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RESEARCH

Pan-cancer analysis to character the clinicopathological and genomic features of KRAS-mutated patients in China

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Abstract

Purpose The Kirsten rat sarcoma viral oncogene (*KRAS*) is the most frequently mutated oncogene in human cancers. Significant advancements have been made in targeted therapy and immunotherapy for this gene in recent years, underscoring the importance of comprehensively understanding the genomic landscape of *KRAS* across various cancer types.

Methods Using next-generation sequencing (NGS) technology and a panel of 520 genes, *KRAS* mutations, tumor mutation burden (TMB), and microsatellite instability (MSI-H) status were investigated.

Results An analysis of 10,820 tumor samples found *KRAS* mutations in 19.97% of cases. Pancreatic cancer showed the highest prevalence of *KRAS* mutations at 73.51%, while colorectal at 41.45%, uterine at 21.23%, and lung cancer at 11.24%. *KRAS* G12D mutation is most common in pancreatic, colorectal, and gastric cancers, while *KRAS* G12V mutation is predominant in uterine cancer, and *KRAS* G12C mutation is most frequent in lung cancer. Significant correlations were found between TMB and *KRAS* G13D/G12V mutations in colorectal cancer. *KRAS* G13D notably affected TMB in uterus cancer, while *KRAS* G12C mutation was linked to high TMB in lung cancer. Moreover, statistical analysis revealed a significant association between *KRAS* G13D/G12V mutations and MSI-H in colorectal cancer.

Conclusions *KRAS* mutations were most frequent in cancers of the digestive, female reproductive, and respiratory systems. Specific *KRAS* mutations are associated with TMB and MSI in various cancer types.

Keywords Malignant tumors · Next-generation sequencing · *KRAS* mutation · Tumor mutation burden · Microsatellite instability

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Abbreviations

KRAS	Kirsten rat sarcoma viral oncogene
NGS	Next-generation sequencing
TCGA	The Cancer Genome Atlas
CHCAMS	Cancer Hospital Chinese academy of medical sciences
PD-L1	Programmed death-ligand-1
EGFR	Epidermal growth factor receptor
TMB	Tumor mutational burden
MSI	Microsatellite instability
ICIs	Immune checkpoint inhibitors
NGS	Next-generation sequencing
PCR	Polymerase chain reaction
FDA	Food and drug administration
NSCLC	Non-small cell lung cancer
PDAC	Pancreatic ductal adenocarcinoma
COADREAD	Colon adenocarcinoma/rectum adenocarcinoma esophageal carcinoma
STAD	Stomach adenocarcinoma
PAAD	Pancreatic adenocarcinoma

ESCA	Esophageal carcinoma
CHOL	Cholangiocarcinoma
LUAD	Lung adenocarcinoma
LUSC	Lung squamous cell carcinoma
UCEC	Uterine corpus endometrial carcinoma
OV	Ovarian serous cystadenocarcinoma
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma
HNSC	Head and neck squamous cell carcinoma
THCA	Thyroid carcinoma
dMMR	Mismatch repair system

Background

RAS gene was the first human oncogene identified (Stephen et al. 2014), comprising three principal subtypes: *KRAS*, *HRAS*, and *NRAS*. Research indicates that mutations in the RAS gene are implicated in 30% of all cancers, with *KRAS* mutations representing 85% of these RAS mutations (Hunter et al. 2015). *KRAS* functions as a critical downstream signaling molecule within the epidermal growth factor receptor (*EGFR*) signal transduction pathway. Activating mutations in the *KRAS* gene are implicated in both tumorigenesis and the aggressive proliferation of tumors. As a result, *KRAS* is considered the most promising target for cancer therapy. *KRAS* mutations are notably prevalent in many types of cancers, including pancreatic cancer, colorectal cancer, and non-small cell lung cancer (Cazzanelli et al. 2018). Moreover, mutation subtypes varied widely across different locations and tumor types. The most frequently observed mutation sites in *KRAS* are predominantly located on exon 2, including G12C, G12V, G12D and other (Kulkarni et al. 2022). However, the differential expression of different *KRAS* mutant isoforms in pan-cancer of Chinese patients has not yet been reported.

In recent years, targeted drugs for *KRAS* G12C mutations, such as Sotolasib, have achieved good efficacy in clinical trials (Nakajima et al. 2022). Moreover, G12D mutation has been identified as a clinical candidate. Recent identification of a non-covalent small molecule inhibitor (MRTX1133) with specificity to the *KRAS* G12D mutant protein has offered an opportunity to evaluate its efficacy directly on *KRAS* cancer cells (Hallin et al. 2022; Kemp et al. 2023). Consequently, the targeting of *KRAS* mutations represents a promising strategy for the treatment of various cancer types, with significant implications for the development of new cancer therapies. *KRAS* mutation also exhibits a broad impact on the tumor microenvironment, and many studies have been carried out investigating the effects of *KRAS* mutations on immunotherapy (Kim et al. 2017). Nevertheless, there is a paucity of comprehensive and robust data pertaining to the impact of *KRAS* mutations on the tumor microenvironment and the efficacy of immunotherapy across a range of cancers.

Here, we performed a comprehensive pan-cancer genomic analysis to identify the incidence of *KRAS* alterations across 16 tumor types in 10,820 Chinese patients. We also analyzed the genomic co-alteration landscapes and immune biomarker profiles associated with various *KRAS* mutations, focusing on tumor mutational burden (TMB), microsatellite instability (MSI), and mutational signatures. This research delineates the landscape of tumors harboring *KRAS* mutations, aiming to furnish critical insights for the design and implementation of clinical trials targeting patients with *KRAS*-mutant tumors in China.

Methods

Patients and data source

The study involved the collection of data from 10,820 patients diagnosed with malignant tumors at the Cancer Hospital Chinese Academy of Medical Sciences (CHCAMS) between 2019 and 2024. The tumor types included those of the digestive system, respiratory system, female reproductive system, head and neck, urinary system, soft tissue, nervous system, and bone system. All patients received hybrid-capture-based next-generation sequencing (NGS) testing after obtaining written informed consent. The NGS data included in this study have been subjected to a rigorous review process by the CHCAMS Ethics Committee, and ethical approval has been granted (NCC2694). The study was conducted in accordance with the ethical principles set forth in the Declaration of Helsinki for research involving human subjects. Prior to participation, all subjects were required to sign an informed consent form, which provided detailed information about the purpose of the experiment, the methods to be employed, the potential risks, the expected benefits, and the subjects' rights. The Cancer Genome Program (TCGA) of *KRAS* mutations-related research data is available from the biological portal platform (https://www.cbioportal.org) (Gao et al. 2013).

NGS

All specimens were initially preserved in standard formalin and subsequently embedded in paraffin. Post-pathological assessment, samples demonstrating a tumor cell content exceeding 20% were selected for further analysis. The paraffin-embedded blocks were sectioned, and genomic DNA was extracted using the QIAamp DNA FFPE Tissue Kit from Qiagen. NGS was employed to analyze critical tumor-associated genes through a targeted NGS approach based on hybrid capture. Furthermore, the microsatellite status and TMB of

each tumor were evaluated. The experimental protocol for NGS was executed as follows: Initially, genomic DNA was fragmented utilizing an ultrasonic disruptor. Subsequently, both termini of the fragmented DNA were amplified through polymerase chain reaction (PCR). The resulting PCR products underwent purification using 75% ethanol, followed by probe capture and additional purification via PCR to construct sequencing libraries. These labeled libraries were then adjusted to the requisite concentration and combined. Variants exhibiting a mutation abundance exceeding 5% were identified as positive using the NextSeq N550 platform (Illumina, San Diego, CA). TMB was quantified by enumerating the non-driver synonymous and non-synonymous mutations within a genomic region spanning 0.8–1.2 megabases (Mb), incorporating computational filtering for germline status, and expressing the results as mutations per megabase. This approach has been previously validated for accuracy in comparison to whole exome sequencing (Chalmers et al. 2017). MSI was assessed by examining intronic homopolymer repeat loci for length variability, with the data subsequently synthesized into a comprehensive MSI score using principal component analysis (Trabucco et al. 2019).

Statistical analysis

The data were analyzed and processed using SPSS version 22.0 and GraphPad Prism version 6.0 (GraphPad Software, La Jolla, CA, USA). The chi-square (χ^2) test was utilized to investigate the association between *KRAS* gene mutations and the clinical and molecular pathological characteristics of the patients. For binary outcomes, polytomous regression was reduced to binary logistic regression. Statistical significance was determined by a two-sided *p*-value of less than 0.05.

Results

KRAS mutations in various tumors

In this study, a cohort of 10,820 patients diagnosed with malignant tumors was enrolled, consisting of 6724 male and 4096 female participants, covering malignancies of the colorectal, gastric, pancreatic, esophageal, biliary tract, and pulmonary systems, among others. *KRAS* mutations were identified in 2161 out of the 10,820 tumor samples analyzed (19.97%). The prevalence of *KRAS* mutations exhibited variability among different cancer types, with the highest frequency observed in tumors of the digestive system (1477 out of 4255 cases, 34.71%), followed by tumors of the female reproductive system (146 out of 982 cases, 14.87%). Furthermore, the frequency of *KRAS* mutations was 11.24% (481 out of 4278 cases) in respiratory tumors, 10.99% (20 out of 182 cases) in soft tissue tumors, and 7.87% (7 out of 89 cases) in bone tumors. In urological tumors, the frequency was 4.19% (9 out of 215 cases), while it was 4.04% (4 out of 99 cases) in neurological tumors, 2.57% (17 out of 662 cases) in head and neck tumors, and 0% (0 out of 58 cases) in breast cancer. The prevalence of *KRAS* mutations varies among different tumor types, with the highest incidence observed in pancreatic cancer (73.51%), followed by colorectal cancer (41.45%), uterine cancer (21.23%), biliary tract cancer (14.56%), lung cancer (11.24%), gastric cancer (8.57%), thyroid cancer (7.41%), ovarian cancer (5.43%) (Table 1).

For analytical purposes, only those cancer types that had been observed in more than 50 cases with a KRAS mutation were considered. And then, this epidemiological distribution was compared with data from the TCGA database. The incidence of *KRAS* mutations was found to be higher in the Chinese NCCN patient cohort compared to the general TCGA population for PAAD (73.51% vs. 63.59%, $p = 0.126$), COADREAD (41.45% vs. 36.70%, $p = 0.562$), UCEC (21.23% vs. 17.62%, $p = 0.592$), and CHOL (14.56% vs. 17.62%, $p = 0.038$). Conversely, the incidence was lower in patients with LUAD (14.10% vs. 30.05%, $p = 0.000$) (Figure 1).

KRAS subtypes in diverse cancers

In pancreatic cancer and colorectal cancer, the G12D mutation was the most common, found in 34.23% and 13.37% of cases, respectively. Uterine cancer predominantly exhibited the G12D and G12V mutations, each accounting for 6.30% of cancer. In contrast, the G12C mutation was most frequently observed in lung cancer, representing 3.37% of cancer. Gastric cancer primarily exhibited the G12D mutation (2.86%) (Fig. 2A). An analysis of *KRAS* mutations subtypes reveals that the *KRAS* G12D mutation is the most prevalent in pancreatic, colorectal, and gastric cancers. In contrast, the *KRAS* G12V mutation predominates in uterine cancer, while *KRAS* G12C mutation is the most frequent mutation in lung cancer. Specifically, in pancreatic cancer, the *KRAS* G12D mutation was identified in 46.5% (115 out of 247) of *KRAS* mutations, followed by *KRAS* G12V mutation in 31.6% (78 out of 247), and *KRAS* G12R mutation in 12.5% (31 out of 247) (Fig. 2B). In colorectal cancer, the distribution of *KRAS* mutations was as follows: G12D mutation in 32.3% (372 out of 1153), G13D mutation in 18.9% (218 out of 1153), G12V mutation in 17.3% (200 out of 1153), and G12C mutation in 6.5% (75 out of 1153) (Fig. 2C). In uterine cancer, the prevalence of *KRAS* mutations was as follows: G12V mutation and G12D mutations were each present in 29.7% (38 out of 128 cases), G13D mutation in 14.1% (18 out of 128 cases), and G12A mutation in 7.8% (10 out of 128 cases) (Fig. 2D). In lung cancer, the distribution of *KRAS* mutations

Table 1 The distribution of KRAS mutations across different systems within the studied sample population

System	Organ	Histological subtypes	Total samples	KRAS mutations	Mutation Percentage (%)
Digestive			4255	1477	34.71
	Colorectal	COADREAD	2782	1153	41.45
	Stomach	STAD	700	60	8.57
	Pancreas	PAAD	336	247	73.51
	Esophagus	ESCA	334	2	0.60
	Biliary tract	CHOL	103	15	14.56
Respiratory			4278	481	11.24
	Lung	LUAD	3355	473	14.10
		LUSC	923	8	0.87
Gynecological			982	146	14.87
	Uterus	UCEC	603	128	21.23
	Ovary	OV	276	15	5.43
	Cervical	CESC	103	3	2.91
Head and neck			662	17	2.57
	Squamous cell carcinoma	HNSC	581	11	1.89
	Thyroid	THCA	81	6	7.41
Urinary	–	–	215	9	4.19
Soft Tissue	–	–	182	20	10.99
Nervous	–	–	99	4	4.04
Bone	–	–	89	7	7.87
Breast	–	–	58	0	0.00
Total	–	–	10820	2161	19.97

COADREAD Colon adenocarcinoma/Rectum adenocarcinoma Esophageal carcinoma; STAD Stomach adenocarcinoma; PAAD Pancreatic adenocarcinoma; ESCA Esophageal carcinoma; CHOL Cholangiocarcinoma; LUAD Lung adenocarcinoma; LUSC Lung squamous cell carcinoma; UCEC Uterine Corpus Endometrial Carcinoma; OV Ovarian serous cystadenocarcinoma; CESC Cervical squamous cell carcinoma and endocervical adenocarcinoma; HNSC Head and Neck squamous cell carcinoma; THCA Thyroid carcinoma.

was observed as follows: G12C mutation in 29.9% (144 out of 481 cases), G12D mutation in 22.4% (108 out of 481 cases), G12V mutation in 18.9% (91 out of 481 cases), and G12A mutation in 7.3% (35 out of 481 cases) (Fig. 2E). In gastric cancer, *KRAS* mutations were identified as G12D mutation in 33.3% (20 out of 60 cases), G13D mutation in 28.3% (17 out of 60 cases), and G12V mutation in 10% (6 out of 60 cases) (Fig. 2F).

Association of TMB with *KRAS* mutations

The TMB values of each tumor were assessed through NGS during the identification of driver gene mutations. A TMB of 10 mutations per megabase (mut/Mb) was categorized as high TMB (TMB-H). TMB values demonstrated variability across different malignancies, with TMB-H being most prevalent in gastric cancer (37%, 22 out of 60 cases), followed by uterine cancer (25%, 32 out of 128 cases), lung cancer (25%, 120 out of 481 cases), colorectal cancer (16%, 186 out of 1153 cases), and pancreatic cancer (2%, 6 out of 247 cases) (Fig. 3). In colorectal cancer, significant associations with TMB were observed for the *KRAS* G13D ($p = 0.016$) and *KRAS* G12V ($p = 0.005$) mutations. In the context of lung cancer, the *KRAS* G12C mutation showed a positive correlation with TMB-H, with a p-value of 0.000. In uterine cancer, the *KRAS* G13D and G12V mutations were found to have a significant impact on TMB. In gastric cancer, the *KRAS* G13D mutation was found to have a significant impact on TMB, indicated by a p-value of 0.025. Conversely, no statistically significant associations were observed in pancreatic cancer (Table 2).

Association of MSI status with *KRAS* mutations

The status of microsatellite instability high (MSI-H) varied across different malignancies, with the highest prevalence observed in gastric cancer (30%, 18 out of 60 cases), followed by uterine cancer (16%, 20 out of 128 cases), colorectal cancer (7%, 77 out of 1153 cases), pancreatic cancer (0%, 1 out of 247 cases), and lung cancer (0%, 1 out of 481 cases)

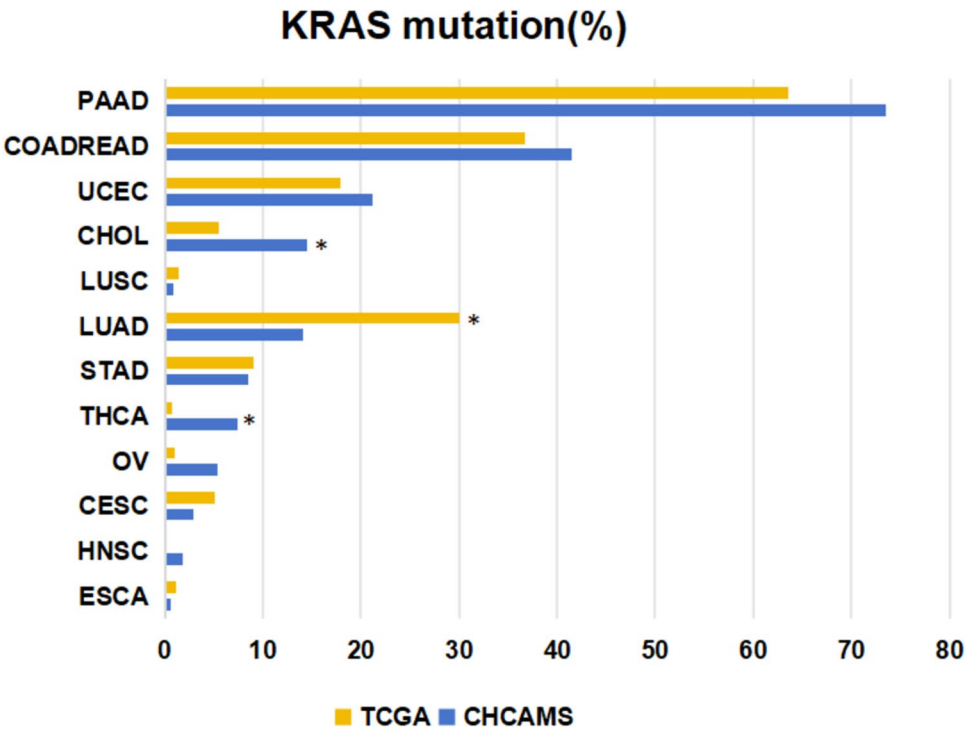

Fig. 1 The frequencies of KRAS were different between current data and TCGA in diverse cancers. *$p < 0.05$

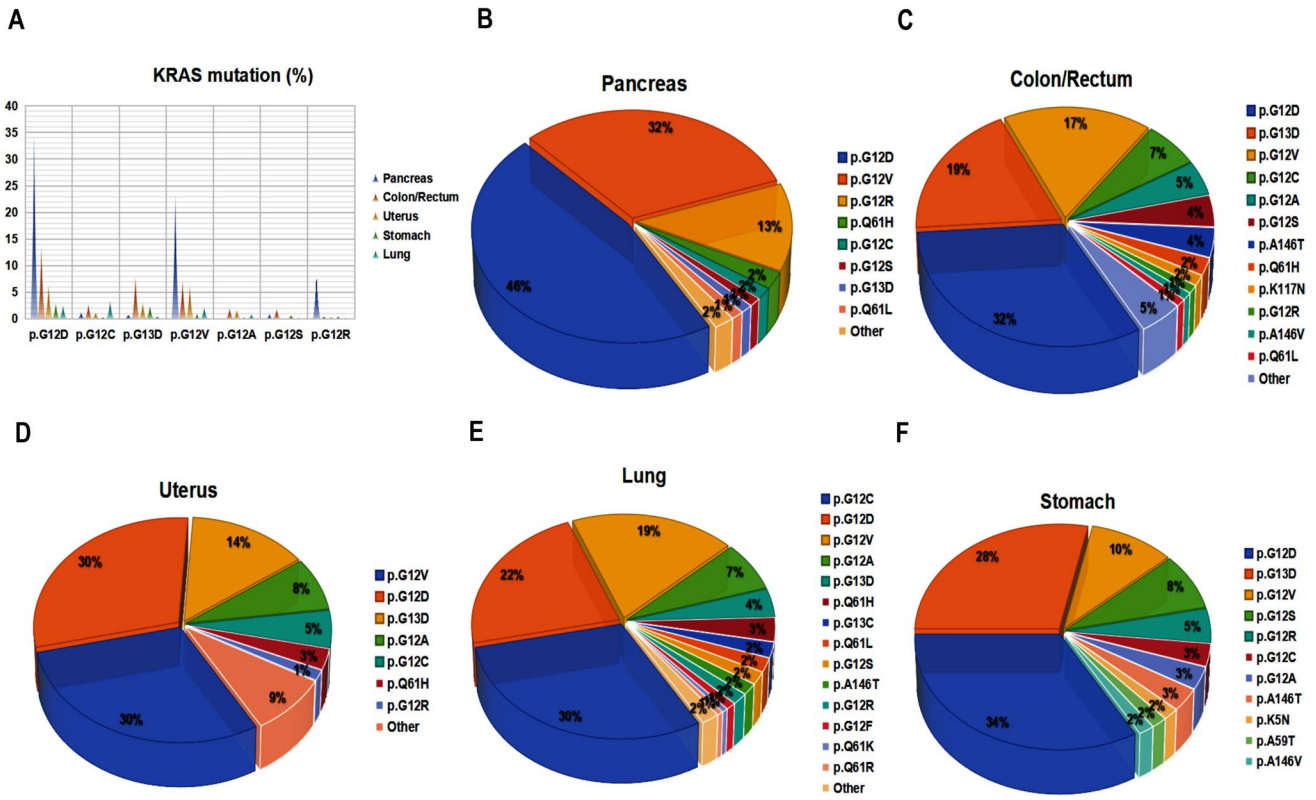

Fig. 2 Distribution of KRAS mutations in all tumors and seven subtypes. **A** Proportional distribution of different mutation subtypes in tumors. Distribution and proportion of KRAS mutation subtypes in pancreatic cancers (**B**), colorectal cancers (**C**), uterine cancers (**D**), lung cancers (**E**) and gastric cancers (**F**), respectively

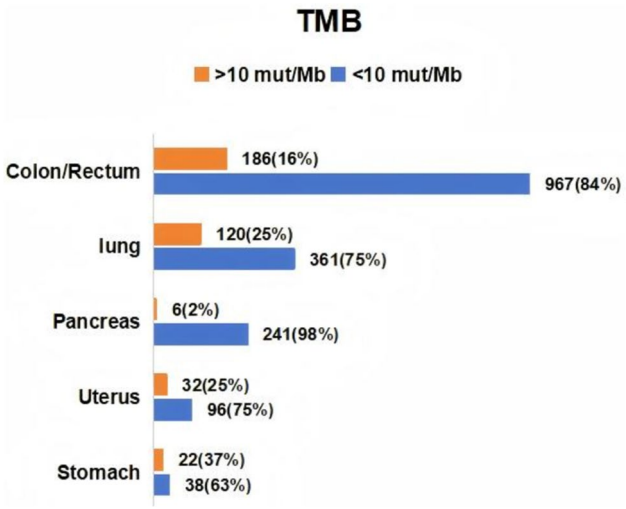

Fig. 3 Distribution of TMB-H in five tumors with KRAS mutations

(Fig. 4). In colorectal cancer, the *KRAS* G13D ($p = 0.000$) and *KRAS* G12V ($p = 0.001$) mutations exhibited significant effects on MSI-H. In gastric cancer, the *KRAS* G13D mutation was found to have a significant impact on MSI, indicated by a *p*-value of 0.015. In contrast, no statistically significant associations were identified in uterine and gastric cancers (Table 3).

Discussion

KRAS is a critical component in the downstream signaling pathways of the *EGFR*, notably within the *RAS-RAF-MAPK* and *PI3K-mTOR* pathways. Extensive evidence indicates that mutations in the *KRAS* gene lead to persistent activation of these signaling cascades. As a result, individuals harboring *KRAS* mutations often exhibit resistance to anti-EGFR targeted therapies (Jiang et al. 2024). Meanwhile, it has been reported that specific *KRAS* mutations are associated with TMB and MSI in various cancer types, and may influence the efficacy of immunotherapy (Hargadon et al. 2018; McGranahan et al. 2016). Here, we analyzed 10,820 cases of prevalent malignant tumors, encompassing gastrointestinal, reproductive system, respiratory, soft tissue, bone, urological, and neurological tumors, using NGS. Approximately 19.97% of Chinese patients with malignant tumors harbored *KRAS* mutations, and the mutation frequency was different from Western populations, particularly in biliary tract, thyroid, and

Table 2 Association of TMB with *KRAS* mutations

Type	TMB (mut/Mb)	Mutation	G12D	G13D	G12V	G12C
Colon/ Rectum	TMB>10	Mutation	51(27%)	47(25%)	19(10%)	12(6%)
		No-mutation	135(73%)	139(75%)	167(90%)	174(94%)
	TMB<10	Mutation	321(33%)	171(18%)	181(19%)	63(7%)
		No-mutation	646(67%)	796(82%)	786(81%)	904(93%)
	p value		0.123	0.016	0.005	0.974
Lung	TMB>10	Mutation	22(18%)	6(5%)	17(14%)	52(43%)
		No-mutation	98(82%)	114(95%)	103(86%)	68(57%)
	TMB<10	Mutation	86(24%)	14(4%)	74(20%)	92(25%)
		No-mutation	275(76%)	347(96%)	287(80%)	269(75%)
	p value		0.212	0.594	0.125	0.000
Pancreas	TMB>10	Mutation	3(50%)	1(17%)	0(0%)	0(0%)
		No-mutation	3(50%)	5(83%)	6(100%)	6(100%)
	TMB<10	Mutation	112(46%)	2(1%)	78(32%)	4(2%)
		No-mutation	129(54%)	239(99%)	163(68%)	237(98%)
	p value		1.000	0.107	.	.
Uterus	TMB>10	Mutation	8(25%)	8(25%)	4(13%)	3(9%)
		No-mutation	24(75%)	24(75%)	28(88%)	29(91%)
	TMB<10	Mutation	30(31%)	10(10%)	34(35%)	4(4%)
		No-mutation	66(69%)	86(90%)	62(65%)	92(96%)
	p value		0.503	0.040	0.025	0.501
Stomach	TMB>10	Mutation	8(36%)	10(45%)	1(5%)	0(0%)
		No-mutation	14(64%)	12(55%)	21(95%)	22(100%)
	TMB<10	Mutation	12(32%)	7(18%)	5(13%)	2(5%)
		No-mutation	26(68%)	31(82%)	33(87%)	36(95%)
	p value		0.705	0.025	0.532	.

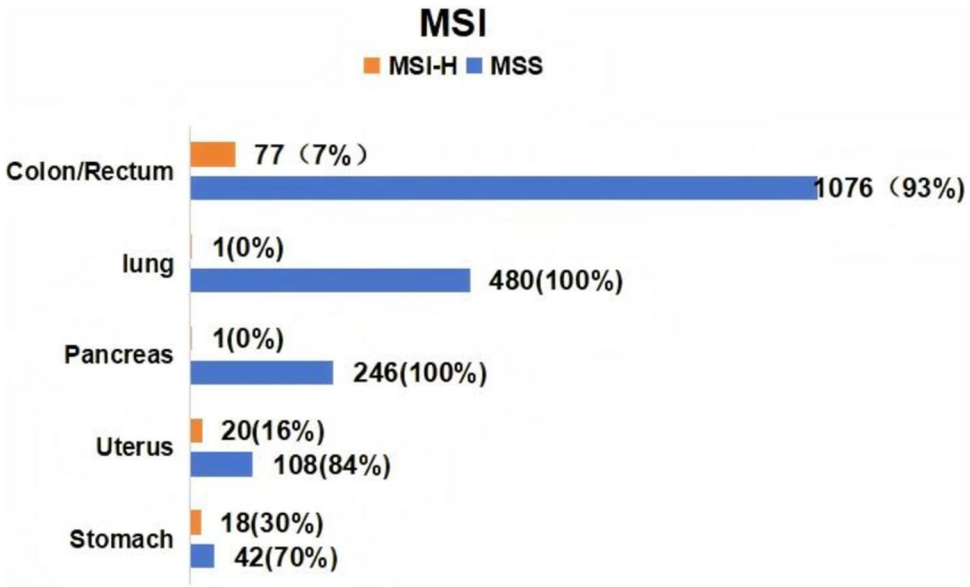

Fig. 4 Distribution of MSI-H in five tumors with KRAS mutations

Table 3 Association of MSI with *KRAS* mutations

Type	MSI	Mutation	G12D	G13D	G12V	G12C
Colon/ Rectum	MSS	Mutation	351(33%)	191(18%)	198(18%)	74(7%)
		No-mutation	725(67%)	885(82%)	878(82%)	1002(93%)
	MSI-H	Mutation	21(27%)	27(35%)	2(3%)	1(1%)
		No-mutation	56(73%)	50(65%)	75(97%)	76(99%)
	p value		0.332	0.000	0.001	0.093
Uterus	MSS	Mutation	33(31%)	13(12%)	35(32%)	4(4%)
		No-mutation	75(69%)	95(88%)	73(68%)	104(96%)
	MSI-H	Mutation	5(25%)	5(25%)	3(15%)	3(15%)
		No-mutation	15(75%)	15(75%)	17(85%)	17(85%)
	p value		0.617	0.126	0.194	0.132
Stomach	MSS	Mutation	14(33%)	8(19%)	6(14%)	2(95%)
		No-mutation	28(67%)	34(81%)	36(86%)	40(5%)
	MSI-H	Mutation	6(33%)	9(50%)	0(0%)	0(0%)
		No-mutation	12(67%)	9(50%)	18(100%)	18(100%)
	p value		1.000	0.015	.	.

neurological cancers. As for *KRAS* subtypes, *KRAS* G12D mutation is most common in pancreatic, colorectal, and gastric cancers, while *KRAS* G12V mutation is predominant in uterine cancer, and *KRAS* G12C mutation is most frequent in lung cancer. Moreover, KRAS-specific subtype mutations, including G13D, G12V, and G12C, had a close association with MSI status and TMB value.

KRAS represents the most frequently mutated oncogene in human malignancies, predominantly impacting epithelial cancers. Mutations are observed with a high frequency in Western populations, accounting for approximately 30% of cancers. Here, we identified a *KRAS* mutation rate of 19.97% within the cohort, with variation in *KRAS* mutation rates depending on the cancer type. These discrepancies in mutation prevalence between Chinese and Western populations may plausibly be attributed to ethnic variations.

Substantial evidence indicates that the *KRAS* gene plays a pivotal role in tumorigenesis and the modulation of tumor immunity. Prior research has shown that mutations in *KRAS* mutation can lead to the overexpression of PD-L1 by activating downstream pathways in NSCLC (Amanam et al. 2020; Sumimoto et al. 2016). And it was observed that the PD-L1 positivity rate was higher in patients with *KRAS* mutations compared to those with the *KRAS* wild-type (Herbst et al. 2020). In the KEYNOTE-189 study, both PD-L1 expression and TMB levels were found to be elevated in patients harboring *KRAS* mutations (Gadgeel et al. 2019). TMB has been shown to enhance tumor

immunogenicity and potentially influence the response to immune checkpoint inhibitors (ICIs). And the findings of multiple studies have indicated that patients with *KRAS* mutations display markedly elevated TMB (Dong et al. 2017; Rizvi et al. 2015). This frequently indicates that patients with *KRAS*-mutated tumors respond better to immunotherapy. The findings of our study indicate that the proportion of high TMB (≥10 mut/Mb) is highest in gastric cancer, at 37%, followed by uterine cancer (25%) and lung cancer (25%). Further Chi-square test yielded statistically significant correlations between the *KRAS* G13D and *KRAS* G12V mutations sites and TMB values in colorectal cancer. Additionally, the analysis revealed that the *KRAS* G13D mutation site had a notable influence on TMB in gastric cancer. Moreover, *KRAS* G12C mutation significantly associated with TMB in lung cancer, which provides a potential explanation for the favourable outcomes of pembrolizumab immunotherapy observed in patients with *KRAS* G12C mutation (Gadgeel et al. 2019; Diaz et al. 2022).

Defects in the mismatch repair system (dMMR) are a primary cause of MSI, which is characterised by a distinct mutant phenotype. The FDA has granted full approval to the use of pembrolizumab in the treatment of patients diagnosed with unresectable and metastatic MSI-H or dMMR solid tumors that have progressed following previous therapeutic intervention. Findings from multiple studies further substantiate the use of pembrolizumab as a primary treatment for Asian patients with mCRC and MSI-H/dMMR (Diaz et al. 2022; Yoshino et al. 2023). In this study, MSI status varied significantly across different malignancies, with the highest prevalence of MSI-H observed in gastric cancer (30.0%), followed by uterine cancer (15.6%), colorectal cancer (6.7%), pancreatic cancer (0.4%), and lung cancer (0.2%). In colorectal cancer, the *KRAS* G13D and *KRAS* G12V mutations demonstrated significant effects on MSI status.

There were several limitations. It must be acknowledged that the present study, which has yielded valuable insights into the role of *KRAS* mutation in tumor development, has been subject to a degree of bias in its sample selection process. The number of samples included in the analysis of breast tumors, bone tumors and neurotumors was comparatively low. Furthermore, this study offers new insights by concentrating on the characterization of *KRAS* mutantion molecular isoforms and their correlation with TMB and MSI. Nevertheless, our study has yet to assess the impact of these molecular characteristics on therapeutic efficacy. While our data provide clues to potential therapeutic targets, the therapeutic efficacy of these in real-world applications remains to be validated by further clinical trials. The association between these molecular features and therapeutic response, as well as the possibility of developing novel therapeutic strategies based on these findings, are avenues that future studies should explore.

In summary, the prevalence of *KRAS* mutations varied among different malignant tumors, with the highest frequency observed in tumors of the digestive, female reproductive, and respiratory systems. The influence of various *KRAS* mutation sites on TMB and MSI in distinct tumors suggests potential implications for immunotherapy. The findings of this study provide valuable insights into the potential applicability of targeted therapies and immunotherapy for KRAS-mutant tumors.

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Author contributions J. Y., W. L. and L. G. contributed to methodology, validation, and project administration. L. W., W. R., F.Z. and W. L. conducted formal analysis and data curation. All authors contributed substantially to the interpretation and writing of the manuscript. L.W. and W. R. were co-first authors and their contributions to the article were equal. The author(s) read and approved the final manuscript.

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Availability of data and materials No datasets were generated or analysed during the current study.

Declarations

Conflict of interests The authors declare no competing interests.

Ethical approval The NGS data included in this study have been subjected to a rigorous review process by the CHCAMS Ethics Committee, and ethical approval has been granted (NCC2694). The study was conducted in accordance with the ethical principles set forth in the Declaration of Helsinki for research involving human subjects. Prior to participation, all subjects were required to sign an informed consent form, which provided detailed information about the purpose of the experiment, the methods to be employed, the potential risks, the expected benefits, and the subjects' rights.

Consent for publication Not applicable.

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