Original Article

KRAS G12C mutations in Asia: a landscape analysis of 11,951 Chinese tumor samples

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Background: Kirsten rat sarcoma vial oncogene (*KRAS*) is one of the most prevalent oncogenes in multiple cancer types, but the incidence is different between the Asian and non-Asian populations. The recent development of *KRAS* G12C targeting drug has shown great promise. It is thus important to understand the genomic landscape of *KRAS* G12C in a specific population.

Methods: Sequencing data of 11,951 tumor samples collected from 11/2016 to 7/2019 from multiple centres in China were analyzed for *KRAS* mutation status. Concomitant genomic aberrations were further analyzed in tumors with *KRAS* G12C mutations, which were sequenced with comprehensive cancer panel including over 450 cancer-related genes. Smoking status and its correlation with *KRAS* were analyzed in 2,235 lung cancer cases within this cohort.

Results: KRAS mutations were identified in 1978 (16.6%) patient samples. Specifically, *KRAS* G12C accounted for 14.5% (n=286) of all *KRAS* mutations. G12C was most commonly seen in lung cancer (4.3%), followed by colorectal cancer (2.5%) and biliary cancer (2.3%). Almost all patients (99.6%) with G12C mutations had concomitant genomic aberrations. These were most commonly associated with the RAS/RTK pathway including *BRAF* and *PI3KCA* mutations. Moreover, *KRAS* mutation was positively correlated with smoking status in lung adenocarcinomas.

Conclusions: The overall incidence of *KRAS* G12C mutations remains low in the Chinese population. The most common tumor types harboring *KRAS* G12C mutations are in patients suffering from lung, colorectal and biliary cancers.

Keywords: *KRAS* G12C; co-aberration; smoking status; pathway analysis; actionable alteration

Submitted Mar 19, 2020. Accepted for publication Jul 16, 2020.
doi: 10.21037/tlcr-20-455
View this article at: http://dx.doi.org/10.21037/tlcr-20-455

Introduction

Kirsten rat sarcoma vial oncogene (*KRAS*), member of the RAS superfamily, is one of the most prevalent oncogenes in cancer (1). Being a GTP-binding protein that links receptor tyrosine kinase activation to intracellular signalling, *KRAS* mutations favour the GTP-bound active state and constitutive activation of downstream effects including differentiation, proliferation and survival. Presence of *KRAS* mutations have been shown to be a negative prognostic factor in multiple cancer types including lung and colorectal cancers (2-5). In addition, presence of *KRAS* mutations is a predictive biomarker for EGFR-directed monoclonal antibodies in patients with colorectal cancer.

The most frequent mutations of *KRAS* occur at codon 12 (6), but the incidence of specific missense mutation at codon 12 are variable among different cancer types. For example, in non-small cell lung cancers (NSCLC), the most common *KRAS* mutation is G12C, whereas G12D is more common in pancreatic cancer (7,8). Incidence of *KRAS* mutations also differs between ethnic groups. Specifically, less than 10% of Asian patients with advanced NSCLC harbor KRAS mutation (9-11), while the incidence of *KRAS* mutations in African-Americans and Caucasians is 19% and 26%, respectively (12). Moreover, distribution of *KRAS* subtypes also varies between ethnic populations. Prior reports have shown *KRAS* G12C as the most common subtype amongst in African Americans (38%) and Caucasians (38%). A smaller cohort study of 218 *KRAS* Chinese NSCLC patients also reported G12C being the most common subtype, accounting for 32.1% of all *KRAS* mutations (9,12). Other reports have illustrated distinct subtypes of *KRAS* mutations between smokers and never smokers (13).

Recent report of a phase I study on AMG 510 is promising. This novel, first-in-class, small molecule specifically inhibits *KRAS* G12C by locking it in an inactive GDP-bound state (14,15). Tumor response rate in 23 patients with *KRAS* G12C positive NSCLC was 48% (16). Based on these preliminary results, the United States Food & Drug Administration has granted "fast-track" designation for AMG 510 (17). Moreover, other *KRAS* G12C specific inhibitors, including MRTX849 have also had promising initial early phase clinical trials data presented at international meetings (18). For future development of this class of agents, it is crucial to understand the comprehensive landscape of *KRAS* GI2C mutation across different tumor types, ethnicities and tobacco exposure. As RAS/RTK is a complex signalling pathway, co-existing genomic aberrations may impact on the clinical outcomes of *KRAS* G12C inhibition. In this study, we aim to study the epidemiologic landscape of *KRAS* G12C mutation in multiple cancer types in a large Chinese population and correlate the incidence of mutation with tobacco exposure in patients with NSCLC. Furthermore, we have also investigated the incidence of concomitant aberrations that may potentially impact on *KRAS* G12C inhibition. We present the following article in accordance with the *STROBE* reporting checklist (Available at http://dx.doi.org/10.21037/tlcr-20-455).

Methods

Patients and sample collection

Total of 11,951 formalin-fixed, paraffin-embedded (FFPE) tumor tissue samples collected between 11/2016 and 7/2019 were analysed by next generation sequencing (NGS) (OrigiMed Ltd, Shanghai, China). This CAP-/CLIA-Accredited Laboratory offered three different types of gene panels commercially, all of which have all known coding exons of *KRAS* included in their analysis. For analysing concomitant aberrations, data from *KRAS* G12C samples were analysed, which were sequenced with Cancer Sequencing YS panel, a validated customized panel targeting over 450 cancer-related genes (19). All tumor samples were reviewed by in-house pathologists and only samples with 20% or more of tumor-cells cellularity were accepted for analysis. Smoking status and clinical characterization were available for 2,223 lung cancer cases within this cohort. Informed consent on plans of further deidentified genomic data analysis were obtained from all patients by test-ordering physicians as part of standard practice at respective institutions.

In order to obtain more representative data, only in diseases of which more than 100 samples have been received during the recruitment period are studied in this report.

All procedures performed in this study were in accordance with the Declaration of Helsinki (as revised in 2013). Due to its multi-institutional, anonymized and retrospective nature of data collection, in conjunction with subjects recruited in this study have already acknowledged and confirmed informed consent in proceeding with genomic testing and for the relevant anonymized used for further studies, ethical approval for this specific study has been waived.

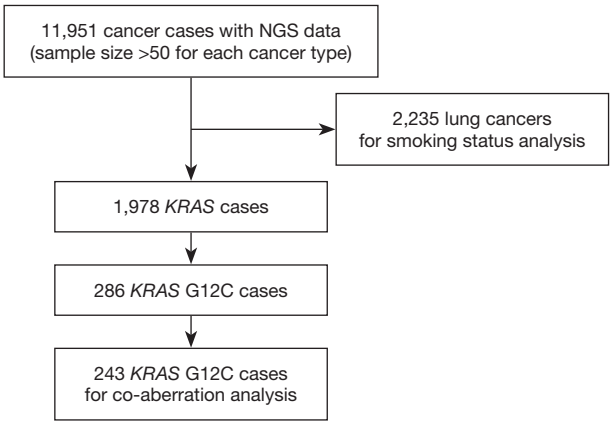

Figure 1 CONSORT diagram of the study design.

Sequencing and detection of genomic alterations

For NGS, 50 to 200 ng DNA was extracted and purified from FFPE samples. Hybridization capture libraries were constructed and sequenced on Illumina sequencing platform (Illumina Incorporated, San Diego, CA), with a mean coverage of at least 700×. Genomic alterations were analysed with bioinformatics tools as reported previously (20). Single nucleotide variants (SNVs), short and long insertions/deletions (indels), copy number variations (CNVs) and gene fusions/rearrangements were analysed. For variant calling, at minimum five reads and variant allele frequency (VAF) of 1% were required.

COSMIC data

KRAS mutations were compared with Catalogue of Somatic Mutation In Cancer data (21) (COSMIC; https://cancer.sanger.ac.uk/cosmic, release v89, 15th May 2019), which provides access to publicly available genomic data of diverse cancers.

Identification of potentially actionable alterations by OncoKB

Four levels of evidence defined by MSK-Precision Oncology Knowledge base (OncoKB; https://oncokb.org/), were used to categorize potentially actionable alterations. Mutational events of each individual were annotated according to the OncoKB criteria.

Statistical analysis

R software was performed for statistical analyses. For comparing the frequency of *KRAS* mutation in Chinese population and COSMIC data, Chi-squared test was used to calculate the significance of differences in each cancer type separately. P value were adjusted through Benjamini and Hochberg (BH) procedure to control the false discovery rate (FDR). For analysing correlation between KRAS mutation and smoking status, Chi-squared test and fisher test was used to calculate the significance of differences. P value smaller than 0.05 were considered significant.

Results

Epidemiology of KRAS mutations

The most common cancer types in our study cohort included lung (42.4%), colorectal (9.3%), liver (8.9%), biliary tract (8.4%), stomach (7.9%), oesophagus (5.5%) and pancreas (3.6%; *Table S1*). We included only the cancer types with more than 50 cases for analysis, thus 11,951 cases were analysed (*Figure 1*). *KRAS* mutations were observed in 1,978 of 11,951 tumor samples (16.6%). Frequency of *KRAS* mutations varies between different cancer types, with highest frequencies observed in pancreatic (81.5%), colorectal (48.9%) and biliary tract (23.5%) cancer. Epidemiologic distribution was compared with the COSMIC database (22) (*Figure 2*). Incidence of *KRAS* mutation is higher in our Chinese patient cohort with pancreatic (81.5% *vs.* 56.8%; P<0.001), colorectal (48.9% *vs.* 33.5%; P<0.001) and gastric cancer (10.3% *vs.* 5.9%; P<0.001), while the incidence is lower in patients with lung cancer (11.7% *vs.* 17.3%; P<0.001).

KRAS G12C mutations

The majority of *KRAS* genomic aberrations were single nucleotide variations (SNVs), accounting for 91.9% of all *KRAS* alterations (*Figure S1*). Gene amplifications were the second most common type of alteration, accounting for a less proportion of only 7.1%. Among SNVs, G12C were detected in 286 samples, accounting for 14.5% of *KRAS* mutations and 2.4% of the entire study population. *KRAS* G12C mutation was more commonly found in lung, colorectal and biliary cancers (*Figure 3*). Out of

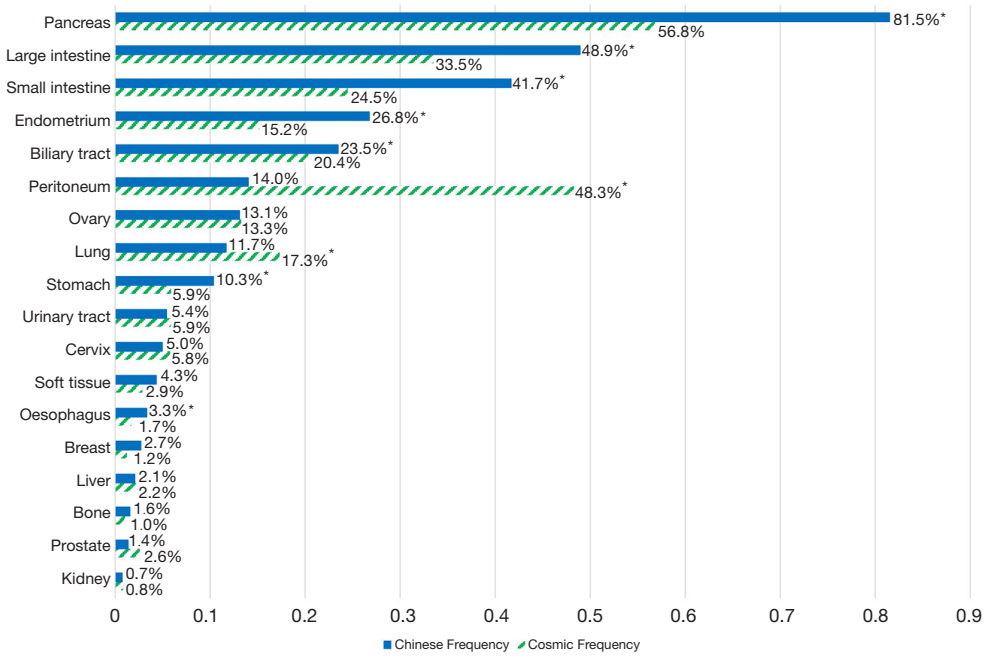

Figure 2 Frequencies of *KRAS* mutations in diverse cancers (N=11,951). Comparison of frequencies between current report and COSMIC. The frequencies of *KRAS* were different between current data and COSMIC in lung, large intestine, stomach, oesophagus and pancreas cancers. *P<0.05.

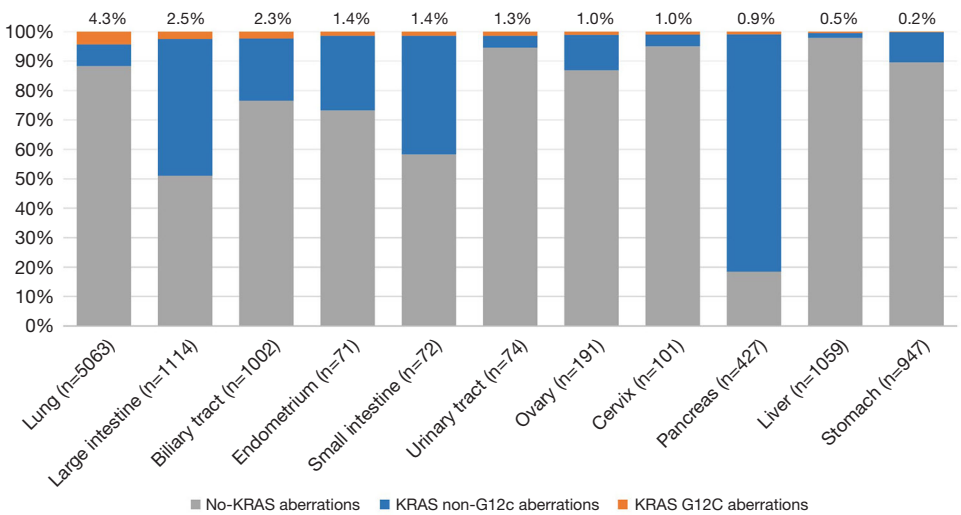

Figure 3 Distribution of *KRAS* G12C alterations.

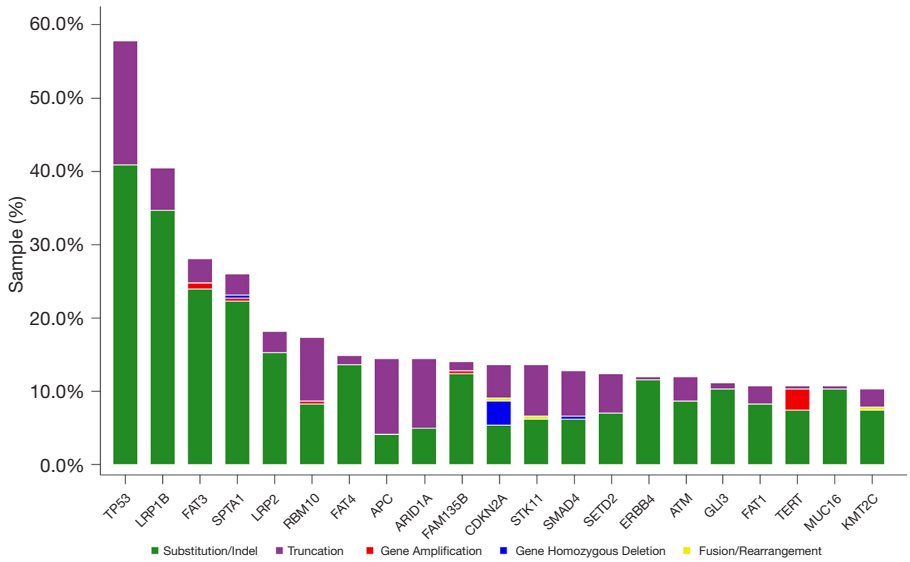

Figure 4 Frequencies of G12C co-occurring aberrations. The top 20 frequent co-aberrations of *KRAS* G12C.

5,063 patients with lung cancer, 218 (4.3%) had *KRAS* G12C mutation and 373 (7.4%) had non-G12C *KRAS* mutations. Distribution of non-G12C *KRAS* mutations is summarized in *Table S2*. Similarly, 28 of 1,114 colorectal cancer patients (2.5%) had G12C mutation and 517 (46.4%) had non-G12C mutations; and 23 of 1,002 (2.3%) biliary cancer patients had G12C mutation and 212 (21.2%) had non-G12C mutations. Ratio of G12C versus non-G12C mutations was 1:1.7, 1:18.5 and 1:9 for lung, colorectal and biliary cancer, respectively. In contrast, only 4 of the 427 patients with pancreatic cancer had G12C mutation, the G12C versus non-G12C mutations ratio was 1:86.

Concomitant genomic aberrations in patients with KRAS G12C

Total of 243 tumor samples with confirmed *KRAS* G12C were analysed with the comprehensive targeted gene panel. One or more concomitant aberrations were identified in 242 samples (99.5%). Median number of concomitant genomic aberrations was 14, ranging from 1 to 122. The most common concomitant aberration was *TP53* (54.7%), *LRP1B* (37.0%) and *FAT3* (25.1%; *Figure 4*). We have also identified 19 (7.8%) cases of co-existence of *KRAS* G12C and non-G12C mutations.

The histological subtypes of the 243 G12C tumors were further analysed (*Table S3*). The most common cancer histological subtype was lung adenocarcinoma (LUAD; N=148), followed by colorectal adenocarcinoma (CRC; N=28) and cholangiocarcinoma (CHOL; N=23). Frequencies of specific concomitant aberrations varied between different cancer subtypes. In LUAD, the most frequently altered genes were *TP53* (50.0%), *LRP1B* (45.3%), and *SPTA1* (30.4%; *Figure S2A*), comparing to CRC with most frequently altered genes at *TP53* (71.4%), *APC* (53.6%), and *FBXW7* (39.3%; *Figure S2B*). In CHOL, the most frequently co-altered genes were *TP53* (60.9%), *SMAD4* (39.1%), and *CDKN2A* (34.8%; *Figure S2C*). Given the number of G12C was low in other cancer histological subtypes, we did not analyse the concomitant aberration of this group.

A Recent large-cohort-study reported detailed driver genes in different cancer types (23). The list of driver genes was obtained as reported in *Table S4*. Concomitant aberrations on driver genes in G12C positive LUAD, CRC and CHOL were analysed respectively (*Figure 5*). The most common co-occurring driver gene in LUAD was *TP53* (50.6%), followed by *RBM10* (19.6%) and *STK11* (18.2%). *EGFR* aberrations occurred in 8.8% of cases. We also observed co-occurring *KRAS* non-G12C aberrations in 10 cases (6.8%), in which eight were *KRAS* amplification. In CRC, the most common co-occurring driver gene was *TP53* (71.4%), followed by *APC* (53.6%) and *FBXW7* (39.3%). Coaberrations in three driver genes were found in CHOL, which were *ARID1A* (26.1%), *PBRM1* (13.0 %), and *EPHA2* (4.4%).

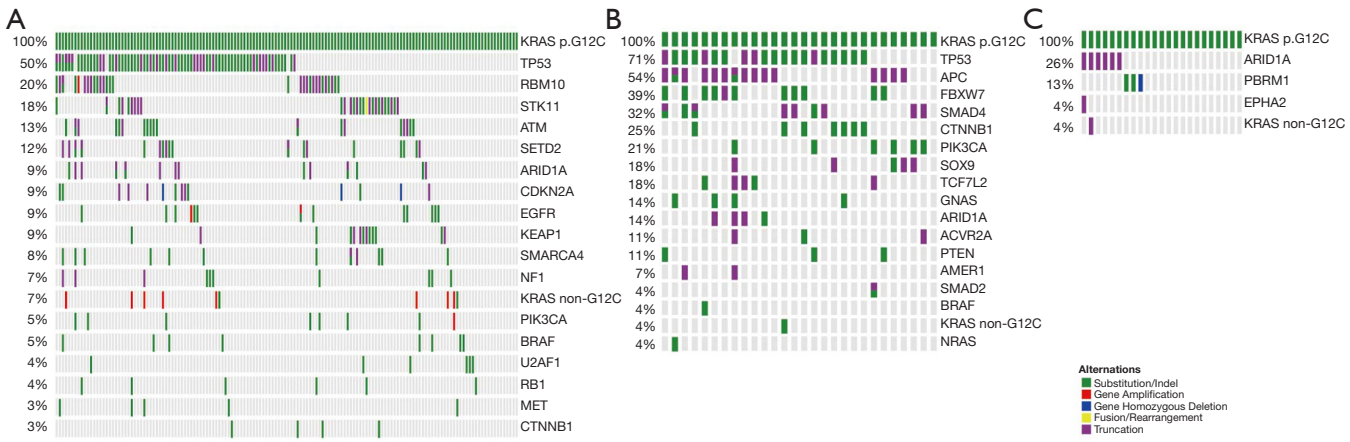

Figure 5 Driver gene analysis in (A) lung adenocarcinoma (LUAD); (B) colorectal cancer (CRC); (C) cholangiocarcinoma (CHOL).

Smoking history and KRAS mutations

Smoking history of 2,235 lung cancer cases was collected. This included 1,582 LUAD and 305 squamous cell carcinoma histologies (*Table 1*). In LUAD, *KRAS* mutations were identified in 20.3% of former smokers, which were significantly higher than that of non-smokers (8.9%; P<0.001; *Table 1*). And in 20.7% of current smokers, significantly higher than non-smokers (P<0.001). On the contrary, smoking history did not play a significant role in the incidence of *KRAS* mutations in patients with lung squamous cell carcinomas (LUSC). Further details on *KRAS* mutation subtypes and respective correlation with smoking status were illustrated in *Table S5*.

Pathway analysis of co-occurring aberrations

Prior studies indicated there were multiple canonical oncogenic pathways among diverse tumor types (24). Concomitant genes associated oncogenic pathways were analysed accordingly (*Figure 6A*). Frequency of mutations in each pathway differed according to tumor subtypes. For patients with LUAD, the most common and impactful oncogenic pathway was RTK/RAS signalling pathway, occurring in 75% of the G12C patients. For patients with CRC, the most impactful oncogenic pathway was WNT signalling pathway (89%). While for patients with CHOL and LUSC, the most common and impactful pathway was *TP53*-associated genes (74% in CHOL and 70% in LUSC). We explored the profile of genomic mutations of RTK/RAS pathway-associated genes (*Figure 6B*) and identified 35 key genes in associated with *KRAS* mutation, and among which, three were tumor suppressor genes.

Most of these identified genes function upstream of the KRAS pathway, while several downstream signalling molecules were also observed (*Figure 7A*). Concomitant non-G12C *KRAS* mutations were observed in LUAD and CRC, occurred in 6.8% and 3.8% respectively. Preclinical data showed that PI3K-AKT pathway inactivation was likely intrinsic resistance mechanism for G12C inhibitors (25). *BRAF* mutations would provide fitness advantage for subclones resistant to G12C inhibition (26). Associated genes on the pathway were analysed in LUAD and CRC. BRAF is on the downstream of KRAS in RTK/RAS pathway. The incidence of BRAF mutation in G12C mutated patients were comparable between LUAD and CRC, occurring in 5.4% and 3.6% patients respectively (P=0.9; *Figure 7A*). PI3K pathway is downstream signalling of RAS. In this study, a higher proportion of PI3KCA mutation was observed in CRC (21.4% *vs.* 8.8%; P=0.04; *Figure 7B*).

Actionable co-alterations analysis

Potential actionable co-alterations were analysed as defined by the OncoKB classification (27). Level 1 actionable alteration was found only in LUAD (*Table 2*). In G12C LUAD cases (*Figure S3A*), 81 (54.7%) cases had at least one potentially actionable alteration in addition to *KRAS* G12C (level 1 to 4). Actionable *EGFR* and *ALK* mutations were identified in eight cases (5.4%), which were recognized as FDA-approved biomarkers for target therapies (level 1). Other cases can also be potentially targetable either with off-label drugs or therapies that are currently in clinical

Table 1 Clinical characteristics of patients according to the *KRAS* mutation status in lung cancer (N=2,235)

Characteristics	All patients	Patients with *KRAS* mutations (N=244)	P
Sex			
Male	1,319	192	2.2E-09
Female	916	52	
Age			
Median	60	61	NA
Range	14–92	33–92	NA
Stage			
0	20	3	
I	680	69	
II	222	20	
III	393	45	
IIIb and IV	756	88	
Unknown	164	19	
Histology type			
Adenocarcinoma	1,582	198	NA
Squamous cell	305	20	0.002
Others	348	44	0.94
Smoking history (adenocarcinoma)			
Never smokers	1,100	98	
Former smokers	217	44	8.059E-07
Current smokers	265	55	4.08E-08
Smoking history (squamous cell)			
Never smokers	83	5	
Former smokers	108	4	0.45
Current smokers	114	11	0.36

trials (level 2B to 4). In colorectal carcinomas (*Figure S3B*), 16 were potentially targetable (57.1%; level 2B to 4). In total, 52.3% of G12C patients with co-aberrations had at least one actionable alteration.

Discussion

Recent discoveries have provided promising therapeutic opportunities for patients harboring *KRAS* G12C mutations. To the best of our knowledge, our report is the largest single-cohort illustrating the genomic and epidemiological landscape of *KRAS* G12C mutations in cancer. Moreover, this dataset represents the largest cohort of Chinese cancer patients with tumors harboring *KRAS* mutations ever assembled.

Higher frequencies of *KRAS* mutation were observed in several common cancers, including colorectal, stomach and pancreatic cancer in our study than reported in COSMIC, most of which were data from western population. This discrepancy may possibly be due to the higher resolution technology being used in our study which mean that mutations with low variation frequency (VAF) were also detectable. In the current study, the full exon of *KRAS* was detected using NGS with a mean coverage of at least 700×, at minimal VAF of 1% could be detected in this study.

On the contrary, the frequency of *KRAS* mutations were lower in our Chinese lung cancer cohort when compared with Western series. Similar findings have been reported in prior studies of Chinese populations (9,20). The imbalance of prevalence of molecular drivers in LUAD between Asian and Caucasian populations has been well documented. Given the fact that *EGFR* mutations are more common in Asians, it is not inconceivable that the relative prevalence of other molecular drivers, including *KRAS* mutations, are altered. The lower proportion of *KRAS* mutations in Asian LUADs in general may explain the overall lower frequency of *KRAS* G12C compared with previous reports among Western populations in lung cancer (7,28). In colorectal and pancreatic cancer patients, the frequencies of *KRAS* G12C were comparable with prior reports (29,30). Interestingly, 2.3% of biliary tract cancer patients were observed with *KRAS* G12C in our dataset. This information on the molecular landscape of *KRAS* G12C mutations may have significant impact on operational aspects in conducting clinical trials with *KRAS* G12C specific inhibitors in the Chinese population.

Moreover, presence of possible co-occurrence of other aberrations aside from *KRAS* G12C were evaluated in all patients within this study. The frequencies of co-altered genes were different in cancers. This has provided us with valuable insight in identifying potential pathways of treatment resistance in patients who are to be treated with KRAS G12C specific inhibitors, as the mechanism of resistance to these inhibitors may have already been established de-novo.

The presence of *KRAS* mutation suggests lack of response to EGFR targeted therapies in non-small lung cancers and colorectal cancers (31,32). As *KRAS* downstream of *EGFR* in

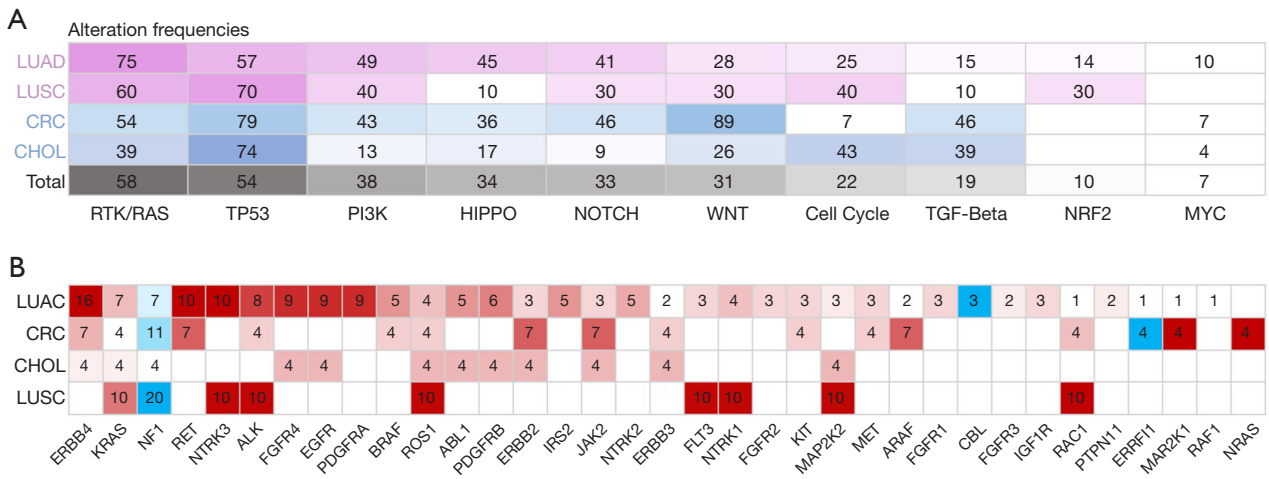

Figure 6 Oncogenic pathway analysis of *KRAS* G12C co-aberrations in LUAD (N=148), LUSC (N=10), CRC (N=28), CHOL (N=23). (A) Frequencies of oncogenic pathways; (B) frequencies of altered genes on RTK/RAS pathway. Red colored the oncogenes, and blue coloured the tumor suppressor genes.

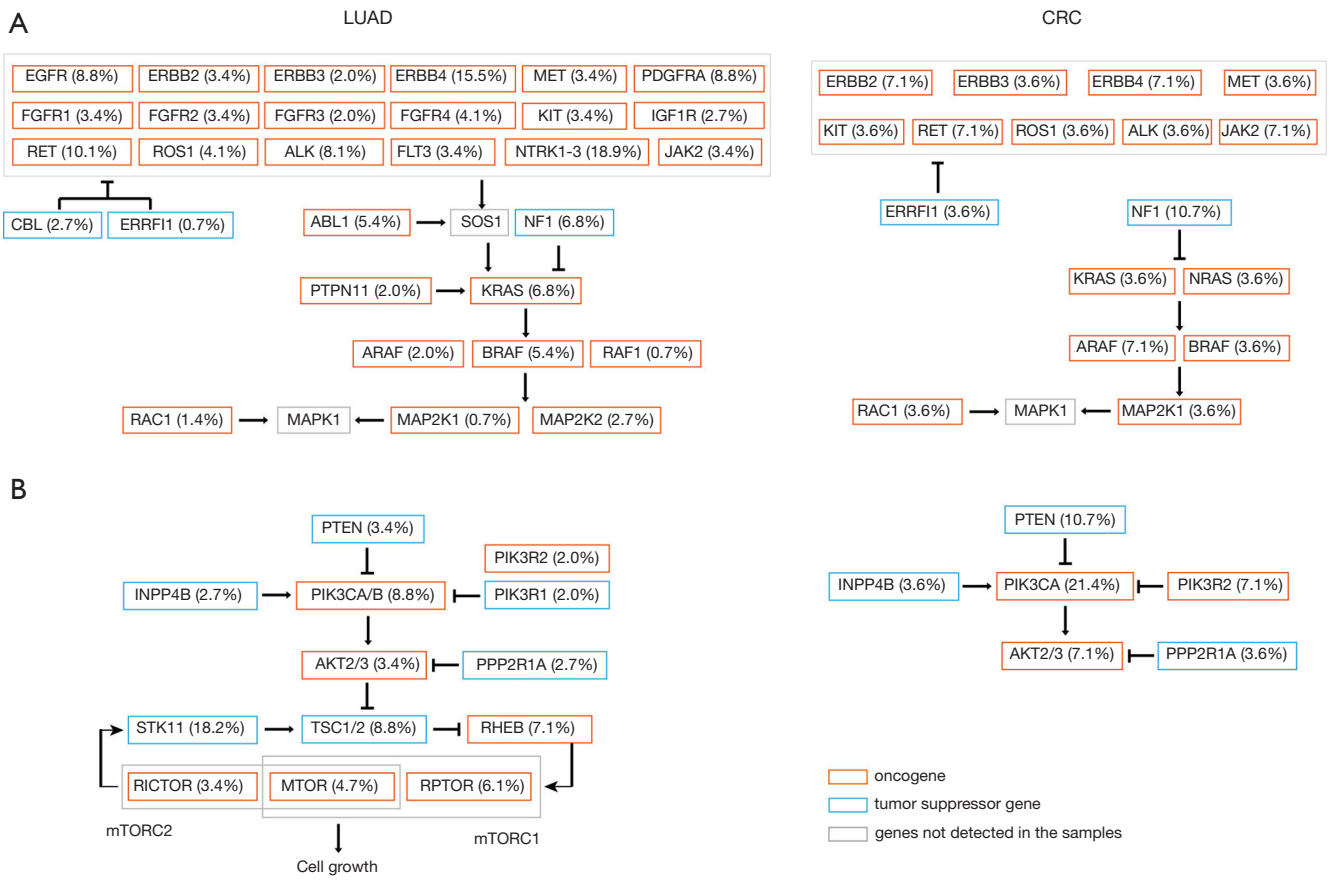

Figure 7 Aberrations on RTK/RAS and PI3K pathway in LUAD and CRC. (A) RTK/RAS signalling pathway in LUAD; (B) PI3K pathway in LUAD and CRC. LUAD, lung adenocarcinoma; CRC, colorectal carcinoma.

Table 2 Number of patients harbored actionable co-alterations as defined by OncoKB (N=243)

Histologies	1	2B	3A	3B	4	Overall[#]
Lung adenocarcinoma	8	35	4	22	43	81
Cholangiocarcinoma		2		2	10	12
Colorectal adenocarcinoma		8		3	8	16
Uterine carcinoma		2		1	2	2
Hepatocellular carcinoma		2			2	4
Lung squamous cell carcinoma		1			3	4
Small intestine neuroendocrine		1			1	1
Gastric adenocarcinoma		1			2	2
Ovarian mucinous carcinoma		1			2	2
Urothelial carcinoma				1		1
Pancreatic carcinoma					2	2

[#], some patient harbored with more than one actionable mutation, so the overall was defined as the number of patients harboring at least one actionable mutation.

the RAS/RTK pathway, there may be potential to consider combination therapies in the rare patients who harbor both of these alterations, or develop KRAS mutations in tumors as secondary resistance to EGFR inhibitors. 52.3% of patients with co-aberrations had at least one actionable alteration in accordance to the OncoKB definition, only 3.3% of these patients had level 1 co-aberrations which were targetable by FDA-approved therapies.

Preliminary data on AMG 510 showed clinical efficacy on *KRAS* G12C mutated NSCLC but not CRC. This might be intrinsic mechanism underlying the difference. Although the genomic mechanisms of intrinsic resistance to kinase inhibitors are complicated, there are usually two categories. One is the secondary mutation on the targeting kinase, second is activation of other molecules on the downstream of the pathway. In the current study, aberrant RAS/RTK pathway were broadly observed in *KRAS* G12C tumors. Downstream genomic alterations, such as *RAFs* and *PI3Ks* alterations, may increase the risk of drug resistance to *KRAS* G12C inhibitors. This is in line with a recent study which demonstrated the presence of BRAF mutations leading to primary resistance to G12C inhibitors (26). Preclinical study is also supportive of the observation of PI3K-AKT as reason of intrinsic resistance to G12C inhibitors (25). The fact that we observed high incidence of *PI3KCA* mutation in patients with CRC may potentially explain the lack of response in this patient group. Considering the genomic difference between populations in study, further investigations are still needed.

This is a retrospective analysis of Real-World data based on a commercial platform; thus, we have limited clinical information from original source documents. Information pertaining to patients' demographics and clinical information were based on the test request form. We are limited by the lack survival outcomes for correlation with the mutation status. However, the large sample collection on a relatively homogenous ethnic population by an identical platform has provided us with valuable and important data for further investigations. It will be extremely challenging to conduct a perspective study with similar sample size. But considering the relative rarity of *KRAS* G12C mutation, a large sample size is mandatory for accurate evaluation.

Our study has demonstrated the genomic landscape of *KRAS* G12C mutations in a large Chinese population and we confirmed the incidence to be relatively low. The most common tumor types harboring the mutation are lung, colorectal and biliary cancer.

Acknowledgments

Funding: None.

Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at http://dx.doi.org/10.21037/tlcr-20-455

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tlcr-20-455). HHFL reports personal fees from Novartis, personal fees from Pfizer, grants and personal fees from MSD, personal fees from Eisai, personal fees from Boehringer-Ingelheim, grants from Mundipharma, outside the submitted work; CC, HL, JY, YX, MY, KW reports employment by Origimed, during the conduct of the study; TSKM reports grants and personal fees from AstraZeneca, grants and personal fees from Roche/Genentech, personal fees from Eli Lilly, grants and personal fees from BMS, grants and personal fees from Boehringer Ingelheim, grants and personal fees from Novartis, grants and personal fees from MSD, grants and personal fees from Pfizer, personal fees from Merck Serono, grants and personal fees from Clovis Oncology, personal fees from Vertex, grants and personal fees from SFJ Pharmaceuticals, personal fees from ACEA Biosciences, from geneDecode, personal fees from Oncogenex, personal fees from Celgene, personal fees from Ignyta Inc, grants and personal fees from Taiho, grants from Eisai, personal fees from Fishawack Facilitate Ltd, grants and personal fees from Takeda, personal fees from Janssen, personal fees from Hutchison ChiMed, grants from XCovery, personal fees from OrigiMed, personal fees from Hengrui Therapeutics, personal fees from Sanofi-Aventis R&D, personal fees from Yuhan Corporation, outside the submitted work. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study were in accordance with the Declaration of Helsinki (as revised in 2013). Due to its multi-institutional, anonymized and retrospective nature of data collection, in conjunction with subjects recruited in this study have already acknowledged and confirmed informed consent in proceeding with genomic testing and for the relevant anonymized used for further studies, ethical approval for this specific study has been waived.

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Cite this article as: Loong HHF, Du N, Cheng C, Lin H, Guo J, Lin G, Li M, Jiang T, Shi Z, Cui Y, Jin X, Yao J, Xing Y, Yao M, Wang K, Mok TSK, Liu L. KRAS G12C mutations in Asia: A landscape analysis of 11,951 Chinese tumor samples. Transl Lung Cancer Res 2020;9(5):1759-1769. doi: 10.21037/tlcr-20-455

Supplementary

Table S1 Comparison of KRAS mutation frequencies between Chinese population and COSMIC

Tissue primary	Chinese cohort, N=11,951		COSMIC data, N=192,981		P value	P adjusted value
	All patients	KRAS mutant patients	All patients	KRAS mutant patients		
Lung	5,063	591 (11.7%)	43,591	7,547 (17.3%)	2.47E-24	2.04E-23
Large intestine	1,114	545 (48.9%)	77,334	25,922 (33.5%)	3.60E-27	6.48E-26
Liver	1,059	22 (2.1%)	3,390	74 (2.2%)	0.84	0.92
Biliary tract	1,002	235 (23.5%)	5,022	1,022 (20.4%)	0.027	0.05
Stomach	947	98 (10.3%)	5,887	347 (5.9%)	2.52E-07	9.07E-07
Oesophagus	662	22 (3.3%)	3,195	55 (1.7%)	1.21E-13	5.42E-13
Pancreas	427	348 (81.5%)	12,635	7,180 (56.8%)	3.4E-24	2.04E-23
Soft tissue	323	14 (4.3%)	4,072	117 (2.9%)	0.14	0.22
Kidney	271	2 (0.7%)	4,219	35 (0.8%)	0.87	0.92
Breast	258	7 (2.7%)	9,828	121 (1.2%)	0.035	0.06
Bone	193	3 (1.6%)	1,163	12 (1.0%)	0.52	0.72
Ovary	191	25 (13.1%)	6,848	908 (13.3%)	0.94	0.95
Cervix	101	5 (5.0%)	2,242	130 (5.8%)	0.72	0.92
Urinary tract	74	4 (5.4%)	2,774	163 (5.9%)	0.86	0.92
Prostate	73	1 (1.4%)	4,808	125 (2.6%)	0.51	0.72
Small intestine	72	30 (41.7%)	1,074	263 (24.5%)	0.0012	0.0031
Endometrium	71	19 (26.8%)	4,520	688 (15.2%)	0.0075	0.016
Peritoneum	50	7 (14.0%)	379	183 (48.3%)	4.49E-06	1.34E-05

Table S2 Distribution of KRAS subtypes in lung cancers

KRAS aberration	Proportions (607 KRAS aberrations in 591 samples)
G12C	218 (35.9%)
G12V	108 (17.8%)
G12D	99 (16.3%)
Amplification	57 (9.4%)
G12A	37 (6.1%)
Q61H	18 (3.0%)
G13C	15 (2.5%)
G13D	13 (2.1%)
G12S	7(1.2%)
A146T	5 (0.8%)
Q61L	4 (0.7%)
G12R	3 (0.5%)
Q22K	3 (0.5%)
G13V	2 (0.3%)
Q61R	2 (0.3%)
G12F	2 (0.3%)
A146V	2 (0.3%)
K117N	2 (0.3%)
R164Q	1 (0.2%)
V8E	1 (0.2%)
L19F	1 (0.2%)
G60V	1 (0.2%)
P34A	1 (0.2%)
T50I	1 (0.2%)
A59G	1 (0.2%)
F156L	1 (0.2%)
E31K	1 (0.2%)
D119H	1 (0.2%)

Table S3 Tumor subtypes of the 243 samples with G12C

Cancer type	Tumor subtype	Number
Lung	Lung adenocarcinoma	148
	lung squamous cell carcinoma	10
	unknown	7
	sarcomatoid carcinoma	2
	Non-small cell lung cancer	1
	Lung clear cell carcinoma	1
	Large cell neuroendocrine carcinoma	1
	Pulmonary mucoepidermoid carcinoma	1
	Poorly differentiated cancer	1
	Large cell lung cancer	1
	Complex small cell lung cancer	1
	Poorly differentiated lung cancer	1
Colorectal	Colorectal adenocarcinoma	28
Biliary tract	Hilar cholangiocarcinoma	4
	Extrahepatic cholangiocarcinoma	7
	Intrahepatic cholangiocarcinoma	11
	Mixed hepatocellular and cholangiocarcinoma	1
Liver	Hepatocellular carcinoma	3
	Hepatic Angiosarcoma	1
	Hepatic adenocarcinoma	1
Pancreas	Pancreatic adenocarcinoma	2
	pancreatic adenosquamous carcinoma	1
	Sarcomatoid carcinoma	1
Gastric	Gastric adenocarcinoma	2
Uterine	Endometrioid adenocarcinoma	1
	cervical squamous cell carcinoma	1
Ovary	Ovarian mucinous adenocarcinoma	1
	Ovarian mucinous carcinoma	1
Small intestine	Neuroendocrine neoplasms of small intestine	1
Cervix	cervical squamous cell carcinoma	1
Urinary	Invasive urothelial carcinoma of bladder	1

Table S4 Cancer driver genes defined by TCGA

Cancer	Gene	Tumor suppressor or oncogene
Cholangiocarcinoma	*ARID1A*	
Cholangiocarcinoma	*BAP1*	tsg
Cholangiocarcinoma	*EPHA2*	tsg
Cholangiocarcinoma	*IDH1*	Oncogene
Cholangiocarcinoma	*PBRM1*	tsg
Colorectal adenocarcinoma	*ACVR2A*	tsg
Colorectal adenocarcinoma	*AMER1*	Possible tsg
Colorectal adenocarcinoma	*APC*	tsg
Colorectal adenocarcinoma	*ARID1A*	Possible oncogene
Colorectal adenocarcinoma	*BRAF*	Oncogene
Colorectal adenocarcinoma	*CTNNB1*	Oncogene
Colorectal adenocarcinoma	*FBXW7*	tsg
Colorectal adenocarcinoma	*GNAS*	Oncogene
Colorectal adenocarcinoma	*KRAS*	Oncogene
Colorectal adenocarcinoma	*NRAS*	Oncogene
Colorectal adenocarcinoma	*PCBP1*	Oncogene
Colorectal adenocarcinoma	*PIK3CA*	Oncogene
Colorectal adenocarcinoma	*PTEN*	tsg
Colorectal adenocarcinoma	*SMAD2*	Possible tsg
Colorectal adenocarcinoma	*SMAD4*	tsg
Colorectal adenocarcinoma	*SOX9*	tsg
Colorectal adenocarcinoma	*TCF7L2*	tsg
Colorectal adenocarcinoma	*TGIF1*	Possible tsg
Colorectal adenocarcinoma	*TP53*	tsg
Colorectal adenocarcinoma	*ZFP36L2*	Possible tsg
Lung adenocarcinoma	*ARID1A*	tsg
Lung adenocarcinoma	*ATM*	tsg
Lung adenocarcinoma	*BRAF*	Oncogene
Lung adenocarcinoma	*CDKN2A*	Possible tsg
Lung adenocarcinoma	*CTNNB1*	Oncogene
Lung adenocarcinoma	*EGFR*	Oncogene
Lung adenocarcinoma	*KEAP1*	Possible tsg
Lung adenocarcinoma	*KRAS*	Oncogene
Lung adenocarcinoma	*MET*	Possible tsg
Lung adenocarcinoma	*MGA*	tsg
Lung adenocarcinoma	*NF1*	tsg
Lung adenocarcinoma	*PIK3CA*	Oncogene
Lung adenocarcinoma	*RB1*	tsg
Lung adenocarcinoma	*RBM10*	tsg
Lung adenocarcinoma	*RIT1*	
Lung adenocarcinoma	*SETD2*	tsg
Lung adenocarcinoma	*SMARCA4*	Possible tsg
Lung adenocarcinoma	*STK11*	tsg
Lung adenocarcinoma	*TP53*	Possible tsg
Lung adenocarcinoma	*U2AF1*	Oncogene

Table S5 The correlation of KRAS mutation subtypes with smoking history in lung adenocarcinomas (N=198)

Smoking history	All patients with KRAS mutation	G12C (N=75)	G12D (N=35)	G12V (N=29)
Never smokers	99	27	25	14
Former smokers	44	25	2	5
Current smokers	55	23	8	10
P value		0.003	0.007	0.627

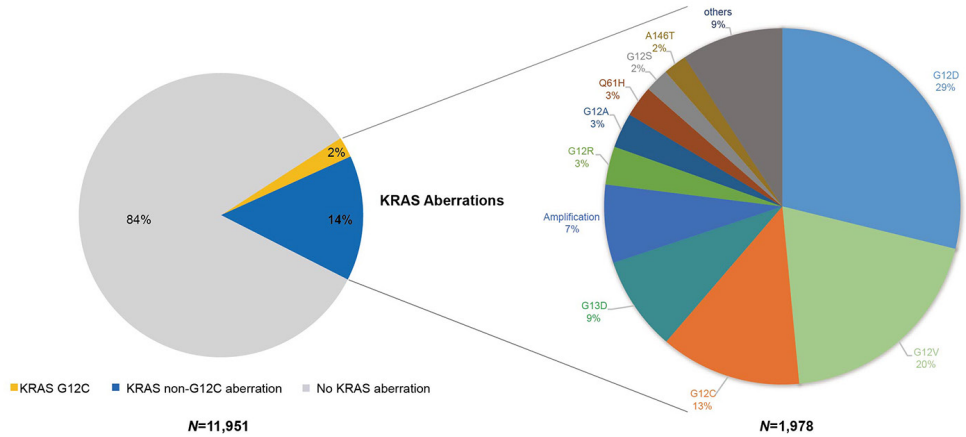

Figure S1 Frequencies of *KRAS* mutation subtypes in diverse cancers (N=1,978).

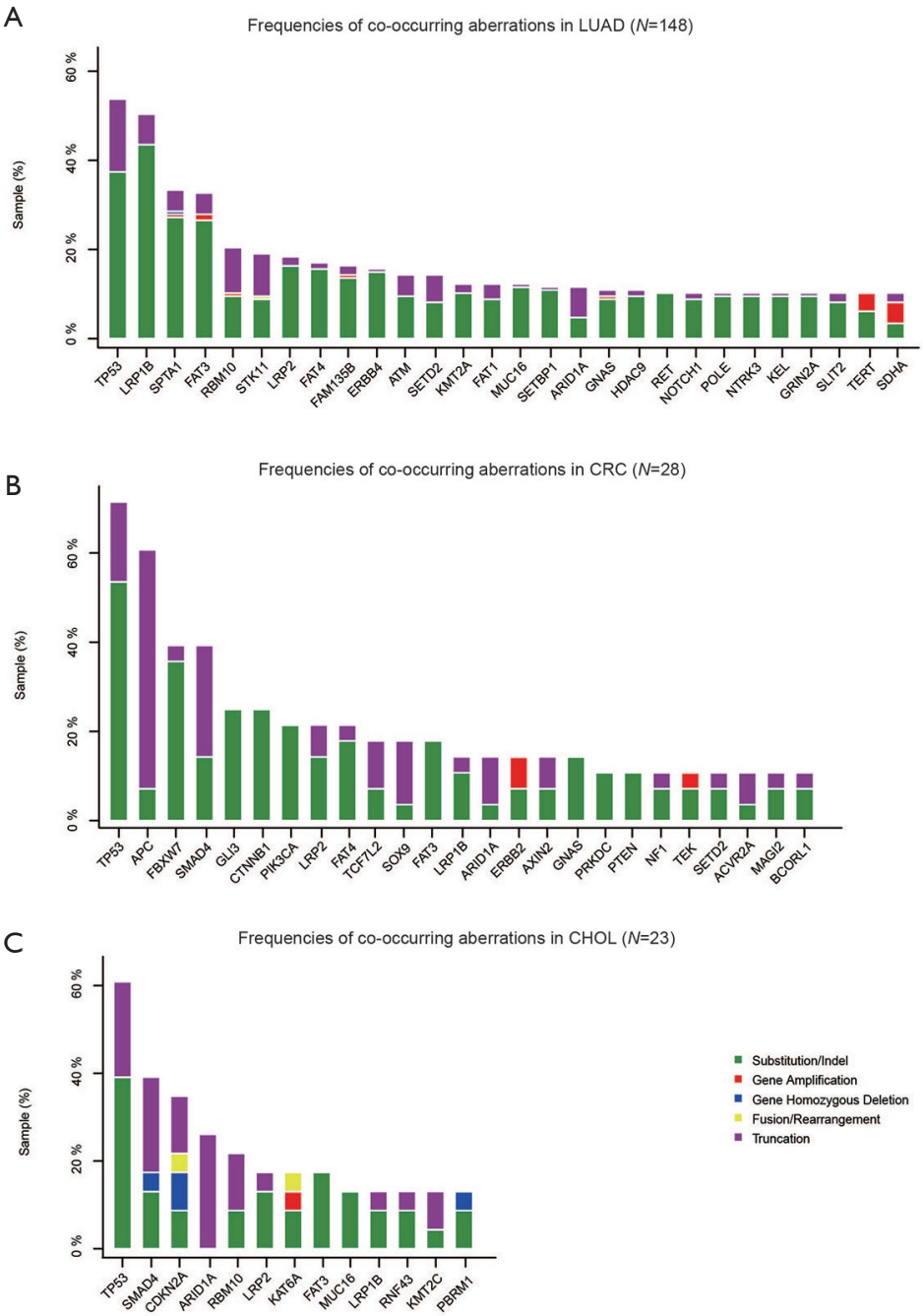

Figure S2 The top 20 most frequent co-alteration of KRAS G12C in (A) lung adenocarcinoma (LUAD), (B) colorectal adenocarcinoma (CRC) and (C) cholangiocarcinoma (CHOL), respectively.

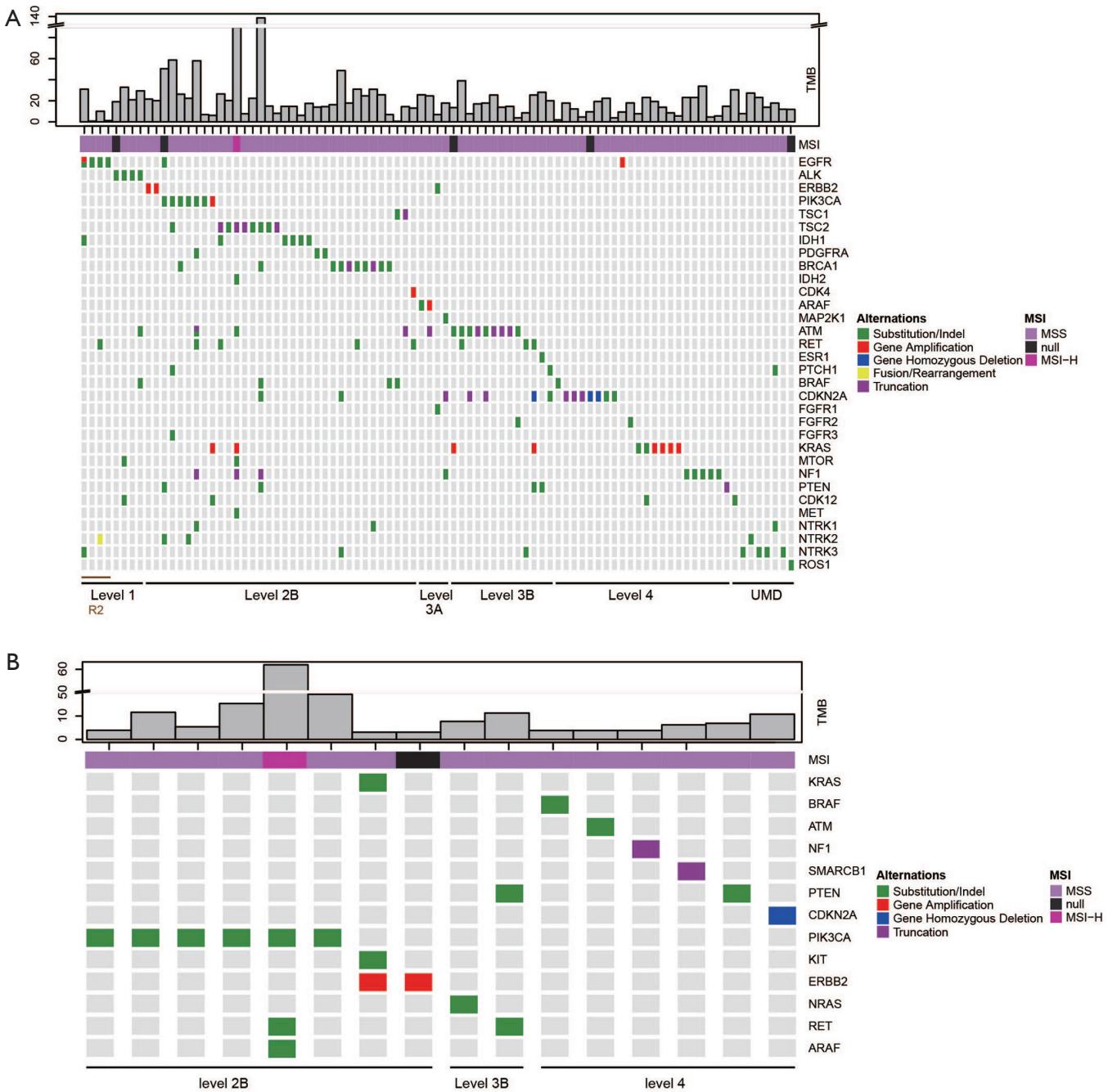

Figure S3 (A) Potential actionable alterations in *KRAS* G12C co-alterations defined as OncoKB level 1 to 4 (uncharacterized alterations, UMD) in lung adenocarcinoma (LUAD); (B) potential actionable alterations among *KRAS* G12C co-alterations in CRC. * denoted as non-G12C *KRAS* alterations. *KRAS* mutation is associated with resistance to EGFR tyrosine kinase inhibitors (TKIs), so patients with *EGFR* oncogenic mutation were also defined as level R2. Tumour mutational burden (TMB) and microsatellite instability (MSI) status were also displayed in the figure.