Abstract

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Transcriptome Engineering

Although genome engineering with the CRISPR-Cas technology has found many applications in basic research, its therapeutic use remains challenging. For certain therapeutic applications, the site-specific conversion of adenosine to inosine (A-to-I) in RNA might be a safer and more effective alternative. Since inosine is biochemically interpreted as guanosine, site-directed A-to-I RNA editing offers the possibility to manipulate protein and RNA function.

A-to-I editing is catalyzed by adenosine deaminases acting RNA (ADARs) which edit millions of sites in the human transcriptome. To convert these enzymes to specific editing machines, the Stafforst group (University of Tuebingen) fused the ADAR deaminase domains with the so-called SNAP-tag that can covalently bind benzylguanine-modified guide RNA (gRNA). Once bound to the SNAP-ADAR fusion, the gRNA directs the enzyme to its RNA target and forms a secondary structure that is considered necessary for efficient and specific A-to-I editing. During my PhD research in the Stafforst lab, I successfully established the editing tool in cell culture by using chemically modified gRNAs. SNAP-ADARs can edit adenosines in endogenous transcripts with yields up to 90%. Additionally, I demonstrated the concurrent editing of two disease-relevant transcripts, KRAS and STAT1, which highlights the potential of this tool to be applied for manipulating entire signaling networks. My PhD work has significantly contributed to the fact that the SNAP-ADAR technology is currently the best characterized editing approach and offers the best balance between editing efficiency and specificity compared with all others approaches using engineered ADAR fusions.

Another possibility to perform site-directed A-to-I RNA editing was recently published by the Stafforst group which directed endogenous ADAR to user-defined target RNAs with antisense oligonucleotides (ASOs). This approach circumvents off-target editing that is associated with the ectopic overexpression of an engineered ADAR fusion, and requires only the administration of a chemically modified ASO. Together with the Stafforst lab, I am currently working on establishing the new editing tool in mouse models, which is an important proof of concept towards its therapeutic application. In first in vivo experiments, we indeed demonstrated the site-specific repair of a disease-causing G-to-A point mutation. We will further work on the optimization of the technology in order to obtain highest possible efficacy in mice. This should bring us a step closer towards the clinical application of site-directed RNA editing in future.