



Prime Editing: An Emerging Tool in Cancer Treatment

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Received: 1 July 2022 / Accepted: 26 September 2022

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Abstract

Prime Editing is a CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) based genome editing technique having promising potential in terms of reducing off target activity. It introduces fragments of DNA sequences into the target site using a guide RNA (gRNA) molecule, composed of both the sequence that is to be inserted into the target site along with an inactive Cas9 nickase and a reverse transcriptase. Prime Editing can cause insertions, deletions, and various point mutations for reverting the phenetic characteristics of a disease specially tested in human adult stem cells and cancer cell lines. The main aim of our review is to explore how Prime Editing and its various forms are being utilized as an emerging tool to cure deleterious diseases like cancer, also as a delivery strategy of the tool into cells. There are almost five generations of Prime Editors (PE) with increasing levels of efficiency from one level to another that have huge clinical potential in correcting mutations; however, the necessity for a pegRNA design is extremely significant. But besides having such advantages, the limitations of this technology particularly include generation of double nicks while optimizing the efficiency of PE3. So, it is important to consider all such consequences and customize PE as per requirements.

Keywords Cancer · CRISPR · Prime Editing · Prime Editors · pegRNA

Introduction

Prime Editing is a novel technology that relies on the use of a precise and efficient technique of genome editing [1]. The tool utilises the property of specificity that is derived from the conventional CRISPR Cas9 yet there is an additional component like the guide RNA, an edit template that is to be incorporated into the target site of the DNA along with a reverse transcriptase enzyme [2]. Prime Editing shares a significant number of similarities with other prominent CRISPR methods. The Prime Editing technology is so

precise that it has the capability to perform highly specified deletions, insertions, and base swapping functions; the outcomes of functions like the ability to delete bases are very important and are considered to be one of the most significant attributes and on the other hand, the errorless insertion of selected nucleotides contributes to knock-in mechanisms. The most special feature of Prime editing that makes it different from other techniques under CRISPR technologies is that even without the creation of dsDNA breaks targeted editing can be achieved very easily. There is no need for donor templates for targeted insertions. Xueli Tian et al. stated that besides being useful, the CRISPR Prime editing technologies find their use in performing site-specific genome editing for curing malignant diseases like Cancer and other oncological studies [3]. Anzalone et al. stated that the Prime editing machinery is composed of two main components—CRISPR Cas9 and reverse transcriptase enzyme [4]. His research group further made an approach to generate three different prime editors (PE1, PE2 and PE3 discussed in the later parts of this section). A fusion protein was created by combining nCas9 and engineered Reverse transcriptase. This fusion protein was further combined with pegRNA or the Prime Editing Guide RNA. The fusion protein-pegRNA complex guided the nCAS9 component of the fusion protein

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to the target where it encodes for its desired edit with the help of its RNA template. After the target is recognized, that is the location where the intended edit has to be incorporated is detected, the strand containing the Protospacer Adjacent Motif (PAM) is nicked followed by attachment of the pegRNA extension binding to this nicked region specifically at the Primer Binding Site (PBS). The 3' flap containing the intended edit is now synthesized by the Reverse Transcriptase domain and this DNA flap is resolved by several repair mechanisms when the PE3 guide RNA nicks the opposite DNA strand in close proximity [4, 5]. The basic mechanism of Prime Editing is illustrated in Fig. 1.

Prime Editing has a huge number of advantages over the conventional CRISPR Cas9 tool for genome editing. For instance, the CRISPR Cas9 introduces DNA double strand break followed by induction of Double Strand Break (DSB) thus carrying a copy of template DNA while Prime Editing does neither require the DNA template nor uses HDR for introduction of the desired mutation of the gene into the target site. There are many other reasons why the Prime Editing has the capability to surpass all other available tools and become the most desirable method for cancer therapy that are discussed in the coming sections of this review. The primary objective of this review is to study extensively the research done on applications of Prime Editing in treating cancer.

Materials and Methods

Recent publications on CRISPR Cas9 based genome editing and Prime Editing involving the mode of action of these tools, applications, advantages and limitations were reviewed. Articles on cancer and its treatment were also taken into consideration while preparing the review. Two databases and one software were utilized to accumulate all related research and review articles. These include Google Scholar, PubMed and Publish and Perish software. As Prime Editing technology is one of the most recently discovered forms of CRISPR Cas systems, most of the articles range from the year 2019 to 2022. Fifty-eight articles were taken as references, out of which nineteen were about conventional CRISPR Cas9 systems, seven were about Prime Editing systems, and the rest were about cancer therapies. The following keywords were used for selecting papers: CRISPR-Cas9, Prime Editing Technology, Traditional Cancer Therapy, Cancer, and pegRNA.

Prime Editors-Underlying Mechanism

Prime editors- a revolutionary and efficient “search and replace” genome editing technology developed by Anzalone et al. [4] is one of the most important, advanced and promising technologies developed in the past few decades

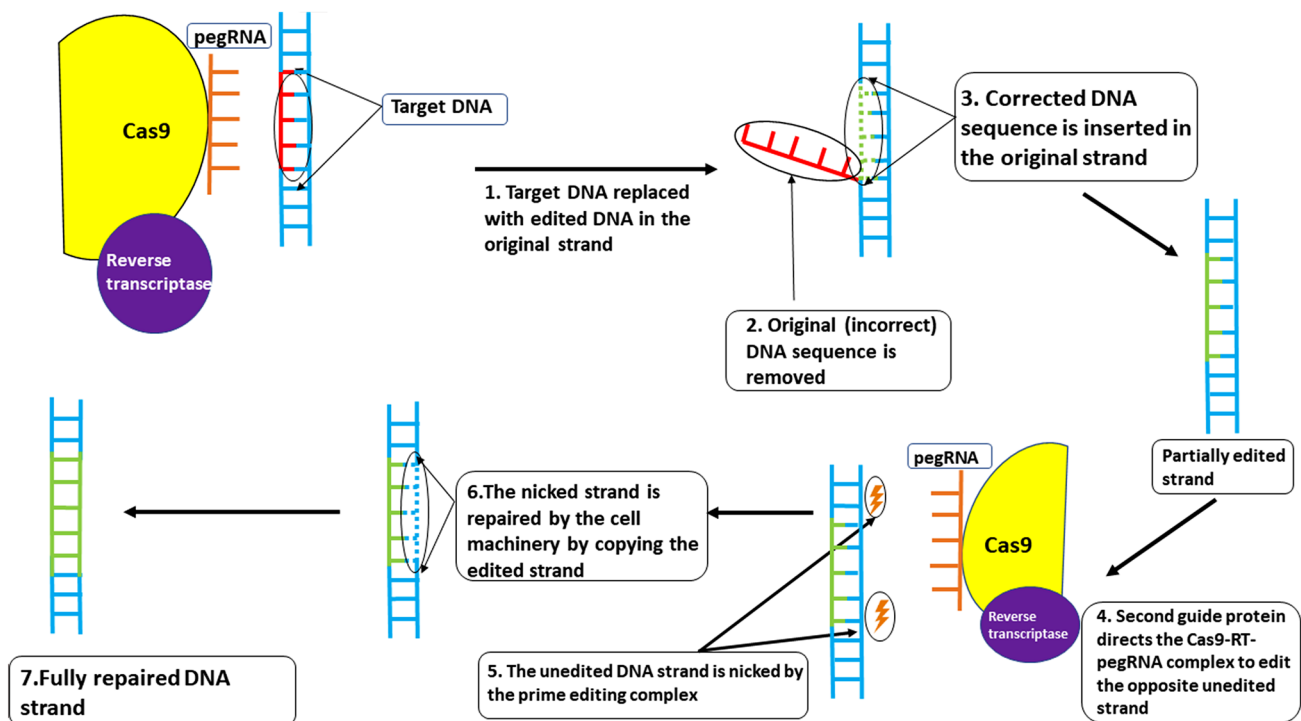


Fig. 1 Basic mechanism of Prime Editing

since it allows researchers to write the genetic code. Three editor devices were designed by Anzalone et al. Prime Editors—PE1, PE2 and PE3 are tabulated in Table 1 and illustrated in Fig. 2. PE1 was developed using the fusion of Cas9 H840A nickase and WT (wild type) Maloney Murine leukaemia virus RT enzyme. The prime editing fusion protein created by the previously mentioned fusion consists of two domains. One domain cleaves a polynucleotide strand nucleotide strand leading to restoration of the same while another domain that is the reverse transcriptase (RT) domain copies the pegRNA (prime editing guide RNA having the desired construct) to generate complementary DNA cDNA. The activities of both the domains restore the nicked DNA segment.

The PE2 device was created by incorporating a pentamutant Reverse Transcriptase enzyme in PE1. The pentamutant nature of the Reverse Transcriptase enzyme was brought about by improving features like thermostability and processivity and substrate specificity of the RT enzyme. PE3 was constructed by the addition of gRNA to the pegRNA. This increased the efficiency of PE3 editor and as labelled PE3b. This gRNA directed the Cas9 H840A nickase element to the prime editing fusion protein. These two components then nicked the genomic strand at a site in proximity to the opposite strand containing the original nick [4].

Role of Prime Editing in Cancer therapy

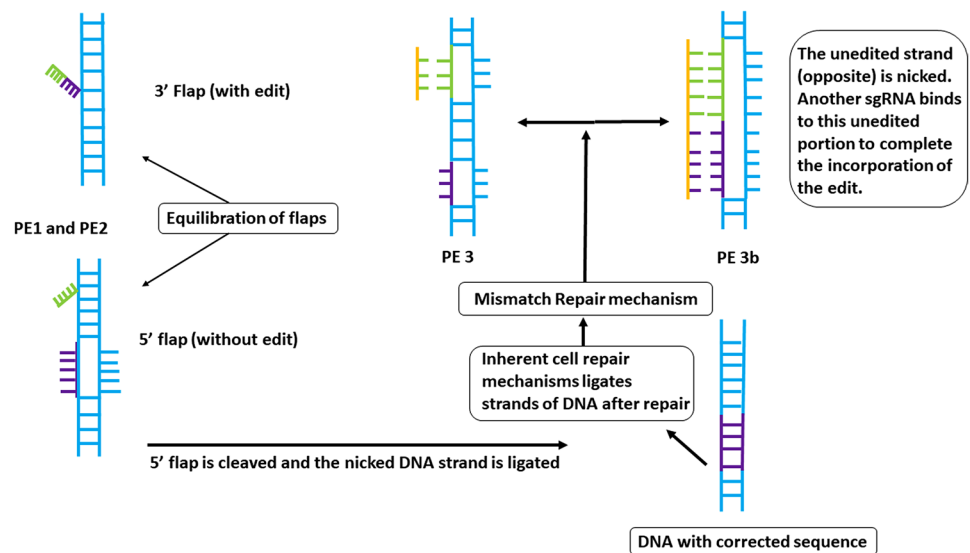
Cancer: A Brief Overview

Cancer (means “crab” in Latin) is described to be a family of diseases in which the growth of tissues and spreading of this abnormal growth in different parts of the body. The abnormal proliferation of cells in an uncontrolled manner and its consequent progress in infecting the rest of the body are the main components of the disease progression. On the basis of origin and the types of cells involved, there are more than 100 types of cancer [6–8]. Several genes are responsible for cancer progression. The most evident genes are the oncogenes and tumor-suppressor genes and alterations in these genes lead to progression of cancer. Rivera and Jacks considered cancer as one of the most malicious and invasive diseases of the past few decades [9]. Uncontrolled growth of abnormal cells followed by the invasion of these cells in the adjacent tissues ultimately leads to organ dysfunction and ultimate failure. Normal cells are transformed into malignant cells followed by the selection of these cells that can promote the disease progression or may impair it. At the terminal stages of the disease progression, the malignant cells consequently lose their cellular uniqueness and result in accumulation of properties like independence in growth,

Table 1 Functional features of the Prime Editors—PE1, PE2, PE3

Sl no	Prime Editor (PE)	Components of the construct	Mechanism involved	Additional features
1	PE1	Cas9 H840A nickase + WT Maloney Murine leukemia virus RT enzyme	Cas9 H840A nickase nicks a polynucleotide strand and RT enzyme generates cDNA (copies the pegRNA carrying the construct) restoring the nicked DNA segment	Additionally increased the length of pegRNA. This device proved to be efficient yet there were presence of detectable genome edits
2	PE2	PE1 + Pentamutant Reverse Transcriptase Enzyme	Improved version of PE1 in terms of thermostability, affinity of DNA–RNA for the substrates, processing capacity	As it is an improvised version of PE1, it increased the ability of PE1 by almost five folds in efficient prime editing point mutation. Led to strategic incorporation of targeted insertions and deletions
3	PE3	An additional gRNA was coupled with pegRNA	gRNA directed Cas9 H840A nickase component of PE1 to nick genomic DNA at a site near to opposite strand nicked portion	Improved efficiency when Anzalone et al. constructed the pegRNA to bring about mutation in the initial Protospacer Adjacent Motif (PAM)

Fig. 2 Role of Prime Editors (PE1, PE2, PE3)



invasiveness. These malignant cells do not respond to cellular mechanisms like senescence and apoptosis [3].

While there are several therapeutic methods available for the treatment of cancer, most of the older methods include surgery [10], and radiotherapy [11, 12]. These therapies serve as effective anticancer therapies. Surgery is one of the most effective methods against localized primary tumors and associated regional lymphatics and this method kills almost a hundred per cent of malignant cells. As new methods like radiation therapy and chemotherapy were brought into light (1920s–1940s), cancer therapy acquired a conservative nature. The chemotherapeutic strategies could only kill a fraction of cells while surgical methods could remove an entire portion of malignant cells. The discovery of new biomarkers as potential candidates for cancer therapies was indeed remarkable yet all the above-mentioned therapeutic methods had several drawbacks as well. Some examples of biomarkers include; Easton et al. developed BRCA1 germline mutation (breast and ovarian cancer) to estimate the risk of developing cancer [13]. CEA (colorectal cancer) is a biomarker developed by Locker et al. to monitor the recurrence of disease [14]. KRAS mutation and anti-EGFR antibody (colorectal cancer) for predicting response to therapy [15]. Ideal targets need “critical for growth” characters that the biomarkers lack. Besides, chemotherapeutic and radiation-based methods present unwanted and critical side effects [16]. To overcome the undesired outcomes of cancer therapeutics, recently scientific communities around the globe are in search of alternative approaches which may alleviate the harsh after effects.

Prime Editing- a technique has been gradually gathering noteworthy efforts in seeking a position in cancer therapeutics. Prime Editing is capable of overcoming major drawbacks of chemotherapeutic and radiation-based strategies.

Prime Editing uses a site-specific mutagenesis approach and presents high efficiency and precision in terms of genome editing [1]. Anzalone et al. demonstrated Prime editing as one of the most promising tools for genome editing. Prime Editing is a versatile and precise editing tool for genomes that is responsible for incorporating new genetic information at a specified location on DNA. Cas9 a catalytically impaired endonuclease coupled to an engineered reverse transcriptase and a prime editing guide RNA (pegRNA) aids to locate the target site and codes for the desired edit. Prime Editing technique was used to edit human cells and treat primary genetic causes of sickle cell disease and Tay-Sachs disease with the generation of fewer by-products. Anzalone et al. research represents the substantial expansion of prime editing as a promising tool for editing genomes and this efficient tool can correct up to 89% of previously discovered genetic variants that are associated with human diseases [4]. Though numerous research is being conducted on the PE in cancer therapy, an in-depth review discussing the different aspects is needed. Hence this review focuses on Prime Editing as one of the most efficient genome editing technology that minimizes undesirable effects of conventional cancer therapeutics and its prospects.

Cancer is a malignant disease which uses blood and lymph as its main vehicle of invasion. Cancer provides multiple genetic and epigenetic alternatives leading to the generation of diverse modifications like that of carcinoma (cancer of tissue lining organs and skin) [17], lymphoma (cancer of the lymphatic system) [16], myeloma (cancer of plasma cells) [18], and sarcoma (a malignant tumor arising from transformed mesenchymal cells) [19]. As per Sánchez-Rivera et al. the impersonation of cancer initiation and progression processes and at the same time influencing the mammalian genome by subjecting it to several

manipulations are important components for shortening a lengthy traditional process. Genomic screening in an extensive and wide range manner is a possible remedy that allows researchers to keep track of incident gene mutations leading to oncogenetic changes [9].

Statistical Data on Cancer Types

A statistical study by Siegel et al. demonstrated that prostate, lung and bronchus; colon and rectum, and urinary bladder cancers are the most common types of cancer associated with men. While in women, breast, lung and bronchus, colon and rectum, uterine corpus and thyroid cancers are very common. The data available from the statistical study proved that breast cancer in women and prostate cancer in men are the most prevalent types of cancer [20]. In 2006, Schottenfeld et al. studied the various cancer types in children. Brain cancers, blood cancers and cancers associated with lymph nodes were common in children [21]. A statistical representation of different types of cancer as per CDC (Centre for Disease Control and Prevention) is illustrated in Fig. 3. Several agents contribute to disease progression and the most prominent ones in the case of cancer are smoking, carcinogenic chemical compounds, viruses, bacteria, radiation rays and other environmental chemical compounds that may have a direct impact on the cytoplasm and nucleus of the cells. These impacts result in genetic mutations and disorders [22].

Molecular Mechanism of Cancer

Kandoth et al. [23] conducted a detailed study on the molecular mechanism behind cancer. The first step of the complex set of events includes cells gathering driver mutations in crucial genes. This leads to the transformation of a non-cancerous cell into a cancerous one by changing the expression of functional proteins. These cells then influence their clonal expansion into tumors due to several signalling system cascades that lead to an increase in proliferation; in other words, a significant decrease in apoptosis takes place. The tumor then derives energy from nutrients by bringing about changes in the cellular metabolism and hence depriving normal cells of the surrounding environment of crucial substrates for their normal growth and development. Angiogenesis [24] leads to the formation of new blood vessels in the tumor (potentially due to anoxic conditions). These blood vessels increase the oxygen supply to the tumors. These set of events are all mediated by Vascular Endothelial Growth Factor (VEGF) [25]. This is followed by the growth of tumor, aggressive invasion and migration to the cell membranes and metastasis to other tissues leading to either continuation of the process or ultimately leading to death. Figure 4 shows the detailed mechanism of cancer development [26].

Fig. 3 Statistical representation of cancer types by CDC (2020)

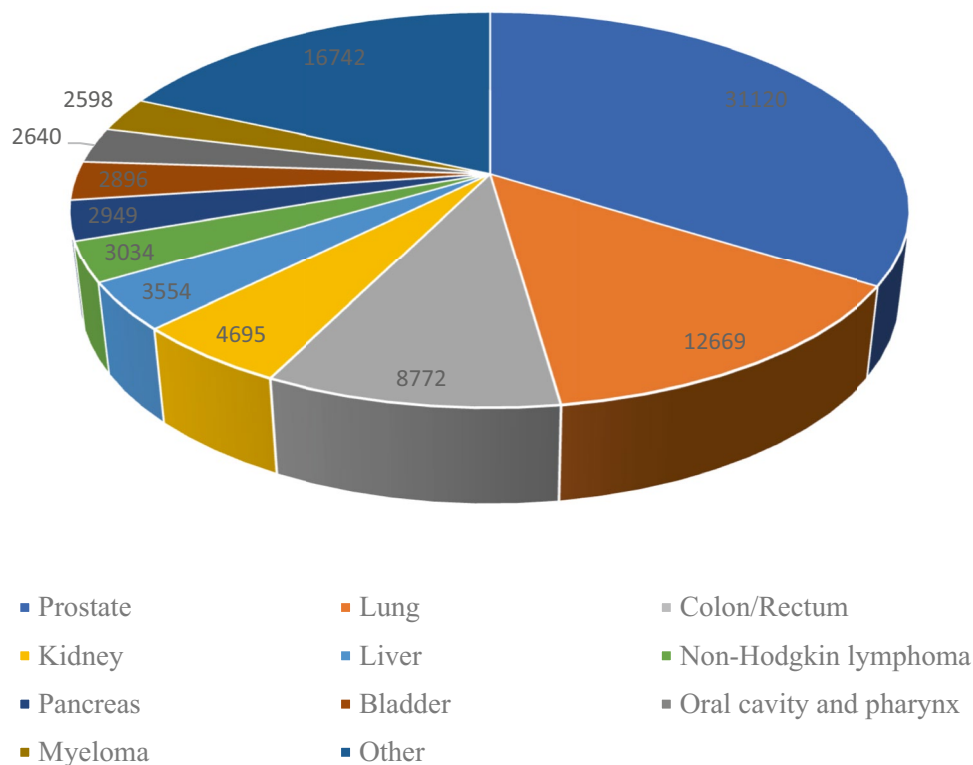
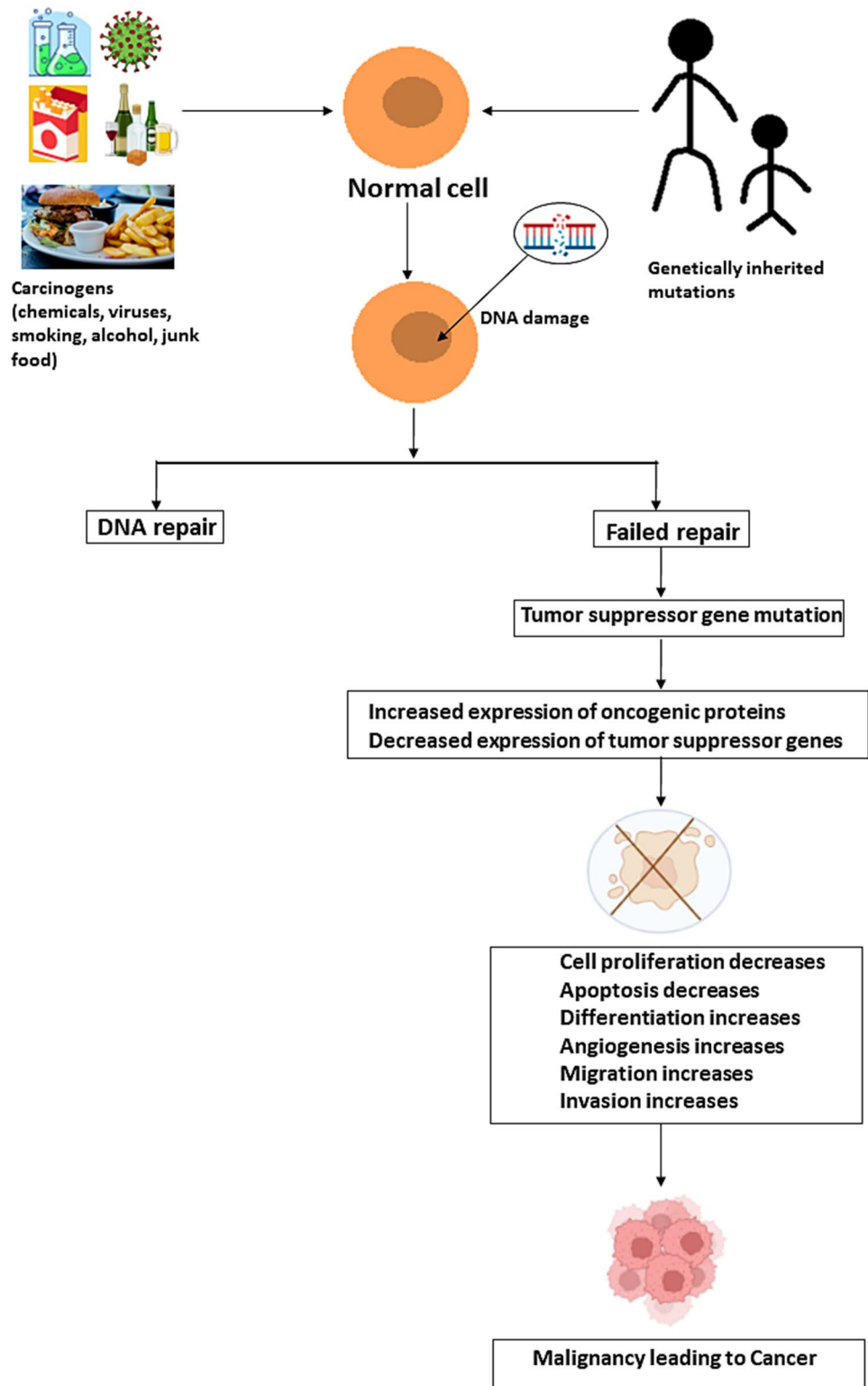


Fig. 4 Depicts the flow chart representation of cancer development



Traditional Cancer Therapies

Cancer treatment methods largely depend upon the type of cancer diagnosed and the stage at which it is detected [27]. No specific method is available for the treatment of

cancer as most of the treatment methods include chemotherapy, radiation therapy, hormonal therapy, targeted therapy (immunotherapy), and surgical procedures or a combination of these mentioned therapies according to the requirement of the treatment [28]. The therapeutic strategy mainly relies

on the fact that the removal of the cancer tissues without harming the adjacent tissues. However, this seems difficult to the metastatic activity of the cancer cells. New treatment plans and strategies are evolving each day as the biological mechanisms behind the cancer signalling mechanisms are being studied. This increases the effectiveness of the therapeutic mechanisms and also improves their precision at the same time enabling better survival rates of the patients [29].

Surgery

Surgical methods are among the widely accepted treatment methods for cancer. Surgical procedures can remove cancerous cells from the organs and are mostly responsible for the removal of non-hematological cancers from the body. The surgical procedures include methods such as mastectomy of breast cancer, brain tumour by neurosurgery prostatectomy for prostate cancer, kidney cancer, lung cancer, liver cancer etc. [30].

Chemotherapy

This therapy includes the use of anticancer drugs that interfere with the growth of tumors and destruction of cancer cells. Chemotherapy is one of the most effective methods of treatment yet it has several side effects. These side effects are largely dependent on the types of drugs used, the target locations and the person's response to such anticancer drugs.

Radiation Therapy

This method is most commonly used to treat cancers like brain, breast, cervix, larynx, lung, pancreas, prostate, skin, stomach and uterine cancers. It is also used to treat leukemia and lymphomas [31]. The most commonly used technique -brachytherapy is an important radio-therapeutic modality for a variety of malignancies, including prostate cancer, cervix cancer, breast cancer, vagina cancer, endometrial cancer, head and neck cancer, and many more [32].

Immunotherapy and Hormone Therapy

Immune check-point based therapy is progressing at a rapid pace and is one of the most important and evident therapies for cancer treatment. Antibodies against T cell checkpoints have revolutionized cancer treatment. However, there is a limitation associated with the same [33]. Only a small portion of patients respond to immunotherapeutic treatments and hence selection of patients is a major point to be kept in mind in order to prevent treatment related toxicity [34]. The disease fighting mechanism of the patient's immune system is stimulated to fight cancer and this therapy is commonly referred to as biological therapy. Monoclonal antibodies can

block specific protein functions by binding to specific cancer cells. This method is quite safe and does not have major side effects. In case of hormone therapy, by changing the hormone levels certain types of cancer can be treated including breast, reproductive system and prostate cancers [29].

Tumor Microenvironment

Tumor microenvironment refers to the cellular location containing tumor inside the body. Several interactions of the tumor with the environment can lead to several impacts. These impacts include release of extracellular signals that promote tumor angiogenesis in turn inducing peripheral immunity tolerance [29]. Apart from malignant cells, the tumour micro-environment contains immune system cells—fibroblasts, pericytes and sometimes adipocytes. Designing a tumor microenvironment system is essential and crucial for disabling or reprogramming the tumor promoting and suppressive immune system. The problematic blood supply is either normalized or completely destroyed leading to the development of new antigens that might be recognized by the immune system [35]. The subtype of each therapeutic strategy is summarized in Table 2.

Prime Editing in Cancer

As cancer is a multifactorial malady causing malignant mutations, the key to the treatment of such a disease has been one of the most significant research interests of CRISPR researchers. The therapeutic potential of CRISPR Cas9 has been investigated to reverse such malignant mutations. Prime Editing has found its applications in organoid production for induction and correction of mutations. This tool has also been used in human induced pluripotent stem cells, human adult stem cells and other cancer lines as well.

A study by Geurts et al. demonstrated the application of nickase Cas9 fused to reverse transcriptase that edited the target site in human organoids in order to introduce mutations related to cancer followed by repairing mutation caused due to cystic fibrosis to the CFTR gene [5]. Their research proved the therapeutic potential of PE in the treatment of diseases in organoid-based models however there were some problems of off-target effects that are discussed in the termination sections of this article.

Oncogenic mutations could be modelled in two organoid models (for TP53 and APC sequences) [39]. However, the results of the experiment by Geurts et al. showed varying levels of efficiency in terms of colonic and hepatocyte organoids [40], thus indicating that the efficiencies were dependent on the type of tissues that were being edited. Undesired mutations were also obtained near the target site. On overcoming the problems associated with off target effects in Prime Editing, the editing efficiencies were considerably

Table 2 Traditional cancer therapies

Therapeutic strategy	Used in (type of cancer)	Site of action (or mechanism)	Advantages	Side effects	References
Methotrexate (MTX) (chemotherapy)	Breast cancer	Competitive inhibitor for dihydrofolate reductase leading to accumulation of folate required for purine synthesis for DNA; cell cycle is arrested	Contributed to prolonged remission of neurological symptoms, increase in survivability and quality of life in patients	Acute meningitis, chronic-delayed encephalopathy, radiculitis, myelosuppression and mucositis	[36]
Brachytherapy (radiation therapy)	Breast Cancer, Prostate Cancer, Vagina cancer, Cervix cancer, Endometrium, head neck and lung cancer	Delivers precise radiation doses to the tumor from the inside out in cells that are in M phases and G ₂ phases that are particularly sensitive to radiation	Radiation dose delivered to the tumor and adjacent tissues is highly conformal and hence reduces the surrounding structure/organ exposure	Long term efficiency and safety issues and may cause collateral radiation exposure to healthy tissue of medical staff	[32]
CTLA-4 based immunotherapy (immunotherapy)	Prostate cancer	Ipilimumab- a fully human monoclonal antibody that decreases the binding of CTLA-4 to B7 this increases antitumor immunity	When combined with prostate cancer vaccines, Ipilimumab appears even more beneficial for the patients	Not any as such	[37]
Tamoxifen hormone therapy (hormone therapy)	Breast cancer	Estrogen agonists, antagonists or both, depending on the tisular target	Effects are continued after treatment termination and is independent in additive to chemotherapy	Side effects are associated with endometrium neoplasm, uterine sarcoma and the thromboembolic risks	[38]

lowered leading to undesired mutations and the introduction of indels as well.

Nucleic acid detection in a rapid manner is one of the most essential and crucial components in the detection of diseases in humans. CRISPR Cas9 has been used to design effective treatment methods to treat patients with hereditary and infectious defects. Till date there are several tools associated with CRISPR Cas9 that have been developed to diagnose infectious diseases like SARS Cov2 as well as non-infectious diseases like cancer. There is a possibility that these tools may assume superior value and replace traditional tools like PCR based diagnostics [41].

A wide variety of applications of CRISPR Prime editing were discovered by pioneers of the field- Feng Zhang [42], James Collins, and Pardis Sabeti of Brad institute [43], and Jennifer Doudna's group at UC Berkeley [44]. Prime Editing efficiently and most significantly helps in precise incorporation of edits on single nucleotides. These strategies used by the promising tool have opened possible avenues in curing diseases like Tay Sach's disease, Sickle cell disease and Duchenne muscular dystrophy [45]. Prime Editing is gradually becoming an efficient tool for genome editing and its therapeutic efficiency in curing malignant diseases like Cancer is being explored to a vast extent. The ability to reverse mutations in Cancer can be detected using this tool. The products of prime editing are namely HPSCs (Human Pluripotent Stem Cells), [46] organoid models for cancer [5], CombiGEM CRISPR study [47]—which provides an approach for synergistic gene study, CRISPRres [48], Drug TargetseqR validation [49], SHERLOCK (*Specific High Sensitivity Enzymatic Reporter UnLOCKING*) and DETECTR (*DNA Endonuclease Targeted CRISPR Trans Reporter*) for diagnosis of the gene. Cancer treatment has become much more personalized and customized with the increase in specificity. This fact is further proved by the discovery of CAR-T cells that are the major game changers of cancer immunotherapy. The Prime Editors can induce as well as correct mutations in organoids. Schene et al. successfully edited primary stem cells using the Prime editing approach matching efficiency standards at par with that of human cancer cell lines. However, their final results indicated that PE can incorporate mutations in intestinal and hepatocellular adult human stem cells with few undesirable outcomes and hence need some improvement in mutational modelling and gene repair mechanisms [40].

Mustafa et al. described the efficiency of SHERLOCK for its single nucleotide specificity that helped to deliver genotyping profiles of cancer patients. This genotyping profiling was achieved by recognizing the associated mutations from circulating cell free DNA, at low concentrations in serum and urine samples [41]. The specificity can be further increased by introducing a synthetic mismatch into the crDNA [50].

Gootenberg et al. in his research article stated that SHERLOCK (Specific High Sensitivity Enzymatic Reporter unLOCKing) is a personalized molecular diagnostic tool that has achieved high specificity in the identification of target RNA sequences. SHERLOCK identifies the RNA of interest by engaging in several mechanisms like RNA binding cis cleavage activity of Cas13a and Cas13b5 RNA endonucleases [51].

Chen et al. described the mechanism of DETECTR, the abbreviated form of DNA Endonuclease Targeted CRISPR Trans Reporter based on CRISPR. Certain enzymes of the CRISPR family have an advantage over others in terms of achievement of sensitive and specific detection of viral nucleic acids, and sequences, from clinical samples. The Doudna lab decided to distinguish between DNA of two varieties of Human papillomavirus (HPV). They isolated anal swabs from 25 patients in a clinical setup and successfully investigated to find out the presence of multiple forms of HPV present in the sample [52]. This was indeed a great technique to detect a mixture of DNA containing impurities. An HPV16 genome-specific guide RNA was used to make DETECTR align correspondingly with Polymerase Chain Reaction (PCR) in those 25 patients. In the case of the HPV18 genome, DETECTR concordant in all but two specific cases showed weaker amplification by PCR [53]. According to Wong et al. CombiGEM-CRISPR, utilizes one-pot cloning steps to empower the get together of combinatorial gRNA libraries, hence improving and speeding up the work process toward the orderly examination of combinatorial gene functions. CombiGEM is exceptionally adaptable and can oblige any hereditary components of interest. It would thus be able to be custom-fitted to address the clients' particular exploration questions. According to Wong et al. CombiGEM has been effectively applied to practically portray combinatorial quality knockouts created utilizing multiplexed gRNA articulation notwithstanding the combinatorial articulation of other hereditary components [54]. According to Santomaso et al. CAR- T cell treatment is a sort of therapy wherein a patient's T cells (a kind of safe immune framework cell) are changed in the research facility so they will assault malignancy cells. Major histocompatibility complex (MHC) atoms assume key parts in the reconnaissance of atypical proteins of tumor cells. White blood cell receptors (TCRs) on the outside of T lymphocytes perceive antigenic peptide parts determined from these distorted proteins in complex with MHCs [55].

Aida et al., in 2020 stated that in an investigation of the utilization of prime editing for a useful fix of changes in human intestinal undifferentiated cells from CF (Cystic Fibrosis) patients [56]. Despite the fact that the right reconciliation of the ideal edits was accomplished on an assortment of targets, undesired edits were additionally uncovered, as has been distinguished before in mice. As per the reports

of Ran et al., the utilization of two sgRNAs that nick contradicting strands is known to produce indels and is even regularly used to build explicitness of CRISPR/Cas9-regulated genome designing [57]. According to Koblan et al., Zafra et al. over the previous years, base editing plasmids have gone through a few rounds of streamlining transforming them into proficient genome editors [58, 59]. Subsequently, prime editing is a flexible instrument that can be utilized for disease demonstration and clinical fix of most sorts of sickness causing changes in human grown-up immature microorganisms yet will require further improvement to permit inescapable use as a method for mutational displaying and for quality fixation of the gene [5].

Conclusions

Prime Editing has been gathering huge momentum in the field of therapeutic genome editing. This tool has tremendous potential to surpass the conventional CRISPR Cas9 system and the Base editing approach to correct and edit genomes. The non-requirement of the Homology Directed Repair (HDR) pathway makes it a better choice than CRISPR Cas9 systems. HDR is less efficient and might cause unwanted indels while DSBs cause a large number of off target mutations. Moreover, PE allows about 12 single base mutations on target sequences rather than the conventional CRISPR Cas9 that hardly allows about small number point mutations only. The Cas9 nickase component of the Prime Editing complex makes it powerful and removes the need for induction of DSB or HDR dependent pathways. The triple hybridization sites for PEs to target sequences increases the specificity of the tool thus yielding correct cleavage and modification of target sequences thus eliminating possible off target effects. Whereas the CRISPR Cas9 has only a single hybridization site to the target sequence thus alteration of function might be seen due to incorrect hybridization leading to chances of genome instability.

While CRISPR Cas9 and Prime Editing is a very promising tool in terms of cancer therapeutics however there are significant disadvantages. There remains a scope of unwanted mutations in case of editing by the PE3. Many components of Prime Editors are still to be optimized for proper execution.

Another limitation associated with the generation of cancer models includes the precise manipulation of particular cell types. Here problem can be addressed by development of a cell type specific nanoparticles that may precisely deliver the functions of CRISPR Cas9 mechanisms [60]. Other drawbacks include accessibility and cost, there is a need for controlled clinical trials with adequate review, and policies for compassionate use. There are regulatory challenges and ethical issues pertinent to CRISPR technologies

to edit somatic and germ line cells. Prime Editing may also introduce undesirable mutations and there are limitations in terms of large DNA insertions. Hence further studies are required to establish the efficiency of the Prime Editing tool as a promising tool for cancer therapeutics with minimized drawbacks [1].

The CRISPR associated methods must be compared to the traditional and conventional already existent methods of cancer therapeutics in order to measure the true potential of these tools. The precise and targeted delivery of CRISPR Cas9 in tissues and their consequent side effects in the surrounding cells still remains to be a major issue to be addressed. The use of CRISPR Cas9 in detecting more complex genetic disorders and cancer related problems still needs to be investigated [61]. A combinatorial approach must be developed for further optimization, reduction of possible off target effects and delivery of the prime editors for making it the most desirable tool in the field of genome editing.

Author Contributions PM contributed in conceptualization of the article; PM and DS contributed in literature search and drafting of manuscript, tables and figures. PM and SS performed proofreading, editing of the data analysed and approved the manuscript for communication. All authors read and agreed to the final version of the manuscript.

Funding This research was not funded.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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