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Harnessing the Therapeutic Potential of RNA-Editing by Human ADARs

Technology Description

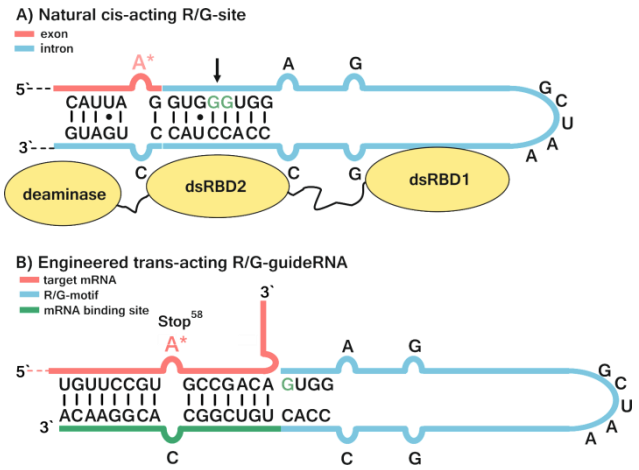

Figure 1
Natural cis-acting R/G-site of the glutamate receptor transcript as a basis for the new engineered trans-acting R/G-guideRNAs. Replacement of the 3' end by new binding sites allows the specific targeting of selected mRNA codons.

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 readdressing of human ADAR2 toward specific sites in selected mRNA targets.
Our design is based on the R/G-hairpin structure of the GluR2 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).

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

IP Status

Patent application filed

Priority date 2015-09-26

We are looking for an industrial partner for research collaboration and licensing.

Applications

- Treatment of genetic diseases caused by G→A point mutations
  Manipulation at the RNA level could complement therapeutic genome editing.
- New Research Tool for reprogramming single amino acids to alter protein function, stability and processing (feasible codon changes see Table 1 next page)

Advantages

- Addressing disease-causing point mutations at RNA-level is more selective and reversible compared to interventions at DNA-level.
- Manipulation of genetic information at RNA-level might surpass DNA-manipulation in terms of safety and ethics.

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SCIENTIST

PROOF OF CONCEPT

Restoring Functionality in a Truncated Version of PINK1

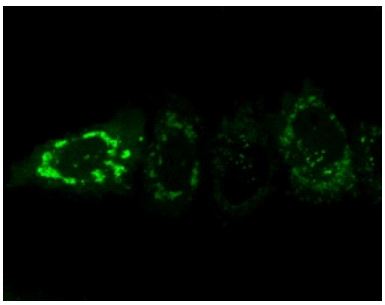

Figure 2A

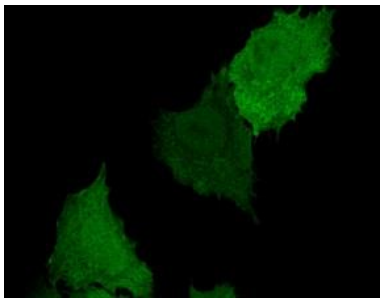

Figure 2B

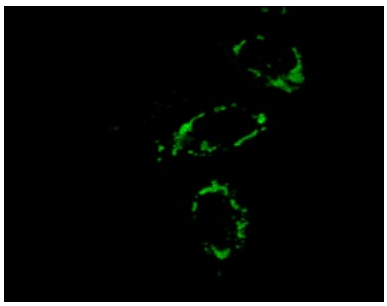

Figure 2C

The *PINK1* W437Stop mutation is linked to an inheritable monogenetic form of Parkinson´s disease[1] 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

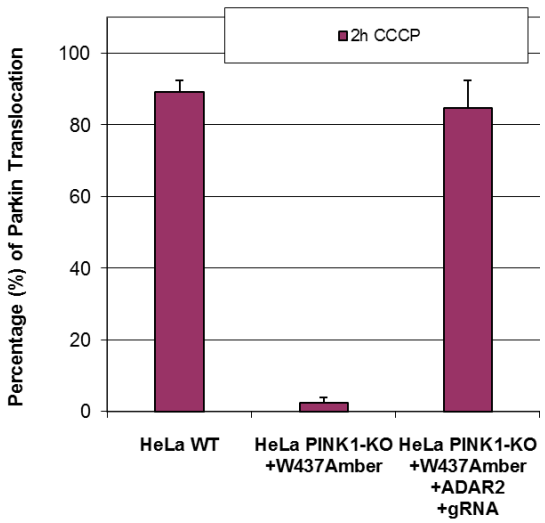

Figure 3
Percentage of Parkin-translocation to mitochondrial membranes. RNA-Editing by ADAR leads to restoration of defective Pink1 and renewed Parkin-clustering under stress conditions.

A Toolbox for Codon Correction

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

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.

REFERENCES

1 J. Wettengel et al. Harnessing human ADAR2 for RNA repair – Recoding a PINK1 mutation rescues mitophagy. (2016) Nucl. Acids Res. 1; doi: 10.1093/nar/gkw911

2 MF Schneider et al. Optimal guideRNAs for re-directing deaminase activity of hADAR1 and hADAR2 in trans. (2014) Nucleic Acids Res. 2014 Jun 1; 42(10): e87; doi: 10.1093/nar/gku272