Research Article

Identifying Genetic Correlates of Colorectal Cancer Through Bioinformatics Analysis

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ABSTRACT

Colorectal cancer (CRC) is a leading cause of cancer-related mortality worldwide, with genetic mutations playing a significant role in tumor progression and overall survival. This study investigates the impact of mutations in *APC, KRAS, BRAF*, and *RNF43* on survival time using the analysis of patient data sourced from Cercek et al. [1] through cBioPortal. The results revealed that *APC* mutations were positively correlated with survival time, while *KRAS, BRAF,* and *RNF43* were negatively correlated with survival time. These findings are consistent with previous literature and the roles of these genes in tumorigenesis and cancer progression. Further research on the importance of genetic mutations and prognostic biomarkers can improve targeted therapies and personalized treatments for colorectal cancer patients.

Keywords: colorectal cancer; bioinformatics; genetics; mutations; Kaplan-Meier analysis; prognostic biomarkers

INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed cancer worldwide and is caused by an overgrowth of cells in the colon or rectum. It typically appears as polyps, abnormal growths that develop in the lining of the large intestine. The risk of CRC development increases with age and environmental exposure. High alcohol consumption, chronic inflammation, physical inactivity, and an unhealthy diet are attributable to more than 50% of CRC cases (2). CRC can occur due to inherited germline mutations or acquired somatic mutations. Less than 5% of CRC cases are caused by inherited mutations, which are often associated with other cancerous syndromes, such as familial adenomatous polyposis, Peutz-Jeghers syndrome, or hereditary non-polyposis colon cancer (3). Mutations can affect oncogenes or tumor suppressor genes, leading to uncontrolled cell growth and invasive malignancies. Treatment plans are often multimodal and utilize surgical resection, chemotherapy, and targeted radiation to reduce tumors (4).

There are many genes associated with CRC. The four genes explored in this study are *APC*, *KRAS*, *BRAF*, and *RNF43*. These genes were selected due to their established or emerging relevance as prognostic biomarkers.

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Accepted August 21, 2025
https://doi.org/10.70251/HYJR2348.34412417

APC is a tumor suppressor gene involved in the WNT signaling pathway. It encodes for the *APC* protein, which negatively regulates gene activation by degrading β-catenin, a transcriptional activator. This prevents uncontrolled cell proliferation and division. Mutations in the *APC* gene lead to the accumulation of β-catenin in the nucleus and promote the formation of adenomas, or polyps, eventually leading to the development of CRC (5). *APC* truncations have also been shown to promote cell survival through mechanisms independent of β-catenin. They decelerate apoptosis-associated caspase cleavage, leading to cell proliferation, a property that CRC cells may use to support tumor growth (5). *APC* can assume zero, one, or two truncating mutations, and single mutations have the most favorable survival. Wildtype *APC* has a worse prognosis, but the presence of multiple *APC* mutation tumors along with mutations in *KRAS* or *TP53* has the poorest survival (6). Alterations in the WNT signaling pathway are also associated with weakened tight junctions and lowered cell adhesion, favoring cell migration and metastasis (4). *APC* is associated with familial adenomatous polyposis (FAP), a syndrome often caused by inherited mutations in the *APC* gene. FAP is also correlated with higher risk factors for other malignancies, such as pancreatic, thyroid, and brain cancers (7). However, nearly 20% of CRC cases associated with FAP are sporadic, meaning patients have no family history or inherited germline mutations. Instead, they display somatic mosaic mutations, which occur after fertilization and are identified using next-generation sequencing (8) *APC* has increased mutation prevalence in cases with a higher adenoma count, and approximately 80% of all CRC cases contain an *APC* mutation (9).

Another gene associated with CRC is *KRAS*, which is an oncogene. *KRAS* is used in signal transduction and initiates the binding of various growth factors to their receptors. It also encodes guanosine triphosphate (GTP) to bind proteins and activate over twenty downstream effectors. These effectors can control apoptosis suppression, cell growth and migration, and angiogenesis (10). Mutations in *KRAS* can lead to the constant activation of MAP kinase, a protein that controls stimuli responses, causing increased cell proliferation. *KRAS* mutations are also associated with poorer prognosis, lower survival rates, and more aggressive tumor behavior. The miRNA involved in *KRAS* mutations causes the induction of cellular proliferation and metastasis, leading to increased CRC development (4). *KRAS* mutations are prevalent in CRC cases, occurring in 35-45% of all cases, and often occur alongside *TP53* and *TGFBR2* mutations. Their decreased response rates to specific targeted therapies also make them harder to treat (10).

BRAF is another gene associated with CRC, and it is also an oncogene involved in the MAP kinase signaling pathway. It codes for a serine/threonine protein kinase involved in the signaling cascade that controls cell proliferation and migration. A variety of substrates and transcription factors are triggered through the activations and phosphorylations of the RAF family proteins, MEK1/2 proteins, and ERKs. These factors control important cellular activities and growth rates (11). Mutations in the *BRAF* gene are linked to high levels of microsatellite instability, which are correlated with lower mortality rates in comparison to microsatellite stable mutations (12). The activation of oncogenes such as *BRAF* also leads to the development of preneoplastic lesions, which frequently cause sporadic CRC cases (10). *BRAF* mutations are also associated with poorer prognosis and therapy resistance, leading to lower survival rates in *BRAF*-mutant cases. The mutation prevalence of *BRAF* in CRC is relatively low, occurring in approximately 10% of cases. However, its increased immune activation and role in pathogenesis make it an important biomarker for possible targeted therapy treatments (13).

RNF43 is a tumor suppressor gene that is also associated with CRC. It encodes a ubiquitin-protein ligase that negatively regulates the WNT signaling pathway by degrading the Frizzled protein receptors to maintain stem cell homeostasis (14). Mutations in *RNF43* frequently occurred alongside other genetic alterations, especially in *KRAS*, *TP53*, and *BRAF*. Genetic alterations in *RNF43* have been associated with both poor prognosis and improved overall survival, depending on the location of the mutation in the N-terminal or C-terminal domains (15). Loss-of-function mutations in *RNF43* lead to enhanced WNT signaling, often resulting in aggressive tumor biology and worse survival. Increased levels of immune-related cells have also been observed in mutated *RNF43* groups compared to wild-type genes, contributing to improved immune environments and overall response rates to treatment (16). *RNF43* mutations are present in 6-18% of CRC cases and are prognostic biomarkers for predicting immunotherapy efficacy and drug sensitivity in CRC treatments (17).

In this study, we assessed the correlations between *APC*, *KRAS*, *BRAF*, and *RNF43* mutations and CRC patient survival. Using a public dataset from Memorial Sloan Kettering Cancer Center, we analyzed patient survival time after diagnosis to determine a correlation between mutation status and overall survival.

METHODS AND MATERIALS

A colorectal cancer dataset (MSK, JNCI 2021) containing data from 1516 patients was downloaded from cBioPortal (Cercek et al., 2021). We accessed patient mutation data and overall survival time in months to analyze various genetic factors. This data was transferred to GraphPad Prism and aligned with the coordinating patient identification codes. Patients were sorted according to mutation status for each gene. Censored and deceased patients were separated, and a Kaplan-Meier survival analysis test was conducted for each gene. Log-rank tests were run to compare the survival curves and the resulting *p* values were used to identify the prognostic significance of mutations in the tested genes.

RESULTS

In order to investigate the relationship between *APC* and the survival of CRC patients, data was exported through cBioPortal sourced from Cercek et al. (2021) that contained clinical and genetic information from 1516 patients. The patients were stratified by *APC* mutation status and we calculated the average survival times after diagnosis for each group (Figure 1a). The average survival time for patients with an *APC* mutation was 41.34 months, while the average survival time for patients without an *APC* mutation was 32.95 months. We generated a Kaplan-Meier curve through GraphPad Prism and conducted a log-rank test to compare the survival of CRC patients based on *APC* mutation status (Figure 1b). The calculated *p* value was 0.002, and it was determined that patients with an *APC* mutation had a significantly increased survival time compared to patients with a wild-type *APC* gene.

We then stratified patients by *KRAS* mutation status and calculated each group's average survival times after diagnosis (Figure 2a). The average survival time was 33.97 months for patients with a present *KRAS* mutation and 43.96 months for patients without a *KRAS* mutation. We conducted a log-rank test on the generated Kaplan-Meier curve and the resulting *p* value was 0.0002 (Figure 2b). We determined that patients with a *KRAS* mutation had a significantly decreased survival time compared to patients with a wild-type *KRAS* gene.

By sorting patients based on *BRAF* mutation status, we calculated the average survival times for each group (Figure 3a). Patients with a *BRAF* mutation had an average survival time of 27.49 months, while patients without a *BRAF* mutation had an average survival time of 40.44 months. A Kaplan-Meier curve was generated and a log-rank test resulted in a *p* value of <0.0001 (Figure 3b). We determined that patients with a *BRAF* mutation

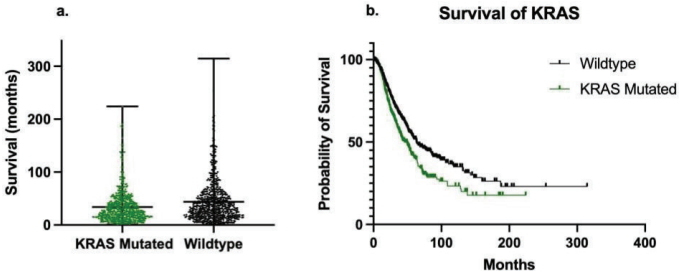

Figure 2. *KRAS*-mutated patients have a decreased survival rate. Data downloaded from cBioPortal and sourced from Cercek et al. (1). **a)** Wildtype (n = 850) and *KRAS* mutated (n = 666); **b)** Kaplan-Meier curve indicates a statistically significant difference in survival between mutated and wildtype *KRAS*. p = 0.0002.

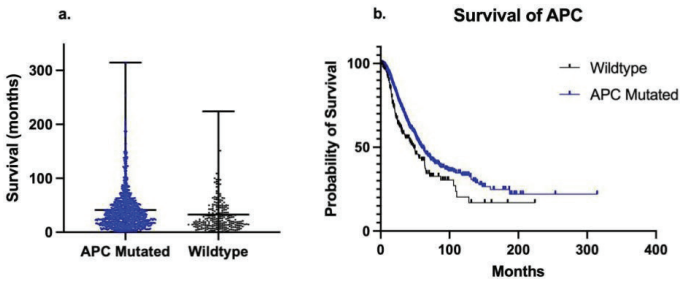

Figure 1. *APC*-mutated patients have an increased survival rate. Data downloaded from cBioPortal and sourced from Cercek et al. (1). **a)** Wildtype (n = 371) and *APC* mutated (n = 1145); **b)** Kaplan-Meier curve indicates a statistically significant difference in survival between mutated and wildtype *APC*. p = 0.002.

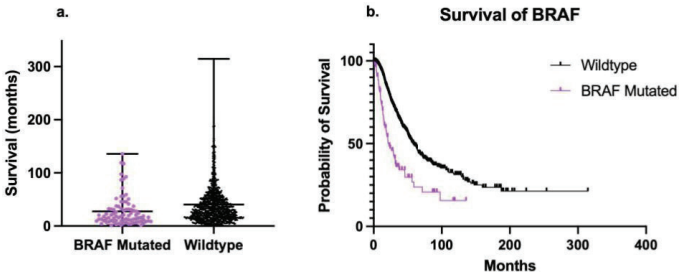

Figure 3. *BRAF*-mutated patients have a decreased survival rate. Data downloaded from cBioPortal and sourced from Cercek et al. (1). **a)** Wildtype (n = 1381) and *BRAF* mutated (n = 135); **b)** Kaplan-Meier curve indicates a statistically significant difference in survival between mutated and wildtype *BRAF*. p < 0.0001.

had a significantly decreased survival time compared to patients with a wild-type *BRAF* gene.

We compared the survival times of patients based on their *RNF43* mutation status and calculated the average survival time after diagnosis of each group (Figure 4a). The average survival time was 24.85 months for patients with an *RNF*43 mutation and 39.92 months for patients without an *RNF43* mutation. Through GraphPad Prism, we generated a Kaplan-Meier survival curve and conducted a log-rank test to compare the survival of patients with and without an *RNF43* mutation (Figure 4b). The calculated *p* value was <0.0001, so we determined that patients with an *RNF43* mutation had a significantly decreased survival time compared to patients with a wild-type *RNF43* gene.

The results for *KRAS*, *BRAF*, and *RNF43* are consistent with published literature that explores the effects of mutations on CRC patient survival. However, the survival results for *APC* mutations may contradict prior studies because of a range of factors like mutation variants. The occurrence of multiple mutations may result in differences in survival due to conflicting mutational effects. The results also do not account for mutation count or type, which could affect patient survival. Other clinical data, like patient demographics and symptoms, were also not included in the analysis, potentially leading to skewed results. While our results show statistically significant differences in patient survival, there is the possibility of selection bias. Although we used a relatively large patient sample to analyze mutation effects, our sample source may not be representative of the general population. Therefore, further research is needed to confirm the generalizability of the results.

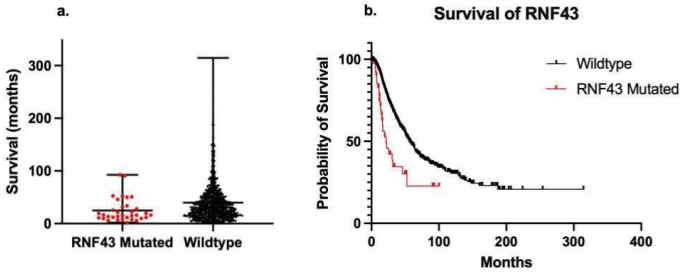

Figure 4. *RNF43*-mutated patients have a decreased survival rate. Data downloaded from cBioPortal and sourced from Cercek et al. (1). **a)** Wildtype (n = 1411) and *RNF43* mutated (n = 105); **b)** Kaplan-Meier curve indicates a statistically significant difference in survival between mutated and wildtype *RNF43*. $p < 0.0001$.

DISCUSSION

The analysis of patient data from Cercek et al. (1) reveals important insights into the relationship between genetic mutations and patient survival in CRC cases. By stratifying patients according to the mutation statuses of *APC, KRAS, BRAF,* and *RNF43*, differences in survival time could be observed, indicating statistically significant results associated with genetic alterations.

APC mutations were associated with an increased survival time compared to wild-type *APC* patients. This finding contradicts previous literature which suggests *APC* mutations are associated with increased tumor growth and decreased survival. However, *APC* truncation counts and other mutational factors may contribute to the increased survival of CRC patients within this dataset. *APC* mutations are also associated with inhibition and crypt homeostasis in the WNT signaling pathway, which opens up possibilities for targeted therapies and improved prognosis (5).

In comparison, *KRAS* mutations were correlated with significantly decreased survival times. The effect of *KRAS* mutations on CRC patient survival has been previously explored due to the central role *KRAS* plays in cell proliferation and tumorigenesis. Many studies support the indication that *KRAS* mutations are associated with decreased survival. Current treatments include *KRAS* inhibitors, which target specific or multiple *KRAS* mutations to target *KRAS* proteins.Through next-generation sequencing, *KRAS* mutations also play an important role in CRC prognosis and personalized therapy treatment (10).

BRAF mutations were associated with a statistically significant decrease in survival in comparison to wild-type *BRAF* genes. *BRAF* mutations have also been associated with increased metastasis and reduced response to therapeutic interventions, according to prior studies. Recent research has also supported the use of *BRAF* as a predictive biomarker for anti-EGFR therapy, although acquired resistance to therapeutic treatment may occur, making it closer to becoming a targetable mutation (13).

RNF43 mutations were also associated with significantly decreased survival times. Although its role in CRC tumorigenesis has been studied less extensively, it has been correlated with both increased and decreased overall survival, complicating the analysis results (16). However, it has been identified as an important prognostic biomarker for immunotherapeutic efficacy and drug sensitivity during CRC treatment, warranting further exploration (17).

These findings highlight the potential for genetic profiling to guide treatment decisions for CRC patients, which has significant implications for patient survival. Future research should explore the effects of genetic mutations on survival and CRC symptoms to design targeted therapies and effective treatment plans that can improve patient prognosis, survival, and recovery.

CONCLUSION

This analysis explores the relationship between genetic mutations and survival times in CRC patients. *APC* mutations are associated with improved survival times, which contradicts existing literature about the role of *APC* in WNT signaling and tumor suppression. However, this conflict may be due to other mutational factors that require further research. In contrast, *KRAS* and *BRAF* mutations are associated with decreased survival times, aligning with previous research and emphasizing the importance of oncogenes in aggressive CRC tumor behavior. *RNF43* mutations are also correlated with lower patient survival time, but prior studies have shown variable prognostic significance, which suggests a need for further investigation. Statistically significant correlations between *APC*, *KRAS*, *BRAF*, and *RNF43* mutations and CRC patient survival times emphasize the role of genetic mutations as prognostic biomarkers and highlight the importance of molecular profiling and immunogenetics in improved CRC treatment. Future research can be used to develop targeted and personalized immunotherapy treatments, leading to improved clinical outcomes and CRC management.

CONFLICT OF INTERESTS

The author declares that there are no conflicts of interest regarding the publication of this article.

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