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Clinical and Biological Impact of KRAS Overexpression in Stomach Adenocarcinoma

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Abstract: This study analyzes Kirsten rat sarcoma viral oncogene (KRAS) expression heterogeneity, and biological and clinical relevance in stomach adenocarcinoma (STAD). The study utilized various tools including UALCAN, GEPIA2, Kaplan-Meier (KM) plotter, cBioPortal, STRING, DAVID, and TIMER 2.0 to conduct this analysis. The results illustrated overexpression of KRAS in STAD and the analysis based on various clinicopathological parameters also verified overexpression of KRAS in STAD. Eventually, this overexpression was linked to poor overall survival (OS) of STAD patients. These results suggested the role of KRAS is involved in the development and progression of STAD. The study also assessed several significant correlations of KRAS expression with promoter methylation tumor purity and immune cell infiltration. Genetic alteration of KRAS revealed to have a strong role in STAD initiation. Gene enrichment analysis highlighted the enrichment of KRAS with various pathways. In conclusion, the findings illustrated the potential of KRAS as a diagnostic, prognostic, and therapeutic biomarker in STAD.

Keywords: KRAS; STAD; Biomarker; Prognosis; Expression variations

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1. Introduction

Cancer is a major devastating disease, leading to millions of deaths annually worldwide. There are above 100 subtypes of cancer based on biological pathways [1–2]. Stomach cancer is the fifth most widespread cancer with 968,350 cases and 659,853 deaths worldwide in 2021 [3]. Adenocarcinoma accounts for roughly more than 90% of all stomach cancer cases. Stomach cancer adenocarcinoma (STAD) has two types which are histologically categorized as diffuse and intestinal, which show variance in epidemiological traits [4–5]. Smoking, alcohol consumption, ethnicity, genetic factors, increasing age, and *Helicobacter pylori infection* are major risk factors for STAD [6–9]. The incidence of STAD varies geographically in the world, with over 50% of cases reported in developing countries. In 2020, the highest number of STAD cases were registered in Asia, and the least were registered in Africa. The incidence rate of STAD is increasing in adults, and males have two times higher risk of developing STAD than females. Surgery, chemotherapy, chemoradiation, and adjuvant therapy are treatments used in STAD. The 5-year survival rate is less than 20% in Asia and varies from 10% to 30% in Europe [10–16]. STAD

diagnosed at higher stages has limited treatment options available, which results in a high mortality rate for STAD. Therefore, it is crucial to evaluate potential diagnostic, therapeutic, and prognostic biomarkers for STAD.

Kirsten rat sarcoma viral oncogene (KRAS) is a member of the RAS family that codes Kirsten rat sarcoma viral oncogene homolog (KRAS) protein. KRAS has a role in various cellular signaling pathways such as the mitogen-activated protein kinase (MAPK) pathway and regulation of cell proliferation. KRAS is highly interactive to GTP because it lacks small molecular binding sites and this results in activation of KRAS. Cell proliferation, cell differentiation, apoptosis, and cellular migration are affected by KRAS, as it initiates the release of signaling molecules facilitating the relay of signals from the cell surface to the nucleus [17–21]. KRAS has a high mutation rate in cancer and mutations are mainly present in, lung adenocarcinoma, colorectal cancer, pancreatic ductal adenocarcinoma, pancreatic cancer, and urogenital cancer [22–23]. KRAS expression is associated with poor prognosis in colorectal cancer and lung cancer [24–25]. Moreover, KRAS mutations are associated with pancreatic cancer, lung cancer, colorectal cancer, and stomach cancer [26–28]. All these data underscore the potential of KRAS as a therapeutic, prognostic, and diagnostic biomarker in many cancers.

This study aimed to conduct a comprehensive analysis of the KRAS gene as a potential biomarker in STAD, as no such analysis has been performed. The study employed different bioinformatics tools to analyze expression, methylation level, mutation, gene enrichment pattern, and prognostic links of KRAS in STAD.

2. Material and method

2.1. UALCAN

UALCAN is a web-based resource utilized for comprehensive cancer OMICS data analysis [29]. The study employed the UALCAN database to analyze KRAS expression in STAD samples. The study investigated promoter methylation OF KRAS and its correlation with expression using the TCGA module of UALCAN. The analysis was based on sample type as well as on different pathological parameters.

2.2. Kaplan-Meier Plotter

Kaplan-Meier (KM) Plotter is an online tool to evaluate the overall survival (OS) of cancer patients based on gene expression [30]. The impact of 54,675 genes on the overall survival of 21 cancers can be evaluated using a KM plotter. The study evaluated the impact of KRAS on the OS of STAD employing a KM plotter. P value < 0.05 is considered significant and the hazard ratio was calculated.

2.3. GEPIA2

Gene expression profiling interactive analysis 2 (GEPIA2) is an online tool that can evaluate gene expression in 84 subtypes of cancer based on GTEx and TCGA databases [31]. GEPIA2 is used to validate the KRAS expression in STAD based on sample types and pathological stages. The prognostic value of KMAS in STAD was also evaluated using GEPIA2.

2.4. cBioPortal

cBioPortal is a public cancer genomic tool intended for interactive investigation of multi-omic cancer datasets [32]. The study employed cBioPortal to evaluate KRAS genetic variation in STAD. The examined information included alteration prevalence, mutation categorization, and copy number variations (CNAs).

2.5. Protein-protein interaction (PPI)

The Search Tool for the Retrieval of Interacting Genes (STRING) is a database that is used for proteomics

analysis [33]. The study employed the STRING database to construct the PPI network of KRAS to observe functional relationships. A *P* value < 0.05 is considered statistically significant.

2.6. Gene enrichment analysis

The Database for Annotation, Visualization, and Integrated Discovery (DAVID) is a web-based comprehensive tool used to elucidate biological functions [34]. The study performed gene ontology (GO) and Kyoto Encyclopedia of Gene and Genomes (KEGG) pathway enrichment analysis of KRAS utilizing the DAVID tool.

2.7. KRAS expression, tumor purity, and immune cell infiltration

The association of gene expression, tumor purity, and immune cell infiltration is analyzed utilizing an online resource TIMER 2.0 [35]. In the current study, the association between KRAS expression, tumor purity, and CD8+ T immune cells is analyzed in STAD using TIMER 2.0. A *P* value < 0.05 is considered statistically significant.

3. Results

3.1. KRAS expression in STAD and normal samples

The study utilized the UALCAN database to analyze the KRAS expression in STAD and normal samples. The analysis demonstrated that KRAS expression was significantly higher in STAD samples in contrast with normal samples (**Figure 1**). The observed *P* value 1.09079967280934E-10 is less than 0.05 which indicates there is a significant difference. This higher expression suggests the role of KRAS in the progression of STAD.

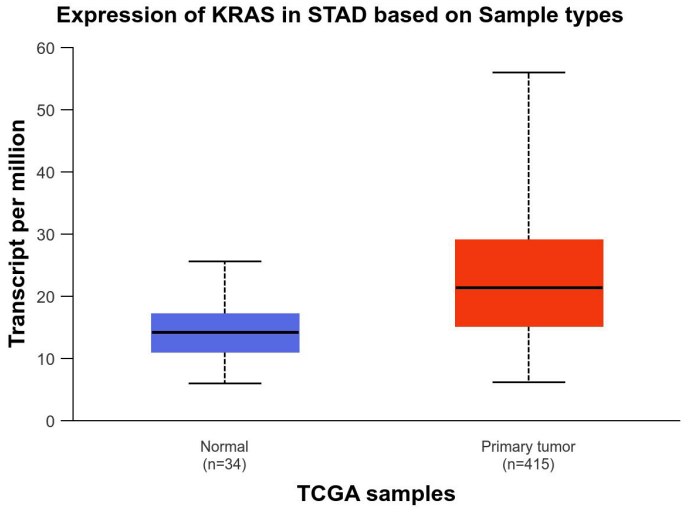

Figure 1: Expression analysis of KRAS in STAD samples and normal samples using UALCAN

3.2. Expression analysis of KRAS in STAD categorized according to various attributes

Simultaneously, the study conducted an analysis of KRAS expression in STAD categorized according to various attributes such as patient's gender, age, race, and pathological stages. First, the study examined expression based on pathological stages and observed that KRAS was statistically up-regulated (*P* value < 0.05) in cancer stages in contrast with the normal sample (**Figure 2A**). Afterward, analysis based on gender revealed that KRAS expression was significantly up-regulated (*P* value < 0.05) in STAD samples as compared to normal samples (**Figure 2B**). Next, up-regulated expression of KRAS was assessed in STAD patient samples of various races (**Figure 2C**). Furthermore, investigation based on STAD patients' age group revealed up-regulation but variation in KRAS expression (**Figure 2D**). KRAS was highly expressed in STAD patient samples of the age

group of 21–40 as compared to the age group of 81–100.

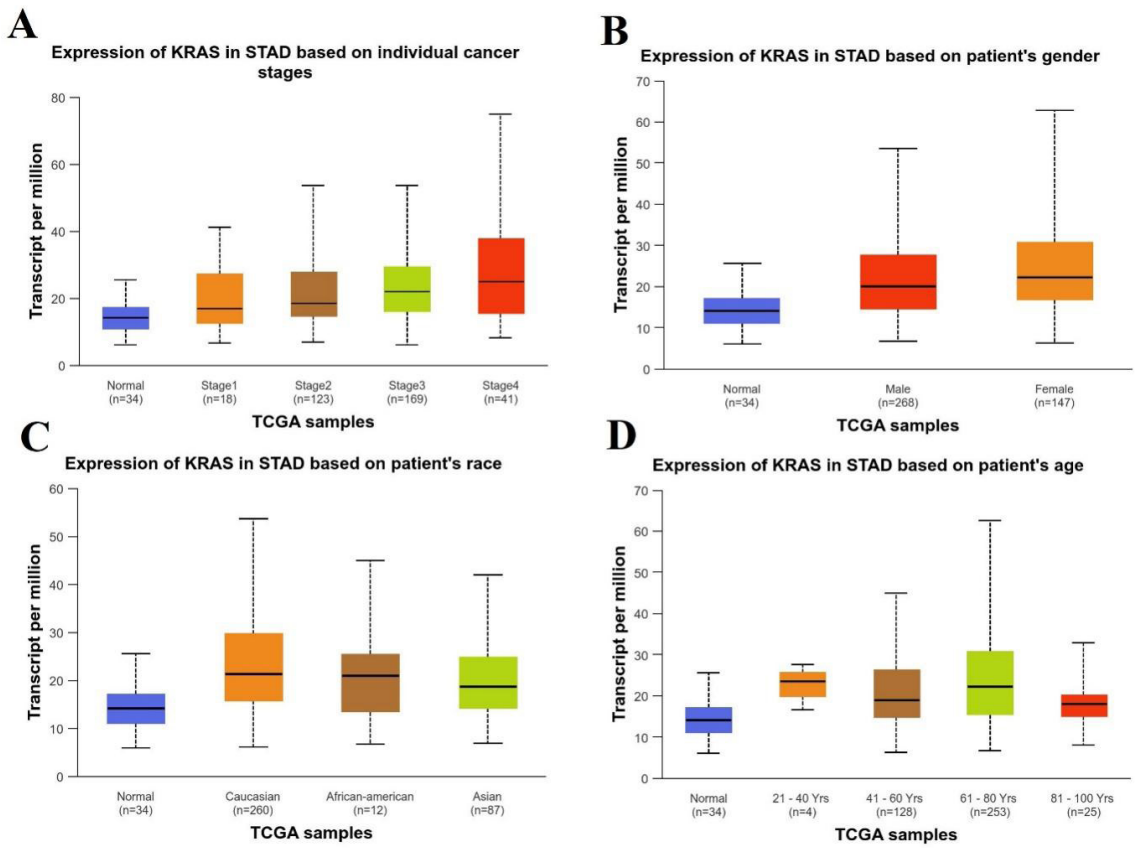

Figure 2. Analysis of KRAS expression in STAD categorized according to various attributes using UALCAN. (A) Analysis of KRAS expression in STAD categorized according to pathological stages. (B) Analysis of KRAS expression in STAD categorized according to the patient's gender. (C) Analysis of KRAS expression in STAD categorized according to the patient's race. (D) Analysis of KRAS expression in STAD categorized according to the patient's age.

3.3. Promoter methylation level of KRAS in STAD and normal

According to prior research, there is an inverse association between expression and promoter methylation. Based on this, the study examined the promoter methylation level of KRAS in STAD using UALCAN [36]. The result highlighted that KRAS was significantly hypomethylated (P value < 0.05) in STAD samples as compared to normal samples (**Figure 3**). This hypomethylation validates the up-regulation of KRAS expression in STAD and reveals that methylation regulates KRAS expression. This investigation indicates the role of KRAS in the progression of STAD.

3.4. Promoter methylation level of KRAS in STAD categorized according to various variables

The analysis of KKRA promoter methylation level in STAD samples was assessed and categorized according to various variables such as patient's age, race, gender, and cancer stages. Assessment based on STAD individual cancer stages revealed that KRAS is significantly (P value < 0.05) hypomethylated in these stages as compared to normal samples (**Figure 4A**). Next, investigation based on gender indicated variation but hypomethylation of KRAS methylation level in STAD. KRAS was highly hypomethylated in STAD male samples in contrast with female samples and vice versa (**Figure 4B**). Furthermore, the analysis revealed that KRAS is significantly

hypomethylated (*P* value < 0.05) in STAD patient samples of different races (**Figure 4C**). Moreover, analysis of KRAS methylation levels in STAD based on patient's age demonstrated variation. KRAS was significantly hypermethylated (*P* value < 0.05) in samples of age group 21–80 and significantly hypomethylated in samples of the remaining age group (**Figure 4D**). This complexity suggests various factors influence the methylation level of KRAS in STAD patient's age samples.

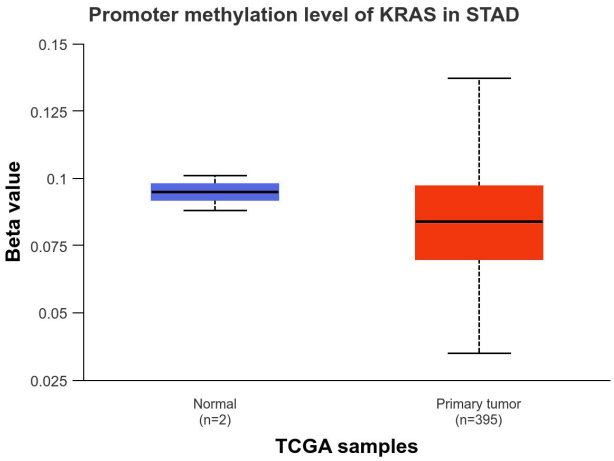

Figure 3. Investigation of the promoter methylation level of the KRAS gene in STAD using the UALCAN database

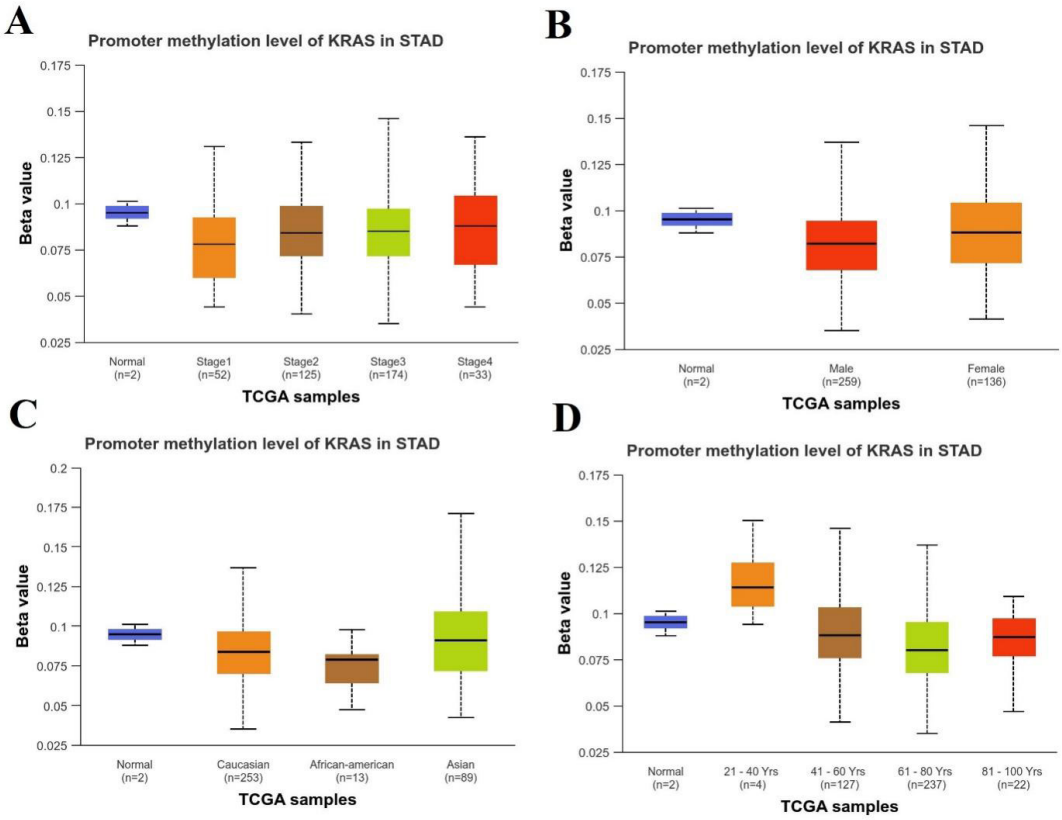

Figure 4. Analysis of KRAS promoter methylation level in STAD categorized according to various attributes using UALCAN. (A) Analysis of KRAS promoter methylation level in STAD categorized according to pathological stages. (B) Analysis of KRAS promoter methylation level in STAD categorized according to patient's gender. (C) Analysis of KRAS promoter methylation level in STAD categorized according to patient's race. (D) Analysis of KRAS promoter methylation level in STAD categorized according to patient's age.

3.5. Prognostic value of KRAS in STAD

The study utilized the KM plotter to examine the impact of KRAS expression on the overall survival (OS) of STAD patients. The investigation indicated that STAD patients with overexpressed KRAS have a low survival rate while patients with lower expressed KRAS have a better survival rate (**Figure 5**). The logrank $P = 0.032$ demonstrates that there is a significant difference and patients with higher KRAS expression have 30% less survival rate hazard ratio HR = 0.7 (0.5–0.97) indicates. Altogether, the results highlight the role of KRAS in the development and proliferation of STAD.

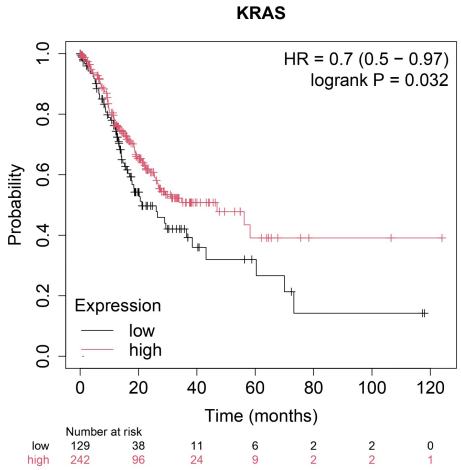

Figure 5. Analysis of the prognostic rate of KRAS in STAD using KM plotter

3.6. Expression and survival analysis using GEPIA2

Simultaneously, GEPIA2 was used to conduct the expression and survival analysis of KRAS in STAD to validate the previous findings. The investigation of KRAS's impact on OS of STAD patients using GEPIA2 demonstrated that there is no significant difference in the prognostic rate of patients with low and high expression (**Figure 6**). The logrank P = 0.9 and HR = 1 suggested no difference.

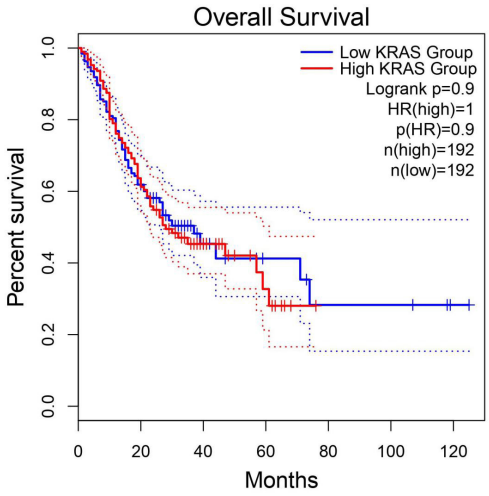

Figure 6. Survival analysis of KRAS in STAD using GEPIA2

The study evaluated the expression of KRAS in STAD in contrast with normal samples using the box plot module of GEPIA2. The study assessed that KRAS was highly expressed in STAD samples compared to normal

samples, but the difference was not significant (**Figure 7A**). Furthermore, the study utilized a stage plot module of GEPIA2 to analyze KRAS expression in STAD pathological stages. The investigation explains that the shape and width of the violin plot are similar, indicating that KRAS expression is equally distributed across these stages (**Figure 7B**). The difference between the KRAS expression is not significant as the *P* value = 0.283.

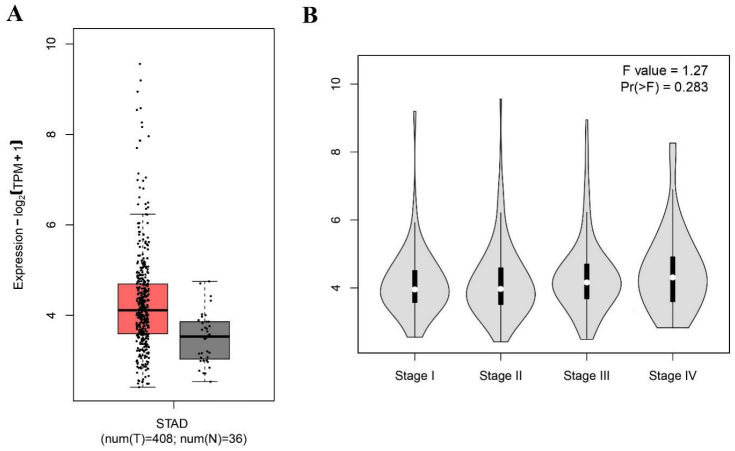

Figure 7. (A) expression analysis of KRAS in STAD using GEPIA2. (B) Expression analysis of KRAS in STAD based on pathological stage using GEPIA2.

3.7. Gene enrichment analysis

The study performed gene enrichment analysis to understand the biological function of KRAS. The study primarily constructed a PPI network using STRING software and assessed 10 strongly associated proteins with KRAS. This explains the diverse association of the KRAS gene and explains its complexity in biological processes (**Figure 8**). Subsequently, employing DAVID software, the study conducted GO and KEGG analysis, evaluating the first four terms for biological process (BP), cellular component (CC), molecular function (MF), and KEGG pathways (**Table 1**).

In GO analysis, the study observed pathways associated with BP, CC, and MF are Ras protein signal transduction, epidermal growth factor receptor signaling pathway, insulin-like growth factor receptor signaling pathway, insulin receptor signaling pathway, cytoplasm, myosin II complex, plasma membrane, cytosol, enzyme regulator activity, calcium ion binding, MAP kinase kinase kinase activity, and protein serine kinase activity. In KEGG analysis, the identified processes are Glioma, neurotrophin signaling pathway, insulin signaling pathway, and Rap1 signaling pathway.

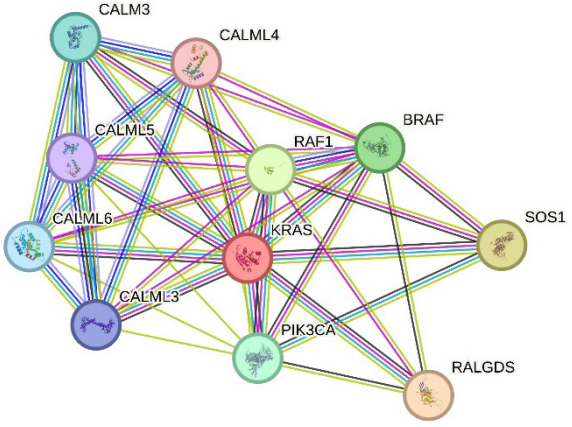

Figure 8. PPI construction of KRAS gene using STRING database

Table 1. GO and KEGG analysis on biological process (BP), cellular component (CC), molecular function (MF), and KEGG pathways

Gene term	Count	Gene	*P* value
BP			
GO:0007265~Ras protein signal transduction	5	BRAF, KRAS, RAF1, SOS1, RALGDS	4.7867092655479335E-8
GO:0007173~epidermal growth factor receptor signaling pathway	4	PIK3CA, BRAF, KRAS, SOS1	1.8957134610072728E-6
GO:0048009~insulin-like growth factor receptor signaling pathway	3	PIK3CA, RAF1, SOS1	9.105920236010638E-5
GO:0008286~insulin receptor signaling pathway	3	PIK3CA, RAF1, SOS1	4.5202316281570294E-4
CC			
GO:0005737~cytoplasm	8	PIK3CA, CALML6, BRAF, KRAS, CALM3, CALML3, RAF1, SOS1	0.002495574955179402
GO:0016460~myosin II complex	2	CALML6, CALM3	0.014132528548431004
GO:0005886~plasma membrane	7	PIK3CA, BRAF, KRAS, CALM3, RAF1, SOS1, RALGDS	0.014211972791745705
GO:0005829~cytosol	7	PIK3CA, BRAF, KRAS, CALM3, RAF1, SOS1, RALGDS	0.014969131029062653
MF			
GO:0030234~enzyme regulator activity	4	CALML5, CALML6, CALM3, CALML3	2.5450179866138044E-7
GO:0005509~calcium ion binding	5	CALML5, CALML6, BRAF, CALM3, CALML3	2.3862462406048352E-4
GO:0004709~MAP kinase kinase kinase activity	2	BRAF, RAF1	0.009750233042020853
GO:0106310~protein serine kinase activity	3	PIK3CA, BRAF, RAF1	0.011817435270777117
KEGG			
hsa05214:Glioma	9	PIK3CA, CALML5, CALML6, BRAF, KRAS, CALM3, CALML3, RAF1, SOS1	1.8264638958926261E-16
hsa04722:Neurotrophin signaling pathway	9	PIK3CA, CALML5, CALML6, BRAF, KRAS, CALM3, CALML3, RAF1, SOS1	8.098283893995263E-15
hsa04910:Insulin signaling pathway	9	PIK3CA, CALML5, CALML6, BRAF, KRAS, CALM3, CALML3, RAF1, SOS1	2.551728046806132E-14
hsa04015:Rap1 signaling pathway	9	PIK3CA, CALML5, CALML6, BRAF, KRAS, CALM3, CALML3, RAF1, RALGDS	8.46507455081279E-13

3.8. Infiltration level of CD8+ T and tumor purity analysis of KRAS

It was proposed that variation in KRAS expression may have an association with infiltration level of CD8+ T and tumor purity, as KRAS regulates various pathways like the epidermal growth factor receptor signaling pathway and insulin signaling pathway. Therefore, the study assessed the correlation among infiltration level of CD8+ T, tumor purity, and KRAS expression within STAD using TIMER 2.0. In the left plot, the investigation of tumor purity demonstrated a weak negative correlation (Rho = 0.035, $P = 0.493$). This reveals that tumor purity and KRAS expression have no significant correlation. However, in the right plot, the assessment of CD8+ T highlighted a weak positive correlation as calculated values were Rho = 0.117 and $P = 0.00223$. This

reveals a significant correlation between up-regulated KRAS expression and the infiltration level of CD8+ T (**Figure 9**). These findings illustrated the potential role of KRAS expression in immune cell infiltrations.

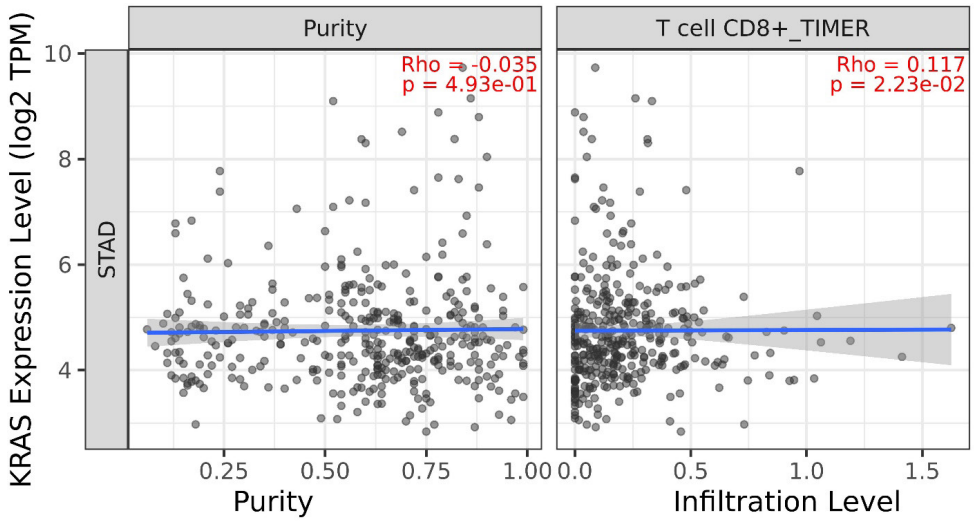

Figure 9. Analysis of correlation of infiltration level of CD8+ T and tumor purity between KRAS expression in STAD using TIMER 2.0

3.9. Genetic alteration of KRAS in STAD

In the study, the assessment of genetic alteration of KRAS in STAD was evaluated using cBioPortal. The analysis revealed that in 16% of genetic mutations of the KRAS gene in STAD, amplification, missense mutation (unknown significance), and missense mutation (putative driver) are observed mutations (**Figure 10**). This suggested that KRAS genetic mutation has a role in the progression and development of STAD.

Figure 10. Genetic mutation of KRAS gene in STAD employing cBioPortal

4. Discussion

Cancer is a major threat with millions of mortalities worldwide [37]. Over 90% of stomach cancers are adenocarcinoma and account for thousands of deaths worldwide. It is the cancer with the fifth highest mortality worldwide. STAD is diagnosed at later stages and limited treatments are available. Therefore, the five-year survival rate is just 10%–30% [38–39]. Thus, there is an urgent need to identify useful diagnostic, therapeutic, and prognostic biomarkers of STAD. KRAS is an oncogene and encodes GTPase transductor protein. KRAS protein can become inactive and vice versa and has a role in cell division [40–41]. KRAS is a highly mutated gene in cancers and is linked with many cancers including pancreatic ductal adenocarcinoma (PDAC), colorectal cancer (CRC), and non-small-cell lung cancer (NSCLC).

The current study started an investigation to evaluate the role of KRAS as a potential biomarker in STAD. The analysis illustrated the up-regulation of KRAS expression in STAD and revealed that the overexpression

is significant (*P* value = 0.05). Overexpression of KRAS was also determined to be associated with poor OS of STAD patients. Collectively, these findings suggested the role of KRAS in the development and progression of STAD. Furthermore, the study examined the expression of KRAS in STAD based on different clinicopathological parameters and overexpression was evaluated.

Furthermore, the study performed an analysis of promoter methylation and genetic alteration as these factors have an impact on the expression of KRAS. The analysis of KRAS promoter methylation using UALCAN revealed a negative correlation with KRAS overexpression, as KRAS was hypomethylated in STAD. Moreover, 16% of KRAS genetic alteration was assessed in STAD by using cBioPortal. This suggested that mutation strongly regulates KRAS expression and has a role in the progression of STAD. Altogether these results suggested that promoter hypomethylation and genetic alteration have a strong role in the overexpression of KRAS in STAD. The study utilized GEPIA2 to analyze KRAS expression in STAD and analyzed that KRAS was overexpressed. This analysis validates that overexpression of KRAS leads to progression of STAD. In recent years, several studies have identified STAD-related biomarkers such as MAGEA11, FASTKD1, IRF7, CHAC1, NOX4, and HIF1A [42–44]. However, to our knowledge, up until now neither these nor other biomarkers have been applied to STAD patients with diverse clinicopathological profiles. This study analyzed significant (*P* value = 0.05) overexpression of KRAS in STAD based on different clinicopathological variables such as patient's age, gender, race, and individual cancer stages. Moreover, KRAS promoter methylation, progression value, and genetic alteration also validate its usefulness as a potential diagnostic, therapeutic, and prognostic biomarker.

Furthermore, the analysis of KRAS's relation with the infiltration level of CD8+ T and tumor purity revealed a significant (*P* value < 0.05) positive correlation with the infiltration level of CD8+ T and a weak negative correlation with tumor purity. These findings highlight the tumor microenvironment of STAD. Moreover, the PPI network illustrated the association of KRAS with 10 other different genes and this explains the diversity of the KRAS gene. Next, enrichment analysis revealed the linked pathways of KRAS and associated genes. Ras protein signal transduction, epidermal growth factor receptor signaling pathway and enzyme regulator activity are some associated pathways. In KEGG analysis, the identified processes are glioma, neurotrophin signaling pathway, insulin signaling pathway, and Rap1 signaling pathway. Some of these pathways such as Ras protein signal transduction, epidermal growth factor receptor signaling pathway, and Rap1 signaling pathway have been associated with various biological functions including cell cycle, cell proliferation, survival, and immunity [45–47]. Dysregulation of these processes is one of the main reasons for cancer progression. Thus, these results highlight the role of KRAS in STAD progression.

5. Conclusion

The present study comprehensively analyzed KRAS expression and the association of expression with different variables in STAD utilizing various bioinformatics tools. The advantages of this in silico study include wide-ranging study samples, cost efficiency, and expansion abilities for comprehensive functional and genomic analysis. The findings highlighted the potential of KRAS as a prognostic, diagnostic, and therapeutic biomarker of STAD. However, further testing is needed before it can be used in clinical practice.

Disclosure statement

The authors declare no conflict of interest.

References

[1] Upadhyay A, 2021, Cancer: An Unknown Territory; Rethinking before Going Ahead. Genes & Diseases, 8(5): 655–661.

[2] Fares J, Fares MY, Khachfe HH, et al., 2020, Molecular Principles of Metastasis: A Hallmark of Cancer Revisited. Signal Transduction and Targeted Therapy, 5(1): 28.

[3] Bray F, Laversanne M, Sung H, et al., 2024, Global Cancer Statistics 2022: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians, 74(3): 229–263.

[4] Deng W, Jin L, Zhuo H, et al., 2021, Alcohol Consumption and Risk of Stomach Cancer: A Meta-Analysis. Chemico-Biological Interactions, 2021(336): 109365.

[5] Yao Q, Qi X, Xie SH, 2020, Sex Difference in the Incidence of Cardia and Non-cardia Gastric Cancer in the United States, 1992–2014. BMC Gastroenterology, 2020(20): 1–7.

[6] Minami Y, Kanemura S, Oikawa T, et al., 2018, Associations of Cigarette Smoking and Alcohol Drinking with Stomach Cancer Survival: A Prospective Patient Cohort Study in Japan. International Journal of Cancer, 143(5):1072–1085.

[7] Alagesan P, Goodwin JC, Garman KS, et al., 2023, Cancer Progress and Priorities: Gastric Cancer. Cancer Epidemiology, Biomarkers & Prevention, 32(4): 473–486.

[8] Zhang R, Li H, Li N, et al., 2021, Risk Factors for Gastric Cancer: A Large-scale, Population-based Case-control Study. Chinese Medical Journal, 134(16): 1952–1958.

[9] Shah D, Bentrem D, 2022, Environmental and Genetic Risk Factors for Gastric Cancer. Journal of Surgical Oncology, 125(7): 1096–1103.

[10] Morgan E, Arnold M, Camargo MC, et al., 2022, The Current and Future Incidence and Mortality of Gastric Cancer in 185 Countries: A Population-based Modeling Study. eClinicalMedicine, 2022(47): 101404.

[11] Sung H, Ferlay J, Siegel RL, et al., 2021, Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians, 71(3): 209–249.

[12] Asplund J, Kauppila JH, Mattsson F, 2018, Survival Trends in Gastric Adenocarcinoma: A Population-based Study in Sweden. Annals of Surgical Oncology, 2018(25): 2693–2702.

[13] Rogers JE, Ajani JA, 2023, Recent Advances in the Management of Gastric Adenocarcinoma Patients. Faculty Reviews, 2023(12): 2.

[14] Ferlay J, Colombet M, Soerjomataram I, et al., 2021, Cancer Statistics for the Year 2020: An Overview. International Journal of Cancer, 149(4): 778–789.

[15] Fitzmaurice C, Allen C, Barber RM, et al., 2017, Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived with Disability, and Disability-adjusted Life-years for 32 Cancer Groups, 1990 to 2015: A Systematic Analysis for the Global Burden of Disease Study. JAMA Oncology, 3(4): 524–548.

[16] Ilic M, Ilic I, 2022, Epidemiology of Stomach Cancer. World Journal of Gastroenterology, 28(12): 1187–1203.

[17] Sheffels E, Kortum RL, 2021, The Role of Wild-type RAS in Oncogenic RAS Transformation. Genes, 12(5): 662.

[18] Kontomanolis EN, Koutras A, Syllaios A, et al., 2020, Role of Oncogenes and Tumor-suppressor Genes in Carcinogenesis: A Review. Anticancer Research, 40(11): 6009–6015.

[19] Fernández-Medarde A, De Las Rivas J, Santos E, 2021, 40 Years of RAS: A Historic Overview. Genes, 12(5): 681.

[20] Stalnecker CA, Der CJ, 2020, RAS, Wanted Dead or Alive: Advances in Targeting RAS Mutant Cancers. Science Signaling, 13(624): eaay6013.

[21] Apken LH, Oeckinghaus A, 2021, The RAL Signaling Network: Cancer and Beyond. International Review of Cell and Molecular Biology, 2021(361): 21–105.

[22] Timar J, Kashofer K, 2020, Molecular Epidemiology and Diagnostics of KRAS Mutations in Human Cancer. Cancer

and Metastasis Reviews, 39(4): 1029–1038.

[23] Won Y, Choi E, 2022, Mouse Models of Kras Activation in Gastric Cancer. Experimental & Molecular Medicine, 54(11): 1793–1798.

[24] Zhou Y, Zeng Z, Li Z, et al., 2024, The Relationship of KRAS Expression with KRAS Status, Prognosis, and Tumor-infiltrated T Lymphocytes in Colorectal Cancer. Therapeutic Advances in Gastroenterology, 2024(17): 17562848241249387.

[25] Pazik M, Zebrowska-Nawrocka M, Wosiak A, et al., 2023, Comprehensive Analysis of the Expression and Clinical Significance of RAS Family Members in Non-Small Cell Lung Cancer Based on Bioinformatics Data and the A549 Cell Line Model. Applied Sciences, 13(1): 166.

[26] Polom K, Das K, Marrelli D, et al., 2019, KRAS Mutation in Gastric Cancer and Prognostication Associated with Microsatellite Instability Status. Pathology & Oncology Research, 25(1): 333–340.

[27] Luo J, 2021, KRAS Mutation in Pancreatic Cancer. Seminars in Oncology, 48(1): 10–18.

[28] Eklund EA, Wiel C, Fagman H, et al., 2022, KRAS Mutations Impact Clinical Outcome in Metastatic Non-Small Cell Lung Cancer. Cancers, 14(9): 2063.

[29] Chandrashekar DS, Karthikeyan SK, Korla PK, et al., 2022, UALCAN: An Update to the Integrated Cancer Data Analysis Platform. Neoplasia, 2022(25): 18–27.

[30] Lanczky A, Gyorffy B, 2021, Web-Based Survival Analysis Tool Tailored for Medical Research (KMplot): Development and Implementation. Journal of Medical Internet Research, 23(7): e27633.

[31] Tang Z, Kang B, Li C, et al., 2019, GEPIA2: An Enhanced Web Server for Large-scale Expression Profiling and Interactive Analysis. Nucleic Acids Research, 47(W1): 556–560.

[32] Ramos M, Geistlinger L, Oh S, et al., 2020, Multiomic Integration of Public Oncology Databases in Bioconductor. JCO Clinical Cancer Informatics, 2020(1): 958–971.

[33] Szklarczyk D, Gable AL, Lyon D, et al., 2019, STRING v11: Protein-protein Association Networks with Increased Coverage, Supporting Functional Discovery in Genome-wide Experimental Datasets. Nucleic Acids Research, 47(D1): 607–613.

[34] Sherman BT, Hao M, Qiu J, et al., 2022, DAVID: A Web Server for Functional Enrichment Analysis and Functional Annotation of Gene Lists (2021 Update). Nucleic Acids Research, 50(W1): 216–221.

[35] Li T, Fu J, Zeng Z, et al., 2020, TIMER 2. 0 for Analysis of Tumor-infiltrating Immune Cells. Nucleic Acids Research, 48(W1): 509–514.

[36] Wagner JR, Busche S, Ge B, et al., 2014, The Relationship between DNA Methylation, Genetic and Expression Inter-individual Variation in Untransformed Human Fibroblasts. Genome Biology, 2014(15): 1–17.

[37] Alzahrani SM, Al Doghaither HA, Al-Ghafari AB, 2021, General Insight into Cancer: An Overview of Colorectal Cancer (Review). Molecular and Clinical Oncology, 15(6): 271.

[38] Tuo JY, Bi JH, Yuan HY, et al., 2022, Trends of Stomach Cancer Survival: A Systematic Review of Survival Rates from Population-based Cancer Registration. Journal of Digestive Diseases, 23(1): 22–32.

[39] Iwu CD, Iwu-Jaja CJ, 2023, Gastric Cancer Epidemiology: Current Trend and Future Direction. Hygiene, 3(3): 256–268.

[40] Chhichholiya Y, Singh HV, Vashistha R, et al., 2024, Deciphering the Role of KRAS Gene in Oncogenesis: Focus on Signaling Pathways, Genetic Alterations in 3'UTR, KRAS Specific miRNAs and Therapeutic Interventions. Critical Reviews in Oncology/Hematology, 2024(194): 104250.

[41] Huang L, Guo Z, Wang F, et al., 2021, KRAS Mutation: from Undruggable to Druggable in Cancer. Signal Transduction and Targeted Therapy, 6(1): 386.

[42] Xiao C, Yang L, Jin L, et al., 2022, MAGEA11 as a STAD Prognostic Biomarker Associated with Immune

Infiltration. Diagnostics, 12(10): 2506.

[43] Yang Y, Gao Y, Liu XS, et al., 2024, FASTKD1 as a Diagnostic and Prognostic Biomarker for STAD: Insights into M6a Modification and Immune Infiltration. Experimental and Therapeutic Medicine, 28(2): 1–16.

[44] Xiao R, Wang S, Guo J, et al., 2022, Ferroptosis-related Gene NOX4, CHAC1, and HIF1A are Valid Biomarkers for Stomach Adenocarcinoma. Journal of Cellular and Molecular Medicine, 26(4): 1183–1193.

[45] Kolch W, Berta D, Rosta E, 2023, Dynamic Regulation of RAS and RAS Signaling. The Biochemical Journal, 480(1): 1–23.

[46] Uribe ML, Marrocco I, Yarden Y, 2021, EGFR in Cancer: Signaling Mechanisms, Drugs, and Acquired Resistance. Cancers (Basel), 13(11): 2748.

[47] Singh B, Kosuru R, Lakshmikanthan S, et al., 2021, Endothelial Rap1 (Ras-Association Proximate 1) Restricts Inflammatory Signaling to Protect from the Progression of Atherosclerosis. Arteriosclerosis, Thrombosis, and Vascular Biology, 41(2): 638–650.