Spatiotemporal control of T lymphocytes using optogenetic CRISPR

Master project
Department of Medicine
Karolinska Institutet
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Background
CRISPR has emerged as a powerful and versatile system for controlling gene activity and introduction precise mutations in the genome. Spatiotemporal control of CRISPR activity could therefore be useful in future therapeutic settings (e.g. to fine-tune the performance of CAR T-cells), and for addressing new biological questions. Current methods for spatiotemporal control of CRISPR systems are Cas-centered and are mainly based on light-induced dimerization of split-Cas. However, these current systems are unable to harness the full potential of CRISPR, e.g. being unable to control base editing and gene expression in vivo. Therefore, we have developed a new more efficient and versatile blue-light based optogenetic system that unleashes the full potential of optogenetic CRISPR. In contrast to current optogenetic systems, our new system is compatible with all Cas proteins and therefore releases the full potential of optogenetic CRISPR, including spatiotemporal control of point-mutations ("base editing") and gene expression. We have recently developed a new experimental system to deliver a genetically encoded optogenetic CRISPR system to primary mouse T lymphocytes, and in this project, we aim to use this system to knockout genes in T lymphocytes in vivo. Since T lymphocytes are critical for the immune defense against tumors, spatiotemporal control of genes in T lymphocytes will provide new insights into anti-tumoral immune responses.

Aims and purpose:
The principal aim of this project is to achieve optogenetic knockouts of genes in T lymphocytes in vivo in mice. The purpose is to generate an experimental system that allows for detailed spatiotemporal interrogation of anti-tumoral immune responses in vivo. The specific aims are:
-Viral delivery of blue-light induced (optogenetic) CRISPR system to primary T lymphocytes.
-To knockout genes in T lymphocytes in vitro using optogenetic CRISPR.
-Establish adoptive transfer of transduced T lymphocytes.
-To knockout genes in T lymphocytes in vivo using optogenetic CRISPR.

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