THERAPEUTIC ADVANCES in
Medical Oncology

Original Research

Characterizing the *KRAS* G12C mutation in metastatic colorectal cancer: a population-based cohort and assessment of expression differences in The Cancer Genome Atlas

Ther Adv Med Oncol

2022, Vol. 14: 1–11

DOI: 10.1177/17588359221097940

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Abstract

Introduction: In metastatic colorectal cancer (mCRC), *RAS* mutations impart inferior survival and resistance to anti-epidermal growth factor receptor (EGFR) antibodies. *KRAS* G12C inhibitors have been developed and we evaluated how *KRAS* G12C differs from other RAS mutations.

Patients and Methods: This retrospective review evaluated patients in British Columbia, Canada with mCRC and *RAS* testing performed between 1 January 2016 and 31 December 2018. Sequencing information from The Cancer Genome Analysis (TCGA) was also obtained and analysed.

Results: Age at diagnosis, sex, anatomic location and stage at diagnosis did not differ by *RAS* mutation type. Progression free survival on first chemotherapy for patients with metastatic *KRAS* G12C tumours was 11 months. Median overall survival did not differ by *RAS* mutation type but was worse for both *KRAS* G12C (27 months) and non-G12C alterations (29 months) than wildtype (43 months) ($p = 0.01$). Within the TCGA, there was no differential gene expression between *KRAS* G12C and other RAS mutations. However, eight genes with copy number differences between the G12C and non-G12C *RAS* mutant groups were identified after adjusting for multiple comparisons (*FITM2*, *PDRG1*, *POFUT1*, *ERGIC3*, *EDEM2*, *PIGU*, *MANBAL* and *PXMP4)*. We also noted that other *RAS* mutant mCRCs had a higher tumour mutation burden than those with *KRAS* G12C mutations (median 3.05 *vs* 2.06 muts/Mb, $p = 4.2e{-}3$) and that *KRAS* G12C/other *RAS* had differing consensus molecular subtype distribution from wildtype colorectal cancer (CRC) ($p < 0.0001$) but not each other ($p = 0.14$).

Conclusion: *KRAS* G12C tumours have similar clinical presentation to other RAS mutant tumours, however, are associated with differential copy number alterations.

Keywords: cancer, colon, metastatic, *RAS*, rectal, signalling

Received: 26 November 2021; revised manuscript accepted: 14 April 2022.

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Introduction

The *RAS* genes code for the RAS GTPase proteins that regulate cellular signalling pathways, activated in the guanosine triphosphate (GTP)-bound state and inactivated in the guanosine GDP-bound state.[1] Many cancers are driven by a *RAS* mutation that favours the GTP-bound state with resultant constitutive activation, proliferation and survival. The *RAS* family of proto-oncogenes includes *KRAS*, *NRAS* and *HRAS*.[1] *KRAS* is the most frequently mutated *RAS* family member in colorectal cancer (CRC).[2] For many years, attempts to develop targeted therapies towards *RAS* have been unsuccessful. Recently, small molecules that specifically and irreversibly inhibit the *KRAS* G12C (glycine-to-cysteine substitution) mutation,

thereby locking KRAS in an inactive GDP-bound state, have shown promising activity.[3]

In CRC, *KRAS* mutations provide resistance to anti-EGFR therapies and are associated with inferior progression-free survival (PFS) and overall survival (OS) compared with wildtype *KRAS* when treated with standard therapies.[4–6] Many studies previously characterized the *KRAS* mutation[7–9] in CRC, but fewer have characterized the impact of the specific *KRAS* G12C mutation,[10] particularly in a North American population-based cohort. The objective of this study was to describe the clinicopathologic characteristics of *KRAS* G12C CRCs and assess their first-line PFS and OS in the metastatic setting to inform comparisons for trials evaluating *KRAS* G12C directed therapies. We also aimed to use publicly available sequencing information from The Cancer Genome Atlas (TCGA) to compare and contrast gene expression and copy number differences between *KRAS* G12C and non-G12C mutations that have not been described in previous studies.

Methods

Cancer care in the Canadian province of British Columbia (BC) is publicly funded and almost exclusively delivered through BC Cancer to the ~4.5 million people living across the province, achieved through a network of six regional comprehensive cancer centres and community sites that provide chemotherapy for patients living remotely. This provides a robust population-based cohort to evaluate health services research. This study was approved by the BC Cancer Research Ethics Board (approval number: H19-02927), performed per the Declaration of Helsinki. A waiver of consent was obtained for this retrospective chart review.

We identified all patients with metastatic CRC in BC between 1 January 2016 and 31 December 2018 who had tumours that underwent testing with the next generation sequencing panel currently used in the province or reflexive *RAS* polymerase chain reaction (PCR) testing when adequate DNA was not available for the panel. All patients throughout the province undergo the same standard-of-care testing. Baseline characteristics obtained included age, sex, tumour location (right-sided CRC was defined as arising proximal to the splenic flexure and left-sided CRC arose at or distal to the splenic flexure), stage at presentation and clinical outcome data. Concurrent molecular profiling data (if available as part of the standard-of-care testing) was also obtained.

Student's *t*-tests and Fisher's exact tests were used to compare between groups and obtain odds ratios with GraphPad Prism (Version 8.4.2, San Diego, California USA). PFS was defined as the time from the first date of chemotherapy to disease progression on the first line of treatment or death. Patients without progression or death at the time of last follow-up were censored. OS was defined as the time from diagnosis of metastatic disease to the date of death or last follow-up. Again, patients who were alive at the time of last follow-up were censored. Survival was estimated using the Kaplan–Meier method and compared using a log-rank test. Comparison of the *KRAS* G12C mutant group was made against *RAS*/*BRAF* wildtype cancers as well as other *RAS* (non-*KRAS* G12C or *NRAS*) mutations.

To understand how *KRAS* G12C mutation affects biology in CRC, TCGA expression, mutation and copy number alteration datasets were obtained[10–12] (the expression (illuminaga_rnaseqv2-RSEM_genes and illuminahiseq_rnaseqv2-RSEM_genes) datasets were downloaded from http://firebrowse.org/ on 18 February 2020 and the MC3 Public MAF mutation data (mc3.v0.2.8.PUBLIC.maf.gz) was downloaded from: https://gdc.cancer.gov/about-data/publications/mc3-2017 on 30 September 2020). Due to limited number of metastatic cancers in the cohort, only primary tumours biopsied from solid tissue were included in this analysis. The obtained samples were also divided into the three cohorts: *KRAS* G12C mutation, *RAS*/*BRAF* V600 wildtype and other *RAS* (non-*KRAS* G12C or *HRAS*/*NRAS*) mutations. The samples that contained a *KRAS* G12C variant and another *KRAS* variant 1 bp away were removed from the analysis since it is possible that one of these variants was miscalled.

Differential gene expression analyses were performed between all pairs of the above groups. For these analyses, gene expression datasets were obtained from both Genome Analyzer (GA) and Hiseq sequencing platforms. Expression values were converted to transcript per million (TPM) to allow cross sample comparison and the log2 of TPMs was found. Samples that existed in both datasets (GA and Hiseq) were removed from the

GA dataset, since Hiseq sequencer is a newer generation of sequencing machines. Then, the datasets were merged, and genes with zero expression across all samples were removed. To obtain a more normally distributed set, the genes with log2 TPM expression less than 2 in at least 25% of samples were filtered out. The distribution of log2TPM values can be found in Supplementary Figure 1. In the next step, batch correction was performed using ComBat function of sva package[13](version 3.32.1). The principal component analysis (PCA) plots of data points were made using ggplot package[14] (version 3.3.2) before and after batch correction (Supplementary Figure 2). For differential expression analysis, Wilcoxon rank-sum test was performed for each pair of *RAS* groups and Benjamini–Hochberg correction was performed to adjust the *p*-values. The heatmap of genes with significant differential expression in at least one of the tests as well as the boxplot of expression of genes with significant differential expression between *KRAS* G12C and wildtype *RAS* groups were made (Figure 2 and Supplementary Figure 3).

The copy number alterations of genes that were significantly differentially expressed between any *RAS* mutant group and wildtype samples were also obtained and visualized. To find the total copy changes per gene, the ploidy was subtracted from the total number of copies found per gene. To find the copy changes for major and minor alleles, the ceiling and floor of ploidy divided by two were subtracted from the number of major and minor allele copies, respectively. The copy change profiles were made for total, major and minor alleles across the *RAS* groups (Figure 3 and Supplementary Figures 4 and 5). Wilcoxon test was performed between each pair of the three *RAS* groups to find the genes with significant total, major and minor copy changes in the set of genes with significant differential expression and the *p*-values were adjusted *via* Benjamini–Hochberg procedure. In addition, to find the genes with significant copy changes across all genes, Wilcoxon test was performed between each pair of the *RAS* groups, and the genes with significant total, major, and minor copy number changes were found. Similar to previous analyses, all the *p*-values were adjusted using Benjamini–Hochberg procedure.

The plot of single nucleotide variants (SNVs) and small insertions or deletions (INDELs) was also made for the genes that were significantly differentially expressed (Figure 4), and Fisher's exact tests were performed on the number of SNVs and INDELs in these genes between all pairs of *RAS* groups. To compare the mutation rate across the *RAS* groups, the tumour mutation burden (TMB) was calculated using the suggested guidelines by Merino *et al.*[15] In summary, the 'frameshift', 'inframe', 'missense', and 'nonsense' mutations located at exons with tumour depth greater than or equal to 25, alternative variant count greater than or equal to 3, and variant allele frequency greater than or equal to 0.05 were filtered. Then, the number of mutations per patient was divided by 33 Mb to obtain the number of mutations per megabase of exome otherwise known as the TMB. The scatter plot of TMB values can be found in Figure 5(b). Finally, the samples were categorized based on their consensus molecular subtype (CMS) subtype using CMS classifier[16] and the number and percentage of samples in each category were obtained (Figure 5(c)).

Results

A total of 643 colorectal cases had available testing data in our population-based cohort with 30 (4%) harbouring a *KRAS* G12C mutation and 359 (51%) harbouring another *RAS* (non-*KRAS* G12C or *NRAS*) mutation. There were 254 (36%) *KRAS*/*NRAS*/*BRAF* wild type cases. Table 1 summarizes the baseline clinicopathological characteristics by mutation status. The median ages at diagnosis and sex distribution were similar between the *KRAS* G12C, other *RAS* mutant and wild type tumours. Anatomic location and initial disease stage were also not statistically significantly different between groups. Left-sided CRC constituted the majority (70%) of *KRAS* G12C tumours. Synchronous metastatic disease was present in 40% of *KRAS* G12C diagnoses, 48% of *RAS*/*BRAF* wild type diagnoses ($p = 0.25$), and 49% of diagnoses with other RAS mutations ($p = 0.45$).

Of the 30 *KRAS* G12C cases in the population-based cohort, 18 were tested for mismatch repair (MMR) status by immunohistochemistry (IHC) and none exhibited deficient MMR (dMMR)/microsatellite instability (MSI). In the *KRAS*/*NRAS*/*BRAF* wildtype cases, 6/128 (5%) were dMMR while in the cases with other *RAS* mutations, 6/212 (3%) were dMMR. There were

Table 1. Baseline clinicopathologic characteristics of patients with *KRAS* G12C and non-*KRAS* G12C tumours in a population-based cohort.

Characteristic	*KRAS* G12C	Other *RAS*	*p*-value	Wildtype *KRAS*, *NRAS*, *BRAF*	*p*-value (*KRAS* G12C *vs* wildtype)
Number of patients	30	359		254	
Median age at diagnosis (interquartile range)	62.5 (54.5–70.5)	63 (55–70)	0.93 (*t*-test)	61 (54–70)	0.46 (*t*-test)
Median survival from metastasis to death in months	27	29	0.29 (log-rank)	43	*0.01 (log-rank)
Sex					
Number of male (%)	18 (60)	204 (57)	OR = 1.14 [95% CI = 0.55–2.42] *p* = 0.85 (Fischer)	180 (71)	OR = 0.62 [95% CI = 0.29–1.36] *p* = 0.29 (Fischer)
Number of females (%)	12 (40)	155 (43)		74 (29)	
Anatomic location					
Number of right-sided (%)	9 (30)	122 (34)	OR = 1.22 [95% CI = 0.56–2.81] *p* = 0.69 (Fischer)	47 (19)	OR = 0.54 [95% CI = 0.23–1.32] *p* = 0.15 (Fischer)
Number of left-sided (%)	21 (70)	234 (66)		203 (81)	
Number of unknown	0	3		4	
Metastasis					
Metachronous metastases (%)	18 (60)	176 (49)	OR = 1.56 [95% CI = 0.75–3.31] *p* = 0.26 (Fischer)	123 (48)	OR = 1.6 [95% CI = 0.75–3.56] *p* = 0.25 (Fischer)
Synchronous metastases (%)	12 (40)	183 (51)		131 (52)	
Microsatellite instability					
Microsatellite instability high (MSI)(%)	0 (0)	6 (3)	OR = 0 [95% CI 0–9.16] *p* = 0.99	8 (5)	OR = 0 [95% CI = 0–4.07] *p* = 0.60
Microsatellite stable (MSS) (%)	18 (100)	206 (97)		142 (95)	
Number of unknown	12	147		104	

CI, confidence interval; MSI, microsatellite instability; OR, odds ratio.
*denotes comparisons where p<0.05.

11/30 (37%) *KRAS* G12C cases that had other co-mutations. Most (64%) only had one other co-mutation. The most common co-mutation was *PIK3CA* (n = 7, 23.3%). Other co-mutations included *APC*, *BRCA2*, *CCND1*, *CIC*, *ERBB3*, *PDGFR*, *SMAD4* and *TP53*. There was also one case with a non-V600E *BRAF* mutation.

Impact of KRAS G12C on clinical outcomes

The median first-line PFS of patients with metastatic *KRAS* G12C tumours was 11 months (Figure 1(a)). The majority of patients (63%) received first-line irinotecan-based doublet therapy with or without bevacizumab (FOLFIRI ± bevacizumab = 13, CAPIRI ± bevacizumab = 4). Most

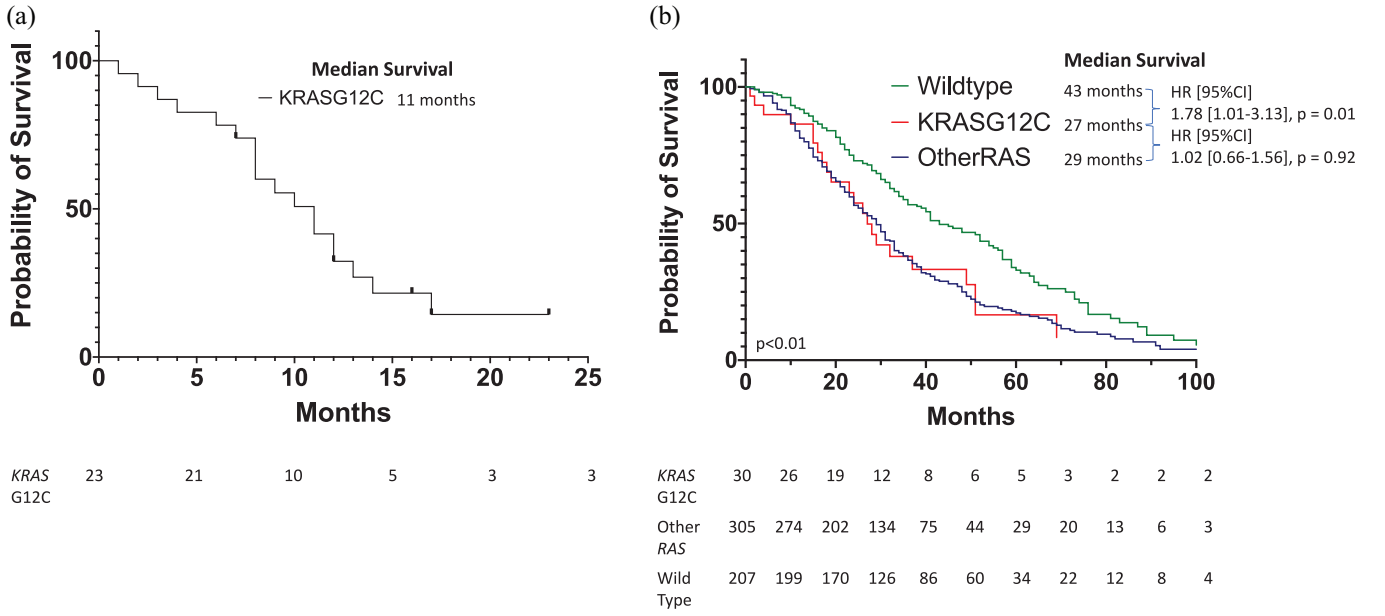

Figure 1. (a) Progression-free survival of KRAS G12C patients on first-line therapy and (b) overall survival of KRAS G12C *versus* non-KRAS G12C patients.

other patients received single agent capecitabine (6). One patient received oxaliplatin-based doublet therapy (CAPOX) and one patient received raltitrexed. The median OS of patients was significantly worse in *KRAS* G12C than *RAS*/*BRAF* wildtype tumours (HR = 1.78; 95% CI = 1.01–3.13; $p = 0.01$) (Figure 1(b)). There was no statistically significant difference in median OS between patients with *KRAS* G12C mutation and patients with other RAS mutations (HR = 1.02; 95% CI = 0.66–1.56; $p = 0.92$). Given the small number of patients with a *KRAS* G12C mutation, we were not able to perform a multivariate analysis with robust statistical power.

RAS mutations in TCGA primary colorectal samples

In total, 505 primary CRCs had both mutation and expression data in TCGA. Fifteen (3%) had a *KRAS* G12C mutation, 256 (51%) carried other *RAS* mutations and 234 (46%) were *RAS*/*BRAF* V600 wildtype. The differential expression analysis between the samples harbouring *KRAS* G12C mutation and the wildtype samples resulted in two genes (*HOXB5* and *HOXB8*) with significant differential expression ($p_{adj} < 0.05$) that are summarized in Supplementary Table 1 and Figure 1. While *HOXB5* was shown to be overexpressed in both normal and tumour colorectal tissues, it has been demonstrated that upregulation

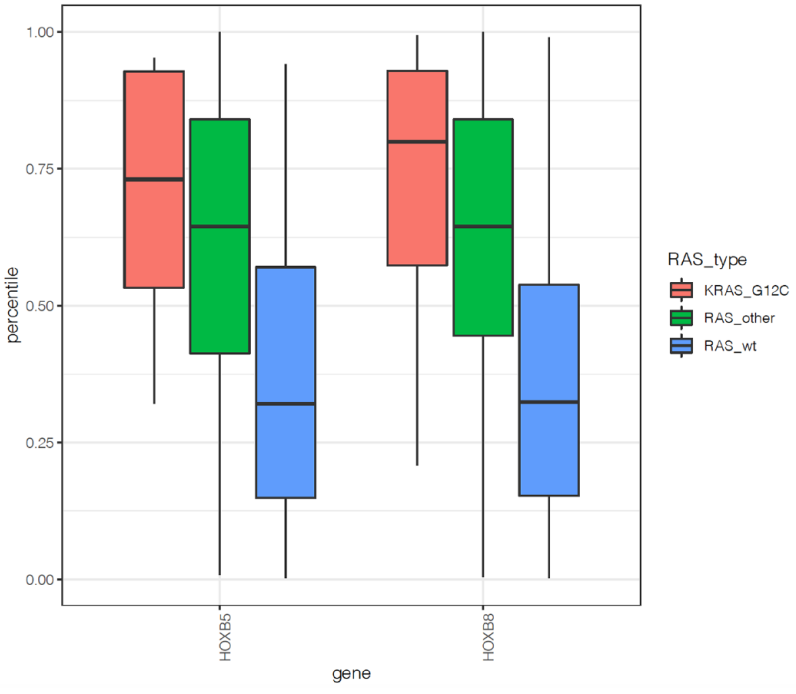

Figure 2. Boxplot of expression percentile of significant genes found in DE between *KRAS* G12C and wildtype *RAS* groups.

of *HOXB8* gene is correlated with CRC development.[17] The differential expression analysis between the samples with other *RAS* mutations and the wildtype samples resulted in 3737 genes

with significant differential expression ($p_{adj} < 0.05$). The top 20 genes of this set ($p_{adj} < 10^{-12}$) are shown in Supplementary Table 2. The considerable difference between the number of significant genes found for the samples with a *KRAS* G12C mutation and the samples containing other *RAS* mutations when compared with the wildtype samples is due to the substantial difference in the number of samples in each group. It has been demonstrated that small sample size can affect the results of differential expression analysis in microarray and RNA-seq studies.[18,19] We could also show that by taking a random sample of size 15 (same as our *KRAS* G12C set size) from the other *RAS* cohort, the number of differentially expressed genes decreases markedly. The number of differentially expressed genes between the other RAS sample and the wildtype set in 10 iterations was on average 31.5 ($\pm$65.85 *SD*). The differential expression analysis between the samples harbouring *KRAS* G12C mutation and the samples with other RAS mutations did not result in any significant genes. Supplementary Figure 3 shows a heatmap of *z*-scores of log2 TPM values of the genes that are summarized in Supplementary Tables 1 and 2 across the *RAS* groups.

Out of 466 TCGA samples with both mutation and copy number alteration data, 12 (3%) had a *KRAS* G12C mutation, 235 (50%) had other *RAS* mutations, and 219 (47%) had wildtype *RAS* and *BRAF* V600. The copy number alteration profile of genes differentially expressed between cancers with any *RAS* mutation or the wildtype group are shown in Figure 3. The copy changes were also obtained for both major and minor alleles separately and are visualized in Supplementary Figures 4 and 5. The Wilcoxon analyses that were performed on the genes of interest showed no significant copy changes between the *KRAS* G12C group and the wildtype *RAS* group. This finding suggests that the genes found to be differentially expressed between these two groups do not have a significant change in their copy number. However, it should be noted that the small sample size of the *KRAS* G12C cohort can affect the results of this analysis. Between the *KRAS* G12C group and the other *RAS* group, eight genes were found with significant copy changes. These genes were *FITM2*, *PDRG1*, *POFUT1*, *ERGIC3*, *EDEM2*, *PIGU*, *MANBAL*, and *PXMP4* with adjusted *p*-values of $\leqslant 0.026$. In addition, 15 genes were found with significant copy changes between the other *RAS* and wildtype groups. These genes were *POFUT1* (adj. *p*-value = 2.0e-13), *ERGIC3* (adj. *p*-value = 2.0e–13), *PIGU* (adj. *p*-value = 2.0e–13), *PXMP4* (adj. *p*-value = 2.0e–13), *PDRG1* (adj. *p*-value = 2.2e–13), *EDEM2* (adj. *p*-value = 2.2e–13), *FITM2* (adj. *p*-value = 3.2e–13), *MANBAL* (adj. *p*-value = 5.6e–13), *HOXB8* (adj. *p*-value = 5.2e–4), *HOXB4* (adj. *p*-value = 5.2e–4), *HOXB5* (adj. *p*-value = 5.2e–4), *HOXB6* (adj. *p*-value = 6.0e–4), *DUSP4* (adj. *p*-value = 2.0e–3), *DUSP6* (adj. *p*-value = 3.4e–3) and *PHLDA1* (adj. *p*-value = 1.3e–2).

The copy number alterations were also analysed across all genes. The Wilcoxon test found no gene with significant copy changes between *KRAS* G12C and the other groups. There were 3278 genes with significant copy changes (adj. *p*-value < 0.05) between other *RAS* and wildtype *RAS* groups. The top 48 genes (adj. *p*-value $<$ 2e–11) found in this analysis are shown in Supplementary Table 3. *EDEM2*, *PDRG1*, *POFUT1*, *ERGIC3*, *PIGU* and *PXMP4* from the top 48 genes found here intersect with the 15 genes with significant copy changes found in the analysis of genes with differential expression between groups. All the 15 genes are found in the list of 3278 genes with significant copy changes (with adj. *p*-value < 0.05).

The plot of SNVs and INDELs was made for genes with significant differential expression (Figure 4). In cases that a sample had two mutations in the same gene, the mutation with the higher importance was selected (frameshift > nonsense > nonstop > missense > inframe > splice > other > silent). As seen in Figure 4, none of the samples in *KRAS* G12C group had a mutation in one of the genes with significant differential expression. To compare the rate of mutation across the *RAS* groups, TMB was calculated and visualized (Figure 5(b)). The TMB median in *KRAS* G12C group was 2.06 (interquartile range (IQR) = 1.86–2.49) compared with 3.05 (IQR = 2.27–4.11) and 2.39 mutations/megabase (muts/Mb) (IQR = 1.76–3.15) in other *RAS* and wildtype *RAS* groups, respectively. The TMB values were also compared between each pair of *RAS* groups using Mann–Whitney test. TMB is significantly higher in other *RAS* groups compared with both *KRAS* G12C and wildtype groups (*p*-values of 4.2e–3 and 5.9e–10, respectively). However, there is no significant difference in TMB between *KRAS* G12C and wildtype group (*p*-value = 0.62).

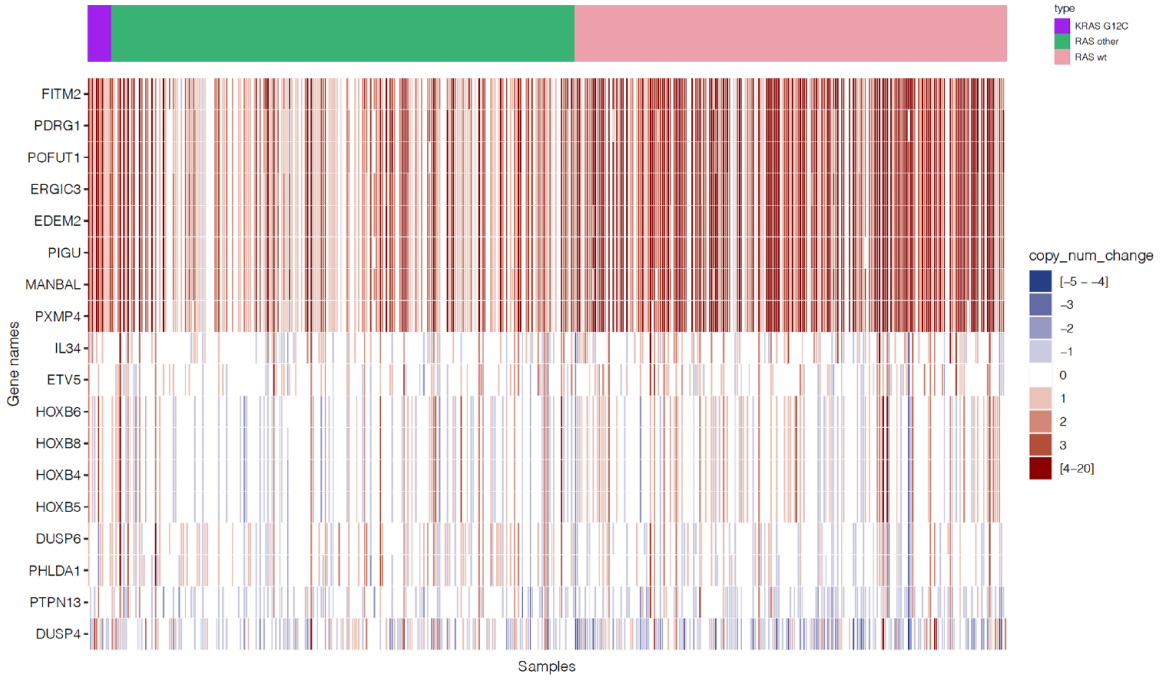

Figure 3. Heatmap of copy number changes of differentially expressed genes.

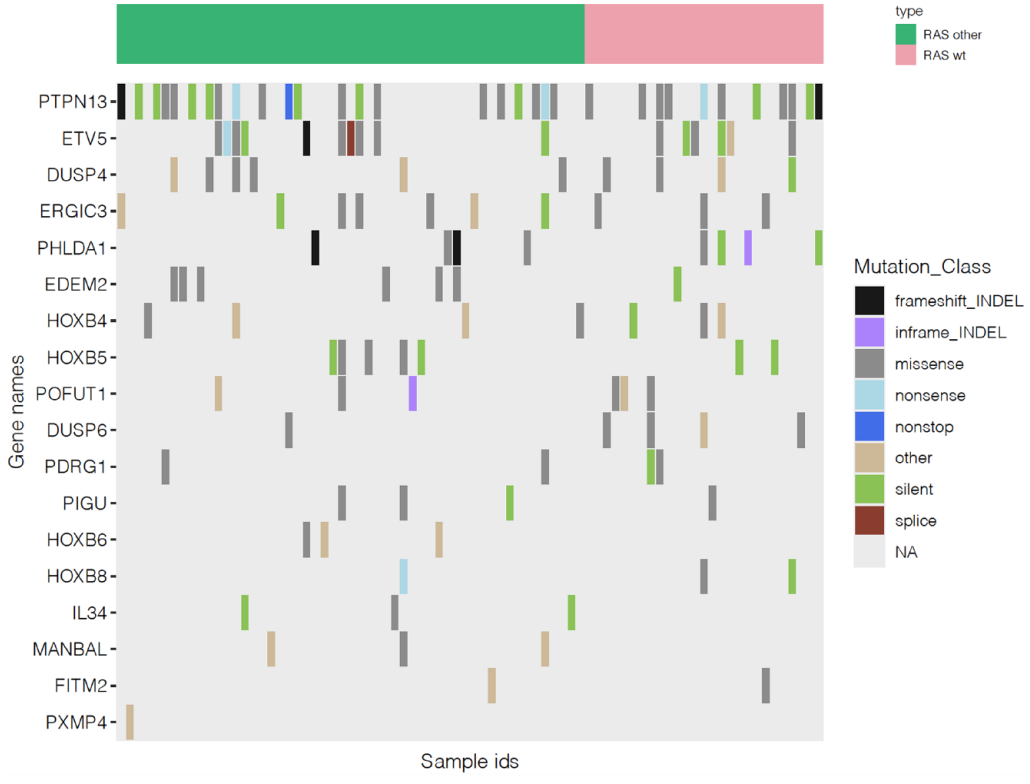

Figure 4. Mutations in differentially expressed genes (none of the *KRAS* G12C tumours has a mutation in these genes).

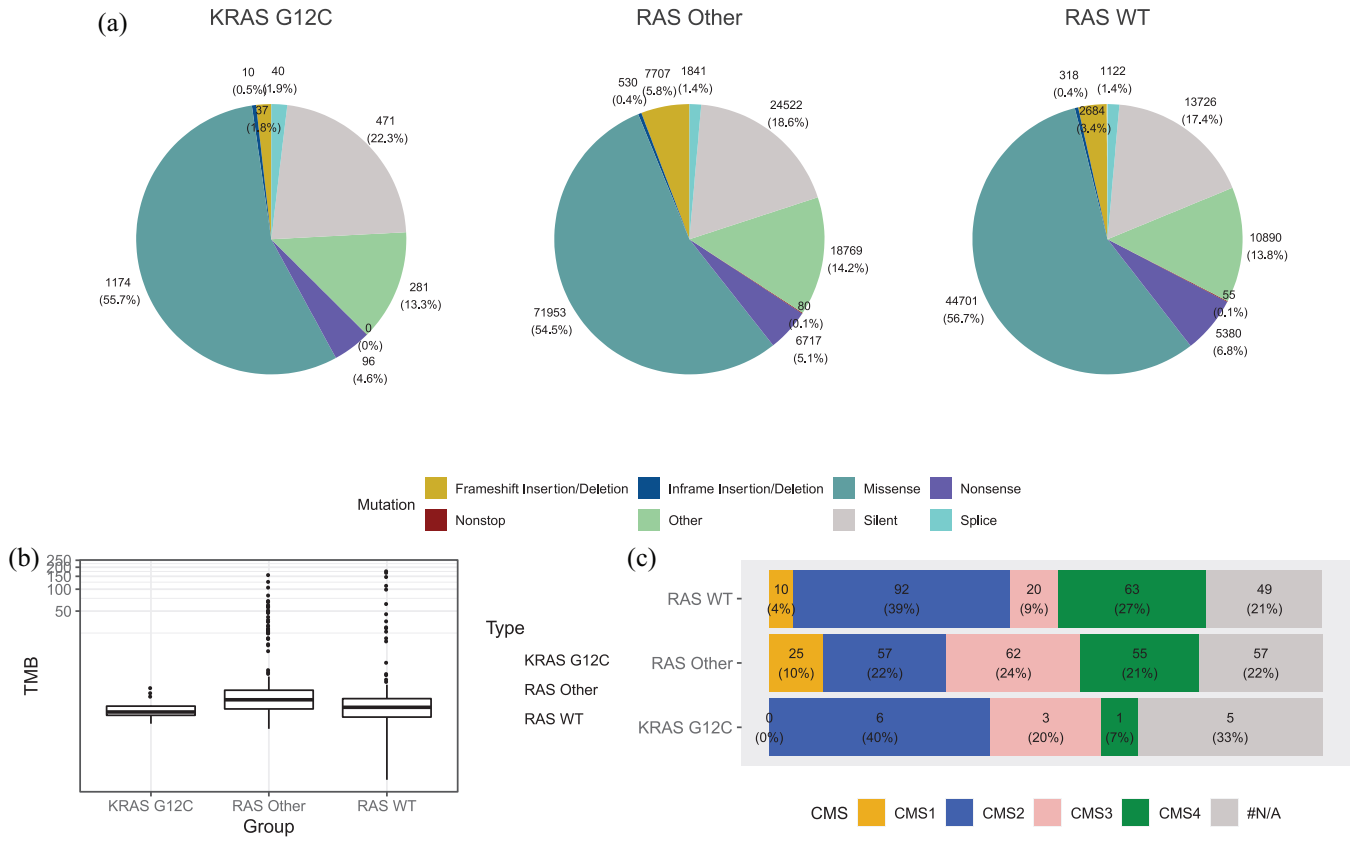

Figure 5. (a) Number and percent of different mutation types, (b) scatter plots and boxplots of TMB values (*y*-axis is log10-transformed), and (c) number and percent of samples assigned to each CMS subtypes across *RAS* groups.

Finally, samples were subtyped by CMS classifier using expression data. The number of samples assigned to each CMS subtype grouped by *RAS* cohorts can be found in Figure 5(c). Only the samples with a probability greater than 50% were assigned to a CMS subtype and the rest of the samples were assigned to the unknown group. As seen in Figure 5(c), most samples in *KRAS* G12C were assigned to CMS2 and a third could not be assigned to a CMS subtype confidently. In the other *RAS* group, samples were more evenly distributed across CMS subtypes. In the wildtype group, approximately 40% of the samples were assigned to CMS2 and 27% of them were assigned to CMS4 subtype. After removing the samples which could not be assigned to a CMS subtype confidently, a chi-square test was performed to compare the distribution of samples across CMS subtypes in the *RAS* groups, and $p < 0.0001$was obtained, however, *KRAS* G12C mutant CRC did not differ in CMS distribution from other *RAS* mutant CRC (p-value = 0.14).

Discussion

Like previous reports, *KRAS* G12C mutation occurred in 4% of CRCs,[7] with the median age of 62.5 years, similar to patients without *KRAS* G12C mutations. Recently, Schirripa *et al.*[10] showed that *KRAS* G12C was significantly more likely to occur in men (71%). We also noted that the *KRAS* G12C mutation was more likely to occur in men (60%), but the sample size in our study may not have been large enough for this finding to reach statistical significance. In a recent study by Nasar *et al.*[20] reviewing the distribution of *KRAS* G12C by cancer type, sex and race (White, Black and Asian) from the American Association for Cancer Research Project Genomics Evidence Neoplasia Information Exchange (GENIE) version 8.0, they found that more female patients harboured *KRAS* G12C than male patients in CRC, but this was only statistically significant in white patients and not in other races (Black and Asian). We did not obtain race characteristics in our current study. No other specific clinicopathological characteristics were

significantly associated with the *KRAS* G12C mutation, including anatomic location and stage at presentation. A Japanese study of mCRC *KRAS* G12C and non-*KRAS* G12C patients also demonstrated no significantly different characteristics.[21] Both *KRAS* G12C and non-*KRAS* G12C mutated tumours were more commonly left-sided in keeping with patterns from previous studies[4,8,9,21] and were almost equally as likely to present as early stage disease as metastatic disease at diagnosis.[6,8]

BRAF is another key mutation contributing to poor CRC prognosis and survival and is usually independent of *KRAS* mutations. Similar to the 0.9% of cases, Imamura *et al.*[4] found of *BRAF*/*KRAS* G12C co-mutation, only 1/30 *KRAS* G12C cases (3.3%) from this study had a *BRAF* mutation found in a 66-year-old female with metastatic grade 3 colorectal adenocarcinoma at diagnosis. However, this was a non-V600E mutation.

In our study, the most common co-mutation with *KRAS* G12C was *PIK3CA* (23%), again, similar to previous co-mutation rates.[4] Like many *KRAS* mutations, *PIK3CA* mutations also occur in the classic adenoma to carcinoma transition sequence of CRC development.[22] In *in vitro* experiments combining a *KRAS* G12C inhibitor with a PI3 K inhibitor, synergistic killing of tumour cells was reported, suggesting that combination therapy may serve as an effective strategy against *KRAS* G12C tumours commonly co-mutated with *PIK3CA*.[23]

Impact of KRAS G12C on clinical outcomes

The median PFS of patients with *KRAS* G12C mutation on first-line treatment for metastatic disease was 11 months, comparable with the 10.1 months PFS demonstrated by Modest *et al.*[5] Chida *et al.*[21] also showed a PFS of 9.4 months, which was significantly shorter than patients with non-*KRAS* G12C mutations. Compared with *RAS*/*BRAF* wildtype cancers, OS in *KRAS* G12C patients was notably inferior as expected, since *KRAS* G12C has been shown to impart adverse prognosis in several previous studies,[4–6] with similar OS reported by Jones *et al.*[8] and Chida *et al.*[21] Schirripa *et al.*[10] and Henry *et al.*[24] reported a similar median OS in patients with *KRAS* G12C mutation but a statistically significant longer OS in patients with other *KRAS* mutations. They evaluated a more discriminated cohort of patients referred to select oncology units in Italy or to a quaternary care centre in the United States, whereas, we evaluated a population-based cohort encompassing diverse patient groups province-wide, accounting perhaps for the worse OS in patients with other *KRAS* mutations in our study that is similar compared with *KRAS* G12C patients.

RAS mutations in TCGA primary colorectal samples

The analyses performed on TCGA primary colorectal samples helped us identify genes with significant differential expression between *KRAS* G12C and the wildtype groups as well as between the other RAS and the wildtype groups. We observed that *HOXB5* and *HOXB8* genes were the only two genes with differential expression analysis in both comparisons. These two genes produce transcription factors that are a part of the developmental regulatory system,[25] and as drivers of growth and proliferation, a higher expression of these genes would be expected in cancer cells.

The copy number analysis across all genes could not detect any genes with significant copy changes in the *KRAS* G12C group compared with the wildtype group. This could be due to the lower number of samples in this cohort compared with the other two groups, especially because many genes with significant copy number changes between the other *RAS* and wildtype *RAS* groups were identifiable. The Mann–Whitney test on the TMB showed that samples in other *RAS* groups have higher TMB values on average compared with the other two groups. Finally, we showed that most *KRAS* G12C samples belong to Canonical subtype (CMS2), while in the other *RAS* cases, samples were approximately evenly distributed across subtypes. Samples with CMS2 subtype are usually associated with upregulation of WNT and MYC downstream targets.[16] Although we did not find any of these downstream targets in the differential expression analysis, further investigations can be performed to find the potential association between these pathways and *KRAS* G12C mutation.

Study limitations

The major limitation of this study was the number of metastatic CRCs detected that harboured a *KRAS* G12C mutation, although not unexpected as the *KRAS* G12C mutation occurs in less than 5% of cases. Therefore, we were not able to perform

multivariate analyses with robust statistical power. However, in this study, baseline patient characteristics did not differ by *RAS* mutation type. Furthermore, the low number of samples did make it more difficult to detect differentially expressed genes and significant gene copy changes. As such, we saw significantly more genes with alterations among the 'other-*RAS*' mutant colorectal cases due to the larger population used as the comparator *versus* wild type samples compared with *KRAS* G12C.

Conclusion

KRAS G12C tumours appear to have similar clinicopathologic features to non-*KRAS* G12C mutated CRC. A fundamental understanding of *KRAS* G12C mutation will further facilitate the clinical development of targeted *KRAS* G12C drugs that will improve the prognosis for these patients. As well, we provided information about first-line PFS to help inform future clinical trials should G12C inhibitors be moved to an earlier line of therapy.

Author contribution(s)

Meredith Li: Data curation; Formal analysis; Investigation; Writing – original draft.

Faeze Keshavarz-Rahaghi: Data curation; Formal analysis; Investigation; Writing – original draft.

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Lucas Swanson: Availability of data and materials; Data curation; Investigation; Writing – review & editing.

Caroline Speers: Investigation; Writing – review & editing.

Daniel J Renouf: Investigation; Writing – review & editing.

Howard J. Lim: Investigation; Writing – review & editing.

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Sharlene Gill: Investigation; Writing – review & editing.

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Funding

This work was supported by funds from the BC Cancer Foundation. Jonathan Loree and Daniel Renouf are supported by Michael Smith Health Professional Investigator Awards which help make their research possible.

Conflict of interest statement

The authors declared the following potential conflicts of interest with respect to the research, authorship and/or publication of this article: Stephen Yip is a member of the advisory boards for Amgen, AstraZeneca, Bayer, Merck, Novartis and Roche. Jonathan Loree is a member of advisory boards for Amgen, Bayer, Novartis, Roche, Ipsen, Eisai and Pfizer and has received research funding from Ipsen, AstraZeneca and Amgen.

Supplemental material

Supplemental material for this article is available online.

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