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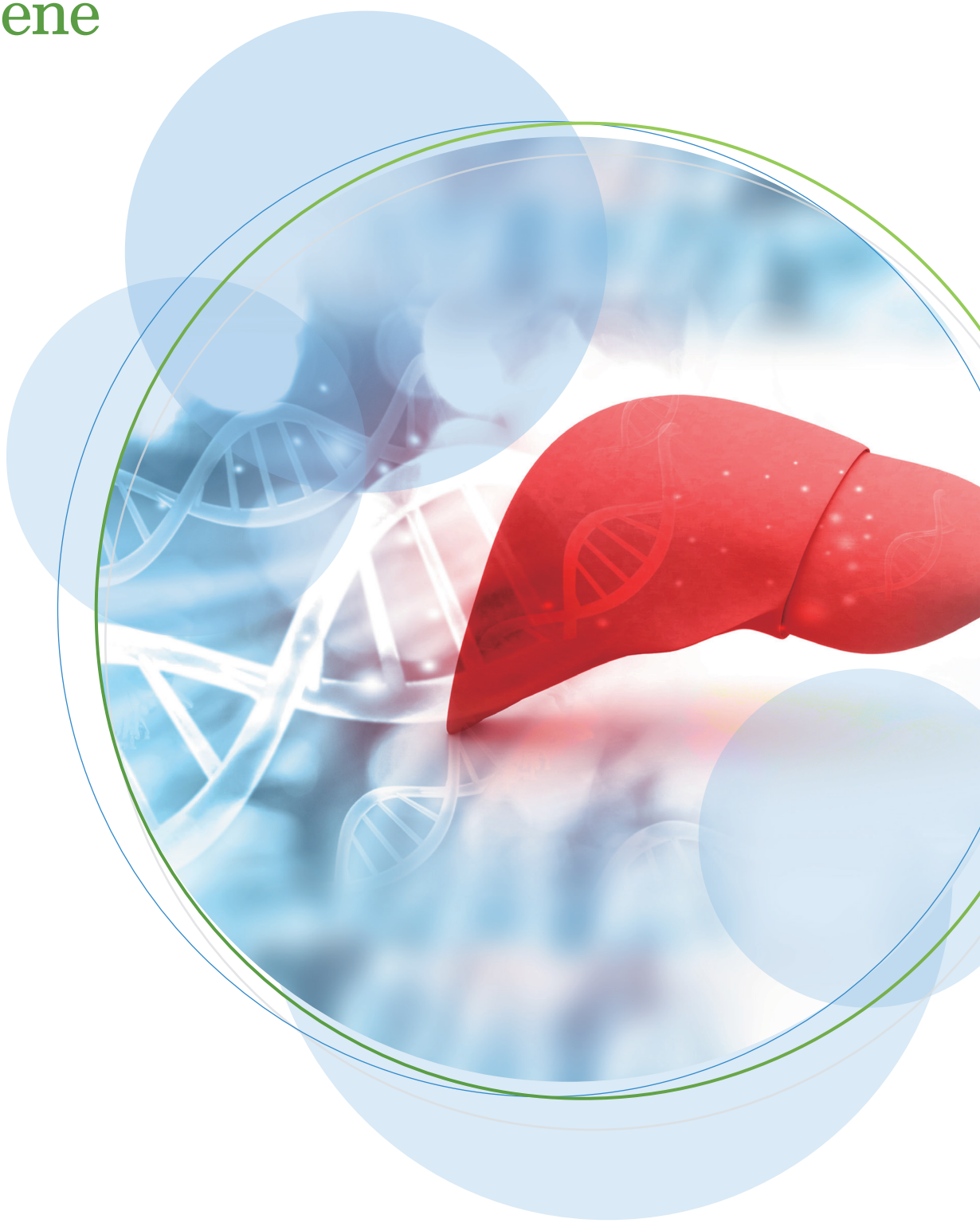


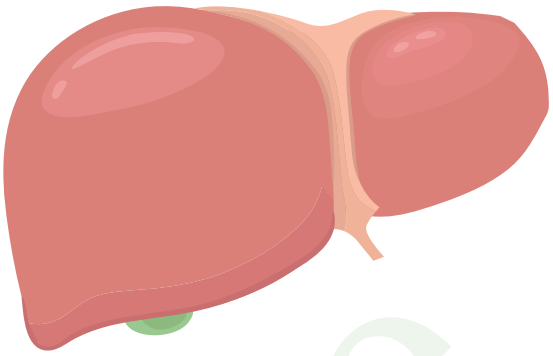
The Genetic Landscape of Hepatocellular Carcinoma:

The Role of One Oncogene.

Novogene

The arrival of next-generation sequencing technologies has allowed researchers from multiple fronts to scrutinize the genetic basis of multiple diseases, one of the most studied ones being cancer. Hepatocellular carcinoma still remains a significantly mortal type of liver cancer, a constant challenge for research teams to understand its intricacies. Here we will introduce the excellent work "***Genomic Sequencing Identifies WNK2 as a Driver in hepatocellular carcinoma and a Risk Factor for Early Recurrence***" of Jian Zhou, who is a researcher with an extensive history in liver cancer research. In this study, they studied particularly in the diagnosis and therapeutic targets in hepatocellular carcinoma, and others, identified a potential oncogenic driver in a large Chinese cohort of HCC patients, with the use of sequencing technology provided by Novogene. Its underexpression might be related, among many others, in the development of this malignant cancer.

Introduction



Hepatocellular carcinoma (HCC) is the most common type of primary liver malignancy and a leading cause of mortality among the different types of cancer. In the United States, it represents the ninth leading cause of cancer deaths [1]. It is more common in men than women and more prevalent in Eastern and Southern Asia and Middle and Western Africa.

Frequently, HCC is associated with chronic liver conditions, originated from several risk factors associated with an increased incidence of HCC, such as chronic viral hepatitis (hepatitis B and C, for example), excessive alcohol intake and environmental exposure to aflatoxin and aristolochic acid.

Despite its importance and significantly high mortality rates, the molecular and genetic basis of this pathology largely remains an enigma. However, genome sequencing services have facilitated the sequencing and amplification of specific areas in the genome of patients with HCC, to understand which genes might be at play (either by upregulation or downregulation)[2].

Identifying a patient's genetic signature (a patient's uniquely characteristic pattern of expressed genes) is pivotal in the current medical landscape to provide specialized care for the patient, as well as to provide an accurate prognosis and the likelihood of developing certain diseases, including (but not limited to) different types of cancer, HCC among them[3].

Research Background of Hepatocellular Carcinoma

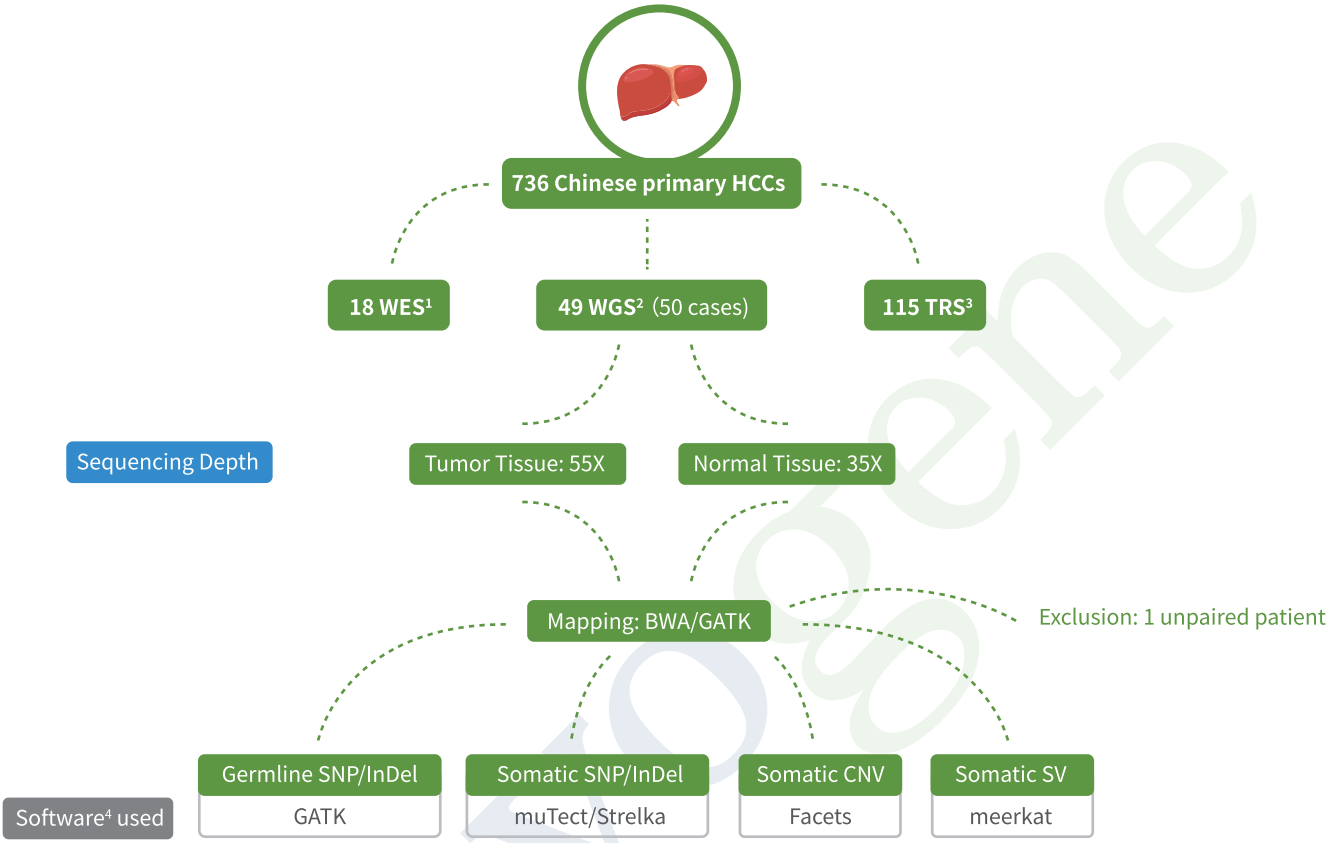
Several studies have allowed a certain insight into the pathophysiological mechanisms that unleash liver carcinogenesis and its possible root causes [4] [5]. In a previous study, Jian Zhoul and *et. al.* had previously researched in the genetic heterogeneity underlying HCC and the clinical significance of potential therapeutical targets, such as the ubiquitin ligase, through the use of Targeted Deep Sequencing and Whole Exome Sequencing, respectively[6] [7].

Some of these past works have identified possible culprits, mutations in several genes, such as: *TP53, CTNNB1, KEAP1, MLL4* and *RAC2*, to name a few. These genes codify cell-membrane proteins (calcium channel subunits), histone methyltransferases, key transcriptional coactivators and many others, participating in a variety of signaling intra and extracellular pathways[8] . These mutations are found in multiple cohorts of HCC. By pinpointing the genetic modification that disrupts molecular pathways, screening tests, prognosis and treatments options will improve as well.

The numerous genes involved in the pathogenesis of HCC may explain the heterogeneous degree of cellular differentiation, as seen in some tumors resected from patients.

Materials & Sequencing Pipeline

Numerous cancerous and non-cancerous tissue samples were used, so their genome could be mapped and described by **Whole Genome Sequencing, Whole Exome Sequencing** and **Targeted Region Sequencing** techniques provided by **Novogene Co., Ltd**.



WES[1]: Whole Exome Sequencing
WGS[2]: Whole Genome Sequencing
TRS[3] : Target Region Sequencing
Software[4] used: the updated software in use can refer to Table 1.

Figure 1. The general research pipeline on next-generation sequencing WGS as example

Each one of the aforementioned sequencing techniques allowed for a depth interpretation of the numerous samples taken. Multiple sequencing software (**GATK, muTect** and others) were used, to identify structural and functional genomic mutations.

These allowed researchers to acquire detailed information about the patients and follow-up as well. For example, the software muTect allowed identification of nucleotides polymorphisms and InDel mutations. Cell lines, animals, cell proliferation and colony formation were implemented to create knockdown models to identify the possible mutated genes.

Analysis of Sequenced Data

The cohort of cancerous tissue samples from patients with HCC demonstrated a high mutational burden, examples of these are somatic **Single Nucleotides Variations (SNVs)**, **InDels**, **Copy Number Variations (CNVs)** and **Structural Variations (SV)**.

Identifying which one of the structural mutations aforementioned, allowed researchers to understand which genomic patterns were most prevalent in a certain subgroup of patients than others. For example, muTech software is highly sensitive and accurate to pinpoint somatic SNPs, or the use of DELLY to detect somatic SVs. Use of high-tech, accurate software allow researchers to easily categorize each mutation for what they are. More examples of Novogene's best practices are present in Table 1.

Table 1. Analytical content Software Comments Version

Analytical content	Software	Comments
Alignment	BWA	Map the sequencing reads to the reference genome, and output the alignment file in the bam format
	Samtools	Sort the bam file
	Picard	Merge all bam files from the same sample and mark the duplicated reads
SNP/InDel detection	GATK	Detect and filter SNPs/InDels
SV detection	DELLY	Detect SVs
CNV detection	control-FREEC	Detect CNVs
Somatic SNP/InDel detection	MuTect/Strelka	Detect and filter Somatic SNPs/InDels
Somatic SV detection	DELLY	Detect somatic SVs
Somatic CNV detection	Control-FREEC	Detect somatic CNVs
Annotation	ANNOVAR	Annotate variants

In this study, the samples sequenced demonstrated certain genetic signatures. Some of said signatures have been previously associated with environmental exposure to certain substances, such as aflatoxin B1 and aristolochic acid[9] [10].

Several amplified genomic segments were identified and, within these, oncogenes such as *CCND1, TERT, MYV*. On the other hand, the study identified certain segments that were lost, especially those harbored tumor suppressant oncogenes, such as *TP53 (17p13)* and *CDKN2A (13q14)*.

Among the different identified oncogenes, the most frequently mutated one was WNK2. This mutation was found in 12 of 101 HCC samples from patients that experienced early recurrence. Additional 554 HCC samples were evaluated and researchers found that, out of the 117 patients with HCC, 17 demonstrated WNK2 somatic mutations. These same 17 patients had also experienced early recurrence of HCC as well.

The carcinogenic role of WNK2 mutations

These results demonstrate that WNK2 underexpression is a driver oncogene of HCC, not only through the overexpression of ERK 1/2, but also through the infiltration of TAMs. WNK2 is also identified as a driver in the early occurrence of HCC after curative resection. Advancement in genetic therapies, through specialized and individualized medicine to help target specific gene signatures, is pivotal to help decrease the high mortality rates associated with HCC.

Novogene helps researchers and other researching organizations or teams by supplying them with high-tech, reliable and accurate genomic profiling technology. We also provide cost-effective and convenient alternatives for Whole Exome Sequencing, since they only target specific coding regions in the human genome, useful for genetic disease studies or cancer research (as demonstrated in this study).

For human Whole Genome Sequencing hWGS, Novogene can provide as well with powerful PacBio Sequel Systems, that allows for specific and accurate characterization of the human genome. This is very useful to detect in small coding regions with a certain mutational burden, cause by polymorphic changes. Target Capture Sequencing services covers other areas of interest, such as identifying rare variants in certain subgroups of genes or specific genomic regions. Novogene’ s human targeted region sequencing (hTRS) is an excellent example of this.

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