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Preliminary characterization of a novel peptide-Fc-fusion construct for targeting amyloid deposits

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Background

Fc-fusion constructs are synthetic biomolecules composed of an Fc immunoglobulin domain synthesized in association with another protein or peptide. The Fc domain endows two major benefits to the peptide of interest: 1) enhancement of the plasma half-life due to its interaction with the neonatal Fc-receptor; and 2) the ability to engage and activate effector immune cells via interactions through membrane-bound Fc-receptors. Numerous Fc-fusion proteins have been approved by the US Food and Drug Administration for indications such as rheumatoid arthritis (etanercept: TNFR) and Cryopyrin-associated periodic syndromes (rilonacept: IL-1R).

Recent advances have resulted in the development of novel amyloid-reactive antibody agents capable of opsonizing amyloid and restoring organ function [1, 2]. These successes indicate that Fc-mediated cellular activation can inspire immune-mediated dissolution of amyloid in patients. To further advance this technology and generate novel, potentially immunotherapeutic, reagents for targeting systemic amyloidosis, we synthesized a murine Fc-fusion construct that incorporates the synthetic amyloidophilic peptide p5 [3] with a murine IgG2a Fc (Figure 1A). The murine variant of the Fcp5 construct was used in this preliminary fusion to allow proof of principle studies in a panel of *in vitro* binding studies and in an *in vivo* mouse model of systemic inflammation-associated (AA) amyloidosis.

Materials and methods

The pFUSE-mIgG2A-Fc vector, expressing the CH2 and CH3 domains of the murine IgG2a heavy chain, was purchased from InvivoGen (San Diego, CA). The cDNA for peptide p5 with a five amino acid spacer added to the N-terminal of the peptide was synthesized and purchased from Integrated DNA Technologies (Coralville, IA). The p5 cDNA was cloned into the vector using In-Fusion cloning techniques. The vector was transiently transfected into HEK293T/17 and CHOK1 cell lines that were cultured in serum free medium. Secreted

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Declaration of interest. SJK, JSF and JSW are inventors on a patent describing the use of Fcp5 for amyloid-targeted immunotherapy.

Fcp5 was purified from the culture medium by affinity chromatography using a protein A-conjugated matrix. Binding of the purified Fcp5 with human AL and ATTR amyloid deposits in formalin-fixed paraffin embedded tissues was demonstrated immunohistochemically. Additionally, reactivity with synthetic fibrils and AL and ATTR amyloid extracts was assessed by using a pulldown assay [see e.g. 4]. Reactivity with systemic inflammation-associated (AA) amyloid deposits in a murine model of the disease [4] was assessed microautoradiographically at 4 h post IV injection of ¹²⁵I-labeled Fcp5.

Results

Fcp5 fusion construct was expressed in both HEK and CHO cell lines at ~1–5 µg/mL of culture medium. In the pulldown assay, ¹²⁵I-Fcp5 bound Aβ(1–40), IAPP, and rVλ6W1l synthetic fibrils with >90% of radiolabeled material in the fibril pellet (data not shown). Additionally, the ¹²⁵I-Fcp5 bound human AL amyloid extracts, albeit with less efficiency than the fibrils (not shown). The Fcp5 fusion specifically localized with amyloid deposits in formalin-fixed paraffin embedded tissue sections and was shown to bind human ATTR (Figure 1B), ALλ (Figure 1C), ALκ, Aβ, and canine AA, confirming that the multi-amyloid reactivity of the p5 peptide was preserved when expressed in the context of the Fcp5 fusion. In mice with AA amyloid, ¹²⁵I-Fcp5 specifically bound the amyloid deposits in all organs and tissues as evidenced by the presence of black silver grains associated with the presence of ¹²⁵I-p5 in microautoradiographs (Figure 1D) which correlated with the pattern of amyloid deposition seen in Congo red-stained consecutive tissue sections (Figure 1E).

Discussion and conclusions

These positive preliminary data indicate that the Fcp5 fusion is capable of binding numerous forms of amyloid *in vitro* and specifically localizes with systemic amyloid *in vivo*. Therefore, this reagent, or a similar construct employing other amyloidophilic peptides, may provide a novel reagent for targeting amyloid to expedite clearance of the deposits in patients. These reagents may perform as effectively as current fibril-reactive antibodies but could additionally provide pan-amyloid reactivity.

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Figure 1. Structure and function of Fcp5 fusion protein. (A) Schematic representation of Fcp5. Immunohistochemical detection of human ATTR in nerve (B) and renal AL λ amyloid deposits (C) using Fcp5 and biotinylated-anti-mouse mAb. Original magnification 160 $\times$. *In vivo* co-localization of ^{125}I -Fcp5 with hepatic AA amyloid was evidenced by the appearance of black silver grains in microautoradiographs (D) that correlated with birefringent amyloid seen in Congo red-stained tissue sections (E).