Z1-3193 Optimizirana tehnika trans-spajanja RNA preko izrezovalno-povezovalnega kompleksa za celično slikanje in terapijo

Optimized spliceosome-mediated RNA trans-splicing for cellular imaging and therapy

Vodja projekta | Project manager: dr. Petra Sušjan

1. VSEBINSKI OPIS PROJEKTA | CONTENT DESCRIPTION OF THE PROJECT:

Medtem ko se pri kanoničnem spajanju RNA po izrezu introna združijo eksoni istega transkripta, je trans-spajanje RNA alternativna oblika spajanja, kjer se združujejo eksoni iz različnih transkriptov in je navdihnila umetno strategijo zamenjave eksonov - tehniko trans-spajanja RNA preko izrezovalno-povezovalnega kompleksa (SMaRT). Osrednjega pomena pri tej tehniki je racionalno načrtovanje RNA molekule, ki se veže na tarčni transkript in v bližino izrezovalno-povezovalnega kompleksa dostavi eksone z željenimi modifikacijami. Trans-spajanje ohranja endogeno gensko regulacijo, omogoča pa zamenjavo tako večjih kot manjših delov gena, zaradi česar je obetavno raziskovalno in terapevtsko orodje. Tehniko trenutno bremeni slaba učinkovitost, vendar študije kažejo, da je le-to možno povečati z optimizacijo konstruktov za trans-spajanje. V tem raziskovalnem projektu želimo uporabiti trans-spajanje na dveh področjih zanimanja naše raziskovalne skupine: pri mikroskopski vizualizaciji inflamasoma NLRP3 in korekciji mutacij, povezanih s sindromom CTNNB1. Na področju inflamasoma NLRP3 želimo trans-spajanje uporabiti kot novo sredstvo za fluorescenčno označevanje endogenih proteinov, na področju sindroma CTNNB1 pa bi radi z novimi pristopi izboljšali učinkovitost trans-spajanja kot sredstva za zamenjavo mutiranih eksonov. Učinkovitost trans-spajanja bomo najprej preizkusili z različnimi poročevalskimi sistemi, kasneje pa z virusno dostavo konstruktov za trans-spajanje v celični in mišji model.

In contrast to canonical splicing, where exons of the same transcript are joined after intron excision, trans-splicing, an alternative form of splicing, combines exons from different transcripts and has inspired an artificial exon replacement strategy – spliceosome-mediated pre-RNA trans-splicing (SMaRT). Central to this technique is the rational design of the RNA molecule, which binds to the target transcript during splicing and delivers exons with the desired modifications into the vicinity of the spliceosome. Trans-splicing maintains endogenous gene regulation and allows the replacement of larger or smaller gene parts, making it a promising research and therapeutic tool. Its breakthrough is burdened by its poor efficiency, however studies show that the efficiency can be increased with optimization of the trans-splicing construct design. In this research project, we want to use trans-splicing in two areas of interest of our research group: microscopic visualization of NLRP3 inflammasome and in the correction of mutations associated with CTNNB1 syndrome. In terms of inflammasome, we intend to use trans-splicing as a new means for fluorescent labeling of endogenous proteins in the NLRP3 inflammasome. In terms of CTNNB1 mutations, we would like to improve trans-splicing efficiency with novel approaches to further the mutated CTNNB1 exon correction. Trans-splicing efficacy will be tested with various reporting systems and later upon the viral delivery of trans-splicing constructs to cells or mouse model.

a. osnovni podatki glede financiranja | basic information on funding:

Projekt sofinancira ARRS s 1700 letnimi urami cenovnega razreda B za obdobje 2 let. Pričetek financiranja je 1. 10. 2021.

The project is co-financed by ARRS with 1700 annual hours of price class B for a period of 2 years. Funding starts on October 1, 2021.

b. sestava projektne skupine s povezavami na SICRIS | composition of the project team with links to SICRIS

Nosilka podoktorskega projekta | Holder of the postdoctoral project:

37644 **dr. Sušjan Petra** http://www.sicris.si/search/rsr.aspx?lang=slv&id=43456

2. faze projekta in njihova realizacija | project phases and their realization

Projekt bo potekal po sledečih fazah:

- Načrtovanje in optimizacija konstruktov za modifikacijo RNA na podlagi trans-spajanja
- Ocenjevanje učinkovitosti in natančnosti trans-spajanja RNA na sesalskih celicah
- Optimizacija dostave konstruktov za trans-spajanje v sesalske celice
- Vzpostavitev sistema za vizualizacijo tvorbe endogenih proteinov inflamasoma
- Spremljanje fenotipa miši z mutacijami CTNNB1 po dostavi konstruktov za trans-spajanje

Project phases are the following:

- Design and optimization of constructs for RNA modification based on trans-splicing
- Assessment of trans-splicing efficiency and precision in mammalian cells
- Optimization of the construct delivery to mammalian cells
- Establishment of visualisation system for inflammasome assembly from endogenous proteins
- Monitoring the phenotype of mice with CTNNB1 mutations upon trans-splicing construct delivery

3. bibliografske reference, ki izhajajo neposredno iz izvajanja projekta | bibliographic references arising directly from the implementation of the project

SUŠJAN, Petra, ŽELEZNIK RAMUTA, Taja, BORŠIĆ, Elvira, OREHEK, Sara, HAFNER BRATKOVIČ, Iva (avtor, korespondenčni avtor). Supramolecular organizing centers at the interface of inflammation and neurodegeneration. Frontiers in immunology. 1 Aug. 2022, vol. 13, str. 1-28, ISSN 1664-3224. https://dirros.openscience.si/IzpisGradiva.php?id=15673, DOI: 10.3389/fimmu.2022.940969. [COBISS.SI-ID 120421379]

MIROŠEVIČ, Špela, KHANDELWAL, Shivang, **SUŠJAN, Petra**, ŽAKELJ, Nina, GOSAR, David, FORSTNERIČ, Vida, LAINŠČEK, Duško, JERALA, Roman, OSREDKAR, Damjan. Correlation between phenotype and genotype in CTNNB1 syndrome : a systematic review of the literature. International journal of molecular sciences. 2022, vol. 23, no. 20, str. 1-24, ISSN 1422-0067. DOI: 10.3390/ijms232012564. [COBISS.SI-ID 126831619]

4. Vir financiranja | Financing agency:

arrs

JAVNA AGENCIJA ZA RAZISKOVALNO DEJAVNOST
REPUBLIKE SLOVENIJE