Resveratrol Treatment Increases Homology-Directed Repair in Primary Human Fibroblasts and iPSCs

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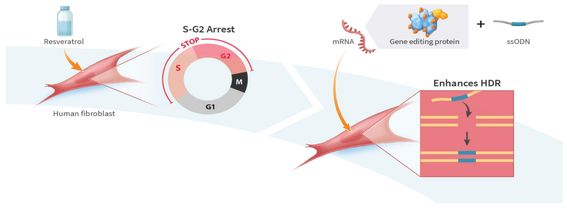
Introduction

Gene editing typically employs sequence-specific endonuclease to create double strand breaks in genomic DNA, and relies on cell's DNA repair mechanisms to apply the desired changes. Precise sequence modifications such as single-base changes or targeted sequence insertions relies on homology directed repair (HDR) mechanism which has considerably lower efficiency than non-homologous end joining (NHEJ) repair mechanism. Here, we evaluate the impact of resveratrol, a small molecule extracted from grape skin that has recently shown to promote the expression of key HDR factors and induce cell cycle arrest at S phase in porcine fetal fibroblasts. We tested effect of resveratrol on single-base editing efficiency in primary human fibroblasts and 1kb sequence insertions efficiency in human iPSCs.



Methodology

Gene editing mRNA & Repair template preparation

Step 1: Design and synthesize **Ultraslice** mRNA pair targeting AAVS1 site

Step 2: Prepare single stranded DNA repair templates with AAVS1 homology arms

Ultraslice mRNA & single stranded repair template delivery / resveratrol incubation

Step 3: Transfect Ultraslice mRNA and desired repair templates using Neon Transfection system to Fibroblasts and iPSCs

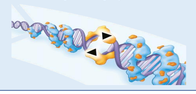
Step 4: Incubate **fibroblasts** (48h) **and iPSCs** (24h) with 25uM resveratrol

AAVS1 locus amplification & SfoI assay

Step 5: Extract gDNA from transfected cells and perform AAVS1 locus amplification

Step 6: Perform RFLP assay (SfoI assay) on AAVS1 PCR amplicon, load SfoI assay product on agarose gel then visualize DNA bands using Chemidoc and Image J software

Ultraslice: Chromatin context-sensitive gene editing endonuclease



Repair templates: Single stranded DNA repair templates synthesized using lambda exonuclease digestion method

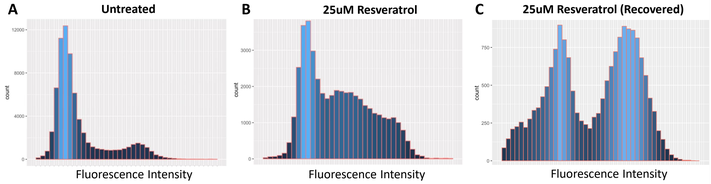
Cell culture:

	Fibroblasts	iPSCs
Media	DMEM + 10% FBS	MteSR+Y27632
Setting	5% CO2 & 5% O2	5% CO2 & 5% O2
Temperature	37 degree C	37 degree C

Resveratrol treatment increases S and G2 population in primary human fibroblasts

Cell cycle analysis

1. **Conditions**
   - **Untreated**: Resveratrol was not added
   - **25uM Resveratrol**: 25uM resveratrol was added 48 hours prior to cell cycle analysis
   - **25uM Resveratrol (Recovered)** : 25uM resveratrol was treated for 48 hours, then removed so that cells are recovered in DMEM + 10% FBS for 8 hours prior to cell cycle analysis
2. **Staining Nuclear DNA / Quantify DNA fluorescence intensity**
   - After staining nuclear DNA with Hoeschst dye, DNA fluorescence intensity was measured using fluorescence microscope
3. **Assigning cell cycle based on relative DNA amount**



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Treatment	S / G2 percentage
Untreated	31%
25uM Resveratrol	60%
25uM Resveratrol (Recovered)	71%

Figure 1: Resveratrol treatment increases S and G2 population in primary human fibroblasts A. Default cell cycle distribution of untreated fibroblasts is shown here. Majority of cell population seems to be G1 phase where relative DNA amount is lower than S and G2 phase. **B.** When treated with 25uM resveratrol for 48 hours, percentage of S and G2 population increased to 60%, indicating that resveratrol treatment induce cell cycle arrest at S and G2 phase. **C.** 25uM resveratrol treatment followed by 8 hour recovery in DMEM + 10% FBS showed even higher percentage of fibroblasts in S and G2 phase (71%). **D.** The table shows S and G2 population percentage in tested conditions.

Resveratrol treatment increases base-editing efficiency in primary human fibroblasts

Resveratrol incubation / Data analysis

1. **Conditions**
   - **Untreated**: Resveratrol was not added
   - **25uM Resveratrol - Prior**: Fibroblasts were incubated with 25uM resveratrol 48 hours prior to transfection and removed at the time of transfection
   - **25uM Resveratrol - Post** : Fibroblasts were incubated with 25uM resveratrol for 48 hours from transfection
2. **RFLP assay**
   - AAVS1 PCR amplicon was digested using SfoI assay
   - SfoI enzyme recognizes and cuts GGCGCC base that is present in AAVS1 locus after successful base editing
3. **Statistical Analysis**
   - Single factor ANOVA was used to compare tested conditions and obtain p-value

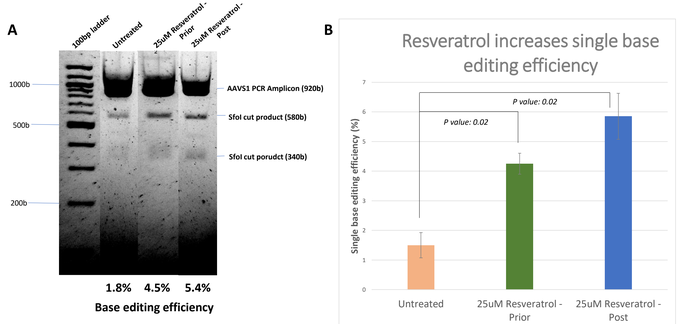


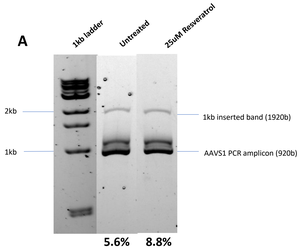
Figure 2: Resveratrol treatment increases single base editing efficiency in primary human fibroblasts A. The gel image shows SfoI assay product bands indicating that there was base editing. Untreated fibroblasts showed 1.8% base editing efficiency while 25uM Resveratrol – Prior condition showed 4.5% base editing efficiency and 25uM Resveratrol – Post condition showed 5.3% base editing efficiency, indicating that resveratrol treatment increases HDR rate. **B.** The graph shows both 25uM Resveratrol – Prior and 25uM Resveratrol – Post treated conditions significantly increased base editing efficiency.

Resveratrol treatment increases 1kb insertion efficiency in iPSCs

Resveratrol incubation / Data Analysis

1. **Conditions**
   - **Untreated**: Resveratrol was not added
   - **25uM Resveratrol**: iPSCs were incubated with 25uM Resveratrol for 24 hours from transfection
2. **Amplicon visualization**
   - 1kb inserted band was directly visualized on 2% Agarose E-gel

*** Clean-cap & Templated-tail iVT-synthesized Ultraslice used for this experiment**



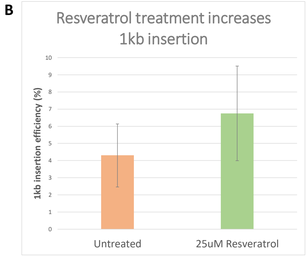


Figure 3: Effect of resveratrol on 1kb insertion efficiency in iPSCs A. The gel image shows 1kb inserted DNA band (1920b) and AAVS1 PCR amplicon (920b). 25uM resveratrol treatment increased 1kb insertion efficiency from 5.6% to 8.8% (1.6 fold increase). **B.** The graph shows 25uM resveratrol treatment increases 1kb insertion efficiency in iPSC.

Sanger sequencing data confirms successful 1kb insertion in AAVS1 site in iPSCs

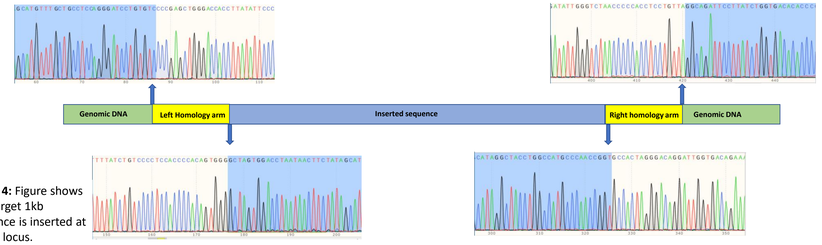


Figure 4: Figure shows that target 1kb sequence is inserted at AAVS1 locus.

Conclusion

We demonstrate that 25uM resveratrol treatment increased S and G2 cell cycle phase population in primary human fibroblasts. Also, data suggest that resveratrol treatment in fibroblasts and iPSCs can increase efficiency of single base editing and targeted 1kb sequence insertion. Resveratrol treatment provides a straightforward way to improve HDR efficiency in primary human fibroblasts and iPSCs, and may potentially serve as a useful tool to develop HDR-based gene-editing therapies.

Acknowledgements

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This work is protected by one or more patents.