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Hinge region modification in sFLT01 to increase the molecular stability and decrease unwanted immune responses incidence

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SFLT01 is an anti-angiogenic Fc fusion protein. AAV2-sFLT01 gene therapy is in the last phase of clinical trial for treatment of exudative (wet form) age –related macular degeneration (AMD) and has shown promising results. Although sFLT01 mainly includes VEGFR1 (FLT1) extra cellular domain 2, it simultaneously binds to VEGF and PLGF with a significant affinity and neutralizes their proangiogenic activities. Due to the significant burden of multiple intravitreal injections of anti-VEGF drugs, gene therapy with possible long lasting effects has been proposed as a therapeutic modality for this disabling disease. Nowadays, Fc fusion proteins play crucial roles in recombinant drug industries. With the accumulating knowledge and experiences via these therapeutics, more efforts have been focused on exploring and extending the variety of antibody structures that improves product quality and efficacy. Upper hinge in Fc is vulnerable to radical attacks that results in breakage of the heavy-light chain linkage and cleavage of the hinge in IgG1. We removed upper hinge and replaced it by a 9 glycine linker. Previous studies have shown that CH3 domain of IgG1 solely is enough for dimerization and the presence of hinge disulfide bonds does not play a crucial role in antibody dimerization. Antibody-dependent cell-mediated cytotoxicity (ADCC) is initiated by the binding of Fcγ RIIIA and C1q to IgG/antigen complexes, respectively. The presence of both middle hinge cysteine residues in human IgG1 is crucial for ADCC activity. The inherent rigidity of the middle hinge predicts the Fc ability to fix C1q. Due to the importance of disulfide bond positions in middle hinge, we removed initial part of the middle hinge to avoid unwanted immune response. Previous studies have shown that, the hinge length/flexibility characteristics do not play a major role in antigen binding for human IgG1. In this study, we generated a few modifications in hinge region to increase the molecular stability and decrease unwanted immune responses incidence. *In-vitro* functional anti-angiogenic analysis through tube formation assay showed remarkable repression in vascular network. To evaluate the anti-angiogenic function *in-vivo*, a stock of high titer AAV2-msFLT01 virus was produced by Agilent AAV helper free systems. Then two to three day old new-born mice received intravitreal injections of the rAAV. Vascular network was visualized two weeks after injections via IHC by using isolectin B4 vascular marker.