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Original Research Article

Explore the dominant factor in prime editing via a view of DNA processing

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A B S T R A C T

Prime editing is a revolutionary gene-editing method that is capable of introducing insertions, deletions and base substitutions into the genome. However, the editing efficiency of Prime Editor (PE) is limited by the DNA repair process. Here, we show that overexpression of the flap structure-specific endonuclease 1 (FEN1) and the DNA ligase 1 (LIG1) increases the efficiency of prime editing, which is similar to the dominant negative mutL homolog 1 (MLH1dn). In addition, MLH1 is still the dominant factor over FEN1 and LIG1 in prime editing. Our results help to further understand the relationship of proteins involved in prime editing and envisage future directions for the development of PE.

1. Introduction

The addition of the PE to the repertoire of gene-editing tools sheds new light on correcting most disease-associated DNA mutations that cannot be restored by Cas9 or Base Editor (BE), as PE is capable of installing base insertions, deletions and all 12 types of base substitutions [1]. Anzalone et al. developed the PE1 protein by fusing a SpCas9 H840A nickase (Cas9n) to the wild-type reverse transcriptase (RT) from Moloney murine leukemia virus (M-MLV). Then, to improve the editing performance of the PE1, Anzalone and co-workers introduced five mutations to the M-MLV RT, yielding the PE2 editor [1]. The Cas9n domain in the PE2 nicks the PAM-containing DNA strand, and then the nicked ssDNA hybrids with the primer binding site (PBS) region of the prime editing guide RNA (pegRNA). The fused RT domain extends the nicked single-stranded DNA (ssDNA) and generates a 3′ ssDNA flap containing designed sequences, which competes with the 5’ ssDNA flap containing unedited sequences for precise genome editing [1]. However, the editing efficiency of the PE2 system is often severely limited [1,2].

To further improve the editing efficiency, an additional sgRNA is used in the PE3 system to nick the non-PAM-containing DNA strand upstream or downstream of the editing locus. The PE3 strategy generally results in a higher editing efficiency than the PE2 system, but generates more indels [1]. To further increase the editing efficiency, the stability of the pegRNA can be improved by fusing specific RNA motifs to the 3′-end of the pegRNA [3,4]. In addition, using a pair of pegRNAs allows PE to process large DNA fragments with greater precision [5–8]. PE fused to additional peptides, such as the Rad51 ssDNA binding domain, chromatin-modulating peptides and peptides derived from DNA repair proteins, shows a higher efficiency than the original PE2 [9–11].

Previous studies have shown that prime editing is severely restricted by the mismatch repair (MMR) pathway [1,12,13]. MLH1 constitutes the MMR complexes hMutLα (MLH1-PMS2), hMutLβ (MLH1-PMS1) and hMutLγ (MLH1-MLH3), whose function is to introduce single-strand breaks following mismatch recognition by hMutSα (MSH2-MSH6) or hMutSβ (MSH2-MSH3) [14]. It has been reported that MLH1 is the most potent factor of the prime editing among the proteins in the MMR complex, such as MLH1, PMS2, MSH2, MSH3 and MSH6, which reduces the efficiency of prime editing [13]. Transient expression of an engineered MMR-inhibiting protein (MLH1dn) enhanced the efficiency of prime editing [12]. In addition, depletion of MMR factors, not limited to MLH1, increased the efficiency and fidelity of prime editing [13].

Besides MLH1, the efficiency of prime editing is also affected by other key proteins involved in different canonical DNA metabolic pathways [12]. FEN1 is a structure-specific nuclease that cleaves the DNA flap structures generated during the replication or repair of DNA, helping to ensure accurate DNA processing and avoiding potential

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mutations or DNA damage. In the long-patch BER pathway and the Okazaki fragments procession, FEN1 removes the 5′ flap generated by single-stranded DNA displacement synthesis [15]. In contrast to the clear function of FEN1, which specifically cleaves the 5′ flap, the exonuclease 1 (EXO1) exhibits both 5′-3′ DNA exonuclease and cryptic 3′-5′ exonuclease activity, catalyzing the removal of individual nucleotides from the ends of DNA strands, specifically from the 3′ ends to the 5′ ends. EXO1 is highly specific for ssDNA, and it does not act on double-stranded DNA (dsDNA) or RNA. The function of EXO1 in MMR is to excise the mismatched DNA strand, starting from a strand nick either 5′ or 3′ to the mismatch generated by the MMR protein complexes. In addition, EXO1 has endonuclease activity against 5′ flap structures, similar to the function of FEN1 [16]. LIG1 is an enzyme that plays a crucial role in the process of DNA replication and repair within a cell. It functions to join, or ligate, single-strand breaks and Okazaki fragments during DNA replication, resulting in the formation of a continuous DNA strand. However, it is still not well understood how these key proteins coordinate to affect the process of prime editing and which protein is the most dominant factor for the efficiency of prime editing. In this study, we explored the relationship between FEN1, EXO1, LIG1 and MLH1, and we found that MLH1 is the dominant factor affecting prime editing among these proteins.

2. Results

2.1. Identification of the role of FEN1, EXO1 and LIG1 in prime editing

Since the EXO1 has been reported to impede prime editing [12], we hypothesized that a dysfunctional EXO1 might improve the prime editing. We engineered a mutated EXO1 (D78A/D173A/D225A) [17] (hereafter referred to as EXO1d) that abrogates double-stranded DNA exonuclease activity and endonuclease activity against 5′ flap structures, and reduces DNA-binding to 5′ flap structures. To test whether overexpression of these proteins affects the efficiency of prime editing, we transfected PE2 with FEN1, EXO1d, LIG1 or MLH1dn and 8 pegRNAs targeting *FANCF*, *RNF2* and *RUNX1* loci in HEK293FT cells, and performed next-generation sequencing to quantify the editing efficiency. We observed that overexpression of FEN1, LIG1 or MLH1dn significantly improved PE2 editing efficiency by an average of 1.8-fold, 2.2-fold, and 3.9-fold, respectively, while overexpression of EXO1d didn't show any significant improvement over the PE2 control (Fig. 1a).

To further test whether the 5' flap exonuclease activity or the binding activity of FEN1 is the primary factor for its increasing effect on the prime editing, we engineered a nuclease-defective FEN1 (D181A/F343A/F344A) [18] (hereafter referred to as FEN1d) and co-transfected it with PE2. We observed no significant differences between overexpression of FEN1 and overexpression of FEN1d for PE2 (Fig. 1b). These results implied that the 5′ flap binding activity of FEN1 may be more essential to promote prime editing than the nuclease activity of FEN1. Next, we fused FEN1 to the N-terminal, C-terminal, and in the middle of PE2. We transfected these FEN1-fused PEs with four pegRNAs targeting the endogenous *FANCF* locus, and observed that the editing efficiency of PE2 with FEN1 overexpression is higher than that of FEN1-fused PEs (Supplementary Fig. 1a). Given that excision of DNA flaps by FEN1 is a key step in the process of prime editing, we wondered whether knocking down FEN1 would affect the efficiency of PE editing. We engineered a stable cell line by infecting shRNA targeting endogenous FEN1 in HEK293FT, which resulted in a ~75% knockdown of endogenous FEN1 expression. Next, we co-transfected PE2 with FEN1 in normal HEK293FT cells and in the FEN1-knockdown cells. We observed no significant differences between the normal and FEN1-knockdown cells for PE2 editing efficiency, while overexpression of FEN1 increased the PE2 editing efficiency by an average of 1.6-fold both in normal and FEN1-knockdown cells (Fig. 1c).

To gain further insight into how EXO1 behaves in the process of prime editing, we co-transfected PE2 with EXO1 or EXO1d, and we observed that neither EXO1 nor EXO1d significantly affected prime editing (Fig. 1d), indicating that the competitive inhibition of EXO1 or overexpression of EXO1 does not directly affect the efficiency of prime editing, although interference of EXO1 by CRISPRi affects the efficiency of prime editing [12].

To evaluate whether overexpression of FEN1, EXO1d, LIG1 or MLH1dn affects the cellular transcriptome, we co-transfected PE2 with FEN1, EXO1d, LIG1, or MLH1dn, while transfected Cas9n as a control, in HEK293FT cells. We performed RNA-seq experiments on these cells and observed that overexpression of PE2 upregulates 6, 0, 6 and 5 genes, respectively, and downregulates 15, 21, 43, and 6 genes, respectively, compared to overexpression of FEN1, EXO1d, LIG1 or MLH1dn with PE2, while upregulates 8 genes and downregulates 17 genes compared to Cas9n (Fig. 1e–i). Indeed, we observed higher cell viability of HEK293FT cells co-transfected with PE2 and FEN1, EXO1d, LIG1 or MLH1dn up to 3 days after transfection, compared to the HEK293FT cells transfected with PE2 only (Fig. 1j). These results suggested that overexpression of FEN1, EXO1d, LIG1 or MLH1dn with PE2 doesn't confer extra toxicity to HEK293FT cells, compared with PE2.

2.2. MLH1 is the main limiting factor of prime editing

It has been reported that depleting MMR-related factors promotes prime editing [13]. To test whether it's possible to regulate proteins not involved in MMR, such as FEN1, and proteins involved in but non-exclusive to MMR, such as EXO1 and LIG1, to achieve an improvement in editing efficiency comparable to the inhibition of MLH1, we co-transfected two or three proteins from FEN1, EXO1d and LIG1 with PE2. We observed that overexpression of FEN1, EXO1d and LIG1 together results in a 7.3% editing efficiency on average, which is higher than that of overexpression of two factors among FEN1, EXO1d and LIG1 (Fig. 2a). However, the editing efficiency of PE2 with MLH1dn, 8.8% on average, was significantly higher than that of the combination of FEN1, EXO1d and LIG1 (Fig. 2a). These results suggested that MLH1 is a dominant factor in prime editing and that regulation of other proteins cannot bypass the dominant effect of MLH1 on prime editing.

With the overexpression of FEN1, EXO1d or LIG1, PE may behave differently in different cell lines with widely varying expression level of MLH1 but comparable expression level of FEN1, EXO1 and LIG1. We aimed to investigate how overexpression of FEN1, EXO1d, LIG1 and MLH1dn would affect prime editing in MLH1-abundant and MLH1-scarce cell lines. Accordingly, we co-transfected PE2 with FEN1, EXO1d, LIG1, or MLH1dn in HeLa, HCT-116, and AN3-CA cells (Fig. 2b) [19]. The endogenous expression level of MLH1 in HeLa cells is 45% higher than that in HEK293 cells. We observed that the editing efficiency of PE2 was reduced by about 46% in HeLa cells compared to HEK293FT cells. Moreover, only the overexpression of MLH1dn improved PE2 editing efficiency by over 2-fold, compared to overexpression of FEN1 and LIG1 respectively, and in combinations (Fig. 2c). In contrast, the endogenous expression level of MLH1 in HCT116 cells and AN3-CA cells is only 10% and 0.31% of that in HEK293 cells. The prime editing efficiency was over 13% on average in HCT116 cells and over 37% on average in AN3-CA cells, which was higher compared to that in HeLa and HEK293FT cells (Fig. 2d and e). Interestingly, overexpression of FEN1, EXO1d, LIG1 and MLH1dn individually or their combinations could not improve the editing efficiency in HCT116 and AN3-CA cells. This finding further indicated that MLH1 is the pivotal factor in the process of prime editing.

3. Discussion

A putative mechanism of prime editing is shown in Fig. 3. After the reverse transcription process by PE2, a 3′ single-stranded DNA flap containing the desired edit forms and equilibrates with the 5′ flap of original DNA. Once the 3′ flap with the desired edit is excised by intracellular nucleases, the prime editing fails. The 5′ flap could be

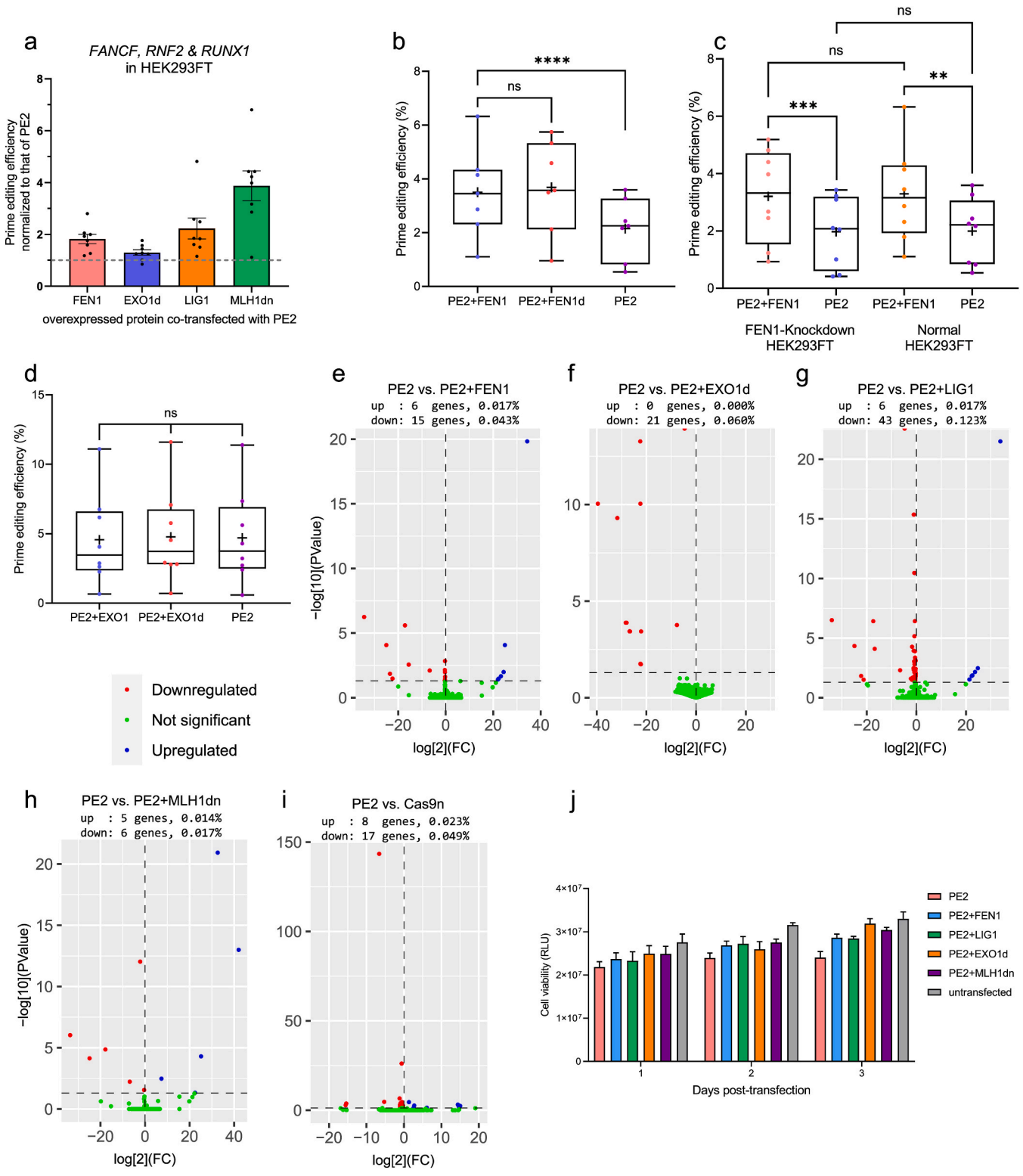

Fig. 1. Evaluation of the influence of overexpression of FEN1, EXO1, LIG1 and MLH1dn. **a** The influence of prime editing efficiency by overexpression of FEN1, EXO1d, LIG1 and MLH1dn at FANCF, RNF2 and RUNX1 loci, normalized by the editing efficiency of PE2. Mean ± s.e.m. of pegRNAs n = 8 was shown. **b** Comparation of influence of overexpression of FEN1 and FEN1d with PE2 on editing efficiency. pegRNAs n = 7. **c** Comparation of influence of overexpression of FEN1 with PE2 on editing efficiency in normal and FEN1-knockdown HEK293FT cells. pegRNAs n = 8. **d** Comparation of influence of overexpression of EXO1 and EXO1d with PE2 on editing efficiency. pegRNAs n = 8. In **b-d**, editing efficiency measured for n = 3 biologically independent replicates for each pegRNA, and data of minimum-to-maximum values are presented. For the boxes, the top, middle, and bottom lines represent the 25th, 50th, and 75th percentiles, respectively. The whiskers indicate the min and max values, and the + mark indicates the mean value. **e-i** Analysis of RNA-seq from HEK293FT cells expressing PE2 only, PE2 with FEN1, EXO1d, LIG1 and MLH1dn, as well as Cas9n. RNAs corresponding to 34,958 genes with nonzero total read count are shown. Biologically independent replicates n = 3 for each group. **j** Cell viability was measured for the bulk cellular population every 24 h after transfection for 3 days by luminescence using the CellTiter-Glo 2.0 assay (Promega). Mean ± s.e.m. of n = 3 independent biological replicates. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

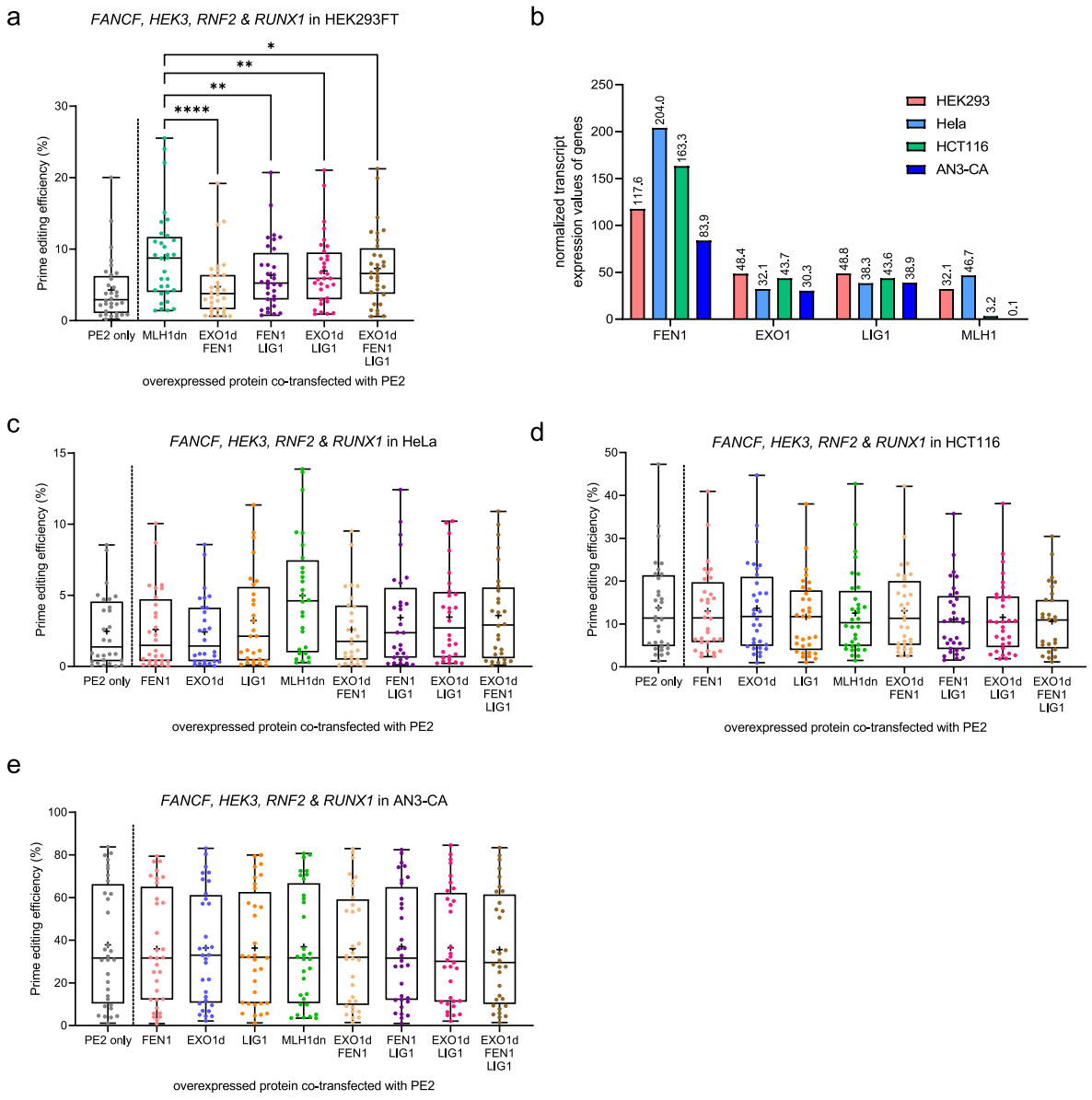

Fig. 2. Comparation of the prime editing efficiency with combinations of FEN1, EXO1d, LIG1 and MLH1dn in four human cell lines. **a** Editing efficiency of PE2 with combinations of FEN1, EXO1d, LIG1 and MLH1dn in HEK293FT. pegRNAs n = 32. **b** Normalized transcript expression values of FEN1, EXO1d, LIG1 and MLH1dn in HEK293, HeLa, HCT116 and AN3-CA. Data are shown in normalized transcript per million (nTPM). **c-e** Editing efficiency of PE2 with combinations of FEN1, EXO1d, LIG1 and MLH1dn in HeLa, HCT116 and AN3-CA. pegRNAs n = 28, 32 and 32 respectively. Data of minimum-to-maximum values are presented in **a, c-e**. For the boxes, the top, middle, and bottom lines represent the 25th, 50th, and 75th percentiles, respectively. The whiskers indicate the min and max values, and the + mark indicate the mean value. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

trimmed by FEN1, after which the 3′ flap is paired to the unedited strand. The nicks on both the edited strand and the unedited strand could be sealed by LIG1, so the additional nicks on the unedited strand cut by the PE3 system is also in a reversible equilibrium. The 3′ flap containing the desired edit is paired with the unedited strand, resulting in a mismatch in the double-stranded DNA. The MMR complexes recognize these abnormal DNA structures and introduce a nick upstream or downstream the mismatched locus. If the MMR-mediated nick is at the 5′ of the desired edit on the edited strand, EXO1 or other 5′-3′ exonuclease will cleave the desired edit, then the DNA will be repaired to the original sequence. In contrast, if the MMR-mediated nick is at the 5′ of the desired edit on the unedited strand, EXO1 exonuclease will cleaves the strand complementary to the desired edit, then the DNA will be repaired to the desired sequence. If the MMR-mediated nick is at the 3′ of the desired edit on the edited strand or the unedited strand, the original DNA will be cut and repaired back to its original sequence. In summary, the process of prime editing consists of many reversible equilibrations and complicated repair processes.

Interfering with MLH1 is by far the most efficient method of improving prime editing efficiency, and no other method of protein regulation shows a comparable effect to interfering with MLH1. However, the inhibition of MMR by overexpression of MLH1dn *in vivo* may lead to mitochondrial dysfunction [20] and Lynch syndrome-like tumors [21], limiting the potential application of MLH1dn for gene therapy. Transient or controllable inhibition of MLH1 may lead to much safer *in vivo* applications. In addition, overexpression of FEN1 has been detected in a variety of cancers and was reported as a driver of genome instability [22]. LIG1 was also reported to cause genetic instability [23]. These risks need to be carefully considered when regulating prime editing. It is interesting to note that FEN1 and LIG1 do not improve editing efficiency in cells with low endogenous MLH1 expression, such as HCT116 and AN3-CA cells, although the editing efficiency is already higher than that

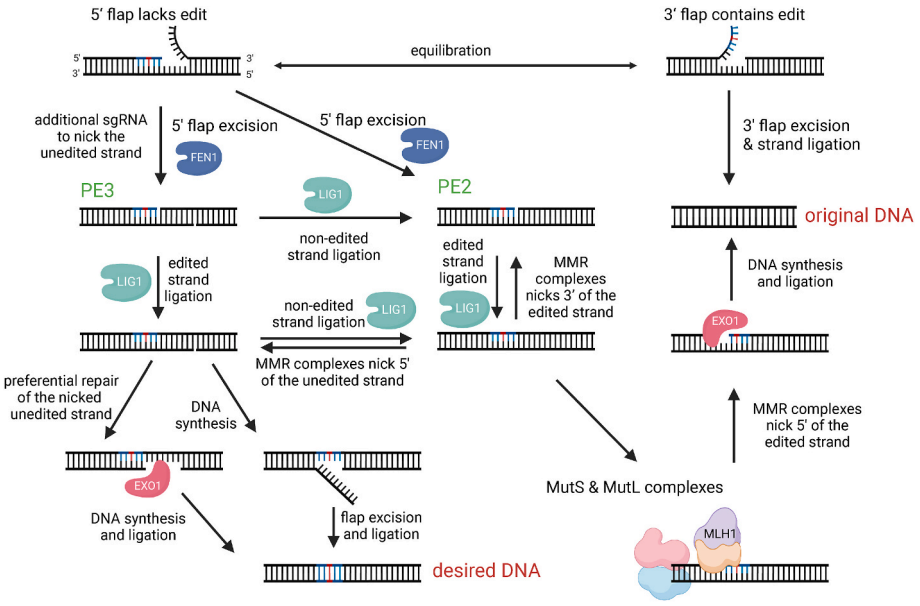

Fig. 3. Schematic of the putative mechanism of prime editing. FEN1, flap structure-specific endonuclease 1. EXO1, exonuclease 1. LIG1, DNA ligase 1. MLH1, mutL homolog 1. The blue single strand DNA indicates the single strand DNA synthesized by the reverse transcriptase, and the red base indicates the mutated base.

in high MLH1-expressing cells, such as HEK293FT and HeLa cells. We hypothesized that FEN1 and LIG1 have less opportunity to participate in the process of prime editing, when fewer flaps were generated in MMR-deficient cells.

In the transcriptome analysis, we observed that overexpression of FEN1, EXO1d, LIG1 and MLH1d only had minimal impact on the genome, compared with PE2 only. The maximum perturbation was about 0.12% of the total gene counts. The statistically significant perturbed genes are listed in the Supplementary Tables. However, these genes didn't exhibit a clear pattern and couldn't be classified into a specific category.

In summary, we performed prime editing experiments to verify the function of FEN1, EXO1d, LIG1 and MLH1dn in the prime editing process. Overexpression of FEN1, LIG1 and MLH1dn increased the PE2 editing efficiency in the MLH1-abundant cells such as HEK293FT and HeLa cells, while barely showed improvement on PE2 editing efficiency in the MLH1-lacking cells such as HCT116 and AN3-CA cells. Among the FEN1, LIG1 and MLH1dn, MLH1dn improved the PE2 editing efficiency most in HEK293FT cells. Our results indicated that MLH1, the key protein of MMR, is also the pivotal factor for prime editing efficiency.

4. Limitations of the study

This study focuses on the dominant factor in the efficiency of prime editing from a view of DNA processing. However, the influence of these factors on other attributes, such as specificity, indels and fidelity, need further investigation.

5. Methods

5.1. Reagents and enzymes

Restriction endonuclease, polynucleotide kinase (PNK), T4 DNA ligase, and Q5 High-Fidelity DNA Polymerase were purchased from New England Biolabs. Oligonucleotides were synthesized by Ruibiotech. PCR enzyme mix was purchased from Tsingke Biotechnology. Protease K and other biochemical reagents were purchased from GenStar Biotechnology.

5.2. Plasmids and pegRNAs

The pegRNA sequences, primers, shRNAs and the DNA sequences of the constructs are listed in Supplementary Tables.

5.3. Cell culture and transfection

The HEK293T, HeLa, and HCT116 cells were cultured in high-glucose DMEM complete medium (Dulbecco's modified Eagle's medium (DMEM), 4.5 g/L glucose, 0.045 unit/mL of penicillin, 0.045 g/mL streptomycin, and 10% FBS (Life Technologies)) at 37 °C, 100% humidity, and 5% CO2. The AN3-CA cells were cultured in AN3-CA-specified medium (Procell Life Science & Technology) at 37 °C, 100% humidity, and 5% CO2. One day before transfection, ~1.2 × 10^5 HEK293FT, HeLa, HCT116 or AN3-CA cells in 0.1 mL of high-glucose DMEM complete media were seeded into each well of 96-well plastic plates (Corning). Shortly before transfection, the medium was replaced with fresh DMEM complete media. For HEK293FT cells, the transfection experiments were performed by using EpFect transfection reagent (SyngenTech) by following the manufacturer's protocol, while for HeLa, HCT116 and AN3-CA cells, the transfection experiments were performed by using lipo8000 transfection reagent (Beyotime) by following the manufacturer's protocol. Each transfection experiment was independently repeated.

For PE2 experiments, 200 ng prime editor plasmid, 66 ng pegRNA plasmid, and 100 ng plasmid for FEN1, LIG1, EXO1 or MLH1dn protein expression (where indicated) were transfected, as well as 100 ng transfection control plasmid (hEF1α-EYFP-2A-puro) that constitutively expressed puromycin resistance gene, into each well of a 96-well plate. To select transfected cells, puromycin (Invitrogen) was added at a final concentration of 4 μg/mL after 1 day, and fresh complete media were replaced after 4 days.

5.4. Editing efficiency quantification by targeted NGS

All of the editing efficiency and indel were measured by targeted next-generation sequencing (Illumina NovaSeq). Cells were harvested 5 days post-transfection and lysed by lysis buffer (0.45% NP40 and 10 mM Tris-HCl, pH8.0) followed by protease K treatment for 2 h. Amplicons were generated by three rounds of PCR to add the illumina adaptor

sequence. Primers used in PCR are listed in Supplementary Tables. After filtering and pairing, editing efficiency is defined by correct edited reads/all reads, indel is defined by (all reads-correct edited reads-original reads)/all reads.

5.5. Lentivirus production and transfection

Lentiviral production was achieved by plating 5×10^6 HEK293FT cells in a 10 cm dish transfected one day post seeding with packaging plasmids (1 μg VSV.G, 2 μg psPAX2 and 4 μg of a U6-shRNA-hEF1a-bla plasmid) using EpFect transfection reagent (SyngenTech). Virus containing supernatant was collected 48 h and 72 h post transfection, cleared by centrifugation and stored at −80 °C. For lentivirus transduction, 1×10^6 HEK293FT cells were seeded on 10 cm dishes and concentrated lentivirus and 8 μg/mL of polybrene (Sigma) were added to the media. The cells were then incubated overnight, after which cells were refreshed with complete medium before adding blasticidin at 48 h and keeping it for minimum of next 5 d to remove untransduced cells.

5.6. Quantification of gene knockdown by RT-qPCR

Total RNA from HEK293FT cells was extracted with Trizol reagent (Life Technology). For each sample, 500 ng total RNA was reverse transcribed by HiScript III 1st Strand cDNA Synthesis Kit (Vazyme), and 1 μL of cDNA was used for each qPCR reaction, using Taq Pro Universal SYBR qPCR Master Mix (Vazyme). The quantitative reverse transcription polymerase chain reaction (qRT-PCR) reaction was run and analyzed in the Light cycler 480 II (Roche) with all target gene expression levels normalized to *GAPDH* mRNA levels. The primers are listed in Supplementary Tables.

5.7. RNA-seq and data analysis

HEK293FT cells were transfected with PE2 and FEN1, LIG1, EXO1d or MLH1dn using lipo8000 reagent (Beyotime). Seventy-two hours after transfection, total RNA was harvested from cells using TRIzol reagent (Thermo Fisher), and purified with RNeasy Mini kit (Qiagen) including on-column DNaseI treatment. Sequencing libraries were generated using NEBNext® UltraTM RNA Library Prep Kit for Illumina® (NEB, USA) following manufacturer's recommendations and index codes were added to attribute sequences to each sample. The clustering of the index-coded samples was performed on a cBot Cluster Generation System using TruSeq PE Cluster Kit v3-cBot-HS (Illumia) according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced on an Illumina Hiseq platform and 125 bp/150 bp paired-end reads were generated. The raw data were trimmed using Trimmomatic. Index of the reference genome was built using STAR and paired-end clean reads were aligned to the reference genome using STAR. RSEM was used to count the reads numbers mapped to each gene. Differential expression analysis of two groups was performed using the DESeq2 R package. Genes with an adjusted P-value <0.05 found by DESeq2 were assigned as differentially expressed. The results were plotted using ggplot in R.

5.8. Cell viability assays

Cell viability was measured every 24 h post-transfection for 3 days using the CellTiter-Glo 2.0 assay (Promega) according to the manufacturer's protocol. Luminescence was measured in 96-well flat-bottomed polystyrene microplates (Corning) using a Varioskan Flash microplate reader (Thermo) with a 500 ms integration time.

5.9. Statistical analysis

There are three individual biological replicates in all experiments, unless otherwise stated. The data with s.e.m. error bars were analyzed with two-tailed, paired t-tests using GraphPad Prism 9, unless otherwise stated. A probability of $p < 0.05$ was considered statistically significant. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

Data and code availability

High-throughput sequencing data have been deposited in the NCBI Sequence Read Archive database under accession PRJNA938776. The Python codes used for counting prime editing efficiency and Source Data were provided in supplementary files.

CRediT authorship contribution statement

Zhimeng Xu: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Dacheng Ma:** Investigation. **Houzhen Su:** Investigation. **Xiaodong Jia:** Investigation. **Yinqing Li:** Writing – original draft. **Yinying Lu:** Conceptualization, Resources, Writing – original draft, Supervision. **Zhen Xie:** Conceptualization, Methodology, Validation, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

A patent application on the method of overexpressing FEN1 or LIG1 for genome editing is pending in State Intellectual Property Office of China.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.synbio.2023.05.007.

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