ApoE, an enigmatic cellular player in neurodegeneration

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STATE OF THE ART

Apolipoprotein E (ApoE) is 299 amino acids long glycoprotein that occurs in 3 distinct isoforms (ApoE2, ApoE3m and ApoE4). Isoforms differ from one to another only **in single amino acid substitution**. ApoE4 is considered the **major genetic risk factor** for the development of Alzheimer's disease (AD), on the contrary, ApoE2 carriers are protected against it. **How such a small difference in amino acid sequence can lead to such a big phenotypical difference?** ApoE is a soluble secreted protein, with N-terminal and C-terminal domains linked by a central hinge region. The N-terminal contains the receptor binding domain, and the C-terminal contains the lipid binding region (**Figure 1**).

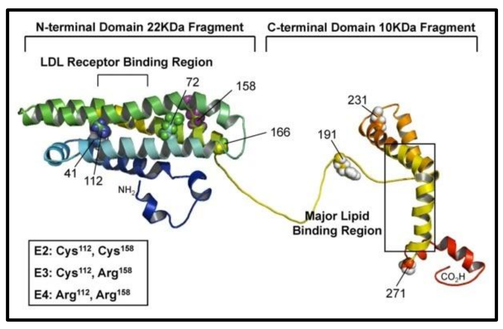

Figure 1:
Structure of ApoE isoforms with highlighted differences. (https://doi.org/10.1186/1423-0127-18-4)

ApoE is **a lipid and cholesterol transporter**, especially in the central nervous system. ApoE4 isoform, however, showed ability to overcome the nuclear membrane, **bind to DNA** in the promoter region of the Sirtuin 1 gene, and act as a non-canonical transcriptional factor for downstream genes.

OBJECTIVES

- **CAN ALL APOE ISOFORMS INTERACT WITH DNA?**
- **IS THE APOE-DNA INTERACTION SEQUENCE SPECIFIC?**
- **CAN BE APOE-DNA INTERACTION INHIBITED BY SMALL COMPOUNDS?**

METHODOLOGY

The main method used to obtain the following results was electrophoretic mobility shift assay (EMSA). This method is based on the fact that in the electrophoretic gel, dsDNA-protein complexes move slower compared to the unbound dsDNA probe.

During experiments human recombinant truncated ApoE proteins were used. They were produced in E. coli BL21(DE3) and purified using TALON resin and size exclusion chromatography. Used isoforms: ApoE2(1-204), ApoE3(1-204) and ApoE4(1-204).

DNA probes for experiments were commercially prepared by gene synthesis. There are two kinds of probes: 1) with specific motif from the promoter region of Sirtuin 1 gene (hypothesis based on the literature) called CLEAR – to confirm sequence-dependent binding and 2) probe with random nucleotide sequence called SCRAMBLED – to confirm sequence-independent binding of the protein. Both probes were around 100 bp long with fluorescent dye Cy.3 on the 5' end.

RESULTS I.

ApoE4(1-204) has an ability to create complexes with dsDNA and this interaction seems to be non-specific as ApoE4(1-204) dsDNA-protein interaction was detected with both dsDNA probes (Figure 2).

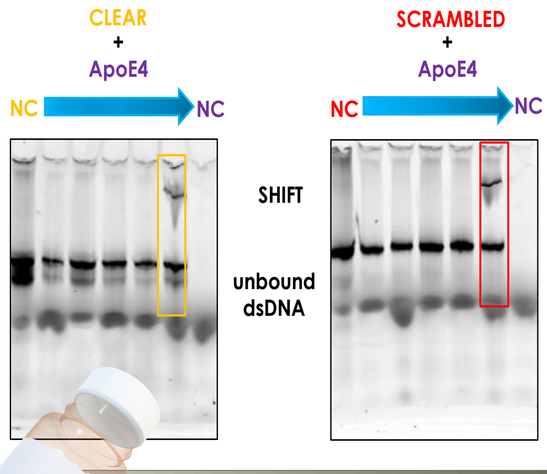

Figure 2:
Native polyacrylamide gels from EMSA experiment. NC – negative control (only dsDNA probe CLEAR), NC – negative control (only dsDNA probe SCRAMBLED), NC – negative control (only protein ApoE4(1-204)). The blue arrow represents the gradually increasing protein concentration within a sample with stable dsDNA concentration..

RESULTS II.

ApoE3(1-204) is also willing to make DNA-protein complexes in a sequence-independent manner similar to ApoE4(1-204).

ApoE2(1-204) does not seem to bind to dsDNA at all (Figure3).

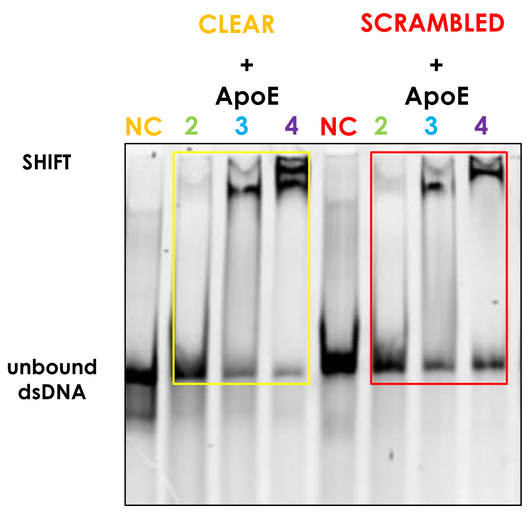

Figure 3:
Native polyacrylamide gels from EMSA experiment. NC – negative control (only dsDNA probe CLEAR), NC – negative control (only dsDNA probe SCRAMBLED). Every sample consisted of the same protein concentration and dsDNA probe.. Truncated ApoE2(1-204), ApoE3(1-204) and ApoE4(1-204) proteins were used.

RESULTS III.

The first potential oral drug for AD tramiprosate (homotaurine) inhibits the formation of Aβ oligomers. This compound also shows the perspective way for the evolution of similar anti-AD molecules for example taurine or 3-sulfur propanoic acid (SPA) (**Figure 4**). Tramiprosate has interesting **ApoE isoform-dependent efficacy**. Clinical trials in the third phase showed that patients' responses to treatment differ in ApoE4 and non-ApoE4 carriers. The most consistent clinical benefits were noticed within **patients homozygous for ApoE4**. Considering the mechanism of action of tramiprosate (multiple molecules of tramiprosate interact reversibly with a single Aβ monomer), interactions between ApoE and tramiprosate molecules could be expected. Hypothesis: **This interaction may inhibit dsDNA-protein interaction.**

Any of the used compounds were able to inhibit dsDNA-ApoE4(1-204) interaction (Figure 5).

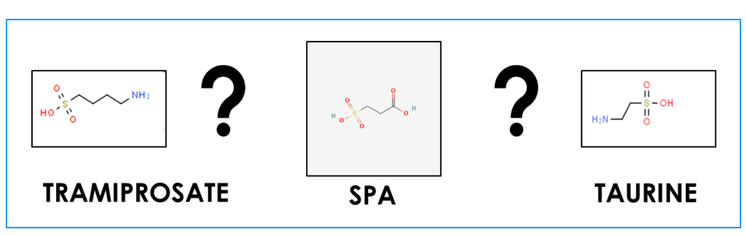

Figure 4:
Structure of potential inhibitors of dsDNA-protein interaction.

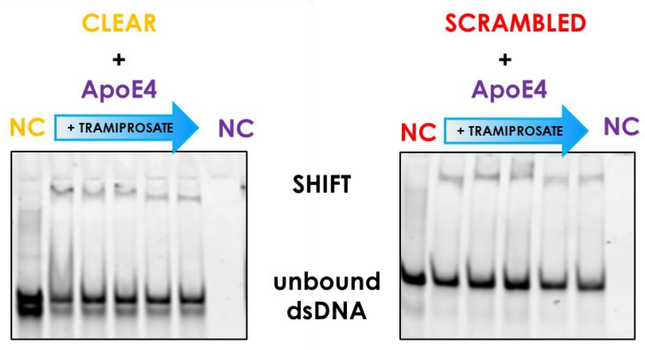

Figure 5:
Native polyacrylamide gels from EMSA experiment. NC – negative control (only dsDNA probe CLEAR), NC – negative control (only dsDNA probe SCRAMBLED), NC – negative control (only protein ApoE4(1-204)). The blue arrow represents the increasing concentration of a small potential inhibitor (tramiprosate) within a sample with stable dsDNA and protein concentrations. Representative picture – same results obtained also using SPA and taurine.

CONCLUSION

- ApoE IS A **CRUCIAL PLAYER IN NEURODEGENERATION,** NOT ONLY IN AD.
- DNA-BINDING ABILITY OF ApoE SHOWS **ISOFORM-DEPENDENT** MANNER.
- INTERACTION BETWEEN ApoE AND dsDNA IS **NON-SPECIFIC.**
- USED SMALL MOLECULES **CAN NOT INHIBIT THE INTERACTION** BETWEEN ApoE4 AND dsDNA**.**

TO BE CONTINUED …
X-RAY CRYSTALLOGRAPHY

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