Scientific Report (December 2018 — December 2019)
Project PN-III-P1-1.1-PD-2016-1942

"Evaluation of the therapeutic potential of non-viral apolipoprotein E gene transfer to limit progression of atherosclerosis"

In the frame of the project **PD20/2018 PN-III-P1-1.1-PD-2016-1942** entitled: **„Evaluation of the therapeutic potential of non-viral apolipoprotein E gene transfer to limit progression of atherosclerosis"** in the period December 2018 – December 2019, we performed the experiments planned in the following activity:

A 2.1: Determine the organs transfected by systemic delivery of C60-apoE/GFP polyplexes. Test the time-course evolution of *in vivo* transfection using these polyplexes.

Apolipoprotein E (apoE) has lipid-lowering and anti-atherosclerotic effects, being involved in the clearance of cholesterol-rich lipoproteins and the cholesterol efflux from cells. Our hypothesis is that an increase in apoE expression induced by fullerene-based nanosystems confer anti-atherosclerotic protection. The goal of this proposal is to generate fullerene-based nanoparticles conjugated with DNA encoding full-length or truncated forms of apoE under CMV promoter to be used for *in vivo* non-viral transfection as a therapeutic tool for limiting atherosclerosis progression. The specific objectives are:

1. To generate non-viral transfection agents for expression of apoE3 or its fragments. We accomplished this objective by cloning apoE3 and its N- and C- terminal regions in pcDNA3.1-DYK vector and then the obtained plasmids were linked to C60-PEI conjugates. We confirmed the functionality of C60-PEI-DNA polyplexes as non-viral transfection agents.
2. To assess the timing and organs accumulation of apoE expression after *in vivo* transfection. We accomplished this objective; we determined the organs transfected by systemic delivery of C60-PEI-DNA polyplexes and we tested the time-course evolution of *in vivo* transfection with these polyplexes.

To identify the organs that were efficiently transfected by systemic administration of nanoparticles and to determine the time course of *in vivo* transfection, C57Bl6 mice were injected with C60-PEI polyplexes and DNA encoding: (**A**) apoE3 (pcDNA3.1-DYK-apoE3) or (**B**) luciferase (pGL3-CMV-LUC). (**A**). Two and five days after *in vivo* transfection of mice with C60PEI-apoE polyplexes, human apoE gene expression was detected by RT-PCR. For this, various organs were harvested from the injected mice and homogenized in TRIzol. The RNA isolated was subjected to DNase treatment and reverse-transcribed into cDNA. The mRNA expression of human apoE gene was detected by PCR amplification with specific primers. (B). The signal emitted by luciferase was monitored daily using the XenoLight D-Luciferin substrate and IVIS Spectrum *In Vivo* Imaging System (PerkinElmer). The results showed that the strongest signal for luciferase was detected in liver, lung and spleen two days after systemic administration of C60-PEI-DNA nanoparticles. Thus, by RT-PCR (A) or by using IVIS Spectrum *In Vivo* Imaging System (B), we determined the organs efficiently transfected with C60-PEI-DNA polyplexes and we tested the time-course evolution of *in vivo* transfection using these polyplexes.

In conclusion, the results obtained in this project showed that C60PEI-DNA polyplexes can be successfully used for *in vivo* transfection. The results obtained within the project are part of a manuscript in preparation.

Dissemination

The results obtained this year within the project were presented as posters at the following scientific events:

- "***Fullerene-based nanoparticles conjugated with apolipoprotein E encoding DNA for gene therapy purposes***", Violeta G. **Trusca**, Ioana M. Fenyo, Mădălina Dumitrescu, Mariana Pinteala, Anca V. Gafencu, poster presented at "The 11th National Congress with International Participation and The 37th Annual Scientific Session of the Romanian Society of Cell Biology", Constanta, June, 2019. This poster received „Special Mention - Award".
- "**Optimization** ***of transfection using fullerene-based nanoparticles for gene therapy purposes***", Violeta G. **Trusca**, Ioana M. Fenyo, Mădălina Dumitrescu, Mariana Pinteala, Anca V. Gafencu, poster presented at Annivrsary Simposium of IBPC "N. Simionescu", " A fascinating journey of 40 years to discover the secrets of the cell for the benefit of human health" - Institute of Cellular Biology and Pathology „Nicolae Simionescu", Romanian Academy, Bucharest, 19-20 September 2019.

Publications:

- „***The Opposite Effect of c-Jun Transcription Factor on Apolipoprotein E Gene Regulation in Hepatocytes and Macrophages***". Int J Mol Sci. 2019 Mar 23;20(6). pii: E1471. doi: 10.3390/ijms20061471. **Trusca** VG, Fuior EV, Kardassis D, Simionescu M, Gafencu AV. PMID:30909560. Impact Factor: 4.3.
- „***The mechanism of Bisphenol A atherogenicity involves apolipoprotein AI downregulation through NF-κB activation".*** **Trusca** VG, Dumitrescu M, Fenyo IM, Tudorache FI, Simionescu M, Gafencu AV. This article was sent to publication to International Journal of Molecular Sciences. Impact Factor: 4.3.
- „***Fullerene-based nanoparticles conjugated with apolipoprotein E encoding DNA used as an anti-atherosclerosis therapy***", manuscript in preparation.