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世界医药学研究前沿丛书

Genome Sequencing

基因组测序

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Preface

Genome editing, or genome editing with engineered nucleases (GEEN) is a type of genetic engineering in which DNA is inserted, deleted or replaced in the genome of an organism using engineered nucleases, or "molecular scissors." These nucleases create site-specific double-strand breaks (DSBs) at desired locations in the genome. The induced double-strand breaks are repaired through nonhomologous end-joining (NHEJ) or homologous recombination (HR), resulting in targeted mutations ("edits"). There are currently four families of engineered nucleases being used: meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector-based nucleases (TALEN), and the CRISPR-Cas system. Genome editing was selected by Nature Methods as the 2011 Method of the Year. The CRISPR-Cas system was selected by Science as 2015 Breakthrough of the Year[1].

In the present book, thirty typical literatures about genome editing published on international authoritative journals were selected to introduce the worldwide newest progress, which contains reviews or original researches on genetic engineering, biotechnology, medical science, etc. We hope this book can demonstrate advances in genome editing as well as give references to the researchers, students and other related people.

[1]https://en.wikipedia.org/wiki/Genome_editing.

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Contents

Chapter 1

Next-Generation Sequencing in Clinical Oncology: Next Steps towards Clinical Validation

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Abstract: Compelling evidence supports the transition of next generation sequencing (NGS) technology from a research environment into clinical practice. Before NGS technologies are fully adopted in the clinic, they should be thoroughly scrutinised for their potential as powerful diagnostic and prognostic tools. The importance placed on generating accurate NGS data, and consequently appropriate clinical interpretation, has stimulated much international discussion regarding the creation and implementation of strict guidelines and regulations for NGS clinical use. In the context of clinical oncology, NGS technologies are currently transitioning from a clinical research background into a setting where they will contribute significantly to individual patient cancer management. This paper explores the steps that have been taken, and those still required, for the transition of NGS into the clinical area, with particular emphasis placed on validation in the setting of clinical oncology.

Keywords: Massively Parallel Sequencing, Next Generation Sequencing, Clinical Oncology, Validation

1. Introduction

On 19 November 2013, the U.S. Food and Drug Administration (FDA) approved the use of four diagnostic devices comprising two cystic fibrosis assays, kit reagents and the Illumina MiSeqDx platform for high throughput gene sequencing, commonly known as next-generation sequencing (NGS)[1]. This decision by the FDA has paved the way for future clinical diagnostic and prognostic use of NGS in a multitude of disease settings, including cancer.

Compelling evidence supports the transition of NGS technology from a research environment into clinical practice. While our knowledge of the relationship between disease pathogenesis and genetic variations continues to expand, the cost to NGS equipment and operation continues to fall. Ultimately this will enable relatively cheap exploration of specific nucleic acid regions, large gene panels, whole exomes, genomes, transcriptomes and the methylome. The expansion and acceptance of NGS technologies in many specialty fields of biological research is providing the evidence and promoting its incorporation into the clinical setting. But before NGS technologies are fully adopted in the clinic, they must be thoroughly scrutinised for their potential as powerful diagnostic and prognostic tools. Due to the massive amounts of data generated from patient genetic material, the potential for detailed and extensive analyses is vast. The importance placed on generating accurate NGS data, and consequently appropriate clinical interpretation, has stimulated much international discussion regarding the creation and implementation of strict guidelines and regulations for NGS clinical use.

Establishment of a validation process of current systems for any NGS facility must be undertaken before sample collection and nucleic acid extractions are initiated. This paper is aimed primarily at clinical diagnostic and/or prognostic use of NGS technologies and not the use of NGS equipment for research purposes. In the context of clinical oncology, NGS technologies are currently transitioning from a clinical research background into a setting where they will contribute significantly to individual patient cancer management.

In this paper, we have chosen to present material that we consider important in the field of oncology generally, and also applicable for head and neck cancer (HNC) in particular as our area of interest and expertise. In considering a venture

into clinical NGS diagnostics/prognostics, we strongly advise consideration of all information presented herein, and contact with local authorising and monitoring organisations before establishing such workflows.

2. Recommendations and Guidelines for Clinical NGS Applications

Internationally, many guidelines and recommendations for regulating and standardising NGS technologies for clinical use have been produced by government, clinical and research organisations (**Table 1**). The documents listed in **Table 1** discuss NGS issues ranging from ethical considerations and patient education through test development and bioinformatics pipeline validation to clinical reporting and data storage. Some documents discuss the entire process comprehensively (*i.e.*, 1, 3, 10) while others focus more on specific areas or relay legal and/or recommended requirements (*i.e.*, 5, 7, 8, 9 and 3, 10). The New York State Department of Health is one government regulatory organisation that has defined and implemented NGS guidelines for validation, quality control and reporting[2]. In combination with this document, laboratories wishing to undertake diagnostic NGS are required to apply for approval, in relation to each specific NGS assay, for genetic testing and oncology purposes[3]. This legislative approach to clinical NGS diagnostics will most likely be common place in the near future. Other guidelines (**Table 1**) propose extensive, similar and overlapping approaches to NGS clinical implementation. For readers interested in establishing high quality standardised NGS pipelines, we recommend perusal of the information found in **Table 1**, with particular attention paid to Groups 1, 3, 8 and 10.

3. Summary of Information Available within Table 1

Platform selection: A selection of the documents listed introduces sequencing technologies, NGS platform differences, characteristics and data generation. These documents also cite more detailed information and together can aid appropriate platform selection for a specific NGS purpose[5][7]–[9][11]. *Terminology*: Organisations such as CLSI (Clinical and Laboratory Standards Institute) and ISO (International Standards Organisation) have aligned the use of terminology and definitions to achieve uniformity for global application of NGS standards and

Table 1. International guidelines and/or recommendations for NGS use.

Group	NGS Document	References	Current Guideline Status
1. Royal College of Pathologists of Australasia (RCPA)	Standards for Massively Parallel Sequencing in Diagnostic Genetic Testing (in development)	[4]	The recommendations presented in this document do not carry regulatory authority.
2. National Association of Testing Authorities (NATA), Australia	Using recommendations in RCPA document for best laboratory practices	[4]	
3. US Centres for Disease Control and Prevention (CDC). Next-generation Sequencing: Standardization of Clinical Testing (Nex-StoCT) workgroup. (USA)	Next-generation Sequencing: Standardization of Clinical Testing (Nex-StoCT) Workgroup Principles and Guidelines. Ultra High Throughput Sequencing for Clinical	[5]	Principles and guidelines designed for NGS incorporation into clinical practice while meeting necessary quality assurance standards [1] The purpose of the meeting was to discuss challenges in assessing
4. U.S. Food and Drug Administration (FDA)	Diagnostic Applications—Approaches to Assess Analytical Validity, June 23, 2011	[6]	analytical performance for ultra high throughput genomic sequencing-based clinical applications.
5. Dutch Society for Clinical Genetic Laboratory Diagnostics (VKGL)	Best Practice Guidelines for the Use of Next-Generation Sequencing Applications in Genome Diagnostics: A National Collaborative Study of Dutch Genome Diagnostic Laboratories	[7]	
6. Whole Genome Analysis group of the Association for Molecular Pathology	Opportunities and Challenges Associated with Clinical Diagnostic Genome Sequencing: A Report of the Association for Molecular Pathology	[8]	
7. American College of Medical Genetics (ACMG)	ACMG clinical laboratory standards for next-generation sequencing	[8]	
8. Wadsworth Centre; New York State Department of Health	"Next Generation" Sequencing (NGS) guidelines for somatic genetic variant detection	[2]	Governed by the Official Compilation of Codes, Rules and Regulations of the State of New York