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Prime Editing the Non-Coding Genome: A Molecular Review of Correcting Regulatory Mutations for Precision Cancer Therapy

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ABSTRACT

Prime editing, a flexible "search-and-replace" genome editing technique, has become a potent instrument for accurately altering the genome without the need for donor templates or double-strand DNA breaks. The vast non-coding regulatory genome, which includes enhancers, promoters, untranslated regions (UTRs), and non-coding RNAs, is now understood to be a major driver of tumorigenesis, despite the fact that the majority of cancer research has concentrated on protein-coding mutations. Oncogene dysregulation, tumor suppressor silencing, and treatment resistance can all result from mutations in these areas. The convergence of these two fields is examined in this review, which also describes the use of prime editing to fix particular, harmful non-coding mutations. In order to restore normal gene expression patterns, we describe methods for focusing on regulatory elements, present a landscape of non-coding drivers in cancer, and talk about the fundamentals of prime editing. Lastly, we look at delivery methods, therapeutic factors, and how prime editing might be incorporated into the precision oncology paradigm going forward. We emphasize how prime editing could help usher in a new era of targeted therapy and functional genomics.

INTRODUCTION

The accumulation of somatic mutations in protein-coding genes that confer a selective growth advantage has historically been used to characterize cancer, which is essentially a genetic disease. However, only around 2% of the human genome codes for proteins; the majority of the genome is non-coding. According to the ENCODE project and other research, a sizable section of the non-coding genome is functional and plays important roles in controlling the expression of certain genes (1). It is now clear that a large number of cancers contain recurrent mutations in these non-coding regulatory regions, which can cause tumor suppressors to be silenced or oncogenes like TERT and MYC to be aberrantly activated (2), (3). Specific mutational signatures and recurrent single-nucleotide variants (SNVs) in regulatory loci under positive selection have been found in whole-genome sequencing studies of pan-cancer cohorts, confirming their functional significance (4). Biomedical research has been transformed by the advent of genome editing based on clustered regularly interspaced short palindromic repeats (CRISPR). But traditional CRISPR-Cas9 depends on causing double-strand breaks (DSBs), which can result in unwanted effects like chromosomal translocations and indels. This is especially dangerous when editing sensitive regulatory regions (5). Although base editors are limited by bystander edits, limited editing windows, and the inability to accomplish all possible nucleotide changes or insertions, they provided a breakthrough by allowing direct chemical conversion of one base pair to another without DSBs. Prime editing's recent advancements mark a significant advancement (6).

The precise correction of point mutations and small indels that are present in the non-coding genome of cancer cells is made possible by this adaptable system, which can mediate all 12 possible base-to-base conversions as well as small insertions and deletions without the need for donor DNA templates or DSBs (7). The present knowledge of non-coding cancer drivers is summarized in this review, which also clarifies how prime editing may be used to functionally examine and therapeutically fix these elusive mutations.

NON-CODING GENOME AND CANCER: A HIDDEN DRIVER

Composition of the Non-Coding Genome

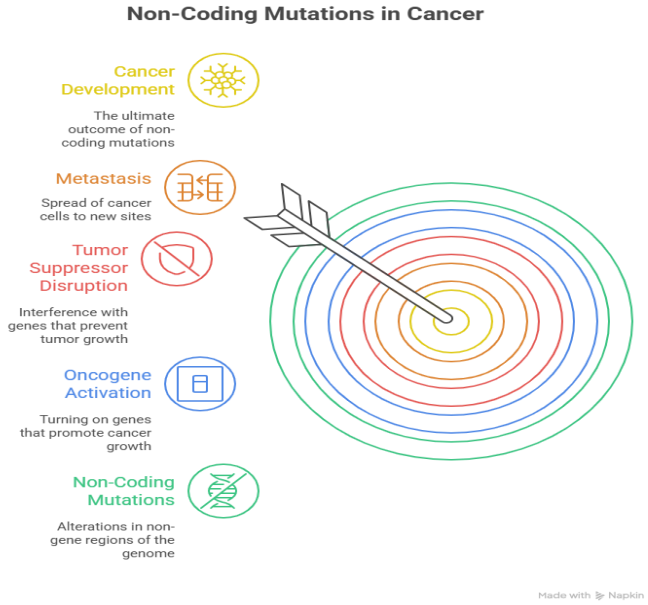
The regulatory landscape of the non-coding genome is intricate. Gene transcription is started by promoters, which are found close to transcription start sites. Distal regulatory elements known as enhancers have the ability to interact with promoters over long genomic distances, significantly increasing gene expression (8). Large clusters of enhancers known as super-enhancers are often dysregulated in cancer and are specifically linked to genes that define cell identity. Insulators and silencers work in opposite ways, preventing enhancer-promoter interactions or suppressing gene expression, respectively. The 5' and 3' ends of mRNA contain untranslated regions (UTRs), which are essential for controlling the stability, localization, and translation efficiency of mRNA (9). Additionally, a broad class of non-coding RNAs (ncRNAs), such as circular RNAs (circRNAs), long non-coding RNAs (lncRNAs), and microRNAs (miRNAs), function as important post-transcriptional regulators, scaffolds, and competitors in a variety of cellular processes, many of which are dysregulated in cancer (10).

Non-Coding Mutations in Cancer

Cancer can be strongly influenced by somatic mutations in non-coding areas. One well-known example is the frequent mutation in the TERT gene's promoter, which generates a de novo binding site for ETS transcription factors and causes abnormal TERT expression and telomere maintenance in about 70% of melanomas and numerous other cancers (11). Similar to this, "enhancer hijacking" events, which are frequently brought on by structural variations, can position potent enhancers close to oncogenes such as MYC or GFI1, causing them to be massively overexpressed (12). Enhancer hijacking is a common mechanism of oncogene activation, according to a pan-cancer analysis. A SNP in the KRAS 3' UTR, for instance, interferes with let-7-mediated repression, raising the risk of cancer (13). Mutations in the 3' UTR of genes can also interfere with miRNA binding sites, increasing the stability and translation of oncogenic mRNAs. Furthermore, splicing patterns or the expression of regulatory lncRNAs, like PVT1 or MALAT1, can be impacted by non-coding mutations. These changes can then have an impact on tumor suppressor networks and the likelihood of metastasis (14). These findings highlight the fact that non-coding mutations are legitimate drivers and possible therapeutic targets rather than just "bystanders." Non-Coding Genome and Cancer: A Hidden Driver is depicted in Figure 1.0. This diagram illustrates the intricate regulatory processes of the non-coding genome, highlighting components such as enhancers, promoters, and different non-coding RNAs (miRNA, lncRNA, and circRNA) that affect gene expression. It emphasizes how these regulatory processes are interfered with by non-coding mutations, like those in the TERT promoter or enhancer hijacking close to MYC. The non-coding genome plays a crucial role in the development of cancer since these disruptions can ultimately result in oncogenic events like unchecked cell growth and tumor formation.

Figure 1

Non-Coding Genome and Cancer: A Hidden Driver



PRIME EDITING: PRINCIPLES AND MECHANISM

Evolution of Genome Editing Tools

Zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and engineered meganucleases were the first to be used in genome editing. These nucleases were difficult to design but provided programmability (15). Because CRISPR-Cas9 relies on DSB generation and the error-prone non-homologous end joining (NHEJ) pathway, it made gene editing easier by using a guide RNA for target recognition. However, this relies on a high frequency of indels, which is unacceptable for correcting precise regulatory sequences (16). Despite its ability to make precise edits, homology-directed repair (HDR) is ineffective and mostly active in dividing cells. Off-target editing, a limited editing window, and the inability to make transversion mutations or insertions were the main drawbacks of base editors (BEs), which were fusion proteins of a deaminase enzyme and a catalytically impaired Cas9 that allowed C•G to T•A or A•T to G•C conversions without DSBs (17).

Mechanism of Prime Editing

To get around these restrictions, prime editing was created. The two main parts of the system are 1) a prime editing guide RNA (pegRNA), which encodes the desired edit in a reverse transcription template and specifies the target site, and 2) a fusion protein that combines an engineered reverse transcriptase (RT) and Cas9 nickase

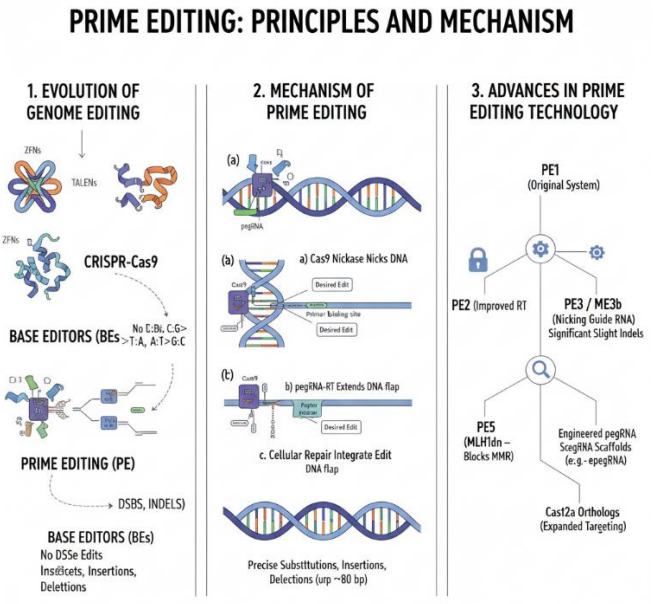
(H840A) (18). Three crucial steps are involved in the process: The RT creates a DNA flap with the desired edit after (a) the Cas9 nickase nicks the non-target DNA strand at the location designated by the pegRNA spacer sequence; (b) the 3' primer binding site (PBS) of the pegRNA hybridizes to the nicked DNA strand; and (c) cellular DNA repair processes resolve this intermediate, integrating the edited strand into the genome. Compared to Cas9, this sophisticated mechanism greatly reduces indel byproducts by enabling precise substitutions, small insertions (up to ~44 bp), and deletions (up to ~80 bp) without DSBs (19).

Advances in Prime Editing Technology

Iterative improvements have been made to the original PE system (PE1). PE2 increases editing efficiency by incorporating an engineered reverse transcriptase (M-MLV RT) with improved processivity and stability. Although there is a slight increase in indel formation, the PE3 and PE3b systems further increase editing rates by nicking the non-edited strand using an additional nicking guide RNA. The PE5 system was created more recently by combining the editor with MLH1dn, a dominant-negative form of the mismatch repair (MMR) protein. This system significantly increases efficiency across a wide range of cell types by blocking the MMR pathway, which frequently corrects pegRNA-directed edits (20). To further improve pegRNA stability and performance, dual-pegRNA strategies and engineered pegRNA scaffolds (e.g., epegRNAs with structured RNA motifs) have been developed (21). The targetable genomic space is also increased by the creation of PEs using various Cas orthologs, such as Cas12a (22). Figure 2.0 highlights the accuracy of genome editing by showing its progression from early tools to Prime Editing. It describes the precise steps involved in Prime Editing, which uses pegRNA and Cas9 nickase-RT to repair DNA in specific areas. Additionally, the figure highlights significant technological advancements in Prime Editing, demonstrating its continuous development and enhanced tactics.

Figure 2

Illustration of Prime Editing: Principles and Mechanism



CORRECTING REGULATORY MUTATIONS WITH PRIME EDITING

Enhancer and Promoter Editing

Reverting single-nucleotide variants (SNVs) in enhancers and promoters is a special application of prime editing. For example, eliminating the de novo ETS binding site and suppressing TERT transcription by fixing the recurrent TERT promoter mutations C228T or C250T may theoretically cause cancer cells to undergo replicative senescence (23). The strategy has been validated by proof-of-concept studies employing CRISPR interference (CRISPRi), which have demonstrated that targeting the mutant TERT promoter can suppress its expression (24). Likewise, it is possible to precisely restore enhancer mutations that drive MYC expression in cancers such as Burkitt lymphoma or medulloblastoma to their wild-type sequence, thereby eliminating a major oncogenic driver (25). On the other hand, prime editing might be used to reactivate the expression of transcriptional repressors by disrupting their binding sites or introducing protective mutations in the promoters of tumor suppressor genes (26).

UTR and Splicing Regulation

Gene expression can be significantly impacted by mutations in UTRs. Increased KRAS protein levels and oncogenic transformation can result from a single-nucleotide mutation in the 3' UTR of the KRAS oncogene, which can interfere with let-7 miRNA-mediated repression. Post-transcriptional control could be restored by using prime editing to restore the native miRNA binding site. Oncogenic isoforms can also result from mutations that impact regulatory splicing elements or splice donor/acceptor sites. For instance, novel splice sites are produced by recurrent intronic mutations in BCOR in hematologic malignancies (27). These mutations can be fixed by prime editing, restoring the synthesis of complete, useful tumor suppressor proteins. For splicing defects, this method has been shown to work in vitro (28).

Non-Coding RNA Modulation

Non-coding RNA sequences are powerful targets in and of themselves. An oncogenic miRNA (oncomiR) such as miR-21 can have its oncogenic function neutralized by editing its seed region to change its target repertoire (29). Similarly, it is possible to edit the promoters of lncRNAs that are frequently overexpressed in cancer and encourage metastasis, like MALAT1 or HOTAIR, to decrease their expression. Altering lncRNAs' structural domains may also reactivate dormant tumor suppressor genes by interfering with their interactions with protein partners like Polycomb Repressive Complex 2 (PRC2) (30). A recent study employed base editing, which is directly transferable to prime editing for increased precision, to disrupt the PVT1 oncogenic lncRNA promoter, lowering its expression and slowing tumor growth (31).

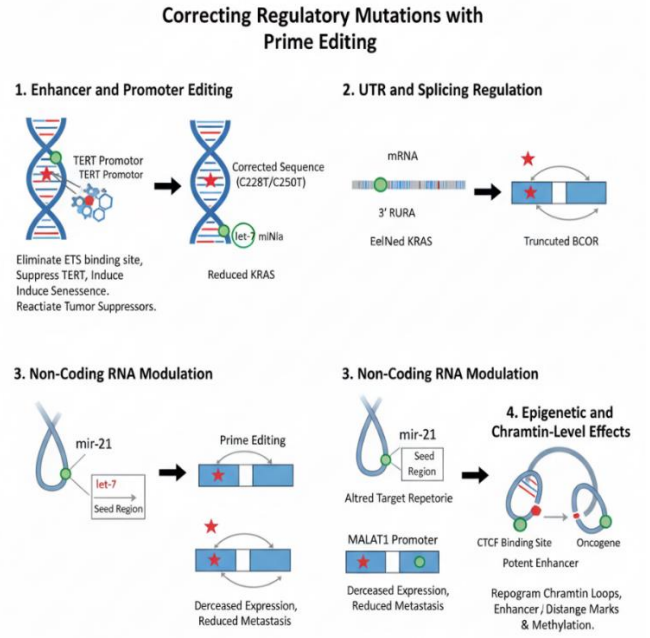
Epigenetic and Chromatin-Level Effects

Prime editing alters the DNA sequence directly, but it can also have a cascading effect on the local epigenetic environment. Histone marks and DNA methylation patterns can be changed by correcting a mutation in a transcription factor binding site within an enhancer, which

will change the recruitment of chromatin modifiers (32). Additionally, chromatin looping and topologically associating domains (TADs) may be reprogrammed by altering boundary elements or insulator sequences, which would physically separate an oncogene from a powerful enhancer (a process referred to as "enhancer dissolution". It has been shown that prime editing can predictably change chromatin architecture and gene expression by introducing particular SNPs into CTCF binding sites (33).

Figure 3

Correcting Regulatory Mutations with Prime Editing



DELIVERY STRATEGIES AND THERAPEUTIC CONSIDERATIONS

Delivery Platforms for Prime Editors

Adeno-associated virus (AAV) vectors have a ~4.7 kb packaging limit, making delivery difficult due to the prime editor fusion protein's large size (~6.3 kb for the PE2 coding sequence). Non-viral techniques or dual-AAV systems that divide the editor are two ways to get around this (34). Lipid nanoparticles (LNPs) are a promising platform for prime editing ribonucleoproteins (RNPs) or mRNA, and they have been successfully used to deliver CRISPR-Cas9 components in vivo (35). Large payloads can be carried by lentiviral vectors, but insertional mutagenesis poses safety risks. Reducing off-target effects in healthy tissues will require tissue-specific targeting with engineered viral capsids or cell-specific promoters (36).

Safety and Off-Target Concerns

Comprehensive analyses have shown that pegRNAs can cause undesirable edits, including off-target prime edits and RNA-dependent edits at related genomic sites, even though prime editing produces far fewer off-target indels than Cas9 (37). Despite having less off-target activity than Cas9, PE3 was still able to produce pegRNA-dependent off-target edits, according to a Circle-seq study. A single nucleotide alteration in a crucial regulatory element carries a high risk of unintentionally activating an oncogene or silencing a tumor suppressor. Consequently, it is crucial to carefully design pegRNAs using sophisticated computational tools and to thoroughly evaluate off-targets using techniques like GUIDE-seq or CIRCLE-seq modified for prime editing. Another major obstacle to clinical translation is immune responses to the delivery vehicle or the bacterially derived Cas9 protein (38).

Preclinical and Clinical Developments

Although prime editing's clinical use in oncology is still in its early stages, encouraging preclinical research is starting to appear. Mouse models of neurological and liver disorders have shown evidence of in vivo prime editing (39). Prime editing has demonstrated its effectiveness as a research tool by being used to produce and fix particular mutations in patient-derived organoids in cancer models in order to examine their functional impact. A TP53 mutation in glioblastoma models was corrected by prime editing in a recent study, showing decreased tumorigenicity. Before moving on to direct in vivo targeting of solid tumors, the initial clinical trials will probably concentrate on ex vivo editing of immune cells or hematopoietic stem cells (40)

INTEGRATING PRIME EDITING WITH PRECISION CANCER THERAPY

Combination with Immunotherapy and Targeted Therapy

Prime editing can work in concert with current treatments. As demonstrated with CRISPR-KO, altering the regulatory regions of immune checkpoint genes such as PDCD1 (PD-1) in CAR-T cells may improve their anti-tumor efficacy and persistence (41). Additionally, it could be used to fix gene promoter mutations that result in resistance to targeted therapies, like those that cause *BRCA1/2* reversion mutations or EGFR T790M mutations, which would re-sensitize tumors to treatment (42). Moreover, tumor immunogenicity may be altered by modifying non-coding regions that regulate neoantigen expression.

Personalized Medicine and Patient-Derived Models

Personalization is the key to the future of oncology. Non-coding driver mutations that are specific to each patient can be found through whole-genome sequencing of tumors (43). By reverting these variants and observing the phenotypic result, prime editing can be utilized in patient-derived organoids or xenografts (PDOs/PDXs) to functionally validate them. This "functional genomic" method can determine which non-coding mutations are actually harmful and should be the focus of treatment. For example, it is possible to screen for drug sensitivity in isogenic PDOs with a corrected enhancer mutation, which allows for the creation of customized combination therapies (44).

Diagnostic and Prognostic Implications

Prime editing is a potent tool for finding biomarkers outside of therapy. Researchers can definitively ascertain a suspected regulatory mutation's role in oncogenesis by allowing its precise installation in an isogenic background. This may result in the creation of diagnostic and prognostic tests based on non-coding mutation profiles

that are more accurate. For instance, even in the absence of gene amplification, a particular 3' UTR variant can be validated as a biomarker for HER2-targeted therapies if it is shown to increase ERBB2 expression (45).

CHALLENGES, ETHICAL CONSIDERATIONS, AND FUTURE PERSPECTIVES

There are still a number of obstacles in the way of its potential. Due to local chromatin states and DNA repair mechanisms, editing efficiency can differ greatly between genomic loci and cell types (46). One of the biggest challenges is getting the drug to reach a significant enough percentage of tumor cells in vivo to have a therapeutic effect. Strict regulatory frameworks are necessary to address the ethical concerns raised by the possibility of germline editing, even if it is unintentional. There are still a number of obstacles in the way of its potential. Due to local chromatin states and DNA repair mechanisms, editing efficiency can differ greatly between genomic loci and cell types (47). One of the biggest challenges is getting the drug to reach a significant enough percentage of tumor cells in vivo to have a therapeutic effect. Strict regulatory frameworks are necessary to address the ethical concerns raised by the possibility of germline editing, even if it is unintentional (48).

Table 1

Applications of Prime Editing in Precision Cancer Therapy

Application	Example / Strategy	Key Outcome	References
Immunotherapy enhancement	Editing regulatory regions of PDCD1 (PD-1) in CAR-T cells	Enhances CAR-T persistence and anti-tumor activity by fine-tuning immune checkpoint expression	(49), (50)
Overcoming drug resistance	Correcting mutations linked to EGFR T790M or BRCA1/2 reversions	Restores drug sensitivity and improves targeted therapy effectiveness	(50), (51)
Neoantigen modulation	Editing non-coding regions controlling neoantigen expression	Increases tumor immunogenicity and response to immune checkpoint blockade	(49), (51)
Personalized therapy development	Reverting patient-specific enhancer or UTR mutations in PDOs/PDXs	Identifies true driver mutations and enables individualized therapy design	(52), (53)
Biomarker discovery	Installing 3′ UTR variants (e.g., ERBB2) in isogenic models	Validates regulatory mutations as diagnostic/prognostic biomarkers	(53), (54)

CONCLUSION

Prime editing offers previously unheard-of precision for genome modification, marking a paradigm shift in genome engineering. It is the perfect tool for targeting the non-coding regulatory genome, a recently recognized frontier in cancer biology, because it can fix point mutations and small indels without DSBs. Prime editing has enormous potential to improve our knowledge of tumorigenesis and create a new class of precision oncology treatments by facilitating the functional dissection and possible correction of harmful mutations in enhancers, promoters, UTRs, and non-coding RNAs. Even though there are still many technical and translational obstacles to overcome, the field's explosive growth indicates that prime editing will soon go from being a potent research tool to a revolutionary clinical cancer treatment modality, ultimately realizing precision medicine's promise by rewriting the non-coding drivers of disease.

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