Expression and epigenetic profile of different CRISPR-Cas9 efficiency clones

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Abstract

CRISPR/Cas9 cleavage efficiency is crucial in a genomic editing experiment. However, the molecular mechanisms that underlie this cleavage difference remain unclear. In our study, we characterized genome-wide gene expression and epigenetic features in CRISPR-Cas9 low and high clones across 3 different cell lines. We show that Cas9 expression level is relatively higher in high efficiency clones. Notably, histone mark ChIP-seq data demonstrate that differentially expressed genes also have a different acetylation of histone 3 at lysine 27 (H3K27ac) level. Finally, we observed that PARVA is an important gene that can enhance CRISPR-Cas9 efficiency.

Background

Clustered regularly interspaced short palindromic repeat (CRISPR) are essential components of nucleic-acid-based adaptive immune systems that are widespread in bacteria and archaea[1]. In 2012, a family of endonucleases that use dual-RNAs for site-specific DNA cleavage was revealed and the potential to exploit the system for RNA-programmable genome editing was highlighted[2]. CRISPR-Cas9 system was used in genome-scale functional screening by two different research groups in 2014[3,4]. Afterwards, another group performed CRISPR/Cas9 screening in HeLa cell line using a small library containing 869 sgRNAs targeting 291 genes[5].

CRISPR/Cas9 is a very versatile tool in genome editing work. Cleavage efficiency matters a lot in a successful experiment. Attempts have been made to improve the efficiency. For example, the use of long single stranded DNA (lssDNA) molecules as donors efficiently yielded founders bearing the conditional allele[6]. CRISPR/Cas9 allows the generation of knockout cell lines and null zygotes by inducing site-specific double-stranded breaks. The null effect could be increased with an sgRNA targeting the splice donor site (SDS) of the chosen exon[7]. However, it is not clear what factors may affect the efficiency. So we did the expression and epigenetic profile in CRISPR-Cas9 high and low efficiency clones in order to illustrate the point.

Results

Cas9 single clone shows differential cleavage efficiency by T7E1 digestion examination

We obtained different CRISPR/Cas9 cell lines from Wei lab. Among them, one is HeLa-sc8 high efficiency clone as they reported previously[5]. Other 2 cell lines are HT1080 and 293T. We used sgRNA targeting CSPG4 gene to validate the cutting efficiency as reported[8]. Different CRISPR editing efficiency clones are showed in gel figure (Fig 1a). The efficiency ranges from 3% to 27%. Then we carried out a genome-wide transcriptomic and epigenetic assessment on these clones (Fig 1b). RNA-seq and H3K27ac ChIP-seq were performed respectively in these clones.

Exploring the genomic expression difference in CRISPR-Cas9 high and low efficiency clones by RNA-seq

On average, we sequenced each sample at ~50 million PE50 fragments and observed ~90% read mappability (Supplementary file 2). Cas9 with OCT1 and BSD were constantly expressed in different clones (Fig 2a). We performed RNA-seq in 293T, HT1080 and HeLa to seek the genes that may correlate with cleavage efficiency. RPKM value was calculated for Cas9, BSD and Oct1 respectively (Fig 2a). Gene expression analysis revealed that Cas9 is higher expressed in high efficiency clone than low efficiency clone (Fig 2b). OCT1 and BSD genes are also showing the same pattern with Cas9 (Fig 2b). A total of more than 200 genes were differentially expressed in high and low clones. Then we performed Gene Ontology analysis for differential expressed genes (Fig 2c). GO analysis showed that differential expressed genes are extracellular matrix organization, extracellular structure organization related genes (Fig 2c). Specifically, antigen processing and presentation of peptide antigen via MHC class-related genes were up-regulated in efficiency high clones in 293T cells. We next analyzed the genes that were differentially expressed between CRISPR-Cas9 high efficiency clone and low efficiency clone. There are 58 up-regulated and 35 down-regulated genes in CRISPR/Cas9 high efficiency clones relative to low efficiency clones (Fig 2d and Supplementary file 1). In addition, UNC5B was up-regulated in high efficiency clones compare to low efficiency clones, and PARVA was down-regulated in high efficiency clones compare to low efficiency clones (Fig 2d).

Exploring the epigenetic difference of CRISPR/Cas9 high and low efficiency clones by H3K27ac ChIP-seq

Epigenetic changes are important features in biological process^[9-11]. We next examined the acetylation of histone 3 at lysine 27(H3K27ac) level of CRISPR/Cas9 high and low efficiency clones. We performed H3K27ac ChIP-seq in 293T low and high CRISPR/Cas9 efficiency cell lines. H3K27ac, which is a marker of actively transcribed genes, showed clear differences between high and low efficiency clones. In total, 77,977 peaks were identified in high efficiency clones, and 77,606 peaks were identified in low efficiency clones. Venn diagram shows that H3K27ac peak overlap by number of 51,107 (Fig 3a). We then profiled H3K27ac signal on differential genes between 293T high efficiency clone and 293T low efficiency clones (Fig 3b). Differential expressed genes also have different H3K27ac level. We separated differential expressed genes into 2 groups, including up genes and down genes. For up genes, H3K27ac level is higher on the nearby peaks in CRISPR-Cas9 high efficiency clone than CRISPR-Cas9 low efficiency clone (Fig 3c, up panel). For down genes, H3K27ac level is higher on the nearby peaks in CRISPR-Cas9 low efficiency clone than CRISPR-Cas9 high efficiency clone (Fig 3c, down panel). Genome browser showed unique peaks in low and high CRISPR-Cas9 efficiency cell lines (Fig 3d). Integration of our epigenetic and transcriptome data showed that 120 genes were up regulated and marked with histone modifications in high efficiency cells, whereas 230 genes were down regulated across the 3 cell lines. Collectively, these results show that high efficiency clones display distinct gene expression and epigenetic changes compared with low efficiency clones.

Identification of the PARVA expression level in improving CRISPR-Cas9 cutting efficiency

We next seek the role of PARVA in improving CRISPR-Cas9 cutting efficiency. We built PARVA overexpression lentivirus vector and transfected CRISPR-Cas9 low efficiency clones. 3 days after

transfection, cells are lysed and total RNA was harvested. RT-PCR shows that PARVA was overexpressed successfully (Fig 4a). We used sgRNA targeting CSPG4 gene to validate the cutting efficiency as reported[8]. The efficiency is enhanced from 3% to 27% by T7E1 digestion analysis (Fig 4b).

Discussion

PARVA encodes a member of the parvin family of actin-binding proteins[12]. Parvins are associated with focal contacts and contain calpomi homology domains that bind to actin filaments. The encoded protein is part of the integrin-linked kinase signaling complex and plays a role in cell adhesion, motility and survival. The infection efficiency of adenovirus is determined by the levels of CAR and integrin on cancer cell[13]. That's why PARVA gene expression level can improve CRISPR-Cas9 cleavage efficiency. PARVA also promotes metastasis by modulating ILK signaling pathway in lung adenocarcinoma[14].

Conclusions

To improve CRISPR-Cas9 gene editing efficiency, we recommend taking Cas9 and PARVA expression level together into consideration.

Methods

Cell culture

The different efficiency CRISPR-Cas9 clones were gifts from our collaborator Wei lab. Cells were maintained in Dulbecco's modified Eagle medium (DMEM) with 10 % fetal bovine serum, glutamine and 1% penicillin/streptomycin.

RNA-seq and analysis

Raw sequence reads were aligned to the hg19/GRCh37 reference assembly using Tophat. Cufflinks was used to calculate RPKM value. Differential expression analysis was performed using the R package limma. Genes with an absolute fold change >1.5 and false discovery rate (FDR)-adjusted $P < 0.05$ were considered significant.

ChIP-seq and analysis

H3K27ac ChIP-seq was performed as described. Raw sequence reads were aligned to the hg19/GRCh37 reference assembly using BWA v0.5.7. Peaks were defined using MACS2 V2.1.0.20140616. HTseq was used to create an input matrix used for detecting differentially accessible peaks. We accessed the significant change of chromatin accessibility between different groups using the DESeq2 R package[15]. The total count of the qualified fragments in each sample was used as the library size. It was defined as significantly changed if the peak showed log2 fold change > 1 and P-value < 0.05. The HOMER tool suite was used for TF motif discovery, by analyzing differential motif enrichment in M238R specific element

datasets against all elements (peaks) background. Binding and Expression Target Analysis (BETA) was used to integrate ATAC-seq data with differential gene expression data to infer direct target genes.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing interests.

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Authors' contributions

Y.L. conceptualized the study, supervised the experiments and data analysis, and wrote the manuscript. Y.L. and S.M. conceived and designed the study. Y.L. performed all experiments including RNA-seq, H3K27ac ChIP-seq, and wrote the manuscript. S.M. performed computational analysis of the data and wrote the manuscript. F.Y. and P.Z. supervised experiments and edited manuscript. H.W., F.W., Y.W., L.N., M.X. and Z.L. helped to do some experiments. W.X., B.Z., Y.W., F.Q., Y.G., R.G. and N.W. participated the discussion. All authors contributed to the discussion and writing of the final manuscript.

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Figures

A

B

Figure 1

T7E1 digestion examined CRISPR/Cas9 efficiency of different clones (a) Indels induced by sgRNA(5'-TTGCCAGACTTGCATCCG-3') targeting the CSPG4 gene in 293T, HT1080 were assayed by T7E1 Enzyme digestion. All cells were incubated for 7 days after sgRNA transfection before assay as indicated. The percentage of cleaved band was measured using the ImageJ software. (b) Schematic representation of the experiment design. RNA-seq and H3K27ac ChIP-seq experiment was performed and integrated in data analysis.

Figure 2

Expression profile of different CRISPR-Cas9 cleavage efficiency clones. (a) Primers designed on the Structure of the lentiviral plasmid expressing 2A-linked OCT1 and Cas9. (b) RPKM of Cas9, OCT1 and BSD genes. (c) Gene ontology analysis for differentially expressed genes in CRISPR-Cas9 high and low efficiency clones across 3 cell lines. (d) Volcano plot showing differentially expressed genes between CRISPR/Cas9 high efficiency and low efficiency cell lines. The horizontal and vertical lines indicate the cutoff (Fold change ≤ 2 ; FDR ≤ 0.05) of differential genes.

Figure 3

H3K27ac modification pattern in different CRISPR-Cas9 cleavage efficiency clones (a) Venn diagram showing the number of H3K27ac peaks overlapped of CRISPR-Cas9 high efficiency and CRISPR-Cas9 low efficiency (b) Heatmap showing H3K27ac level across differential expressed genes in 293T high efficiency clone and 293T low efficiency clone (c) H3K27ac signal on down genes nearby peaks(10kb) and up genes nearby peaks(10kb). Red indicates CRISPR-Cas9 high efficiency clone. Black indicates CRISPR-Cas9 low efficiency clone. (d) H3K27ac profile of PARVA gene in CRISPR-Cas9 high efficiency clone and CRISPR-Cas9 low efficiency clone (up panel). H3K27ac profile of DNM1 gene in CRISPR-Cas9 high efficiency clone and CRISPR-Cas9 low efficiency clone (down panel).

Figure 4

PARVA and UNC5B improved CRISPR-Cas9 cleavage efficiency (a) RT-PCR analysis for PARVA gene mRNA level before and after low efficiency clones are transfected by lentivirus containing PARVA. (b) Indels induced by sgRNA (5'-TTGGCCAGACTTGCATCCG-3') targeting the CSPG4 gene in control and PARVA overexpressed clones were assayed by T7E1 Enzyme digestion.

Supplementary Files

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