Harnessing the Therapeutic Potential of RNA-Editing by Human ADARs

**Technology Description**

Adenosine deaminases that act on RNA (ADAR) are a class of enzymes that catalyze the conversion of adenosine to inosine in RNA-molecules during translation. Since inosine is read as guanosine ADAR activity formally introduces A-to-G point mutations. Here we describe the first design of genetically encodable trans-acting guideRNAs that enable the readressing of human ADAR2 toward specific sites in selected mRNA targets.

Our design is based on the R/G-hairpin structure of the Glutamate 2 transcript. At the R/G-site of the natural transcript, a cis-located intronic sequence folds back to the exon under formation of a bulged stem loop structure that recruits ADAR2 via its two dsRNA Binding Domains (dsRBDs, see Figure 1A).

We decided to cut the native R/G-site between the two guanosines five and six nucleotides downstream of the editing site. The complementary RNA strand for targeting a desired mRNA can be added to the 3’end of the shortened RNA stem loop (Figure 1B).

**Figure 1**

Natural cis-acting R/G-site of the glutamate receptor transcript as a basis for the new engineered trans-acting R/G-guideRNAs. Replacement of the 3’ end by new binding sites allows the specific targeting of selected mRNA codons.

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**Innovation**

**Up to now:** Use of ADAR technology in living cells only possible via transgenic expression of chemically altered ADAR enzymes (SNAP-tag technology)

**Now:** Versatile redirection of ADAR enzymes to any given RNA target via application of specifically designed guideRNAs

**Applications**

- Treatment of genetic diseases caused by G→A point mutations
- Manipulation at the RNA level could complement therapeutic genome editing.

**Advantages**

- Addressing disease-causing point mutations at RNA-level is more selective and reversible compared to interventions at DNA-level.
- New Research Tool for reprogramming single amino acids to alter protein function, stability and processing (feasible codon changes see Table 1 next page)

- Manipulation of genetic information at RNA-level might surpass DNA-manipulation in terms of safety and ethics.
Proof of Concept

Restoring Functionality in a Truncated Version of PINK1

The PINK1 W437Stop mutation is linked to an inheritable monogenetic form of Parkinson’s disease characterized by a loss of mitophagy under stress conditions. A central player in this cellular process is the protein Parkin that has to be activated by a second protein, PINK1.

As proof of concept we have prepared HeLa cells expressing a Parkin-GFP fusion-protein and different PINK1-mutants from added vector plasmids. Fluorescence signals of cells were read out 2 h after adding the mitochondrial uncoupler CCCP (Carbonyl cyanide m-chlorophenylhydrazone).

- Figure 2A) Positive Control. Wildtype HeLa cells with Parkin-GFP and PINK1. Note the typical clustering of Parkin near the mitochondrial membrane 2 h after stress induction.
- Figure 2B) Negative Control. HeLa cells with Parkin-GFP, PINK1-Knockout and transgenic PINK1 with an amber stop codon in the coding sequence (W437X PINK1). No Parkin-clustering around mitochondrial membranes.
- Figure 2C) Editing. Addition of ADAR2 together with a guide RNA that addresses the amber stop codon restores a functional PINK1 protein by A→I editing. The typical Parkin-clustering around mitochondrial membranes occurs once again.

Quantitative Measurement of Restoration A Toolbox for Codon Correction

Figure 3

Percentage of Parkin-translocation to mitochondrial membranes. RNA-Editing by ADAR leads to restoration of defective Pink1 and renewed Parkin-clustering under stress conditions.

<table>
<thead>
<tr>
<th>Prior to Editing</th>
<th>After A→I Editing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ser (AGU/C)</td>
<td>Gly (IGU/C)</td>
</tr>
<tr>
<td>Tyr (UAU/C)</td>
<td>Cys (UIU/C)</td>
</tr>
<tr>
<td>Thr (ACA/C/G/U)</td>
<td>Ala (ICA/C/G/U)</td>
</tr>
<tr>
<td>Ile (AUA/C/A)</td>
<td>Val (IUU/C/A)</td>
</tr>
<tr>
<td>Start/Met (AUG)</td>
<td>Val (IUG)</td>
</tr>
<tr>
<td>Stop (UAA)</td>
<td>Stop (UAI, UIA)</td>
</tr>
<tr>
<td>Stop (UAG, UGA)</td>
<td>Trp (UIG)</td>
</tr>
<tr>
<td>His (CAU/C)</td>
<td>Arg (CIA/G)</td>
</tr>
<tr>
<td>Arg (AGA/G)</td>
<td>Gly (IGA/G)</td>
</tr>
<tr>
<td>Lys (AAA/G)</td>
<td>Arg (AIA/G); Glu (IAA/G)</td>
</tr>
<tr>
<td>Asn (AAU/C)</td>
<td>Asp (IAU/C); Ser (AIU/C)</td>
</tr>
<tr>
<td>Gln (CAA/G)</td>
<td>Arg (CIA/G)</td>
</tr>
<tr>
<td>Asp (GAU/C)</td>
<td>Gly (GIA/G)</td>
</tr>
<tr>
<td>Glu (GAA/G)</td>
<td>Gly (GIA/G)</td>
</tr>
</tbody>
</table>

Table 1

RNA-Editing by ADAR permits the manipulation of many codons. One example is the change of a premature amber stop codon to a Trp codon.
