DOCTORAL THESIS

Generation of Cytotoxic Aptamers Specifically Targeting Fibroblast-like Synoviocytes by CSCT-SELEX for Treatment of Rheumatoid Arthritis

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by systemic inflammation, synovial hyperplasia, and the progressive destruction of bone and cartilage. Despite the availability of various immunosuppressive disease-modifying antirheumatic drugs (DMARDs), a significant proportion of RA patients continue to experience symptoms. Fibroblast-like synoviocytes (FLSs) undergo a phenotypic transformation into tumor-like cells and play a critical role in RA pathogenesis, making them a promising non-immune cellular target for RA treatment. However, the absence of RA-FLS-specific molecular signatures has impeded the development of therapies that selectively ablate these cells.

Objectives: 1) To develop and validate the cell-specific and cytotoxic (CSCT-SELEX) strategy: Establish a novel method for screning and isolating aptamers that specifically target RA-FLSs with cytotoxic effects. 2) To evaluate the therapeutic effects of the selected aptamers: Assess the efficacy of these aptamers in inhibiting tumor-like behaviors and inducing apoptosis in RA-FLSs. 3) To identify and characterize target proteins: Discover and understand the specific proteins targeted by the aptamers, such as nucleolin (NCL), and their role in RA-FLS pathology. 4) To investigate the mechanism of action of the aptamers: Explore how the selected aptamers modulate key signaling pathways, including p53/Bcl-2, to exert their therapeutic effects. 5) To assess the in vivo efficacy: Evaluate the therapeutic potential of the aptamers in animal models of arthritis, determining their effectiveness in targeting inflamed joints and enhancing current RA treatments.

Methods: To achieve the study's objectives, the CSCT-SELEX strategy was developed and validated as a novel method for isolating aptamers with selective cytotoxicity against RA-FLSs. The therapeutic effects of selected aptamers, particularly SAPT4 and SAPT8, were evaluated through *in vitro* assays, assessing their binding affinity, cytotoxicity, and ability to inhibit tumor-like phenotypes in FLSs. The molecular targets of these aptamers were identified using pull-down assays followed by mass spectrometry, with a focus on

NCL. The mechanism of action was explored by examining how the aptamers interact with their targets and modulate key apoptotic signaling pathways, such as p53/Bcl-2. Finally, the *in vivo* efficacy of SAPT8 was assessed in both collagen-induced arthritis (CIA) mouse models for RA and destabilization of the medial meniscus (DMM) models for OA.

Results: We reveal that molecular target of SAPT8 is NCL, which is highly expressed on surface of RA-FLSs and participates in tumor-like transformation of RA-FLSs. Mechanistically, SAPT8 directly interacts with cell-surface NCL and is internalized into RA-FLSs to degrade NCL via a lysosome-dependent manner,and altered expression of NCL target genes involved in cell apoptosis, including an upregulation of pro-apoptotic p53 and a downregulation of anti-apoptotic Bcl-2. When systemically administrated into arthritic mice, SAPT8 is mainly accumulated in activated FLSs of inflamed joints. SAPT8 monotherapy is proven to effectively relieve symptoms of arthritis. Further, SAPT8 combined with a TNF-targeted biological DMARD synergistically ameliorates arthritis.

Conclusion: CSCT-SELEX could be a promising strategy for developing cell-targeting and cytotoxic aptamers. SAPT8 generated by CSCT-SELEX selectively ablates RA-FLSs via modulating NCL-p53/Bcl-2 signaling, representing a potentially alternative or complementary therapy to the DMARDs for RA.

KEYWORDS: CSCT-SELEX, aptamer, fibroblast-like synoviocytes, nucleolin, rheumatoid arthritis.