-affinity is lacking.

However, paralleling the challenges faced by natural lymphocytes during their development and antigen receptor randomization, basic questions concerning the physical expression, stability, and binding capacity of CAR sFv regions remained unanswered. To address these, Fujiwara et al., in the March 2020 issue of Biochemical and Biophysical Research Communications, examined sFv structural influences on antigen-binding and expression.1 They constructed four anti- Kinase insert Domain Receptor (KDR) CARs from the variable fragments of antibodies with a range of antigen affinities. Four distinct CAR T Cells were made from these constructs, which each included a GFP reporter to ensure equivalent transduction efficiencies. One construct exhibited significantly decreased surface expression efficiency, suggesting that it was intracellularly degraded. Two others had higher-than-expected molecular weights, suggesting post-translational modifications.

Only one construct bound KDR, as evidenced by specific lysis of a KDR-expressing B Cell line, L1.2. Immunofluorescent staining was done to observe the distribution of the CARs in each of the sFv types; it was found that CAR1-L3H was evenly distributed about the cell surface, while CAR2-L3H and CAR4-L3H formed clusters of aggregate. This provides further evidence for post-translational modifications that could be the cause of intermolecular adherence and aggregate formation.

When these L1.2 cells were transduced with the CAR constructs to assess differences in surface expression regulation, each exhibited high surface expression, including CAR3-L3H, which previously exhibited low expression in T cells. This suggests that the chaperone machinery in B cells could be better suited to expression of sFv- containing constructs.

To assess structural influence on sFv surface expression and antigen specificity, Fv order and linker structure was modified. CAR1-L3H, which previously was shown to be highly expressed and have strong affinity for KDR, was unaffected by either Fv order or linker structure, which suggests that these properties do not significantly affect sFv expression or binding. Alternatively, it was found that the framework regions (FRs) of the sFv have a potent influence on membrane stability. FRs and complementary-determining regions (CDRs) are the two components that make up the Fv. CDRs are the component that determine antigen binding affinity, while FRs are the scaffolding that orient the CDRs. CDR grafting is a technique where the CDRs of one antigen-binding region are grafted into the FRs of another (**Figure 1**). This has traditionally been done to reduce the immunogenicity of antibody-based therapies derived from murine antibodies by grafting their CDRs into human immunoglobulin. This allows for retention of antigen specificity while maintaining the properties of the antibody.10 Fujiwara *et al.* utilized this technique in the context of CAR sFv's.

Sequence analysis revealed that CAR3-L3H possesses a high similarity to CAR4-L3H. Thus, to increase surface expression efficiency of CAR3-L3H, the CDRs of CAR3-L3H were grafted into the FRs of CAR4-L3H. Interestingly, this construct, termed CAR5-L3H, exhibited significantly improved surface expression from CAR3-L3H. Another construct using the FRs of CAR1-L3H and CAR3-L3H, termed CAR6-L3H, was not expressed on T cells. While CAR1-L3H originally had high surface expression, it does not have much sequence similarity with CAR3-L3H. These data suggest that the FRs of sFv's is a potent determinant of surface expression, and that CDR grafting is an effective method to express s sFv's of low surface expression efficiency. It also appears that expression of grafted chimeras requires high sequence similarity between the two sFv's .

These findings provide valuable information in the progression of CAR efficacy. While the order of Fv's and linker structure do not appear to significantly affect CAR surface expression or antigen specificity, the FR's seem to have a major influence. Post-translational modifications and intracellular degradation may be important considerations in the avidity of CAR constructs, as evidenced by aggregation or low expression in some sFv's . CDR grafting represents a potential step forward in tuning CAR-mediated T cell activation by conferring variable antigen-specificity to FR's of high membrane stability. B cell proteins involved in the biosynthesis and trafficking of antibodies may also be an important area of further investigation, as it was found that B cells exhibit high surface expression efficiency, even in CAR constructs that previously were found to be minimally expressed in T cells. Further research into the structural influence of sFv's is necessary to achieve optimal clinical outcomes in CAR T cell therapies.

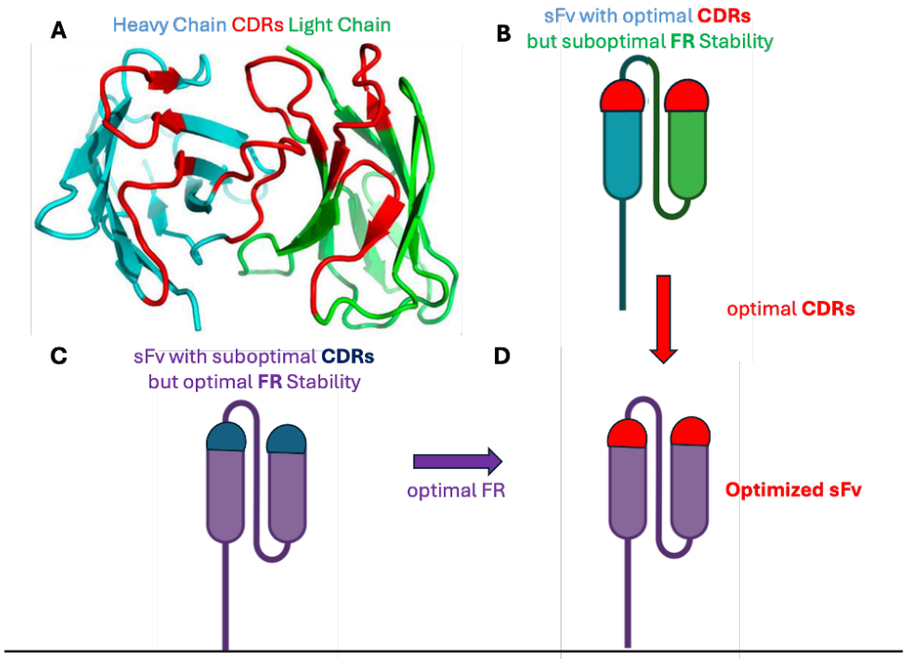

Figure 1 | Grafting of optimal affinity CDRs to FRs generates fine-tuned sFv. A) Ribbon Diagram of sFv with FR and optimal CDR elements highlighted. B) A Cartoon of the same sFv from A with region coloration preserved. C) An sFv with suboptimal CDR, but stable FR region. D) A chimeric sFv with optimal CDR and FR regions.

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