MASTER'S THESIS

Screening and Characterisation of High Affinity Aptamers Targeting Microglia Using Cell-SELEX

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Date of Award:
2024

ABSTRACT

Microglia, the resident macrophage in the central nervous system (CNS), play crucial roles in regulating brain development, maintaining homeostasis, synaptic integrity, and immune surveillance. They are responsible for the release of proinflammatory cytokines and the phagocytic clearance of harmful substances in CNS such as tissue debris and protein aggregates. This clearance process aids in resolving inflammation and promoting tissue repair. However, accumulating evidence from genetic and functional studies indicates that hyperactivated microglia are closely associated with the pathogenesis of various neurological diseases, including stroke and neurodegenerative diseases. Targeting hyperreactive microglia has recently emerged as a potential therapeutic strategy for the treatment of neurological diseases. Nonetheless, there is currently a lack of reliable markers for distinguishing microglia from peripheral macrophages and monocytic subsets due to their shared myeloid lineage, and these markers may be modulated during disease development. Furthermore, the existing molecular tools for specifically targeting microglia remain limited, predominantly relying on antibodies. This limitation raises concerns regarding their capability to penetrate the blood-brain barrier effectively and the increased expenses associated with developing targeted delivery system.

Aptamers are single-stranded nucleic acids DNA or RNA that can selectively recognize specific targets through their unique conformations. Their remarkable binding properties are attributed to their binding interaction and diverse structures, enabling them to distinguish target molecules with subtle differences. Moreover, aptamers can be selected without prior knowledge of a specific target. They generally exhibit the dissociation constants within the picomolar to nanomolar range, indicating their effectiveness at low concentrations. Owing to their small size, non-immunogenicity and

ease of chemical modification, aptamers and their applications are rapidly emerging as promising interventions for diagnostic and therapeutic in the CNS.

In this study, single-stranded DNA aptamers were screened using nine rounds of Cell-Systematic Evolution of Ligands by Exponential Enrichment (Cell-SELEX) techniques and were analysed through next-generation sequencing. Ten aptamer candidates were selected based on their occurrences in the library pool and unique structures. Binding affinity assays were performed to evaluate the interaction between cells and each aptamer candidate. Among them, Apt-15 exhibited the most potential interactions with microglial cells and weaker affinity towards other neuronal cells. The dissociation constants (K_d) of the aptamer candidates targeting microglia were also determined. The results demonstrated that Apt-15 had the lowest K_d value (52.24 ± 28.7nM) and relatively high fluorescence intensity, indicating its binding effectiveness to microglial cells at low concentrations. In addition, in vitro fluorescence imaging was conducted to assess the uptake of aptamers by microglia, and the results were consistent with the binding affinity measured by flow cytometry. The functional properties of the selected aptamer candidates on microglial activity were rigorously characterized by using migration, proliferation, immunostaining, and cytotoxicity assays. These findings revealed that Apt-15 displayed inhibitory effects on microglial migration and proliferation in the presence of LPS stimulation. Moreover, Apt-15 exhibited remarkable serum stability over an extended period. In summary, our findings suggest that Apt-15 holds significant promise for potential applications in the treatment of neurological diseases.

Key words: Aptamer; Cell-SELEX; Microglia; Immune response