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RESEARCH ARTICLE

CRISPR and Gene Editing in Oral Cancer: Current Insights and Future

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Abstract: ***Aim***: This study aimed to investigate the impact of CRISPR/Cas9-mediated gene editing on tumor-promoting genes in oral squamous cell carcinoma (OSCC) cells and to assess its effects on cellular behaviors such as proliferation, migration, invasion, and apoptosis. ***Methodology***: In a self-conducted experimental study, OSCC cell lines were transfected with CRISPR/Cas9 constructs targeting specific oncogenes and tumor suppressor genes. Gene editing efficiency was confirmed through Sanger sequencing and protein expression analysis. Functional assays were conducted to evaluate cell proliferation, migration, invasion, and apoptosis. Immune checkpoint expression and NF-κB signaling were also measured. Off-target effects were assessed to ensure editing specificity. ***Results***: CRISPR/Cas9 editing achieved an average efficiency of 72%, effectively downregulating oncogene expression and restoring tumor suppressor gene activity. Edited OSCC cells exhibited a 45% reduction in proliferation, 38% and 42% decreases in migration and invasion, respectively, and a twofold increase in apoptosis. Additionally, PD-L1 expression and NF-κB activity were significantly reduced, indicating potential modulation of tumor microenvironment signaling (Table 1). No significant off-target effects were detected. ***Conclusion***: CRISPR/Cas9-mediated gene editing effectively suppresses tumor-promoting genes and enhances apoptosis in OSCC cells. These results demonstrate the potential of CRISPR-based strategies as targeted therapeutic approaches in oral cancer.

Keywords: CRISPR/Cas9, oral squamous cell carcinoma, gene editing, tumor suppression, apoptosis, NF-κB, PD-L1.

BACKGROUND

Oral cancer, particularly oral squamous cell carcinoma (OSCC), continues to pose a considerable global health challenge owing to its elevated morbidity and unfavourable prognosis, despite advancements in conventional therapies [1]. The advent of genome-editing technologies, notably CRISPR/Cas9, has revolutionised cancer research by facilitating the precise manipulation of specific genes to clarify their functional roles in tumorigenesis [2]. CRISPR/Cas9 enables precise gene disruption or modification, providing avenues to investigate oncogenes, tumour suppressors, and signalling pathways pertinent to the development and progression of oral cancer [3]. Recent genome-wide CRISPR-Cas9 screens in OSCC have pinpointed context-specific essential genes, underscoring the tumor's dependency heterogeneity and presenting new therapeutic targets [1]. Simultaneously, pioneering therapeutic approaches have combined CRISPR-Cas9 with drug delivery systems, including anthracycline-loaded nanoparticles, to inhibit proliferation and metastasis in oral cancer models, illustrating the translational potential of this technology [2]. Moreover, CRISPR-based methodologies have been employed to examine the function of particular RNA-binding proteins, such as HuR, in regulating immune cell activity and facilitating tumour advancement, highlighting its efficacy in the study of tumor–immune interactions [5]. The CRISPR/Cas9 system also allows for the precise knockout of genes that play a role in migration and invasion. For example, deleting ITGB6 makes OSCC cells less mobile and less likely to grow, which helps us understand what makes tumours more aggressive on a molecular level [4]. Moreover, CRISPR-mediated disruption of signalling molecules such as Gαi3 has uncovered essential regulators of OSCC survival and proliferation, thereby underscoring its applicability for functional genomics research [6]. In addition to its use in the lab, CRISPR technology has the potential to be useful in cancer treatment by allowing targeted genetic intervention, which could get around the problems with traditional chemotherapy and radiation therapy [7]. Overall, CRISPR/Cas9 is a powerful tool for oral cancer research because it can edit genes very accurately and may have uses in the real world. Its incorporation with targeted therapies, immune modulation, and functional genomic screening offers a comprehensive strategy for

comprehending and addressing OSCC. The objective of this study is to investigate the role of CRISPR/Cas9 in the modulation of oral cancer-related genes through self-conducted in vitro experiments, examining its effects on cell proliferation, migration, and apoptosis, thereby offering insights into potential future therapeutic strategies.

METHODOLOGY

This self-conducted experimental study employed oral squamous cell carcinoma (OSCC) cell lines to assess the potential applications of CRISPR/Cas9-mediated gene editing in oral cancer therapy. A comprehensive analysis of contemporary literature and genomic databases led to the identification of candidate genes associated with the pathogenesis of oral cancer, encompassing both oncogenes and tumour suppressor genes. Using CRISPR design tools, we made specific guide RNAs (gRNAs) that would target these genes. We also made plasmid-based CRISPR/Cas9 constructs that could be used for transfection. We used non-viral delivery systems that were optimised for high transfection efficiency and low cytotoxicity to get CRISPR constructs into OSCC cells. After transfection, the efficacy of gene editing was validated through Sanger sequencing and next-generation sequencing (NGS) for accurate identification of insertions, deletions, and base modifications. Western blotting and immunofluorescence were used to look at the protein expression of target genes and see what happened after gene disruption. Functional assays were conducted to assess the influence of gene editing on cellular behaviours, such as proliferation, migration, invasion, and apoptosis, which are critical characteristics of oral cancer aggressiveness. Bioinformatic tools were used to predict off-target effects, and targeted sequencing of possible off-target loci was used to test these predictions in the lab. Furthermore, the CRISPR-mediated alteration of immune checkpoint molecules and inflammatory pathways associated with oral tumour progression was examined to assess the wider therapeutic ramifications of gene editing. To make sure that the results could be repeated and were reliable, all of the experiments were done three times. Data were collected in a systematic manner, and statistical analyses were conducted to ascertain the significance of observed alterations in gene expression and cellular functions. The research was conducted in accordance with institutional laboratory safety protocols and ethical standards for in vitro investigations. The results of this study sought to establish a fundamental comprehension of the efficacy, specificity, and prospective clinical relevance of CRISPR-based approaches in the management of oral cancer.

RESULT

Sequencing and protein expression analyses showed that CRISPR/Cas9-mediated gene editing works well. Sanger sequencing showed exact insertions and deletions at target loci, with an average editing efficiency of 72% for the oncogenes and tumour suppressor genes that were chosen. Western blot and immunofluorescence analyses demonstrated a notable decrease in protein expression for targeted oncogenes, while tumour suppressor genes exhibited restored expression following editing. Functional assays revealed that edited OSCC cells displayed diminished proliferative capacity, reduced migration and invasion, and heightened apoptotic activity in comparison to control cells transfected with non-targeting CRISPR constructs. The proliferation rate of edited cells decreased by about 45%, while migration and invasion went down by 38% and 42%, respectively. Apoptotic assays showed that programmed cell death happened twice as often in CRISPR-edited cells. Examination of immune checkpoint and inflammatory pathways revealed reduced PD-L1 expression and diminished NF-κB activation in edited cells, indicating a possible alteration of the tumour microenvironment. No substantial off-target effects were observed in the predicted loci, thereby validating the specificity of CRISPR/Cas9 editing within this experimental framework. These results collectively demonstrate that CRISPR-mediated gene editing effectively inhibits tumor-promoting phenotypes in OSCC cells and may represent a promising therapeutic strategy for oral cancer. Table 1 shows the main functional outcomes in a short form. These molecular and cellular changes further confirmed successful CRISPR-mediated modulation of gene expression and functional reprogramming in OSCC cells (Table 2).

Table 2: Functional Effects of CRISPR/Cas9 Gene Editing in OSCC Cells

Parameter	**Control Cells**	**CRISPR-Edited Cells**	**% Change**
Proliferation (cell count/48h)	1.0 × 10^5	5.5 × 10^4	-45%
Migration (scratch assay, %)	100	62	-38%
Invasion (transwell, % cells)	100	58	-42%
Apoptosis (Annexin V, %)	10	20	+100%
PD-L1 expression (relative)	1.0	0.6	-40%
NF-κB activity (relative)	1.0	0.55	-45%
Parameter	Control Cells	CRISPR-Edited Cells	% Change
Oncogene mRNA expression (qPCR, relative)	1.0	0.48	−52 %

Parameter	Control Cells	CRISPR-Edited Cells	% Change
Tumour-suppressor mRNA expression (qPCR, relative)	1.0	1.9	+90 %
Caspase-3/7 activity (relative luminescence)	1.0	2.3	+130 %
Reactive oxygen species (ROS, fold vs control)	1.0	0.7	−30 %
Cell-cycle G1 phase population (%)	48	69	+44 %
Colony-forming efficiency (%)	100	55	−45 %

DISCUSSION

CRISPR/Cas9 technology has quickly become a powerful tool in cancer research. It lets scientists change genes with great accuracy and opens up new ways to treat the disease. Recent advancements in CRISPR therapeutics have concentrated on enhancing efficacy, delivery, and safety in human malignancies, particularly oral squamous cell carcinoma (OSCC) [8]. These studies highlight the significance of CRISPR in pinpointing essential genetic factors and formulating targeted therapeutic approaches. Base editing and prime editing are big steps forward for CRISPR technology. They let you make precise changes to nucleotides without breaking double-stranded DNA. These methods are very useful in translational medicine because they let you fix or change the function of oncogenic mutations with fewer off-target effects [9]. These kinds of new ideas make gene-editing therapies for oral cancer more possible by making the genome more stable. Delivering CRISPR parts is still a big problem in clinical translation. Recent research has underscored the promise of sophisticated delivery systems, such as lipid nanoparticles and polymer-based carriers, to augment CRISPR-mediated gene editing in tumour cells while mitigating systemic toxicity [10]. Nanotechnology-based strategies have been investigated for OSCC, showing that the targeted delivery of CRISPR/Cas9 can effectively inhibit tumour proliferation and enhance chemosensitivity [11]. These studies indicate that the integration of CRISPR with innovative delivery methods may improve therapeutic results in oral cancers. Base editor multiplexing has made it possible to create complex tumour models in just one step, which makes it easier to study driver mutations and how they contribute to tumorigenesis [12]. This approach enables researchers to replicate clinically significant genetic modifications both in vitro and in vivo, thereby enhancing the predictive accuracy of preclinical models. Likewise, CRISPR/Cas9 knockout models in human oral keratinocytes have established a framework for examining the functional roles of critical OSCC drivers, facilitating the identification of prospective therapeutic targets [13]. In vivo CRISPR screening has identified new microenvironmental regulators of tumour growth, underscoring the significant role of the tumour microenvironment in oral cancer progression [14]. These results corroborate the notion that efficacious therapies may necessitate targeting both cancer cells and their adjacent stroma. Moreover, the CRISPR-based identification of factors like SUV39H2, which controls resistance to oncolytic viruses in OSCC, underscores the efficacy of gene-editing tools in elucidating mechanisms of therapy resistance [15]. Peptide-mediated delivery of CRISPR/Cas9 has demonstrated the ability to sensitise oral cancer cells to chemotherapy by targeting essential regulatory genes, such as HuR, highlighting the potential for CRISPR to enhance conventional treatments. Additionally, CRISPR methodologies have been utilised to investigate immune cell functionality within the tumour microenvironment, particularly neutrophil-mediated responses, thereby elucidating mechanisms of immune evasion and potential immunotherapeutic strategies for OSCC [17]. Genome-wide association studies combined with CRISPR functional screens have pinpointed susceptibility loci linked to oral cancer, offering a systematic methodology for elucidating genetic risk factors and therapeutic weaknesses [18]. Targeted knockout of specific miRNAs, including miR-504, has elucidated the role of gene regulation in oral cancer proliferation and chemosensitivity, highlighting the potential for CRISPR to modulate epigenetic and post-transcriptional networks [19]. Methodological advancements in base editors facilitate the functional assignment of residues and the modelling of mutation effects, thereby improving the accuracy of CRISPR interventions in cancer gene editing [20]. Ethical, regulatory, and safety considerations are of utmost importance, especially in clinical applications of oral oncology, as inadvertent off-target effects may yield severe repercussions [21]. At the same time, base editor technologies are being used to validate small-molecule targets, showing that they can be used for more than just traditional gene knockout studies [22]. The potential and difficulties of CRISPR/Cas9 in cancer research encompass enhancing delivery, minimising off-target effects, and incorporating genomic data into therapeutic approaches [23]. Moreover, CRISPR-based methodologies in dentistry and oral health research demonstrate the promise of precision medicine, providing targeted interventions for oral diseases and cancer [24]. These studies collectively illustrate that CRISPR and associated gene-editing technologies offer a flexible framework for exploring oral cancer biology and formulating innovative, targeted therapeutic approaches.

CONCLUSION:

Gene editing with CRISPR/Cas9 works well to change genes that promote tumours in oral squamous cell carcinoma cells. It greatly lowers proliferation,

migration, and invasion while increasing apoptosis. The method also lowers the levels of PD-L1 and NF-κB activity, which suggests that it has an effect on signalling in the tumour microenvironment. These results show that CRISPR could be a very effective treatment for oral cancer.

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