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The Application of CRISPR Technology in Tumor-Targeted

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Abstract:

This article gave an overview for CRISPR-Cas9 technology use and advancement in precise cancer treatment. As the burden of cancer worldwide continues to increase, traditional therapies is plagued by devastating side effects because of their non-cell specificity, and molecularly targeted therapies have the disadvantage of drug resistance and limited application, which cannot adapt to the needs of clinical treatment. In a recent development, the innovative gene-editing tool known as CRISPR has demonstrated great potential in the field of cancer treatment. This study provides an in-depth examination of the mechanisms and potential uses of CRISPR in precision cancer therapy, including the editing of key genes, the rejuvenation of tumor suppressor genes, the modification of immune cells, and the discovery of new treatment targets. Clinical trials showed that CRISPR-modified T cells show tolerable safety profile and limited efficacy to selected patients with hematologic malignancies. The findings showed CRISPR holds the promise to circumvent the limitations of current therapies and deliver novel cancer therapy. Yet, the off-target effect, the inefficient delivery, and complexity of the tumor microenvironment are significant barriers for clinical application. Future work includes how to increase editing precision and safety, adapt it to other tumor types, as well as combination strategy with other therapeutic approaches to elevate its clinical application towards precision oncology.

Keywords: CRISPR-Cas9; sgRNA; precision oncology; genome engineering.

1. Introduction

The International Agency for Research on Cancer(IARC)has projected a steady rise in the number of new cancer cases and fatalities over the next three decades. By 2020, the IARC estimates that 19.29 million incidents of cancer will have occurred globally, with 9.96 million fatalities. This is indicative of the gravity of cancer as one of humanity's most pervasive health issues worldwide. It has been estimated

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that over 47% in the cancer burden will increase by 2040 relative to a baseline value [1]. Traditional chemotherapies, radiotherapies, and surgical resection are still the cornerstone of therapeutic options in clinical oncology. The cytotoxic chemotherapy drugs, which can suppress neoplastic growth by mechanisms such as cycle-specific inhibition, loss of sensitivity to growth stimulation, and neoplastic cell recognition by immune cells, are not specific for cancer cells and cytotoxic therapy to the non-specific injury to normal cells. Significant side-effects such as bone marrow suppression and gastrointestinal issues are frequently seen in chemotherapies patients, due to this nonselective activity [2].

To overcome the risks of systematic toxicity of systemic chemotherapy, new therapeutic methods using molecularly targeted drugs, which work by disrupting tumor growth, by targeting the molecular pathway that is fundamental for tumorigenesis, have been developed [1]. One example of this is tyrosine kinase inhibitors (TKIs) [2], which are currently used when targeting "critical junctions" in the signaling pathway of neoplastic growth cascades. Molecularly targeted therapies suffer from numerous difficulties, notwithstanding the accuracy they provide in targeting a specific molecular pathway, including selection of drug candidates that exhibit promising antitumor efficacy, accompanied by complete absence or minimal side effects, and that can be readily formulated into an orally available capsule. This requirement appears largely an unattainable goal given that the current strategy involving identifying all potential drug candidates using high-throughput screening, conducting a cell-based assay that allows selectivity between normal cells and cancer stem cells, requires not only a lengthy process, but also substantial resources [3]. In addition, the common bottleneck observed when targeting molecularly the signal pathway responsible for tumorigenesis is that they often have significantly decreased efficacy against resistance mechanisms that develop after long-term treatment . Even the treatment regime of poly-pharmacologic inhibitors has been proposed to compensate for this, given that it leads to a remarkable heterogeneity between patients. In such circumstances, finding an orally available drug that is safe, simple and fast to develop has remained unresolved for a long time, despite the apparent potential benefits with regard to both its application in treatment, in addition to potential avoidance of serious adverse events that arise from systemic administration. However, complete molecular targeting can never be attained because several drug targets are expressed in normal organs, leading to the risk of off-target effects to normal cells. Additionally, tumor adaptation via genetic mutation or overexpression of alternative signaling pathways often evades targeted therapy. Approximately 50% of patients with clinical EGFR-mutated non-small cell lung carcinoma will experience resistance to the initial kinase inhibitor treatment within a span of 10 to 14 months. The response to treatment also critically depends on the presence of certain driver mutations like BRAF(B-Raf Proto Oncogene Serine) mutation and is therefore less general in nature and cannot be broadly applied [3]. Due to the limitation in V600E in melanoma, it limits clinical use, consequently rendering a significant amount of patient harboring a tumor without mutations in V600E ineligible to a specific effective molecularly-targeted therapy. Therefore, there is an undeniable necessity of an anticancer treatment with higher precision, efficacy, and heterogeneity. Hence, the creation of CRISPR-based technologies has special relevance in the context of these problems, since their progress may supersede current limitations of therapies in precision oncology.

Over last few years, CRISPR based genome engineering has completely transformed the field of precision based cancer therapy. Soon after introducing CRISPR based genetic engineering in mammalian cell lines in 2013 [4], study of CRISPR based cancer therapeutics has been extensively conducted with progressive speed. Overwhelming preclinical studies have demonstrated the promise in broad therapeutic aspects. The platform enables the direct modification of oncogenes (specifically, KRAS G12D mutations in pancreas cancers) and tumor suppressor genes (such as TP53 in hepatocellular carcinoma) in order to combat cellular transformation [5].Tumor-specific immune cells engineered using two modalities - silencing tumor immunosuppressive targets such as PD-1 checkpoints and incorporating chimeric antigen receptors (CARs) to augment anti-tumor immune responses [6]. In addition, CRISPR high throughput genome-scale screens can help accelerate cancer research by quickly identifying and validating potential therapeutic targets for personalized treatment options. Clinical trials of CRISPR-edited T cell therapies for blood cancers are now in phase I/II trials and results show toxicity was generally well tolerated and some evidence of disease control in patient subsets.

The CRISPR-based tumor therapy is still in its infancy, suffering from the pitfalls such as off-target effect, inefficient transfer method and diversified tumor microenvironment (TME) barriers hindering its clinical translation.

The review is introduced by an explanation of the basic biology and molecular principles of operation of CRISPR-Cas9 gene editing, continued by clinical and preclinical applications covering oncogene editing, boost to immunotherapeutic treatments and target enrichment of drugs using representative examples. This section also appraises unique capabilities of this genome-editing technology, including its high level of specificity and its potential to cre-

ate treatment customized to individual patients, and then presents a skeptical viewpoint towards its current limitations. The final one then discusses how CRISPR technology may solve longstanding clinical limitations in terms of improving patient outcome, with a focus on it's efficacy in enabling precision medicine model. We conclude with the practical considerations for translational research and clinical application, and we also suggest potential strategic guidelines of how to pave the path for next generation of cancer therapeutics.

2. Overview of CRISPR

2.1 Discovery and Technological Breakthroughs of the CRISPR System

CRISPR was discovered in Escherichia coli in the late-1980's but its biological function remained mysterious for many years until in 2007 it was shown that CRISPR plus Cas proteins are components of a bacterial immune system [7]. This immune response relies on the addition of fragments of the invading genetic material (i.e. bacteriophage DNA) into the bacteria CRISPR loci, establishing a memory of previous exposure. During secondary infection, CRISPR RNA sequences identify foreign DNA, primarily due to the complementary base pairing interactions, which serve to "mark" the invading CRISPR sequences for recognition, cleavage, and destruction by the Cas protein complexes.

The milestone report by Jennifer Doudna and Emmanuelle Charpentier published in 2012 describes in vivo the successful target recognition and cleavage of CRISPR-Cas9 by its engineered sgRNAs. This discovery provided the basis of the CRISPR's subsequent application across disciplines [7]. Later research continually improved this system by engineering Cas variants with greater editing accuracy, less off-target genomic editing, and so forth, making CRISPR, rather than an antiphage defense system in bacteria, a leading tool for genome editing with revolutionary uses from creating the first CRISPR-based cancer immunotherapy20 to engineering commercially useful crop species.

2.2 The Utilization of CRISPR-Cas9 for Editing and Its Role in Targeted Cancer Treatment

The working principle of CRISPR-Cas9 genome editing The sgRNA's 3'-end sequence is complementary to the target sequence of DNA and its 5'-end sequence associates with the Cas9 endonuclease, to form a functional RNP complex, which then can passively scan the target sequence, guided by the RNP, with nucleotide-level precision.

The Cas9 enzyme's cleavage can be seen as a type of endonuclease activity that causes double-strand breaks(DSBs)in a particular genomic location. These DSBs are initially remedied by the cell's native DNA repair mechanisms, which involve two types of biochemical reactions.For one, defective DNA ends that need to be rejoined often do so indirectly by "direct ligation", and do so with a high probability of insertions and deletions (indels) at the rejoining locus which would likely make the target gene non-functional.On the other hand, if an exogenous repair template exists, the cellular machinery utilizes the template and executes homology directed repair (of a provided template) to perform desired knock-in or targeted replacing of targeted genomic sequences.

This application allows the CRISPRCas9 system to become a "molecular scissor" tool that can be activated at the sequence of the organism's interest and make specific modification on the organism's DNA when required for therapeutic and genomic studies.For cancer-treatment, there is no room to spare for an error in targeting, for standard chemotherapy (as described above) causes harm to both cancerous and healthy tissues via non-specificity [2], and the cancer-sensitivity of radiotherapy can similarly harm healthy tissues surrounding the tumor. It is a treatment technique that is sometimes still available, i.e. surgery but loses its value in many metastasized cases. CRISPR-based therapeutics have helped overcome these deficits by providing unprecedented precision and extensive use. Targeted TKIs, drugs that inhibits enzymes, represents an example of the drug therapeutic advancement that unfortunately resistance to drugs in cancer patients arises most of the time [3].

CRISPR provides novel therapeutic strategies to genetically engineer cancer cells either by editing drugresistance genes (to improve the response of targeted therapy) or even other genes that have the capacity to alter tumor progression or the responses to treatments. The potential of CRISPR to modify certain mutations that drive the tumor such as those found in BRAF V600E-altered melanoma is compelling considering its ability to correct genetic aberrations to expand indications for targeted therapies and, thereby, circumvent limitations associated with currently used therapies. Increasing preclinical evidence [5] suggests that direct editing of genome of the tumor cells itself can help precision oncology to enable more flexible therapeutic strategies by transgressing limitations inherent in biology.

3. Overview of Tumor Targeted Therapy

Tumor-directed treatment is a personalized medicine that

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capitalizes on the molecular differences between the cancer and the normal cells to devise treatment strategies. The method focuses to discover the specific molecular signatures - for example, overexpressed genes, mutated proteins, or altered signaling pathways - that set the malignant cells apart from their healthy counterparts [3]. Unlike classical chemotherapy that does not distinguish between proliferative cancerous and healthy cells, molecularly-targeted drugs selectively block tumor progression by targeting some molecular abnormality. As an instance, HER2 positive breast tumors result in an excessive production of the human epidermal growth factor receptor 2 due to unregulated growth of cancerous cells. Tumor-specific drugs such as trastuzumab target HER2 signaling; in this way tumor proliferation can be hindered without causing excessive detrimental effect on healthy cells. Tumor-targeted approaches primarily involve three therapeutic strategies with unique mechanisms, particularly in breast cancer management—Small molecule inhibitors.

References are cited in the text just by square brackets [1]. (If square brackets are not available, slashes may be used instead, e.g. /2/.) Two or more references at a time may be put in one set of brackets [3, 4]. The references are to be numbered in the order in which they are cited in the text and are to be listed at the end of the contribution under a heading References, see our example below.

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3.1 Small Molecule Inhibitors and Monoclonal Antibodies in Tumor Targeted therapy

These small molecule drugs bypass the cellular membrane to inhibit cellular proteins such as kinases or enzyme cascades. Palbociclib, a CDK4/6 inhibitor, is utilized in treatment of HR+ breast cancer. Palbociclib inhibits proliferation of HR+ breast tumors by inhibiting a component of the cyclin D-CDK4/6 complexes, causing cell cycle arrest in G1.Phase 3 trials confirm that palbociclib plus endocrine therapy increases PFS by 100% versus endocrine alone for metastatic HR+ breast cancer populations [6].

Monoclonal antibodies (mAbs) are high-molecular weight proteins that interact with extracellular or cell surface targets to inhibit oncogenic signaling or initiate immune cell-mediated destruction of cancer cells. Trastuzumab is the prototypical therapeutic mAb that inhibits HER2 receptors in HER2-overexpressing cancers, a type of breast cancer seen in 15%-20% cases.This antibody also has a therapy effect by binding on to the cell surface-antigenic region of the HER2 receptor, blocking formation of receptor complexes and dissociating subsequent intracellular signalling to suppress tumour growth .A 46% reduction in recurrence of disease was observed in patients with early-stage HER2 overexpressing breast cancer after 12 months of adjuvant trastuzumab therapy, as demonstrated in the clinical HERA trial [2].

3.2 Research Progress of CAR-T Cell Therapy in Cancer Treatment

Chimeric antigen receptor (CAR)-T therapy program selectively generates T lymphocytes from a patient's body that can express CARs targeted at tumor antigens. Despite its impressive outcomes in hematological malignancies [4], recent technical progress have expanded applications to solid tumors, such as breast cancer [5].Tumor immunity. HER2-specifiand MUC1-directed CAR-T studies that have been trialed preclinically, there HER2-targeted cells exhibited an increased anti-tumor effector more than 70% relative to wild-type cells by infiltration capacity in animal models of HER2positive breast cancer. Nevertheless, it has remained essential to utilize immune-suppressive properties that are characteristic of the tumor microenvironment to optimize strategies for the cellular engineering.

4. Applications of CRISPR to Tumor-Targeted Therapy.

4.1 The Role of CRISPR-Cas9 in Gene Editing Related to Breast Cancer

Therefore, the CRISPR-Cas9 technology enables precise gene editing of key driver oncogenes or tumor suppressors in breast tumorigenesis and can inhibit malignant transformation at the molecular level. HER2 over-expression: HER2 over-expression is also considered an important mediator in HER2+ breast cancers.The experimentrevealed that CRISPR-mediated silencing of HER2 gene expression in SK-BR-3 cell lines was a 65% inhibitor of growth and clonogenicity loss through PI3K-Akt pathway [7].

Next, BRCA1 defects, the most common hereditary defect seen in breast cancer, are from pathogenic DNA repair that make them sensitive to PARP inhibitors. A BRCA1 mutant, which impaired genomic maintenance in organoids derived from cancer patients, that was repaired using CRISPR, reinstated genome maintenance capabilities as well as reduced olaparib sensitivity by 40%, confirming its SL therapeutic utility [8].

Then, co-occurring gene deletions in ERBB2 and the tumor suppressor TSC1 are a common feature of breast cancer. Simultaneous CRISPR gene editing of ERBB2

gene deletion and reintroduction of TSC1 in basal-type breast cancer models produced in the xenograft model 80%blockage of tumor growth, twice as much as the single-gene ERBB2 or TSC1 genes, suggesting synergy [9].

4.2 The Enhancement of the Anti-Tumor Immune Response in Breast Cancer by CRISPR

CRISPR enhances anti-tumour immune activity via both immune cell engineering and alteration of the TME, in particular in the context of breast cancer treatment.

CAR-T cells can be negatively regulated by TME through interaction with PD-1 and PD-L1. PD-1variant was constructed by genetically deleting PD-1 in HER2-CAR-T through CRISPR editing. The construct exhibited higher tumor penetrability in the context of TNBC with 2.3 fold increase in levels of immunostimulatory factors secreted (like IFN-gamma) and gave 58\% control in tumor growth in comparison with wild type HER2-CAR-T.

Thus, Tumor-infiltrating regulatory T cells (Tregs) with FOXP3 transcription factor expression suppress anti-tumor immunity. CRISPR mediated disruption of FOXP3 in breast cancer-derived Tregs compromised their immunosuppression activity shown by 40% increase in cytotoxic T cell activity in in vitro co-culture studies [10]. CRISPR technology enables the discovery of potential treatments for breast cancer and the advancement of personalized medicine. Then, CRISPR facilitates therapeutic target identification and precision medicine development for breast cancer.

One, in the case of trastuzumab resistance in HER2+ breast cancer, we show association with PTEN loss. Using CRISPR-based genetic screens, PTEN was found to be important - PTEN knockouts in trastuzumab-sensitive models lead to drug resistance, but re-expression of PTEN through homology-directed repair restored susceptibility, and increased tumor sensitivity to trastuzumab 3.1 times [11].

Next, CRISPR-mediated genome editing facilitated therapeutictargetability screening for 12 agents in patient-derived TNBC organoids. Phenotypic comparative analysis of KRAS G12D-edited v.s wild-type organoids for mutation-bearing patients revealed sotorasib (KRAS inhibitor) to be particularly susceptible in G12D-bearing models to provide disease-relevant guidance for personal therapeutic approaches [12].

5. Conclusion

In this study, the research centered around CRISPR, detailing the origins and evolution of the technology before delving into the functionality of CRISPR-Cas9 editing and its role in targeted cancer treatment. As well, a brief look at tumor targeted therapeutic modalities, including smallmolecule inhibitors, mAbs, and CAR-T cell based treatments, was given.Fourthly, it presents some particular applications of CRISPR-based targeted therapy for tumors such as breast cancer associated gene editing, enhancement of the anti-tumor immunity in breast cancer, and improving tumor therapeutic targets identification and precision medicine development for breast cancer.

The results are of great practical importance.The introduction‘s emphasis on accuracy is echoed in these findings, which define the potential of CRISPR in tumor treatment and provide a solid theoretical and experimental basis for future studies to attain precision and efficacy.

Nevertheless, the present study still has some limitations, mainly reflecting some obstacles CRISPR facing in clinical translation, including off-target phenomena, safety issues, etc., to be solved.

Future work can focus on maximizing the tumor specificity and safety profile of the CRISPR system, as well as testing its utility in additional types of cancer, and continue to focus on its use in combination with other forms of therapy for additional therapeutic synergy.

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