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Review

Hallmarks of cancer: The CRISPR generation

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Abstract The hallmarks of cancer were proposed as a logical framework to guide research efforts that aim to understand the molecular mechanisms and derive treatments for this highly complex disease. Recent technological advances, including comprehensive sequencing of different cancer subtypes, have illuminated how genetic and epigenetic alterations are associated with specific hallmarks of cancer. However, as these associations are purely descriptive, one particularly exciting development is the emergence of genome editing technologies, which enable rapid generation of precise genetic and epigenetic modifications to assess the consequences of these perturbations on the cancer phenotype. The most recently developed of these tools, the system of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), consists of an RNA-guided endonuclease that can be repurposed to edit both genome and epigenome with high specificity, and facilitates the functional interrogation of multiple loci in parallel. This system has the potential to dramatically accelerate progress in cancer research, whether by modelling the genesis and progression of cancer *in vitro* and *in vivo*, screening for novel therapeutic targets, conducting functional genomics/epigenomics, or generating targeted cancer therapies. Here, we discuss CRISPR research on each of the ten hallmarks of cancer, outline potential barriers for its clinical implementation and speculate on the advances it may allow in cancer research in the near future.

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1. Introduction

Cancer is a complex, heterogeneous and highly dynamic disease with multiple evolving molecular constituents. Dissecting the underlying events driving the genesis and progression of cancer remains a major challenge for researchers and clinicians. In 2000, Hanahan and Weinberg [1] suggested a comprehensive logical framework for the functional study of cancer. They described six capabilities acquired during tumourigenesis and tumour development—sustaining proliferative signalling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis and activating invasion and metastasis.

Since then, technological developments have led to significant advances in understanding cancer hallmarks. Hanahan and Weinberg [2] extended their original six hallmarks to encompass the 'next generation' of hallmarks in 2011. They identified two characteristics that enabled cancer development and progression—inflammation, and genomic instability and mutation. The deregulation of cellular energetics and avoidance of immune destruction were also recognised as 'emerging' cancer hallmarks. In addition, Hanahan and Weinberg highlighted the extensive cellular heterogeneity of tumours. Aside from cancer cells themselves, non-immortalised stromal cells were acknowledged to encompass part of a complex tumour microenvironment, contributing to cancer progression.

In the years since these seminal reviews, cancer genetics has progressed remarkably, with further development of comprehensive sequencing techniques to profile genetic mutations associated with certain hallmark capabilities and clinical outcomes [3]. The landscape of mutations in cancer has been integrated with transcriptome, epigenome, and metabolome maps [4,5], and single-cell profiling has provided further insights into tumour heterogeneity and clonal evolution [6,7]. However, until recently, it has been difficult to probe the insights gained from next-generation sequencing, owing to a lack of molecular tools to provoke specific genetic and epigenetic mutations and assess their resulting contribution to the disease phenotype.

The field of genome engineering experienced a revolutionary advancement with the discovery and adaptation of the system of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) proteins [8–11]. Gene editing technology had existed for more than two decades in the form of zinc fingers (ZFs) [12], and more recently transcription activator-like effectors (TALEs) [13]. However, these protein-based DNA-binding domains had to be customised *de novo* for each DNA target, which was expensive and inefficient. In addition, ZFs displayed widespread binding at unintended sites throughout the genome, limiting their clinical applicability [14]. In contrast, the RNA-guided CRISPR platform was much easier to tailor to any desired target sequence, facilitating cheap, high-throughput and multiplex genetic and epigenetic editing.

In this review, we first outline the molecular components of CRISPR systems. We then focus on key applications of CRISPR research that have advanced our understanding of the ten cancer hallmarks [1, 2]; [Fig. 1]. CRISPR systems have expedited the creation of complex cancer model systems and facilitated genome-wide screens for oncogenic drivers or therapeutic targets by gene knockout, activation and repression. In addition, CRISPR has opened the door to a new generation of cancer treatments that directly modify the genome, transcriptome and epigenome with high selectivity. Finally, we comment on potential obstacles to the implementation of CRISPR technology in cancer research and in the clinic.

2. CRISPR-Cas systems: prokaryotic immune system turned gene editing tool

CRISPR systems in prokaryotes had been studied since the 1990s [15,16]. However, key publications in 2012 demonstrated that a CRISPR system in *Streptococcus pyogenes* could be repurposed for highly efficient DNA editing, thus opening a new era of technological advances in the field of genome engineering [8,9]. CRISPR systems represent an acquired immune defence mechanism in bacteria and archaea that recognises and destroys DNA from invading viruses and plasmids [17–19]. In the case of the *S. pyogenes* CRISPR system, the nuclease Cas9 is the protein responsible for cleaving foreign nucleic acids [Fig. 2]. Cas9 is guided to the target DNA sequence by a complex of two short RNA molecules, CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA) [8]. Each crRNA is complementary to a different fragment of viral or plasmid DNA of approximately 20 base pairs [20]. For genome engineering purposes, crRNA and tracrRNA were combined into a chimeric single-guide RNA (sgRNA) that was easier to design and synthesise than the crRNA-tracrRNA complex [8]. For recognition by Cas9, the target DNA sequence must also lie immediately upstream of the protospacer-adjacent motif (PAM) sequence NGG (where N is any nucleotide). Cas9 protein, delivered with custom sgRNAs, constituted a programmable nuclease that could be directed to any PAM-adjacent site within the genome to create a double-strand break.

It was soon shown that CRISPR-Cas9 enabled efficient editing of endogenous genes in mammalian cells [10,11]. Cas9-mediated double-strand breaks could be used for gene editing via two mechanisms [Fig. 2]. First, when the DNA strand was repaired by the inherently error-prone non-homologous end-joining pathway, random insertions and deletions often resulted, leading