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CRISPR-Cas9 mediated efficient PD-1 disruption on human primary T cells for adoptive therapy

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Background

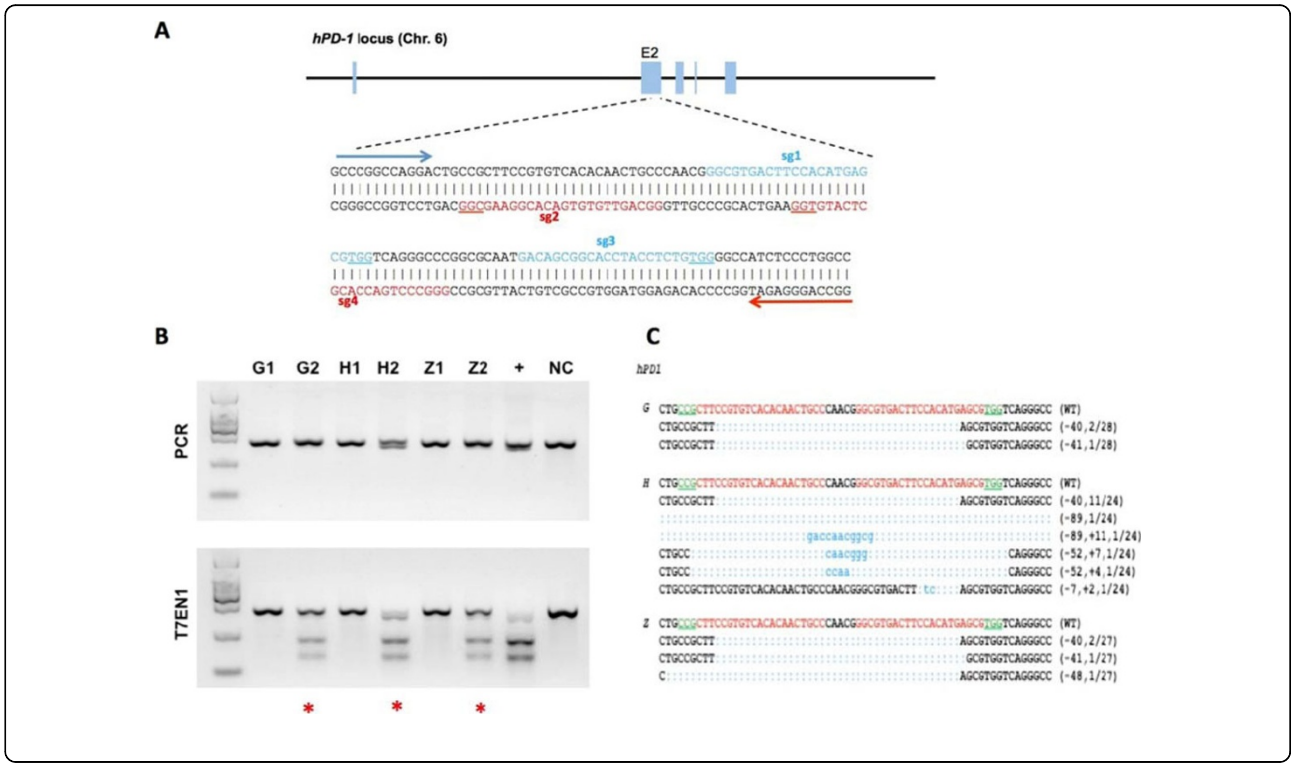
Strategies that enhance the function of T cells are critical for immunotherapy.

Methods

Here we described for the first time a non-viral mediated approach to reprogram primary human T cells by disruption of PD-1.

Results

We showed that the gene knockout of PD-1 by electroporation of plasmids encoding sgRNA and Cas9 was technically feasible. The disruption of PD-1 resulted in significant reduction of PD-1 expression but didn't affect the viability of primary human T cells. Cellular immune response of the gene modified T cells was characterized by up-regulated IFN-γ production and enhanced cytotoxicity.



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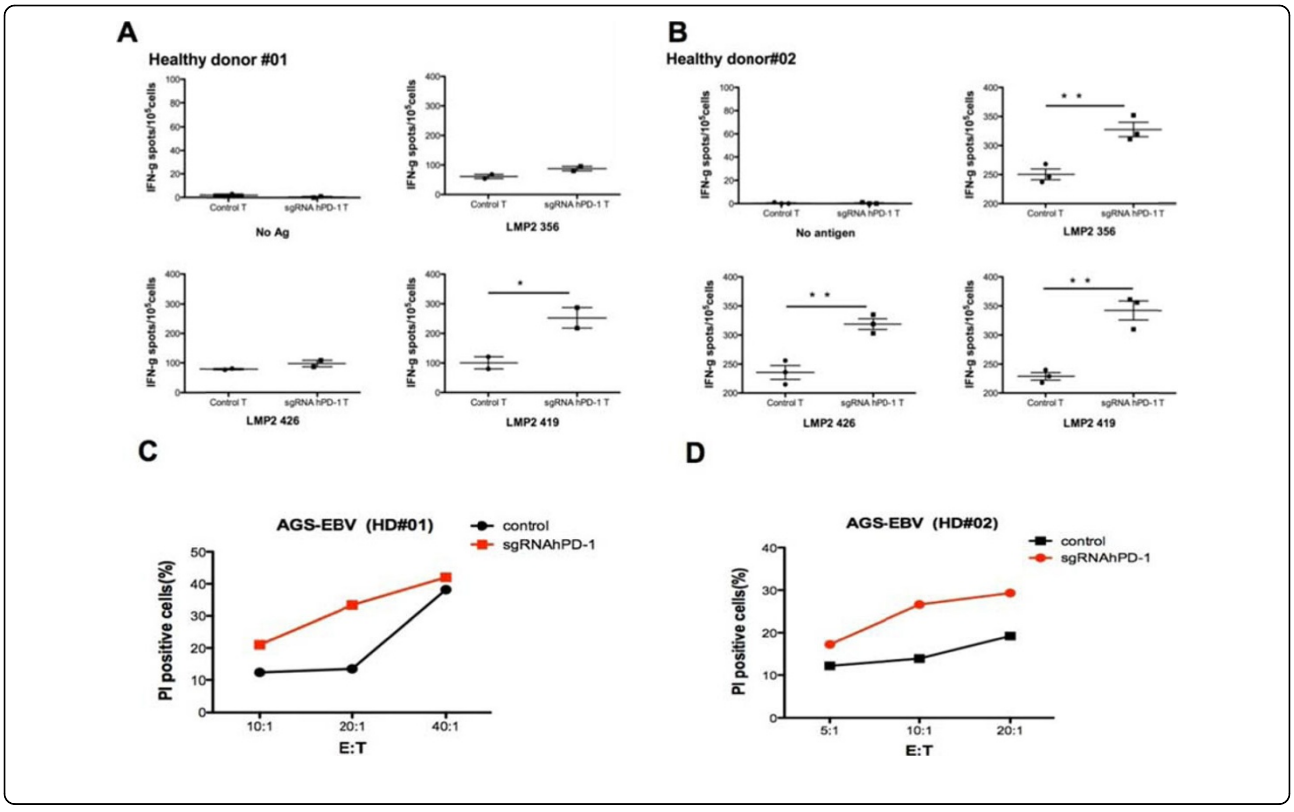
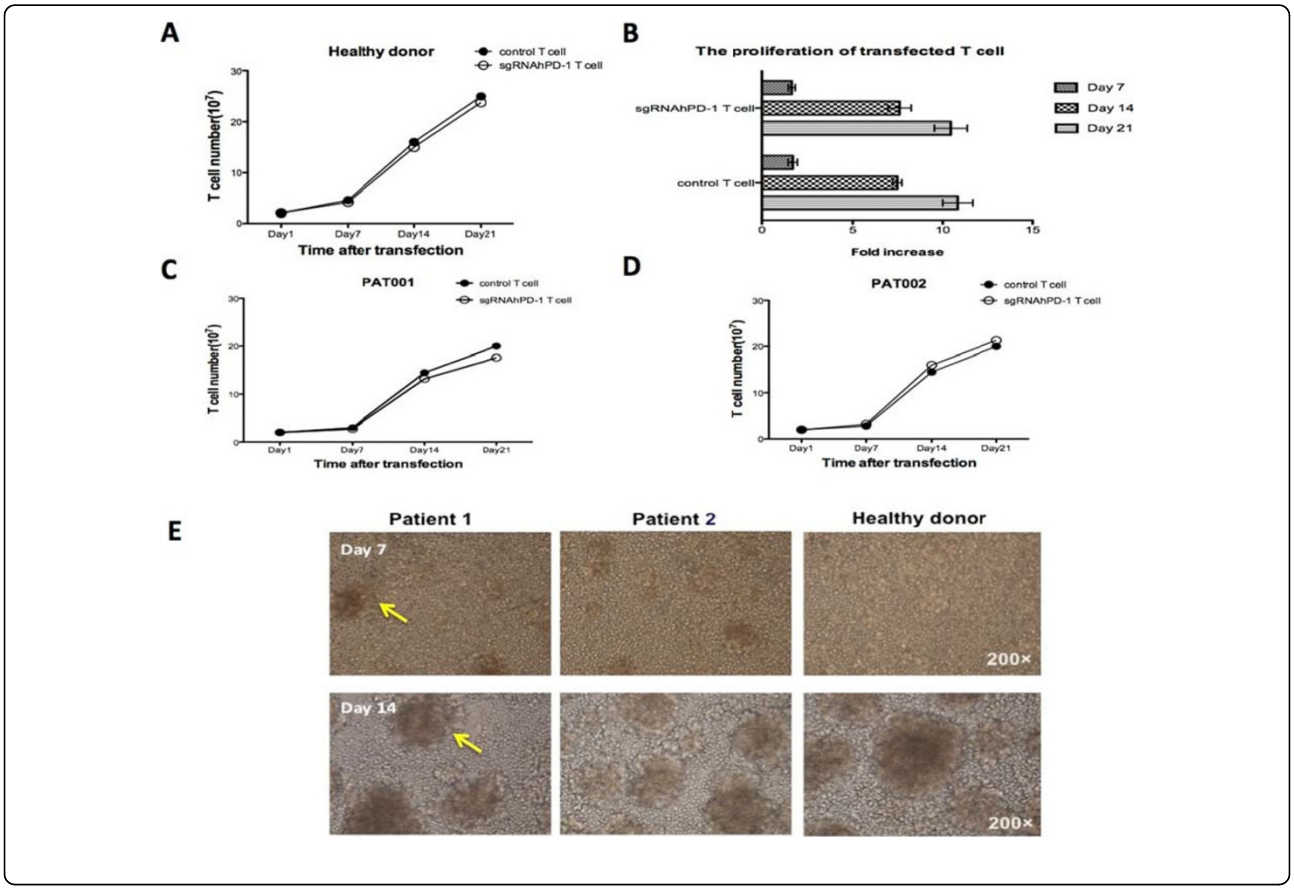

A
Healthy donor #01
IFN-g spots/10^5cells
Control T
sgRNA hPD-1 T
No Ag
LMP2 356
LMP2 426
LMP2 419
B
Healthy donor#02
No antigen
C
AGS-EBV (HD#01)
control
sgRNAhPD-1
PI positive cells(%)
10:1
20:1
40:1
E:T
D
AGS-EBV (HD#02)
5:1


A
Healthy donor
control T cell
sgRNAhPD-1 T cell
T cell number(10^7)
Day1
Day7
Day14
Day21
Time after transfection
B
The proliferation of transfected T cell
Day 7
Day 14
Day 21
Fold increase
C
PAT001
D
PAT002
E
Patient 1
Patient 2
Healthy donor
Day 7
Day 14
200×

Conclusions

These results suggest that we have established an approach for efficient checkpoint inhibitor disruption, providing a new strategy for targeting checkpoint inhibitors to improve the efficacy of T cell based adoptive therapies.

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