A Study of Immune Subtypes and Neoantigen-related Immune Evasion in Advanced Colorectal Cancer

MGI's DNBSEQ-G50 Genetic Sequencer Facilitates the Discovery of a New Subtype of CRC and Prognostic Molecular Biomarkers

The research team led by Tatsuhiko Tsunoda at the Medical Research Institute of Tokyo Medical and Dental University in Japan used the DNBSEQ-G50 genetic sequencer from MGI to explore the mechanism of tumor development and prognosis evaluation of advanced colon cancer patients using techniques such as whole-exome sequencing, and explained the correlation between the tumor microenvironment and immune evasion of the disease, identifying a subtype of cancer with poor prognosis and two related molecular markers. Meanwhile, a reference treatment plan was proposed. The research was published on *iScience*, a subsidiary journal of *Cell*, in 2022, entitled with "Immune subtypes and neoantigen-related immune evasion in advanced colorectal cancer"[1].

Recommended application: Cancer Genomics (Colorectal Cancer)

Recommended model: DNBSEQ-G50RS.

- ### Rapid detection and identification of cancer subtypes and biomarkers

  The excellent low-frequency mutation capture capability of the DNBSEQ-G50 genetic sequencer can effectively support the detection of cancer mutation genes and draw gene mutation map with high resolution.

- ### Automatic operation compatible

  MGI provides automation solutions for experimental processes, which can greatly save labor costs and improve efficiency.

Background

In the treatment of colorectal cancer (CRC), surgery, chemotherapy, and radiotherapy have achieved good results in recent years, improving the prognosis of CRC significantly[2], with an overall 5-year survival rate reaching 64%[3]. Among patients undergoing surgical treatment, although 25%-30% will experience metastasis and recurrence[4], half of them can be cured through multidisciplinary treatment[5]. However, the overall 5-year survival rate for stage IV CRC is only 14% (Figure 1), indicating that the disease is difficult to treat in the late stage and has a poor prognosis.

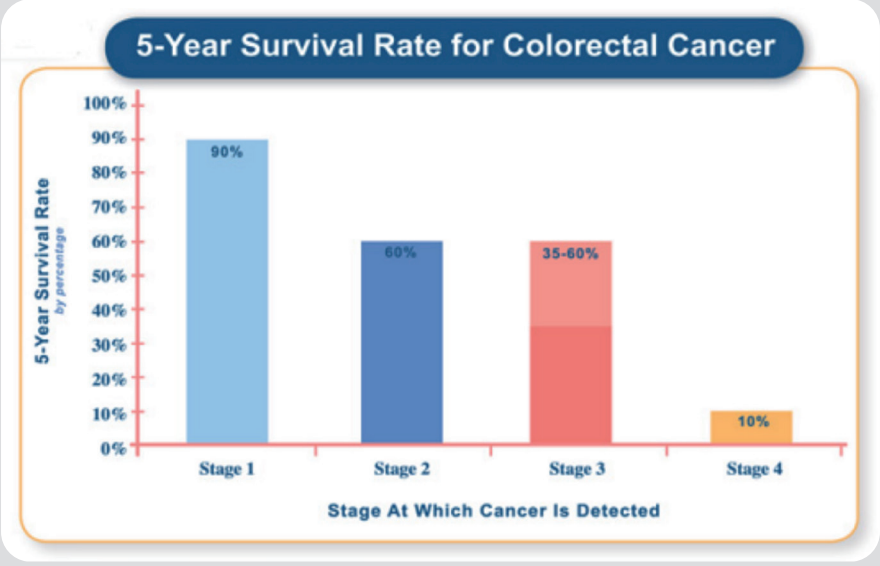

Figure 1. 5-year survival rate of CRC patients at different stages[6]

In recent years, cancer immunotherapy has become an active field in cancer research. The most intriguing immune checkpoint inhibitors (ICI) therapy has brought a transformation in cancer treatment (such as lung cancer and melanoma), but effectively applying it to CRC still poses challenges. Currently, only a few ICI therapies (4%-5%) are recognized. Only half of them respond to treatment and the reasons are unknown[7], so it is particularly important to find good predictive molecular markers.

The uniqueness of CRC lies in the possibility of treating the metastasis of cancer cells even after the tumor has been removed at a certain stage in stage IV[8]. This allows researchers to collect untreated samples from advanced primary tumors. Genotyping analysis was performed on these samples to determine the indications for anti-epidermal growth factor receptor therapy and ICI therapy.

Study Description

The role of tumor microenvironment (TME) status and neoantigen formation in advanced CRC has attracted the attention and interest of Tatsuhiko Tsunoda team. Tumors will evolve during immune infiltration and escape, and advanced cancer is a systemic disease, so high responsiveness to chemotherapy is crucial for prolonging long-term survival rates of patients. Previous research has identified several mutations related to poor prognosis[9], demonstrating the important role of tumor-infiltrating lymphocytes in CRC[10].

The Tatsuhiko Tsunoda team has conducted a focused study on how the interaction between TME and the neoantigen status and their balance affecting the prognosis of advanced cancer. The analysis proves that TME and immune evasion characteristics are important prognostic factors, especially in an immune "cold" subtype of tumors, which have strong immune evasion and poor overall survival (OS). At the same time, the consumption of neoantigens caused by immune editing and high clone neoantigens is associated with good OS.

Materials and Methods

A. Sample collection

The team collected 89 tumor samples from 89 CRC patients at the affiliated hospital of the Tokyo Medical and Dental University, all of which were primary colon tumors surgically removed and did not undergo neoadjuvant systemic chemotherapy. At the same time, normal rectal samples were used as a control. Among 89 patients, 66 were in stage IV colorectal cancer. Total DNA was extracted from fresh frozen tumors and normal tissues using magnetic beads phenol-chloroform. Total RNA was extracted from 10 mm sections of formalin-fixed paraffin-embedded blocks using FFPE extraction kit.

B. Whole exome sequencing and data analysis

The DNBSEQ-G50 genetic sequencer from MGI was used in combination with the Agilent SureSelect Human All Exon V7 reagent kit to target and capture the exon regions of various samples, followed by library preparation and paired-end 100bp (PE100) sequencing. When dealing with a large sample size, the automated extraction and library construction workflow provided by MGI can greatly reduce labor costs, shorten time, and improve efficiency.

The Burrows-Wheeler Alignment (BWA-mem) of version 0.7.17 was used to align sequencing data to the human reference genome (hg38). The Genotype Analysis Toolkit (GATK) of version 4.1.2.0 was used to remove duplicate data and recalibrate base quality scores with default settings. Afterwards, Mutect2 was used to identify somatic mutations and annotate genes using ANNOVAR. R package "maftools" was used to calculate the total number of non-synonymous mutations. SigProfiler was used to perform mutation feature analysis.

C. RNA sequencing and data analysis

For all tumor samples, total RNA was prepared using TruSeq RNA Exome to construct RNA-seq libraries, which were then sequenced. The STAR-2.7.1a software was used to align sequencing data to the human reference genome (hg38). Subsequently, the data that was mapped to the rRNA and transfer RNA (tRNA) regions was removed. The FeatureCount from the Bioconductor package Rsubread was used to assign aligned reads to genes (Ensembl database annotation version GRCh38.98). Then, the R package neuMatIdx was used to normalize the generated FeatureCounts to transcripts

per million or fragments per kilobase million. DESeq2 was used to perform differential expression analysis.

D. Data analysis

Copy number analysis, immune cell population evaluation, T cell and B cell abundance analysis, HLA type I matching, neoantigen detection, neoantigen clonality detection, immune editing index calculation, neoantigen immune evasion detection, and detection of mutation and heterozygosity of HLA Type I region were performed based on the sequencing results above.

Sample collection	Library preparation	Bioinformatics analysis	Result analysis
Samples of tumors from 89 patients with CRC.	 SureSelect Human All Exon V7 reagent kit TruSeq RNA Exome DNBSEQ-G50 gene sequencer	BWA-mem GATK Mutect2 ANNOVAR Maftools STAR Bioconductor DESeq2	Copy number analysis, Immune cell population evaluation, T cell and B cell abundance analysis, HLA type I matching

Results

CRC driver gene mutations were identified through whole exome sequencing, suggesting the production of biomarkers

As shown in Figure 2, the research team firstly identified 167 non-synonymous mutations and some known CRC driver gene mutations in somatic cells through whole-exome sequencing of tumor samples, such as *APC (79%)*, *TP53 (76%)*, and *KRAS (44%)*, indicating that there were some obvious biomarkers produced in the patient's body, providing a targeted window for immunotherapy of the disease.

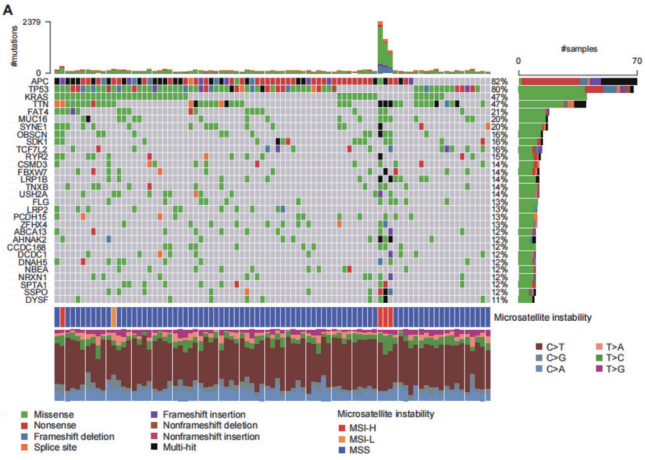

Figure 2. Cancer location map and microsatellite instability status of 85 samples.

The RNA sequencing results show that the intermediate type immune cells and protein levels are decreased, indicating a reduced immune response.

Afterwards, the team obtained the T-cell inflammatory gene expression profile through RNA sequencing. According to the degree of enrichment of *IFNγ* responsive genes, it was divided into hot, intermediate, and cold types, as shown in Figure 3. It was found that the intermediate type had higher expression levels of *PD-L1* and *PD-L2* and lower gene expression of HLA II level as well as fewer levels of immune cells such as B cells. By further analysis, it was found that the intermediate type appeared to have the lowest abundance of predicted B cells and lower BCR clones, as well as a lack of highly expressed genes, while low expression of six genes, CD74/HLA-DRA/HLA-DPA1/HLA-DRB6/C1QB/VIM-AS, suggesting that the intermediate type reflects adaptive resistance to lymphocytes compared to the other types.

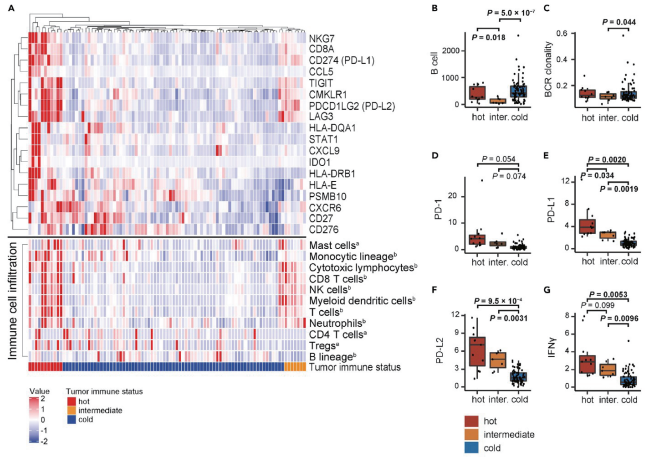

Figure 3. Situation of TME. (A) T cell inflammatory gene expression profile heat map; (B-G) Predicted abundance of B cells, *PD-1*, *PD-L1*, *PD-L2* and *IFN γ*

Antigen detection evaluation

Based on sequencing, the author conducted a predictive neoantigen evaluation based on non-synonymous mutations that form tumor protein changes. As shown in Figure 4, more immunogenic clones with high expression of neoantigens were found in the hot type, which may be the reason for its immune "hot". On the contrary, among intermediate and cold tumors, most tumors lose highly expressed neoantigens (HiNeo) and gained more subclonal neoantigens, which suggests the occurrence of immune editing. At the same time, the author quantified

the chromosomal instability and found that the intermediate and cold types have higher chromosomal instability, which is prone to immune evasion.

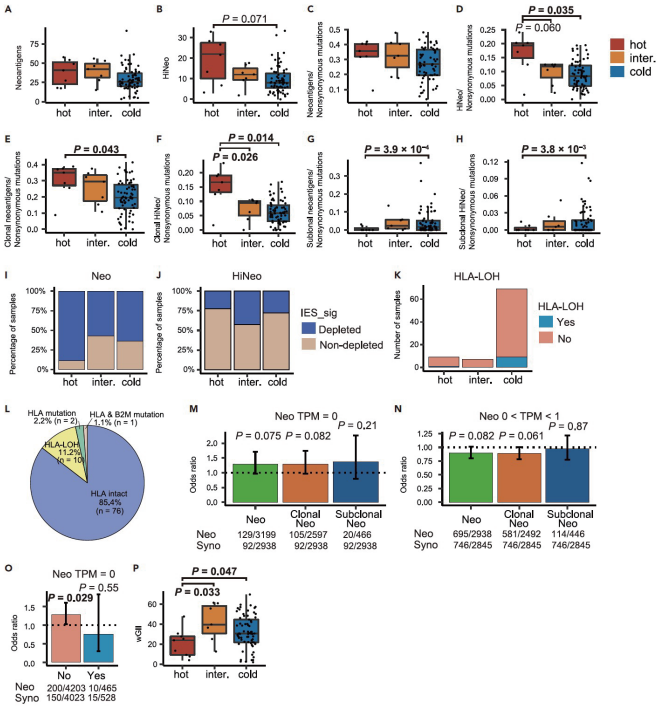

Figure 4. Immune evasion guided by neoantigens: loss, expression and presentation of neoantigens.

Prognosis evaluation

As shown in Figure 5, the research team based on the CRC immune cancer map, combined with the consideration of the immune system microenvironment and prognosis evaluation of CRC patients, found that the intermediate prognosis was poor, while the HiNeo-exhausted type had a good prognosis.

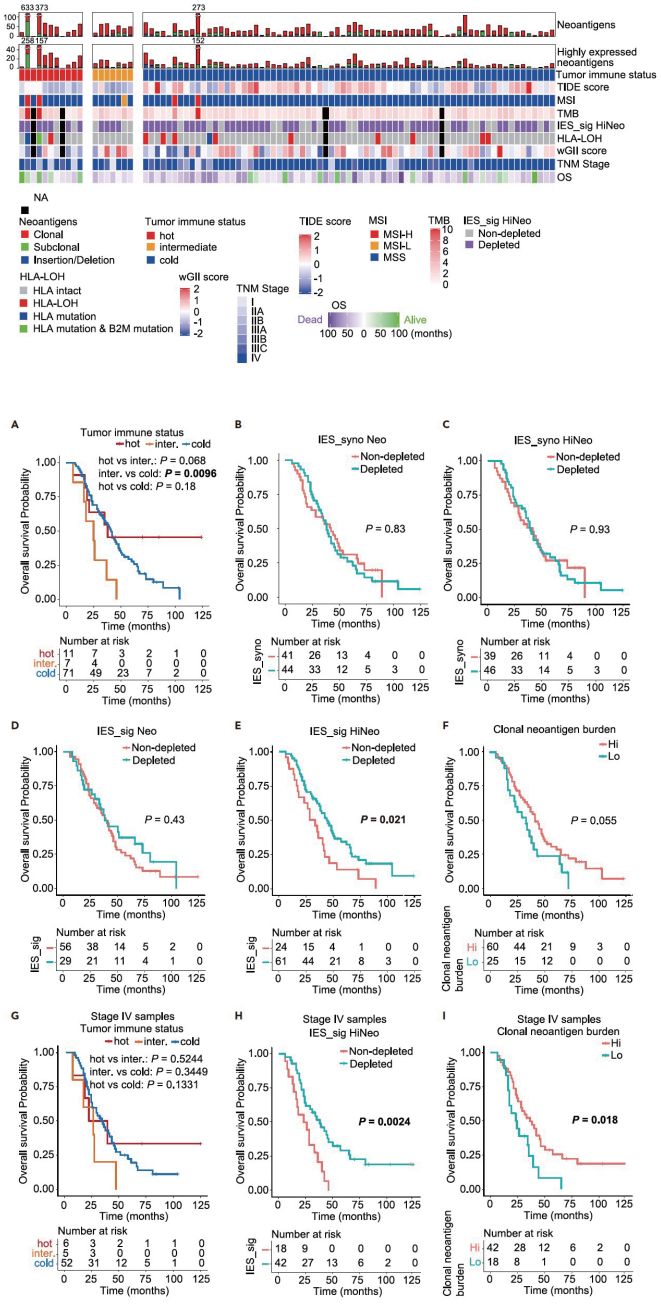

Figure 5. 1) Cancer immune profile of progressive CRC; 2) Survival analysis in cancer immunotherapy

Summary

This study, based on the samples from patients with CRC, comprehensively described the immune evasion pattern of CRC, and linked newly discovered subtypes to differences in patient prognosis. It found that the prognosis effect of intermediate type was poor, and two biomarkers of CRC with prognostic ability: TME status and neoantigen were formed, providing a reference for optimizing treatment.

This study is based on the DNBSEQ-G50 sequencing platform independently designed and developed by MGI, in which, using targeted exon sequencing technology combined with RNA sequencing, a subtype of CRC with immune evasion and low OS characteristics, as well as two new CRC prognostic biomarkers, were discovered. The DNBSEQ-G50 genetic sequencer is a small and flexible sequencing instrument that can meet the needs of low-depth whole-genome sequencing. It can detect low-frequency mutations as low as 0.5%, with high accuracy, low repeat sequence rate, and low label jumping rate. It meets the scientific research or clinical application needs of different application scenarios for time and throughput.

DNBSEQ-G50 Genetic Sequencer

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Recommended Ordering Information

Category	Product	Cat. NO.
Instruments	Genetic Sequencer DNBSEQ-G50RS	900-000353-00
	MGISP-100RS Automated Sample Preparation System	900-000206-00
	MGISP-960RS Automated Sample Preparation System	900-000146-00
Software	MegaBOLT Bioinformatics analysis accelerator	900-000555-00
Library Prep	MGIEasy Exome Capture V5 Probe Set（16 RXN）	940-000187-00
	MGIEasy Duplex UMI Universal Library Prep Set（16 RXN）	1000018643
	MGIEasy Dual Barcode Exome Capture Accessory Kit（16 RXN）	1000018647
	MGIEasy RNA Library Prep Set（16 RXN）	1000006383
Sequencing Reagents	DNBSEQ-G50RS High-throughput Sequencing Set (FCL PE100)	1000019859
	CPAS Barcode Primer 3 Reagent Kit	1000020834

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