LentivirusProduction

Improved cell lines for the production of Lentivirus II

Programm / Ausschreibung	Life Sciences, Life Sciences, Life Science Ausschreibung 2023	**Status**	abgeschlossen
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Projektbeschreibung

Lentiviren sind wichtige Werkzeuge in der Molekularbiologie, werden aber auch zunehmend als Genfähren in der humanen Gentherapie eingesetzt. Noch immer ist die Produktion von Lentiviren in hoher Konzentration eine große Herausforderung. In diesem F&E Projekt wollen wir unsere CRISPR Screening Plattform einsetzen, um Gene und Stoffwechselwege zu identifizieren, die die effiziente Produktion behindern oder steigern können. Durch die Modifikation dieser Stoffwechselwege in Produktionszelllinien soll so eine verbesserte Produktion von Lentiviren ermöglicht werden.

Endberichtkurzfassung

Lentiviruses are of significant importance in molecular biology due to their use in the delivery of cDNA expression cassettes to cells. They can infect a broad spectrum of cells and, upon integration into the genome of the host cell, facilitate stable expression of the cargo. In addition to their utilization within molecular biology, lentiviruses are also employed as vectors for cell and gene therapy, such as for the generation of CAR-T cells.

The aim of the project was to employ CRISPR screens for the identification of genes that can be modulated to enhance lentiviral production pipelines. This is important because current workflows are often not scalable and are resource-intensive, which can have a negative impact on the cost of gene therapies. The enhancement may be achieved by increasing the infectivity of individual viral particles or by engineering producer cells to generate more viral particles in total. In both scenarios, more infection-competent particles could be produced per cell, thereby increasing the scale and reducing the cost of lentivirus manufacturing processes. To achieve this objective, multiple genetic screens have been performed to dissect the lentiviral life cycle. Initially, a pooled CRISPR screening workflow was established and optimized to study lentiviral production This method was subsequently validated in a small proof-of-concept screen and resulted in a patent application. Thereafter, an unbiased genome-wide CRISPR screen was performed to assess the involvement of all protein-coding genes in this process, followed by a validation screen of the top 400 hit genes.

ZC3HAV1 (ZAP) and TRIM25, two well-known antiviral proteins that function by inhibiting viral replication, were consistently among the top hit genes. This finding underscores the efficacy and robustness of our screening workflow. Notably, the

unbiased approach additionally identified genes that have not been previously described to be involved in the lentiviral life cycle. To thoroughly assess the impact of these genes, their function was systematically investigated by generating gene knockouts in an arrayed format, followed by the generation of monoclonal knockout cell lines. This methodology was implemented to circumvent the potential masking of effects by clonal variance. Strikingly, the knockout of ZAP and another novel host factor identified in our screening campaign led to a substantial enhancement in lentiviral production in a widely used HEK293 producer cell line, with an increase ranging from 1.5 to 2fold. This finding signifies an advancement in the field, demonstrating a major improvement in the efficiency of lentiviral production.

To complement the gene knockout screens described above, we planned to additionally perform an unbiased genome-wide CRISPR activation screen, but these experiments proved to be more challenging than anticipated. This is mainly because the CRISPR machinery required to perform these screens is considerably larger than that required for CRISPR knockout screens. As a result, it is more difficult to create cell lines in which the CRISPR activation components are stably integrated and active. To overcome this issue, we investigated a recently published novel CRISPR activation system in which the activation module is separated from Cas9 and hence smaller in size. Initial experiments show robust upregulation of selected cell surface receptors, and these results pave the way to perform the originally planned genome-wide screens in the near future.

Projektpartner

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