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Precision Oncology at the Intersection of CRISPR–Cas9 and Tumor Neoantigens

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Abstract. Malignant tumors remain one of the leading causes of mortality worldwide. Conventional therapies, though effective at killing cancer cells, are accompanied by systemic cytotoxicity. It highlights the priority for precision oncology. Tumor neoantigens represent promising targets for selective immunotherapy, arising from cancer-specific mutations. At the same time, CRISPR–Cas9 genome editing provides remarkable accuracy in genetic manipulation, opening new avenues for cancer research. This paper reviews the emerging convergence of CRISPR–Cas9 technology and neoantigen-based interventions. It showcases recent progress in CRISPR-based neoantigen validation and the engineering of immune effector cells, as well as their application in personalized therapies. Additionally, this paper focuses on several important translational challenges that need to be overcome before broad clinical application, including tumor heterogeneity, off-target effects, and clinical safety. By assembling the current evidence and future scenarios in this field, this review aims to highlight that the combination of CRISPR genome editing with neoantigen targets would be a new direction in the field of precision oncology that may shape the future of cancer immunotherapy.

Keywords: Neoantigen, CRISPR–Cas9, Precision Oncology

1. Introduction

Cancer is still a major threat to human health, due to its genetic heterogeneity, drug resistance, and the serious side effects of conventional therapies. In recent years, tumor neoantigens have been considered as a revolutionary concept in oncology. Neoantigens are new peptide sequences produced by somatic mutations. They are not present in normal tissues, but expressed in malignant cells [1]. Because of their tumor-specific origin, these antigens can be recognized by the immune system as 'non-self'. It provides highly selective opportunities for therapeutic escape. This characteristic has promoted the development of new antigen-based approaches and the emergence of personalized vaccines in particular. Pre- and early clinical studies have demonstrated that neoantigen-specific immunotherapy can induce potent cytotoxic T lymphocyte responses, and even durable tumor regression in some patients [2]. However, antigen escape and validation still limit the broader application of this technology.

Parallel to the development of tumor immunology, genome editing technologies have revolutionized life sciences. Clustered regularly interspaced short palindromic repeats (CRISPR)

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and CRISPR-associated protein 9 (Cas9) constitute the CRISPR–Cas9 system. This platform represents one of the most versatile and revolutionary technologies in modern biology. Originating from bacterial adaptive immunity, CRISPR–Cas9 uses a single-guide RNA (sgRNA) to direct the Cas9 nuclease to complementary DNA sequences, and then introduces cleavage at specific sites [3]. Compared with previous genome-editing tools, such as zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), the cleavage sites of CRISPR-Cas9 are easier to design. It is more cost-effective and generally applicable to different organisms [4]. Its application has promoted advances in functional genomics, disease modelling, and treatment development.

The unparalleled precision of CRISPR–Cas9 is particularly suited to oncology. The improved Cas9 of CRISPR can even perform single-base re-editing on the target fragment. This ability allows investigators to faithfully replicate the mutations caused by cancer in experimental systems, thereby verifying the functions of candidate targets [5]. This accelerates the process from genomic sequencing prediction to functional testing. It plays a key role in clinical translation in biomedicine. Moreover, CRISPR-Cas9 has been used to modify immune effector cells, including T cells or natural killer (NK) cells. These cells have specific receptors that can recognize mutated-derived neoantigens [6,7]. The strategy of CRISPR-Cas9 targeting neoantigens is expected to enhance the efficacy of immunotherapy as well as reduce off-target toxicity.

The intersection of neoantigen-based therapies and CRISPR–Cas9 editing marks the emergence of a distinct domain in precision oncology. Neoantigens represent tumor-specific targets and CRISPR–Cas9 represents a precision tool to validate neoantigens at the genomic level. These advances in crossover research help us understand how tumors initiate an immune response, creating further avenues for future treatments that may be more effective with fewer associated side effects.

2. CRISPR-Cas9-mediated modeling of tumor neoantigens

Tumor neoantigens, somatically mutated antigens exclusively expressed by malignant cells, are potential targets for cancer immunotherapy. Absence of these antigens from normal tissues reduces the potential for off-tumor cytotoxicity and renders them ideal candidates for developing precision therapies. Accurate preclinical models recapitulating the generation, development, and immunorecognition of clinically predicted neoantigens, however, are needed to translate neoantigens into clinically actionable targets. Traditional cancer models have employed the introduction of high-risk oncogenic drivers or tumor tissue transplantation as valuable tools to investigate tumor initiation and progression, which need to interrogate individual antigenic events.

The development of CRISPR–Cas9 genome editing has revolutionized cancer research by enabling precise editing of single genes. CRISPR–Cas9 can generate knock-in mice with point mutations, small insertions, or deletions linked to potential neoantigens. According to this hypothesis, CRISPR could be used to edit genes in adult mice. Cas9/sgRNA plasmids have been delivered into certain organs, such as the lung and liver, and tumor formation in these specific organs has been induced [8]. On the other hand, Cas9 with isogenic sgRNAs targeting specific mutations has been introduced into fertilized embryos by microinjection, electroporation, or viral vectors, and mice with heritable mutations have been obtained. These germline models stably transmit specific neoantigen variations [9]. These models allow us to examine antigen specificity and the overall efficacy of the treatment.

While these approaches are valuable, patient-derived and engineered organoids represent ex vivo systems that recapitulate the genetic, histological, and phenotypic heterogeneity of human tumors, forming a useful complement to in vivo approaches. CRISPR-Cas9 can be used to evaluate the

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function of putative new antigens rapidly in well-characterized in vitro 3D cultures [10]. Organoids capture key features of tumors and are amenable to a number of high throughput applications. Edited organoids expressing defined variants of interest can be co-cultured with autologous lymphocytes or genetically engineered effector cells to assess the kinetics of T-cell activation, cytokine secretion, cytotoxicity, and antigen presentation separately [11]. These systems offer a controlled yet physiologically relevant means of studying antigen–immune cell interactions, allowing the parallel assessment of multiple antigenic candidates and thereby facilitating the discovery pipeline.

These two approaches provide a complementary set of tools to query the immunobiology of tumor neoantigens. Physiologically relevant mouse models can be combined with the advantages of the scalability of organoid systems. CRISPR-Cas9-mediated models enable the systematic evaluation of candidate antigens, the optimization of immunogenic targets, and the precancerous assessment of personalized immunotherapies. This convergence will give rise to the next generation of precision oncology strategies, where therapies are tailored to the genetic drivers of cancer and the antigenic profiles of each tumor.

3. CRISPR–Cas9 in engineering immune cells for cancer therapy

The CRISPR–Cas9 editing technology holds transformative potential for adoptive cell therapy by enabling site-specific engineering of the human immune cell genome with high specificity [12]. Human immune cells display anti-tumor activity. However, the function of human immune cells is impaired in the immunosuppressive microenvironment generated by the tumor. The CRISPR-Cas9 system enables the editing of loci encoding tumor-specific neoantigens, resulting in the activation of human immune cells in the tumor recognition and clearance process. In addition, the scalability of CRISPR offers an opportunity to generate 'off-the-shelf' products with controlled immunogenicity, which is an advantage over autologous products. In summary, CRISPR–Cas9 is a breakthrough technology for the next generation of engineered immune cells, enabling them to overcome the suppressive context generated by tumors.

Chimeric antigen receptor (CAR)-T cell therapy has attained significant success in hematologic malignancies, while failing in solid tumors due to the inhibitory tumor microenvironment. CRISPR–Cas9 unleashes mighty tools to conquer these inhibitory tumor microenvironments. Knockout of inhibitory receptors such as CTLA-4 or TIM-3 could improve CAR-T persistence and effector function under chronic antigen stimulation. It has been reported that TRAC-integrated CAR-T could enhance its cytotoxicity and persistence in vivo. The deletion of endogenous TCR and HLA could eliminate all HLA and TCR, making it a universal allogeneic CAR-T platform that could reduce graft-versus-host disease and immune rejection [13]. In addition, the preliminary results of clinical application of CAR-T cell therapy for lung and esophageal cancer have achieved satisfactory efficacy without off-target editing [12]. Thus, CRISPR engineering not only refines the safety and performance of CAR-T but also extends its applicability from hematologic cancers to long-standing challenges in solid tumors.

Unlike CAR-T cells, which recognize surface antigens that are unrestrictive by MHC, TCR-engineered T cells (TCR-T) recognize intracellular tumor antigens presented by MHC. Amidst the promise of TCR-T, limitations have impeded its clinical translation, including limited avidity, tumor escape, TCR mispairing, and potential autoreactivity. CRISPR–Cas9 directly targets these problems by disrupting the endogenous TCR α and β chains, reducing mispairing and lowering the risk of autoreactivity. Further, tumor-specific TCRs can be targeted to the native locus, improving both expression fidelity and physiological signaling. Critically, CRISPR engineering enables the recognition of tumor-specific neoantigens that are otherwise inaccessible to CAR-T cells. Here,

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recent melanoma and lung cancer studies that have demonstrated proof-of-concept, where tumor-derived mutations provide natural targets for TCRs. In addition, editing of checkpoint regulators, such as PD-1, ameliorates T cell exhaustion and increases the persistence of TCR-T in hostile tumor microenvironments [6]. These studies demonstrate how CRISPR enables the development of durable and antigen-specific TCR-T therapies.

NK cells exhibit inherent cytotoxic activity against malignant cells without prior sensitization, rendering them ideal candidates for cancer immunotherapy. However, the immunosuppressive cytokine milieu and inhibitory signaling present in the tumor microenvironment dampen NK activity. CRISPR–Cas9 can eliminate intrinsic checkpoints to enhance the proliferation and persistence of NK cells, while also enabling their redirection towards tumor neoantigens. For instance, the knockout of CISH/CIS, a negative regulator in the IL-15 signaling pathway, has been shown to improve the anti-tumor activity of NK cells [7]. CRISPR enables the stable knock-in of neoantigen-specific CAR constructs into the NK cell genome, thereby equipping NK cells with the capacity to recognize naturally expressed tumor targets with engineered specificity.

Combining CRISPR–Cas9 and immune cell engineering represents a new frontier in adoptive cell therapies. CRISPR–Cas9 not only enables checkpoint disruption and receptor knock-in, but also offers a distinctive platform for modeling and targeting tumor-specific neoantigens. CRISPR–Cas9 technology with neoantigen-targeted immunity represents a new inflection point for precision oncology. It is anticipated that cooperation can lead to a new therapeutic strategy of personalized and durable cancer immunotherapies enabled by genome editing and antigen specificity.

4. Discussion

There are two major remaining barriers to the use of CRISPR–Cas9–based cellular therapies: efficient delivery and decrease of off-target activity [14]. Existing delivery platforms based on viral vectors, lipid nanoparticles, or extracellular vesicles all have associated limitations. Viral vectors, such as adeno-associated viruses (AAV) or lentivirus, can achieve high transduction efficiencies but are associated with risks including immunogenicity, limited cargo capacity, and insertional mutagenesis. In contrast, non-viral carriers like lipid nanoparticles or exosomes are generally safer and more scalable, but have much lower efficiencies and should be further optimized. Even when efficient delivery can be achieved, general concerns remain about on-target editing and off-target edits that occur at loci in the genome that share partial homology with guide RNAs, which can result in oncogenic mutations, chromosomal rearrangements, or targeting of prohibitive genes. Correspondingly, these risks can be mitigated by engineering high-fidelity Cas9 isoenzymes, the use of orthogonal CRISPR systems, and the engineering of specific guide RNAs. What is essential in assessing CRISPR–Cas9 specificity is the enforcement of unbiased whole-genome experimental analyses to screen for and predict off-target events rigorously.

As other powerful gene editing technologies, CRISPR–Cas9 has stirred enduring and fervent controversy regarding its ethical and social implications. Controversy surrounding in vitro scandal edits of human embryos, in particular, has amplified concern surrounding potential misuse and off-label use as well as permanent changes in the human gene pool [15]. While CRISPR enables exact genome editing with potential abroad, this does not diminish the need for appropriate regulation. An increasingly vocal consortium has called for policy clarity, active public engagement, and international consensus on responsible use of gene-editing technology.

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5. Conclusion

CRISPR–Cas9 brings the frontier of precision oncology one step closer to linking validation of genetic drivers to design of personalized immunotherapies by enabling accurate single-gene editing at the level of individual tumor neoantigens to interrogate and engineer tumor–immune interactions in mice and organoid models with an unprecedented resolution to probe immune recognition and response. In contrast, CRISPR–Cas9 enables precise editing of engineered immune cells, which provides a completely new capability to break tumor immune evasion. As with any emerging biotechnology involving gene editing, safety qualms and regulatory considerations will need to be addressed to facilitate widespread thoughtful and ethical use.

In the future, computational prediction of CRISPR-mediated editing may enable reshaping personalized cancer immunotherapy on a large scale by targeting neoantigens. However, translating these approaches into the clinic will face many challenges. This emphasizes that CRISPR–Cas9 is not only a novel tool for new mechanistic studies, but also a test case for responsible and ethical development of next-generation therapies.

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