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Using CRISPR/Cas9 Technology to Target TP53 Related Cancers

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Abstract. Tumor protein 53 (TP53) is a tumor repressor gene that is highly responsible for activating cell cycle arrest in the event of DNA damage, stopping the replication of damaged cells, which may cause cancer. However, when mutated, TP53 is an oncogene, with mutated variants of it being seen in over half of all cancers diagnosed including the aforementioned four. With groundbreaking CRISPR-Cas9 gene editing technology coming to the forefront of biotechnological research, the applications to cancer research and gene therapy have been increasing. This review explores CRISPR-Cas9 and its uses for treatment and medication investigation in various cancers, including lung, prostate, breast, and bone cancer through the lens of the TP53 gene. Due to TP53's commonality in cancer, CRISPR-Cas9 gene editing technology can be used to its maximum potential in this context as a treatment or investigative tool, with it yielding positive results in reverting tumor growth, as well as great versatility when it comes to screening related genes that inhibit or encourage tumor growth which can be targeted by drugs, or even the screening of the drug's effectiveness themselves. Thus, CRISPR-Cas9's applications in TP53-related cancer treatment and chemotherapeutic fields prove this technology to be successful.

Keywords: TP53, cancer, CRISPR/Cas9.

1. Introduction

Cancer is a disease caused by uncontrollable cell growth caused by genetic mutations which eventually manifest into tumors that harm your body [1]. Cancer cells typically have undergone multiple bouts of DNA damage, causing them to accumulate several generations' worth of mutations that cause them to form tumors [1]. DNA damage can be caused by specific triggers, named carcinogens, with 90-95% of them originating from a person's poor lifestyle choices including tobacco, alcohol (which when metabolized by the liver create carcinogenic acetaldehyde), and poor diet choices like red and processed meat [2]. DNA damage usually occurs in the form of DNA strand breaks as a consequence of carcinogens, where either one or both strands of the cell's genetic code get cleaved and end up being incorrectly repaired, allowing for the DNA to be highly susceptible to mutations [1].

Tumour Protein 53 (TP53) is a gene that encodes p53, a critical protein crucial in tumor suppression during the cell cycle [3]. These tumor suppression proteins regulate cell division and ensure that cells do not divide with mutations caused by damaged DNA, therefore stopping the possibility of an accumulation of genetic defects from occurring after multiple generations of cell division. The activation of TP53 due to DNA damage leads to either cell cycle arrest, cell senescence, or even apoptosis when it

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is found that the DNA within the cell is beyond repair [4]. Being such a crucial gene in preventing cancer by regulating the cell cycle, TP53 is considered a proto-oncogene and aptly named the "guardian of the genome" [3]. However, in its mutated state where it loses its function or doesn't operate correctly to regulate cell division, it becomes an extremely common oncogene, present in 50% of all cancers diagnosed [5].

CRISPR-Cas9 gene editing technology utilizes single guide RNA (sgRNA) instead of the crRNA:tracrRNA complex [6]. sgRNAs can be sequenced and synthesized artificially to recognize the specific gene and cleave it [6]. CRISPR-Cas9 gene editing technology has been used in *TP53* gene editing in multiple models, including genetic screening, testing for drug resistance, and gene mutation repairing.

This review focuses on how CRISPR-Cas9 editing revolving TP53 can be used to develop treatments or increase our knowledge of various types of cancers.

2. Cancer-related signaling and TP53 gene function

2.1. Characteristics of cancer

The characteristics of cancer cells and resulting tumors can be most commonly seen in their immune system repression, programmed ignorance of apoptosis commands, and even angiogenesis so that the tumor can redirect the body's resources to itself so that it can spread [1]. An especially common characteristic that cancer cells have is their ability to spread and create secondary tumors around the body, a process named metastasis. This is completed through the primary tumour's cancer cells breaking off and using the body's blood vessels or lymph nodes as pathways to other organs and their respective tissues [7].

2.2. TP53 gene

Tumour Protein 53 (TP53) is a gene that encodes p53, a critical protein crucial in tumour suppression during the cell cycle. These tumour suppression proteins regulate cell division and ensure that cells do not divide with mutations caused by damaged DNA which may be inherited in the next generation of cells [1].

TP53 is expressed when DNA damage occurs within the cell, producing the p53 protein, a transcription factor that induces the production of another protein named p21 [8]. Then, the p21 protein inhibits the cell cycle checkpoint at G1/S phases by inhibiting cyclin/CDK complexes, halting the retinoblastoma's phosphorylation, and blocking the release of E2F [8]. This important transcription factor binds to the promoter regions of the genome for DNA replication during the S phase, thus pausing DNA replication and inducing an overall pause in the cell cycle [8]. If the DNA is damaged beyond repair, TP53 also causes cells to senesce due to the formation of heterochromatin on the E2F target genes, repressing any further cell division. Alternatively, TP53 may order cells to undergo apoptosis through p53's signalling of pro-apoptotic genes [8]. TP53's molecular mechanism is illustrated in a diagram in Figure 1.

TP53 is one of the most common genetic mutations found in cancers being found in 50%-60% of them, with 90% of these TP53 mutations being missense mutations which cause the protein it produces, p53, to lose its function [9]. For example, missense mutations like R175H, R248Q, and R273C cause TP53 to produce p53 that is unable to bind to DNA, removing its ability to transcribe p21, and overall stopping the process of inducing cell cycle arrest leading to the division of cells with DNA damage [10].

The proliferation of cells with mutated TP53 causes cells to replicate without a proper mechanism to check mutation causing DNA damage, leading possible cancer-causing mutations to accumulate more easily in later generations of cells which may end up in abnormal cell growth and finally tumours. However, TP53's ability to be incredibly useful in tumour suppression due to its all-encompassing DNA protective properties, along with its pathological mutation's pure commonality in a diverse set of cancers, warrants it to be studied more closely for its strong relation with the disease.

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3. CRISPR/Cas9 technology

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is a specific loci found in bacteria and serves as a bacterial immune system. This loci is characterized by short palindromic repeating sequences of bacterial DNA interspaced by fragments of DNA originating from past viral invaders that the bacteria has encountered before [6]. When the CRISPR loci get transcribed, it creates pre-CRISPR RNA (pre-crRNA). Further upstream past the CRISPR loci, transcription occurs to create trans-activating RNA (tracrRNA), a type of RNA that creates a complex with pre-crRNA so that tracrRNA can be used to mature the pre-crRNA, as well as cleave the short palindromic repeats out of the pre-crRNA so that it only isolates the now transcribed viral RNA, creating mature crRNA [6]. This complex then binds with Cas9, a protein that contains nucleases that cleave DNA when it recognizes DNA which is complementary to that of the crRNA:tracrRNA complex [6]. Now, whenever a virus that has invaded a bacteria in the past invades it again, the viral DNA that has been injected into the bacteria is identified through its complementarity with the crRNA and gets cleaved by the Cas9 protein, causing DNA damage which essentially neutralizes the virus' biological attack on the bacteria [6].

Outside of the context of bacterial immune systems CRISPR-Cas9 gene editing technology utilizes single guide RNA (sgRNA) instead of the crRNA:tracrRNA complex [6]. sgRNAs can be sequenced and synthesized artificially, meaning that the Cas9 protein can recognize any strand of DNA and cleave it as long as an appropriately complementary sgRNA commands it to. Donor template DNA is also inserted into the Cas9 protein, such that it allows for the cleaved DNA to be repaired with accuracy through homology-directed repair (HDR), essentially controlling how the cleaved gene can be remade and therefore edited [6].

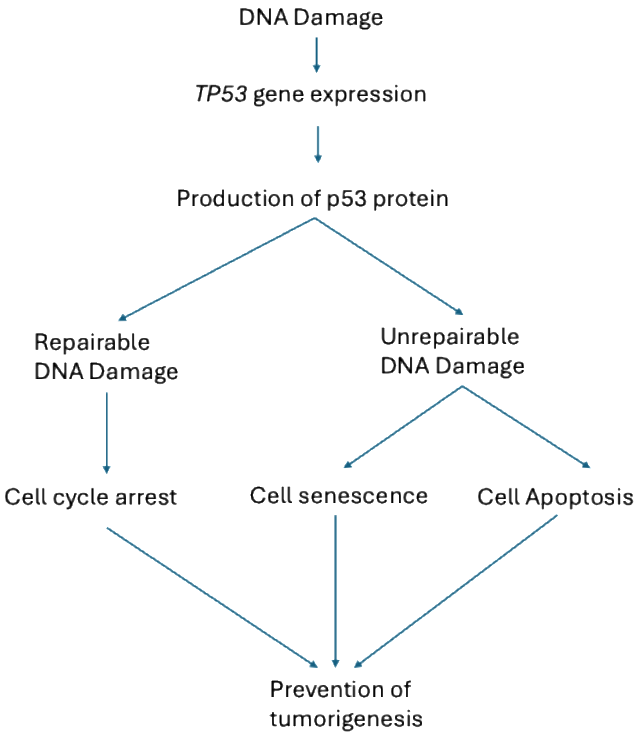

Figure 1. Molecular mechanisms of TP53 in response to DNA damage to prevent tumorigenesis. Figure credit: original.

4. Applications

Targeting TP53 gene with CRISPR/Cas9 technology has been widely used in multiple cancers, including lung cancer, prostate cancer, breast cancer, Osteosarcoma, etc. (Table 1).

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4.1. Lung cancer

4.1.1. CRISPRko screening in wtTP53-RTK NSCLC. CRISPR-Cas9 can be used for screening purposes through the CRISPR knockout (CRISPRko) Screening technique. It can be greatly exemplified in a study by Wang et. al, where CRISPRko was used to identify MDM2 as a therapeutic gene target for non-small cell lung cancer (NSCLC) patients which have both wild-types of TP53 and RTK genotypes [11].

This is first done by obtaining the wtTP53-RTK NSCLC cell line, NSCLC cancerous cells containing the wild types TP53 and RTK genotypes. lentiCRISPR plasmid library v2 was then inserted into HEK239T cells, which create lentiviruses containing over 123,000 sgRNAs spanning over 19050 genes [8]. After these lentiviruses transduce the cell line with the plasmid library, Cas9, and the sgRNAs form complexes, with the Cas9's nucleases knocking out genes in the cell using the sgRNA as an identification mechanism. After a period of incubation, cells and the number of specific sgRNAs that relate to genes are analyzed and quantified through the MAGeKFLUTE system. It is here that and this is where the MDM2 gene's sgRNAs were found to be depleted, with depleted sgRNAs showing that the gene it is related to when knocked out leads to anti-growth behaviours, as the cells containing the sgRNA have stopped/slowed down in proliferation or even underwent apoptosis, decreasing the sgRNA concentration [11]. Mechanistically, the MDM2 protein is a p53-down regulator that typically ubiquitinates it such that it is marked for proteasomal degradation [12]. However, when knocked out, the protein is not present to mark the p53 for degradation, leading to an increased concentration of the tumor-repressing protein, fortifying its cell cycle arrest and apoptotic signaling [12].

After confirming MDM2 as a gene therapy target for NSCLC with the specific wtTP53-RTK genotype, the study then successfully used pharmacological inhibitors of MDM2 such as RG7388 and pemetrexed *in vivo* to cause anti-tumor behaviors, applying the knowledge of MDM2 as a gene therapy target to the greatest degree. In the future, more treatments that target the MDM2 gene in wtTP53-RTK NSCLC cancer patients should be investigated through rigorous clinical trials and further development of drugs.

4.1.2. TP53KO in adenocarcinoma cells to investigate drug resistance. CRISPR-Cas9 can also be used in the context of investigating different types of chemotherapeutic drug resistance. In the study by Hou et. al, CRISPR technology was used to knockout the TP53 gene in the A549 adenocarcinoma cell line to investigate how chemotherapeutic PI3K inhibitors may be less efficient in mutant TP53 adenocarcinomas [13].

PI3K is a family of enzymes that are critical in the PI3K/Akt signalling pathway. This pathway is responsible for cell proliferation, growth, and survival, as PI3K phosphorylates PIP2 into PIP3 a recruiter for Akt [14]. Akt has many downstream effects, including blocking BAX apoptosis signals, promoting protein synthesis through mTOR, and even ubiquitinating the tumour repressor FOXO so that it can be degraded by a proteasome [14].

To create the mutant TP53 cells, PX461 double-nicking CRISPR-Cas9 vectors were used with an inserted pair of sgRNAs which include the 490th nucleotide of the TP53 gene located in the 4th exon [13]. This specific location was picked as it would create a double-strand break at the DNA binding domain (DBD) of the TP53 gene, causing the most authentic type of TP53 mutation in which the p53 it creates is unable to bind to p21's DNA receptors to initiate the cell cycle arrest pathway. This vector was then amplified in E. Coli and then transfected into the A549 cells, with successfully transfected cells emitting fluorescence and sorted by flow cytometry [13]. Next, these cells are treated with PI3K-specific inhibitors, PI3K-associated inhibitors, PI3K-non-related inhibitors, and finally protein-based stimuli, where their rates of proliferation inhibition, and thus inhibition efficiency, are measured [13].

From the results, it can be seen that PI3K inhibitory drugs are less resistant to TP53 negative cells compared to TP53 positive cells. For example, when the PI3Kδ isoform inhibitor HS-173 [15] is administered to TP53 negative cell samples, the effectiveness of inhibition is less than half of that found in TP53 positive cell samples among four different dosages. This means the PI3K inhibitors were mostly

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unsuccessful in decreasing PI3K's proliferation and tumorigenic behaviours in the adenocarcinoma cell line [13]. This is because the mutated DBD of p53 disrupts the pathway PI3K inhibitors through their inability to initiate transcription, decreasing the effect of inhibition.

In the future, these studies should be replicated in vivo to observe the drugs being administered in a more complex system. Furthermore, specific drugs that do carry some inhibitory promise in the presence of TP53-negative cells like the CZC24832 PI3Kγ inhibitor at the specific concentration above 0.05μM [13] should be further investigated in vivo and in clinical trials.

4.2. Prostate Cancer

4.2.1. Inside PC-3 cells with guide blocking mutations. The PC-3 cell line is a prostate cancer cell line that is often used in prostate cancer research. This specific cell line contains a TP53 mutation named TP53 414delC, a gene-altering frameshift mutation that sees the 414th nucleotide cytosine being deleted, leading to the creation of truncated and ultimately non-functional p53 when the gene is translated, halting the ability for p53 to successfully activate its cell cycle arrest pathway [16]. In a study by Batir et. al, a pair of sgRNAs, as well as two single-stranded oligodeoxynucleotides (ssODNs), were used in combination to increase the gene editing efficiency of repairing the TP53 414delC mutation through the implementation of guide-blocking mutations [16].

Guide-blocking mutations are single nucleotide mutations induced into a gene, in this study, delivered by the ssODNs, that are used to prevent the Cas9 system from re-cleaving the DNA after homologous directed repair, increasing gene editing efficiency as edited genes are more likely to stay edited [16]. These single nucleotide mutations are made to be silent mutations, meaning that they do not change the amino acid that is being produced in the gene, ensuring that they are not going to cause further damage to it and rather being present as a safety mechanism.

In this utilization of CRISPR-Cas9, four main constructs were created to restore function to the mutated TP53 414delC gene [16]. These include 1)sgRNA1, a sgRNA helps the Cas9 cleave the DNA to create a double-strand break 7 nucleotides away from the 414delC site; 2) sgRNA2, a sgRNA helps the Cas9 cleave the DNA to create a double-strand break 3 nucleotides away from the 414delC site; 3) ssODN1, an 80-nucleotide long sense single-stranded oligo which contains the genetic code to re-insert the previously deleted C nucleotide in the 414th position of TP53 as well as a guide-blocking G nucleotide mutation; 4) ssODN2, an 80-nucleotide long anti-sense single-stranded oligo which contains the genetic code to insert two guide-blocking nucleotides C and G.

PC-3 cells were transfected with different combinations of sgRNAs (through the help of plasmids sgRNA1px459 and sgRNA2px459 which contain sgRNA1 and sgRNA2 respectively) and ssODNs. The combination of sgRNA2 and ssODN2 was found to be the most effective, being able to reach a gene editing efficiency of 26% [16]. In this case, sgRNA2 leads the Cas9 protein to cleave the TP53 gene 3 nucleotides away from the 414delC site, which then leads to the anti-sense ssODN2 to conduct homologous directed repair of the gene and additional insertion of two guide-blocking C and G nucleotides. The high efficacy of gene editing can be attributed to how sgRNA2's proximity to the 414delC site was closer compared to sgRNA1, allowing for a greater likelihood of a successful edit, how the guide-blocking mutation presented any re-cleaving to occur, and how ssODN2 as an anti-sense oligo was able to stabilize the homologous directed repair TP53 gene after being cleaved by Cas9 [16]. The repaired TP53 gene through this specific combination of sgRNA2 and ssODN2 can be exemplified in how it inhibited cell proliferation in the PC-3 cell line and essentially restored p53 expression through qPCR [16].

In the future, these experiments can be executed in vivo to understand how the use of ssODNs may be influenced in more complex organisms. Moreover, the use of anti-sense ssODNs should be explored more in treatments due to their stabilization of homologous directed repair after sgRNA-directed cleavage, which when combined with guide-blocking mutations, can overall increase the efficiency of CRISPR-mediated gene repair in many different oncogenes.

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4.2.2. Inside PC-3 cells using PEI-GQD vectors. An alternate way to remedy the TP53-414delC which has been explored is the use of polyethyleneimine-graphite quantum dots (PEI-GQDs) as a vector for the Cas9 protein. This was researched by Lee et. al, who showed how the use of GQD nanocarbon vectors has a high level of targeted efficiency when combined with CRISPR-Cas9 for the use of gene editing [17].

To create graphite quantum dots, polyethyleneimine with the help of a precursor material named glucosamine reacts hydrothermally with the assistance of microwaves to create nanocarbons named graphite quantum dots that carry a negative charge [17]. To create the gene editing complex, first, a sgRNA is designed such that, when complexed with the slightly positively charged Cas9 ribonuclease protein (RNP), it will cleave the 1100bp long TP53-414delC gene into 400bp and 700bp fragments [17]. Then, a ssODN containing the genetic code of correct genetic code surrounding the 414delC locus will be used as a template DNA to facilitate homologous directed repair, therefore restoring function into the previously mutated TP53 gene.

After creating a complex between the PEI-GQD and the RNP+ssODN through their electrostatic attraction seen through the negative GQD and positive RNP+ssODN, it will be administered to cells to start the gene editing process. This is where PEI-GQD's delivery efficiency shines. It can induce the protein sponge effect on the PC-3 cell membrane's endosome, rupturing them and therefore allowing the PEI-GQD/RNP+ssODN to transfect the cell. As the complex enters the cell, PEI-GQD gets left in the cytoplasm as seen through how GQD's presence of carbon allows it to be fluorescently imaged. Then, the RNP+ssODN, tagged with an enhanced green fluorescent protein (eGFP), translocates into the nucleus, initiating the gene edit highlighted above [17].

In terms of efficiency, it was measured through the staining apoptosis marker trypan blue, which reports cells that express the apoptosis signal caspase 3/7. Through this, it was revealed that 40% of PC-3 cells expressed caspase 3/7 [17], meaning that TP53's promotion of senescent and apoptotic behavior was restored due to the repairing of the 414delC mutation. When applying this PEI-GQD/RNP+ssODN complex to HEK239 cells, it rendered little to no effect [17], meaning that this gene editing system is highly targeted towards cancer cells containing the TP53-414delC mutation like PC-3.

In the future, PEI-GQDs should be implemented in CRISPR-Cas9 gene editing in vivo so that the efficiency of DNA delivery can be further confirmed and fully taken advantage of in targeted gene therapy. Furthermore, due to its highly scalable method of top-down synthesis, more methods of GQD production should be investigated so that it can be made even more efficient and cost-effective without sacrificing efficiency.

4.3. Breast cancer

4.3.1. Prime editing in T47D breast cancer cells. The CRISPR-Cas9 gene editing system is typically composed of three parts, the Cas9 nuclease which induces a double-strand break, sgRNA so that a strand can be recognized for cleavage, and finally, donor DNA to facilitate homologous recombination. However, prime editing, a gene editing system that was derived from CRISPR-Cas9's core principles, simplifies the system by eliminating the use of donor DNA and instead fuses reverse transcriptase (RT) enzyme onto Cas9n to facilitate the editing of one strand of DNA a time without the use of double-strand breaks. The prime editing system was utilized in a study by Abuhamad et. al, with its efficiency being tested in breast cancers containing a mutant TP53 [18].

Firstly, the T47D human luminal A breast cancer cell line was used in this study, a breast cancer cell line that has the L194F missense mutation present in which the 194th amino acid created is phenylalanine instead of the supposed leucine [18]. The workflow includes the transfection of three plasmids containing these three constructs into the T47D cell line: 1) PE2, which encodes the Cas9n nuclease, an engineered version of Cas9 that deactivates one of the two nucleases that only allows it to perform single-stranded nicks, fused to an RT enzyme; 2) Prime editing guide RNA (pegRNA), which encodes a strand of RNA complementary to the targeted locus in the 5' to 3' strand. In this study, the pegRNA is called p53 T>C pegRNA as it will repair the missense mutation by substituting the mutated

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T nucleotide with the supposed C nucleotide found in wild-type TP53; 3) PE3, which encodes a nicking-sgRNA that is complementary to the complementary strand of the targeted locus, or the 3' to 5' strand relative to the code inside the pegRNA.

After transfection and expression, the workflow starts with the Cas9n-RT using the p53 T>C pegRNA to identify the targeted locus of mutation at the L194F missense mutation. Then, a nick is created, allowing for the RT to use pegRNA as a template to develop and assemble a restored strand through the assembling of nucleotides by the enzyme, with an additional emphasis on substituting the T nucleotide for C to repair the mutation [18]. Next, the nicking sgRNA guides the Cas9n-RT to the complementary strand of the targeted locus, once again creating a single-stranded nick. Through the DNA's single-stranded break repair mechanisms, nucleotides that are complementary to the initial nick on the 5' to 3' strand of the targeted locus will reform and on the 3' to 5' strand to finalize the gene editing of the L194F missense mutation found in the T47D human luminal A breast cancer cells [18].

The main advantage of prime editing is that it removes the need for donor DNA in gene editing, as the RT utilizing the pegRNA synthesizes the supposed donor DNA during the process of prime editing itself, thus streamlining the processes. Post-transfection, it was found that, though using prime editing to repair the L194F TP53 mutation was possible, the efficiency of gene editing was low, as seen in how only 0.043% of edits were deemed as successful when the T47D cell's DNA was sequenced via Sanger sequencing [18].

In the future, studies should implement the systematic improvements highlighted in this paper to remedy the technical mishaps that have caused such a low level of gene editing efficiency such as adjusting the length of the pegRNA.

4.4. Osteosarcoma

4.4.1. Mutant TP53KO in Osteosarcoma Cells. Previous studies that have been reviewed in this paper only see various mutated versions of TP53 being restored through the CRISPR-Cas9 gene editing system's inclusion of homologous directed repair or prime editing; however, in a study by Tang et. Al [19], the direct CRISPR-Cas9-mediated knockout of mutated TP53 was performed instead of restoration, which did not just show promising effects of causing anti-proliferation behaviour of the cancer cells, but also supports the fact that TP53 should be a seriously considered target gene in osteosarcoma clinical prognosis and treatment.

The study focused on osteosarcoma cell lines KHOS and its multi-drug resistant counterpart KHOSR2, which both specifically exhibited the TP53-R156P missense mutation, in which the 156th amino acid is changed from arginine to proline. To knock the TP53-R156P gene out, the pSpCas9(BB)-2A-GFP (PX458) with a ligated sgRNA sequence which contained the loci surrounding the R156P m0.utation, was transfected into the two cell lines using lipofectamine 3000. When the plasmid undergoes transcription and translation, the Cas9 protein will be expressed along with a GFP tag to track transfection efficiency and then will create a complex with the sgRNA, which starts to identify and cleave the mutant TP53 genes to knock them out [19].

From the cell proliferation assay of the TP53-R156P knock-out KHOS and KHOSR2, it was found that their rates of proliferation were decreased compared to the controlled KHOS and KHOSR2 cells [19]. This can be attributed to the various oncogenic pathways being inhibited as a consequence of the gene knock-out, as through western blotting of the TP53-R156P knocked-out cells, genes like Bcl-2 and IGF-1R were expressed at a decreased rate, therefore lessening their behaviours of proliferation in cancerous cells. This is further supported by the cell migration assay that was performed, in which the wound that was created in the TP53-R156 knockout sample of cells healed the slowest compared to the other non-knocked-out control samples of KHOS or KHOSR2 cells [19]. Finally, it was found that the knockout of the mutated TP53-R156P led to the decreased resistance of doxorubicin, an osteosarcoma chemotherapeutic drug, from an LC50 of 0.71μm to 0.33μm [19], which, with the previous results in this study, strengthens the relationship between mutant TP53 and osteosarcomas.

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This important relation has also caused the experimenting of the NSC59984 small molecule TP53 mutant inhibitor drugs as an alternative treatment to CRISPR-mediated knockouts [19], with it yielding success in terms of decreasing cell proliferation and tumorigenic activity with similar efficiency as shown in several assays. In the future, this small-molecule inhibitor NSC59984 should be tested in vivo and eventually in clinical trials regarding osteosarcoma in pursuit of a reliable treatment for this specific type of cancer.

Table 1. The applications of CRISPR-Cas9 gene editing.

Cancer	TP53 Target Mutation	Use of CRISPR-Cas9 Gene Editing	Results	Ref (s)
Non-small cell lung cancer (NSCLC)	Wildtype TP53 - (wtTP53)	CRISPRko screening to find a therapeutic gene target in patients with the wtTP53-RTK genotype of NSCLC.	The MDM2 oncogene, which degrades p53 through ubiquitination, was identified as a target gene in NSCLC with this specific genotype.	[11]
Lung Adenocarcinomas	490th nucleotide, disrupting p53 DBD	Knocking out a specific nucleotide to investigate its effects on drug resistance.	Chemotherapeutic PI3K isoform inhibitors were less effective in cells that contained p53 with a dysfunctional DBD.	[13]
Prostate Cancer	TP53-414delC	Repairing mutation using CRISPR-Cas9 with the help of guide-blocking mutations to decrease the chances of re-cleavage and therefore increase overall editing efficiency.	A sgRNA closer to the 414delC site and an anti-sense ssODN which repairs the 414delC mutation while containing a guide-blocking mutation reduced cell proliferation the most.	[16]
Prostate Cancer	TP53-414delC	Repairing mutation using CRISPR-Cas9 delivered by polyethyleneimine-graphite quantum dot (PEI-GQD) vectors and investigating its efficiency.	The PEI-GQD vectors delivered the CRISPR-Cas9 gene editing complex at a high rate of 40%.	[17]
Breast Cancer	L194F missense mutation	Using prime editing — a gene editing technique that still uses Cas9 nucleases but utilizes a reverse transcriptase (RT) enzyme to repair DNA instead of donor template DNA — in breast cancer cell lines and investigating its efficiency.	Only 0.047% of edits executed by prime editing were successful. More investigation into increasing its success rate is needed.	[18]
Osteosarcoma	R156P missense mutation	Knocking out the TP53 gene containing the R156P mutation to see its effects on cell proliferation.	The knockout of TP53-R156P caused cell proliferation in the cancer cell line to be inhibited.	[19]

5. Conclusion

This review has shown that CRISPR-Cas9 is promising in its ability to investigate mutant or wildtype TP53's interactions between other chemicals, oncogenes, and signalling pathways, all contributing to developing treatments or gaining knowledge to prevent TP53-related cancers from progressing and harming patients. A common use of CRISPR-Cas9 during this review is its mechanism to identify specific genes through its sgRNA identification system followed by its subsequent knockout through

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the double-strand breaks it induces. For example, this application of CRISPR-Cas9 gene editing can be used as a screening method to see how alternative genes are affected in CRISPRko screening, or even to knock out the TP53 gene itself in a cell line to observe how its creation of dysfunctional truncated p53 protein affects other signalling pathways. Furthermore, the integration of donor DNA within the CRISPR-Cas9 gene editing system allows for the restoration of previously mutated genes or the alteration of genes after double-strand breakage through homologous recombination. This application of gene editing has been critical in exploring TP53-related therapies, with investigations into adding guide-blocking mutations, multiple pairs of donor DNAs named ssODNs, its refining into prime editing, and the experimentation of novel vectors have shown that CRISPR-Cas9 is a very useful tool to treat cancers. However, more investigations into reducing CRISPR-Cas9's small yet possible margin for error creating accidental and unintended genetic alterations during the gene editing process, along with more effective vectors for this gene editing technology which increase the rate of delivery, is needed for this technology to become mainstream. Moreover, its inclusion in more clinical trials and in vivo experiments have to be conducted for these gene editing tools to be tested in more complex systems like the human body.

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