

Šifra projekta / Project code J4-4563

Uporaba izboljšanega sistema CRISPR/Cas za ne-virusno produkcijo celic CAR-T
Use of an improved CRISPR/Cas system for non-viral production of CAR-T cells

Vodja projekta:

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1. VSEBINSKI OPIS PROJEKTA:

V predlaganem projektu želimo uporabiti naš izboljšan sistem CRISPR/Cas, ki se je izkazal za zelo učinkovitega pri modifikacijah genoma, za produkcijo celic CAR-T na ne-virusni način, torej z namenom izogib stranskim učinkom virusne priprave celic CAR-T. Izboljšan sistem CRISPR/Cas (CCExo) temelji na paru heterodimernih peptidov, ki tvorijo obvite vijačnice. Določen peptid para je povezan s proteinom Cas9, drug par peptida, ki tvori obvito vijačnico pa je povezan z eksonukleazo. Nastanek obvite vijačnice tako pripelje eksonukleazo v bližino DNA dvojno-verižnega preloma. Eksonukleaza na mestu preloma dodatno cepi DNA konce, tako da ustvari komplementarne enoverižne konce DNA. DNA matrica, ki nosi zapis za želeni transgen bo tudi modificirana. Proces cepitve DNA koncev na genomu in modifikacija DNA matrice omogoči lažjo tarčno vstavev transgena in končni višji odstotek celic CAR-T.

In the proposed project, we want to use our improved CRISPR/Cas system, which has proven to be very effective in genome modifications, for the production of CAR-T cells in a non-viral way, i.e. with the aim of avoiding the side effects of viral preparation of CAR-T cells. The improved CRISPR/Cas system (CCExo) is based on a pair of heterodimeric peptides that form coiled helices. A certain peptide from the heterodimeric peptide pair is associated with the Cas9 protein, and the other peptide from the pair of peptides forming a coiled helix is associated with an exonuclease. The formation of the coiled helix thus brings the exonuclease close to the DNA double-strand break. The exonuclease further cleaves the DNA ends at the break site, creating complementary single-stranded DNA ends. The DNA matrix that carries the record for the desired transgene will also be modified. The process of cleavage of the DNA ends on the genome and the modification of the DNA template allows for easier targeted insertion of the transgene and a final higher percentage of CAR-T cells.

a. osnovni podatki glede financiranja:

Projekt financira ARRS v okviru cenovne kategorije D za obdobje treh let v obsegu 1971 letnimi urami za obdobje 3 let. Pričetek financiranja je 1. 10. 2022.

The project is co-financed by ARRS with 1971 annual hours of price class D for a period of 3 years. Funding starts on October 1, 2022.

b. sestava projektne skupine s povezavami na SICRIS

Na Kemijskem inštitutu v projektne skupini sodelujejo / At the National Institute of Chemistry the project group includes:

Duško Lainšček; SICRIS št. 34069 <https://cris.cobiss.net/ecris/si/sl/researcher/38518>

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2. faze projekta in njihova realizacija

V projektu načrtujemo uresničitev naslednjih faz (ciljev):

Cilj 1: Sestava CCExo komponent za ne-virusno dostavo

Cilj 2: Ne-virusni nastanek celic CAR-T z določitvijo fenotipa, genotipa in imunoterapevtskih lastnosti

Cilj 3: Testiranje funkcionalnosti celic CAR-T, proizvedenimi z ne-virusnim načinom na različnih mišjih modelih raka

In the project, we plan to implement the following phases (goals):

Objective 1: Build CCExo components for non-viral delivery

Objective 2: Non-viral generation of CAR-T cells by determining phenotype, genotype and immunotherapeutic properties

Objective 3: Testing the functionality of CAR-T cells produced by a non-viral method in different mouse models of cancer

3. bibliografske reference, ki izhajajo neposredno iz izvajanja projekta

LAINŠČEK, Duško, GOLOB URBANC, Anja, MIKOLIČ, Veronika, PANTOVIĆ, Jelica, MALENŠEK, Špela, JERALA, Roman. Regulation of CD19 CAR-T cell activation based on an engineered downstream transcription factor. *Molecular therapy oncolytics*. Jun. 15, 2023, vol. 29, [article no.] p77-90, str. 77-90, ISSN 2372-7705. <https://www.sciencedirect.com/science/article/pii/S237277052300030X>, DOI: [10.1016/j.omto.2023.04.005](https://doi.org/10.1016/j.omto.2023.04.005). [COBISS.SI-ID [154300419](https://cobiss.si/id/154300419)]

4. logotip ARRS in drugih sofinancerjev



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