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Exploring KRAS Expression Heterogeneity In Esophageal Carcinoma: Implications For Prognosis, Therapy, And Diagnosis

Syed Sabir Ali Shah[1], Madiha Zaynab[2], Jallat Khan[3], Sammina Mahmood[4], Bushra Munir[5], Hafsa Ilyas[6], Nimra Arshad[7], Abdullah Sethar[8], Tamseel Saleem[9], Kashif Prince[10], Rabab Akhtar[11], Muhammad Arif Rizwan[12*], Anam Aftab[13]

[1]Livestock and Fisheries Department, Government of Sindh, Pakistan
[2]Institute of Biological Sciences, Khwaja Fareed University of Engineering and Information Technology, Rahim Yar Khan, Pakistan
[3]Institute of Chemistry, Faculty of Natural and Applied Sciences, Khwaja Fareed University of Engineering & Information Technology, Rahim Yar Khan, Punjab, Pakistan
[4]Department of Botany, Division of Science and Technology, University of Education, Lahore, Pakistan
[5]Department of Chemistry, Institute of Chemistry, University of Sargodha, Sargodha, Pakistan
[6]Institute of Physiology and Pharmacology, University of Agriculture Faisalabad, Faisalabad, Pakistan
[7]Department of Biochemistry, Institute of Chemistry, GC University Faisalabad, Pakistan
[8]Livestock Breeding Services Authority (LBSA) Sindh, Livestock & Fisheries Department, Government of Sindh, Pakistan
[9]Department of Biochemistry and Physiology, Faculty of Animal Husbandry and Veterinary Sciences (AHVS) , Sindh Agriculture University, Tandojam, Pakistan
[10]Department of Medicine, Cholistan University of Veterinary & Animal Sciences, Bahawalpur, Pakistan
[11]Department of Food Science and Technology-Faculty of Food Science and Nutrition Bahauddin Zakariya University, Multan, Pakistan
[12*]Institute of Continuing Education and Extension, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Punjab, Pakistan
[13]Ikram ul Haq institute of industrial Biotechnology, Government College University, Lahore, Katchery Rd 54000, Pakistan

Corresponding Author: Muhammad Arif Rizwan
*Institute of Continuing Education and Extension, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Punjab, Pakistan, marifrizwan@cuvas.edu.pk

Abstract
Esophageal carcinoma (ESCA) accounts for the seventh-highest global cancer mortality rate; the elevated mortality rates are attributed to treatment resistance and recurrence. This study aimed to analyze the molecular and clinical significance of the Kirsten rat sarcoma virus oncogene (KRAS) expression heterogeneity in ESCA. This bioinformatics investigation demonstrated KRAS overexpression in ESCA based on sample analysis and confirmed overexpression based upon various clinicopathological characteristics. Furthermore, the poor overall survival (OS) of ESCA patients is linked with this overexpression of KRAS. Next, KRAS was identified to be enriched with a number of pathways via gene enrichment analysis. Additionally, the correlation of KRAS expression and methylation level, immune cell infiltration, and genetic modification was examined. Overall, these evaluations demonstrated that KRAS is implicated in both the progression and development of esophageal cancer (ESCA). In conclusion, our results demonstrated that KRAS has potential in ESCA as a prognostic, therapeutic, and diagnostic biomarker.

Keywords: Cancer: Therapy: Diagnosis: Biomarker

Introduction

Cancer has grown into universal health problem with steadily increasing cases and mortalities. In 2022, about 20 million cancer cases were registered with 9.7 million mortalities. Among the most widespread gastrointestinal tract (GI) cancers is esophagus cancer (EC). In accordance with previous studies EC eleventh most prevalent cancer and seventh cancer with high mortalities (1-5). Lately, there has been an overall decline in the incidence rate of EC. Its 5-year relative survival rate is only 20%, the survival rate ranks second lowest, following pancreatic cancer (10%), although its mortality rate is still significant (6-9). Esophageal carcinoma (ESCA) accounts for more than 90% of EC, which is divided into two subtypes adenocarcinoma and esophageal squamous cell carcinoma (SCC). The Prevalence and mortality rates among the sexes by Ranges from two to five times, with males accounting for about 70% of cases (10-12). Gastroesophageal reflux disease (GERD), obesity, poor diet, alcohol consumption and tobacco are some major risk factors of ESCA (130). Furthermore, middle-aged and older populations have higher incidence of ESCA (10, 14). The principle sites of tumor involvement are exophagogastric junction, thoracic esophagus and cervical esophagus, with the thoracic esophagus having higher 86% prevalence (15). ESCA have higher mortalities and a complex malignancy recognized for its treatment difficulty. Together with

a deeper comprehension of genetics and the tumor microenvironment (TME), numerous cutting-edge therapies and methodologies are employed, including target therapy, endotherapy, staging, surgery, and scientific analysis. Tumor DNA in circulation is one promising biomarker (16-19). Despite progress, no significant therapeutic benefit is seen, leading to negligible increases in survival. Therefore, it's a crucial need to identify significant therapeutic, prognostic and diagnostic biomarker for EASC. Consequently, the identification of important therapeutic, prognostic, and diagnostic biomarkers for EASC is imperative. KRAS, or Kirsten Rat Sarcoma Viral Oncogene, belongs to the RAS family and codes for The Kirsten rat sarcoma 2viral oncogene homolog protein (KRAS) (20-22). Most well-known oncogene, KRAS, has the greatest mutation rate of any cancer and is linked to several extremely deadly diseases, such as colorectal cancer (CRC), non-small cell lung cancer (NSCLC), and pancreatic ductal adenocarcinoma (PDAC) (23, 24). It is estimated that KRAS gene mutations account for around 25% of all cancers. Whereas, On the other hand, prostate cancer shown to have lower mutation rate (7%), whereas pancreatic tumors have high mutation rates (90%). The KRAS gene may simultaneously have several mutations that heighten tumor-promoting activity (25, 26). It codes K-Ras protein that is GTPase and act as a switch that coverts GTP to GDP. The paucity of tiny molecule binding sites in KRAS causes it to be extremely interacting with GTP, which in turn activates KRAS. KRAS triggers the release of signaling molecules that enable the transmission of signals from the cell surface to the nucleus, hence affecting cell proliferation, differentiation, apoptosis, and migration. Several biological signaling pathways, including the mitogen-activated protein kinase (MAPK) pathway and the control of cell proliferation, are mediated by KRAS (27, 28). The prognosis was poorest for the colon cancer patients with a KRAS mutation (29). It is stated that KRAS amplifications linked to poor prognosis of ESCA (30). Non-small-cell lung cancer has been linked to KRAS expression (31).
These data demonstrate the potential of KRAS as a diagnostic, therapeutic, and diagnostic biomarker. We planned to analyze KRAS in ESCA using bioinformatics in this work. Since no such analysis has been done before, the goal of this work was to do a thorough examination of the KRAS gene as a possible biomarker in ESCA. We evaluated KRAS expression, mutation, survival and gene enrichment analysis in ESCA using bioinformatics methods.

Methodology

Expression analysis of KRAS

The UALCAN website is widely used for multi-omics analysis of TCGA cancer data; it is based on the TCGA OMICS data (32). In this work, we used the UALCAN default settings to assess the expression and methylation profiling of KRAS expression patterns in ESCA. Additionally, we examined methylation and expression separately according to other parameters such as ESCA patient's age, gender, race and clinical stages. The Scores with a p-value less than 0.05 were deemed significant.

Survival analysis

A n easy-to-use web application called the Kaplan-Meier (KM) plotter can calculate the survival values of the gene(s) in human cancer (33). In current study, we assess the overall survival (OS) analysis of KRAS in ESCA based on GEO and TCGA datasets using the KM Plotter with default parameters. There was also a determination and display of a p-value, 95% confidence interval (CI), and hazard ratio (HR).

Ratification using GEPIA2

GEPIA2 database provides a trustworthy comprehensive study of TCGA data associated to cancer (34). We used GEPIA2 to further ratify expression and survival analysis of KRAS in ESCA. We utilized box plot and stage plot modules of GEPIA2 to evaluate KRAS expression in ESCA based on normal samples and clinical stages. Moreover, using GEPIA2, a survival analysis of KRAS was also assessed. $P < 0.05$ was set as statistically significant.

Genetic variation of KRAS

An online resource that helps researchers analyze large amounts of data connected to cancer is the (35). In current study, cBioPortal was used to assess the KRAS-related genetic mutational and copy number variation (CNV) patterns in ESCA.

Protein–Protein Interaction Network Development and Pathway Analysis

In our study, The PPI network of KRAS-enriched genes was constructed using the STRING (36) database. Thereafter, the DAVID tool(37) was employed to conduct the pathway enrichment analysis of genes involved in the KRAS network. $P < 0.05$ was considered statistically significant.

KRAS expression and immune cell infiltration

A database called Tumor Immune Estimation Resource (TIMER) 2.0 is designed to assess immune cell infiltration in over 30 different cancer subtypes(38). This tool is user friendly and allows you to examine the Spearman association between immune infiltrate and tumor gene expression by utilizing TCGA. In this study, employing the TIMER2.0 database, we assessed the Spearman association between the infiltration of CD8+ T immunological cells, B cells, and macrophages and the KRAS expression tumor purity in ESCA**.**

Results

Analysis of KRAS expression in ESCA and normal control samples

We analyzed KRAS expression in ESCA samples contrasting with normal control sample using UALCAN database. The investigation demonstrated that, ESCA samples had elevated levels of KRAS expression than normal samples did (**Figure 1**). The computed p-value of 5.604400E-04 indicated that there was a significant difference between the two components. This up-regulation revealed the role of KRAS in ESCA progression.

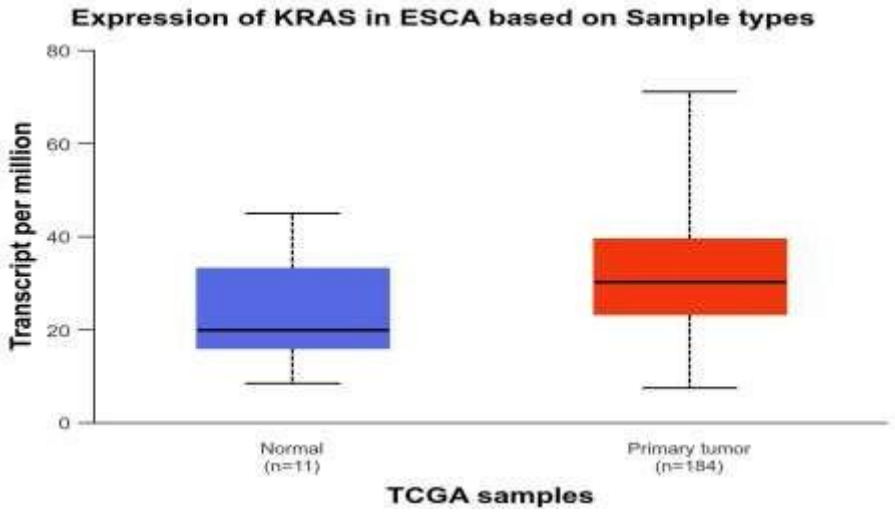

Figure 1: The KRAS gene expression analysis in ESCA and normal control samples using UALCAN.

KRAS expression analysis in ESCA segmented according to numerous factors

Continuing our analysis, KRAS expression was examined in ESCA segmented based on numerous factors such as patient's gender, races, age and clinical stages. Primarily, analysis of KRAS expression based upon clinical stages demonstrated variation but significant overexpression than normal samples (**Figure 2A**). We observed variations as KRAS was highly overexpressed in HNSC stage 4 samples in contrast with stage 1 samples and vice versa. Thereafter, analysis based on the age group of ESCA patient's revealed significant up-regulation (p-value<0.05) in KRAS expression compared to normal samples. The investigation also showed variance as KRAS expression in patient's samples of age group 21-40 was significantly overexpressed than the samples of age group 61-80 and others (**Figure 2B**). Following this, assessment based upon ESCA patient's gender and race disclosed significant overexpression in tumor samples contrasting with normal samples (**Figure 2C-D**).

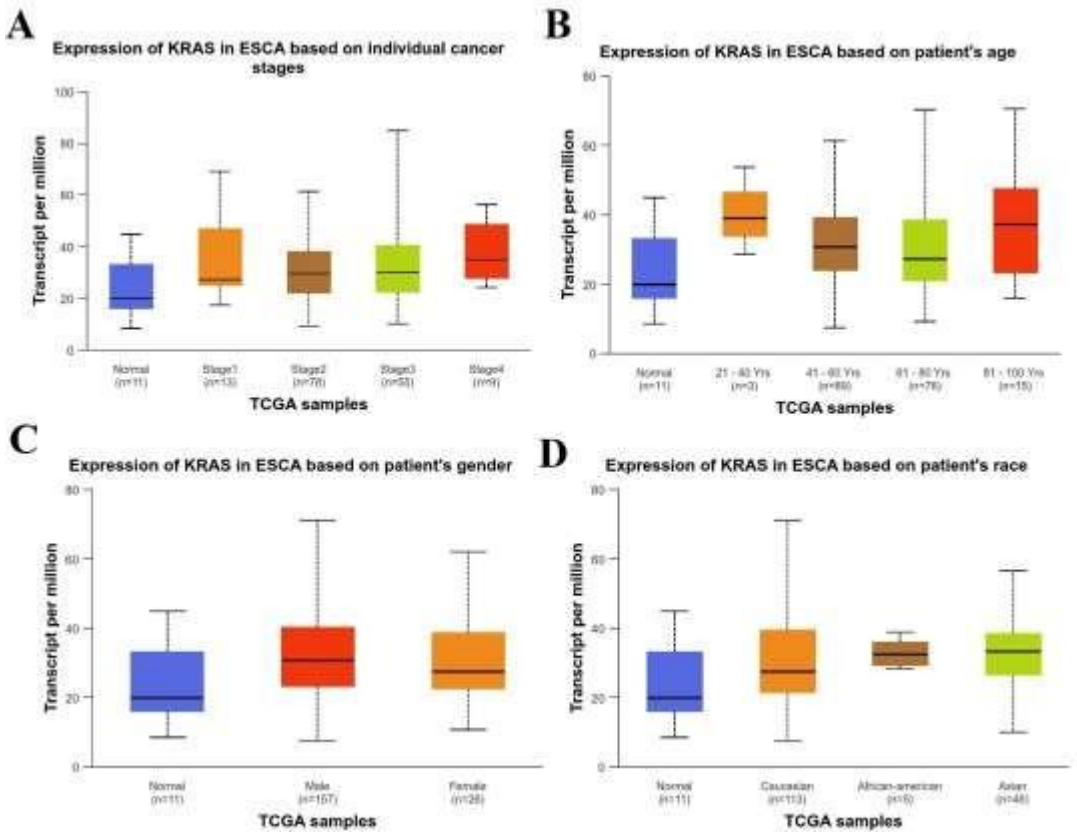

Figure 2: UALCAN was used to evaluate and characterize KRAS expression in ESCA based on several criteria. (A)Evaluation of KRAS expression in ESCA stratified according to pathological phases. (B) Evaluation of KRAS expression in ESCA stratified according to patients age. (C) Evaluation of KRAS expression in ESCA stratified according to patients gender. (D) Evaluation of KRAS expression in ESCA stratified according to patients races.

Analyzing promoter methylation level of KRAS in ESCA and normal samples

We employed UALCAN database to assess promoter methylation level of KRAS in ESCA samples in comparison with normal samples. The investigation indicated that tumor samples had lower levels of KRAS methylation than normal samples (**Figure 3**). Since the computed p-value (9.609200E-01) is more than 0.05, the difference is not significant. Previously it's been stated that gene promoter methylation and expression are negatively correlated (39).Hence, the hypomethylation of KRAS in ESCA samples corroborate the overexpression of KRAS in those same samples.

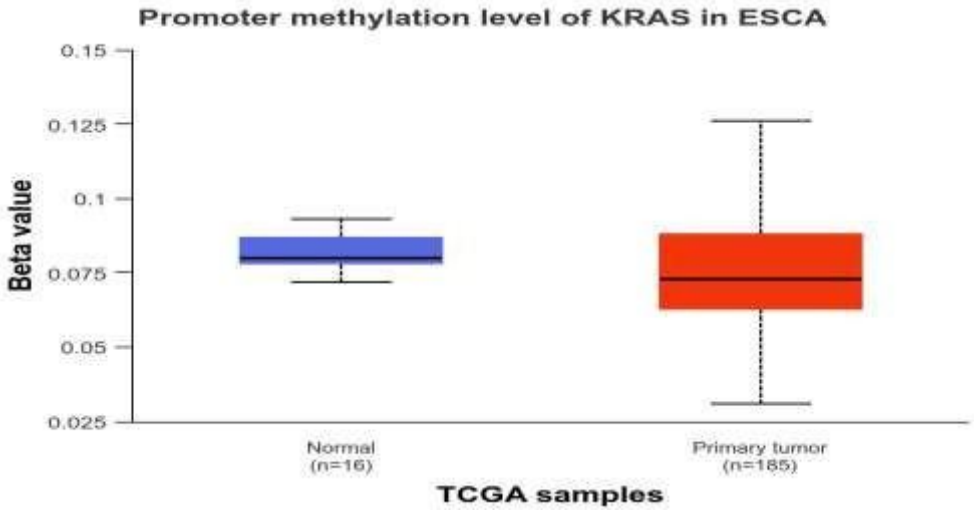

Figure 3: The KRAS promoter methylation level analysis in ESCA and normal control samples using UALCAN.

Analyzing KRAS promoter methylation level in ESCA segmented according to numerous factors

Simultaneously, a variety of factors, including the patient's age, race, gender, and cancer stage, were examined in the course of the analysis KRAS promoter methylation level in ESCA samples. Primarily, we assessed promoter methylation level of KRAS in. We identified that KRAS was relatively hypomethylated in samples of ESCA clinical stages than normal samples **(Figure 4A**). Afterward, variation in of KRAS was assessed in ESCA samples of different age groups in comparison with normal samples. We assessed that KRAS was hypomethylated in samples of age group 21-40 to 61-80; however, in samples of ESCA age group 81-100 KRAS methylation is identical to normal samples (**Figure 4B).** Following this, analysis based upon the samples of ESCA patient's gender and race revealed hypomethylation of KRAS methylation level (**Figure 4C-D).**

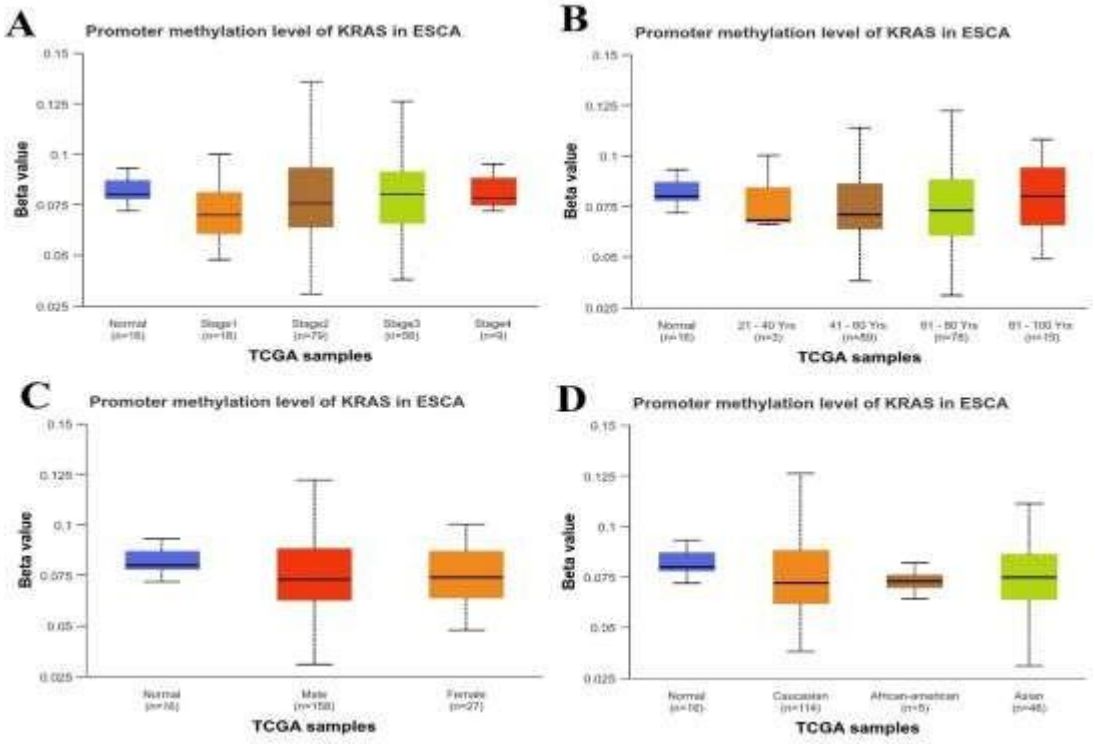

Figure 2: UALCAN was used to evaluate promoter methylation level of KRAS in ESCA based on several criteria. (A) Evaluation of KRAS e promoter methylation level in ESCA stratified according to pathological phases. (B) Evaluation of KRAS promoter methylation level in ESCA stratified according to patients age. (C) Evaluation of

KRAS promoter methylation level in ESCA stratified according to patients gender. (D) Evaluation of KRAS promoter methylation level in ESCA stratified according to patients races.

Analyzing prognostic value of KRAS in ESCA

KRAS expression's impact on ESCA patients' overall survival (OS) was explored by employing KM plotter. The assessment demonstrated a correlation between low expression of KRAS and improved OS in ESCA patients, and a correlation between overexpression of KRAS and poor OS (**Figure 5**). There is a considerable difference between the two values, as demonstrated by the computed logrank P= (P = 9e-04). However, ESCA patients with higher KRAS expression had 3.25 times higher probability of mortality than those with lower expression, according to the computed hazard ratio HR= 0.7(0.5-0.97).

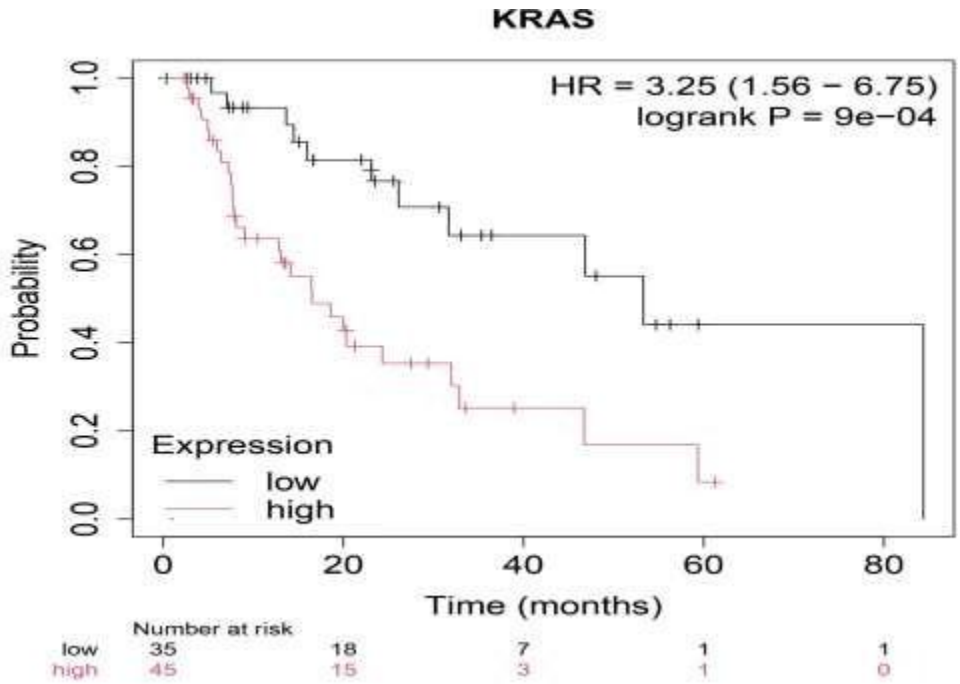

Figure 5: Analysis of prognostic value of KRAS in ESCA by employing KM plotter.

Validating expression and survival analysis of KRAS

Concurrently, aiming to validate our earlier findings, we also used GEPIA2 to examine KRAS expression and prognostic value. We used box plot module of GEPIA2 to evaluate KRAS expression in ESCA and normal control samples. The analysis revealed that KRAS expression are up-regulated in ESCA samples than in normal samples; although, the difference is not significant(**Figure 6A**) t. Afterward, we used stage plot module of GEPIA2 to assess KRAS expression based on ESCA clinical stages. The plot demonstrated the distribution of a KRAS expression across distinct cancer stages, indicated that the values of the variable could differ across stages (**Figure 6B**). But these differences don't seem significant as computed p-value is 0.0667.

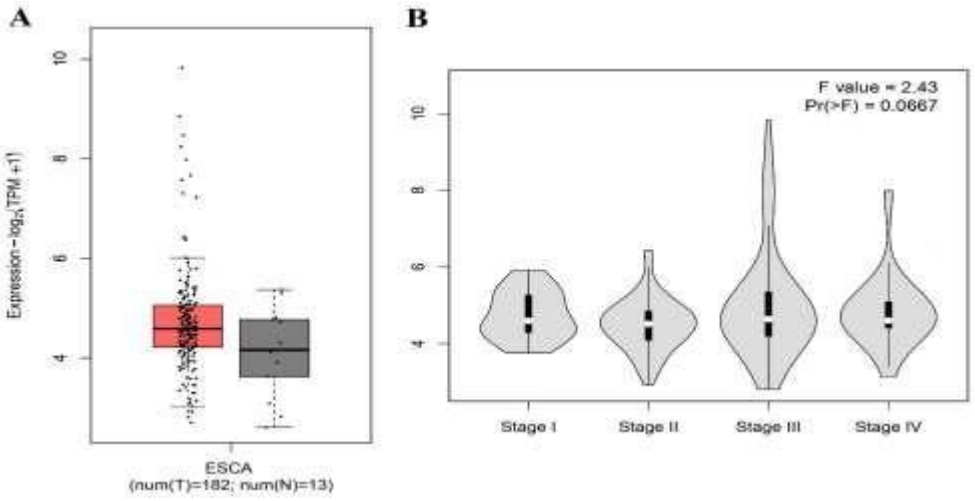

Figure 6: (A) Analysis of KRAS expression in ESCA using GEPIA2. (B) Analysis of KRAS Expression in ESCA based on pathological stage using GEPIA2

Following this, we employed GEPIA2 to evaluate prognostic value of KRAS in ESCA. The analysis demonstrates that patients with lower KRAS expression in contrast to individuals with higher KRAS expression had shorter OS (**Figure 7**). However, Statistical examination, shows that this difference p-value is not significant (p-value<0.05) as the calculated p-value is 0.0667.

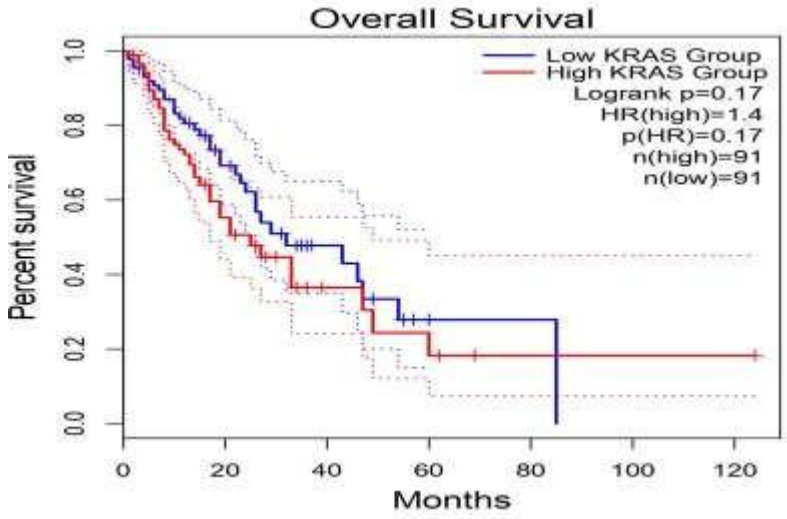

Figure 7: Analysis of prognostic value of KRAS in ESCA by employing GEPIA2.

Analysis of KRAS genetic mutation

The cBioPortal was utilized in our investigation to analyze the genetic modification of KRAS in ESCA. The 15% of KRAS genetic mutations ware identified in ESCA. The investigated KRAS Mutations included amplification, missense mutation (putative driver), and deep deletion (**Figure 8**).This demonstrated that the development and progression of ESCA are regulated by the KRAS genetic mutation.

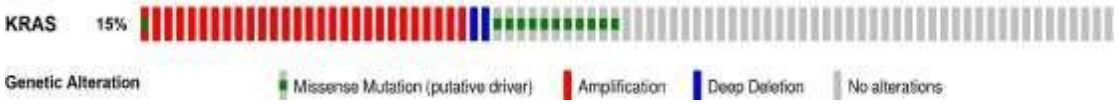

Figure 8: the assessment of KRAS genetic mutation in ESCA using cBioPortal.

Pathway enrichment analysis

In an effort to comprehend the biological significance of KRAS, we executed gene enrichment analysis. First, utilizing the STRING database, the PPI network of KRAS was created and 10 significant associated genes with KRAS were evaluated (**Figure 9**). This illustrated the diverse associations of the KRAS gene and reflects its complexity in biological mechanisms. Henceforth, GO and KEGG analysis was performed using DAVID tool, with the first four keywords for biological process (BP), cellular component (CC), molecular function (MF), and KEGG pathways were documented (**Table 1**).

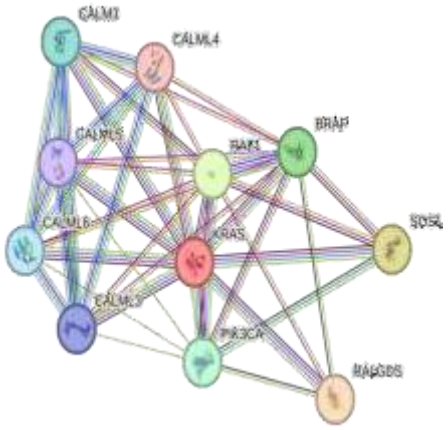

Figure 8: PPI network of KRAS constructed utilizing STRING database.

TABLE-1

Gene Term	Count	Genes	P-value
BP			
GO:0007265~Ras protein signal transduction	5	BRAF, KRAS, RAF1, SOS1, RALGDS	7.950366930491517E-8
GO:0007173~epidermal growth factor receptor signaling pathway	4	PIK3CA, BRAF, KRAS, SOS1	2.702606522439439E-6
GO:0048009~insulin-like growth factor receptor signaling pathway	3	PIK3CA, RAF1, SOS1	1.1370870484216097E-4
GO:00 08286~insulin receptor signaling pathway	3	PIK3CA, RAF1, SOS1	5.637140844961271E-4
CC			
GO:0016460~myosin II complex	3	CALML6, CALM3, CALML4	1.0807031435670904E-4
GO:0005737~cytoplasm	8	PIK3CA, CALML6, BRAF, KRAS, CALM3, CALML3, RAF1, SOS1	0.006335712779545026
GO:0005886~plasma membrane	7	PIK3CA, BRAF, KRAS, CALM3, RAF1, SOS1, RALGDS	0.027541381107253786
GO:0005829~cytosol	7	PIK3CA, BRAF, KRAS, CALM3, RAF1, SOS1, RALGDS	0.028925177934980464
MF			
GO:0030234~enzyme regulator activity	5	CALML5, CALML6, CALM3, CALML3, CALML4	8.569519895774077E-10
GO:0005509~calcium ion binding	6	CALML5, CALML6, BRAF, CALM3, CALML3, CALML4	1.8229073197385505E-5
GO:0005509~calcium ion binding	2	BRAF, RAF1	0.010827986091960612
GO:0106310~protein serine kinase activity	3	PIK3CA, BRAF, RAF1	0.014588116963143239
KEGG			
hsa05214:Glioma	10	PIK3CA, CALML5, CALML6, BRAF, KRAS, CALM3, CALML3, CALML4, RAF1, SOS1	1.5626712077303663E-18
hsa04722:Neurotrophin signaling pathway	10	PIK3CA, CALML5, CALML6, BRAF, KRAS, CALM3, CALML3, CALML4, RAF1, SOS1	1.1411917564597253E-16
hsa04910:Insulin signaling pathway	10	PIK3CA, CALML5, CALML6, BRAF, KRAS, CALM3, CALML3, CALML4, RAF1, SOS1	4.173739554861045E-16
hsa04015:Rap1 signaling pathway	10	PIK3CA, CALML5, CALML6, BRAF, KRAS, CALM3, CALML3, CALML4, RAF1, RALGDS	2.1670447519528147E-14

Analysis of association between KRAS and immune cell infiltration

Previously, It's been identified that immune cell infiltration play a critical role in tumor progression (40). We investigated the Pearson correlation between KRAS and immune cell subtype infiltration, such as CD8+ T cells, B cells, and macrophages, in ESCA. The analysis unveiled that KRAS features weak positive correlation with B cells, macrophages and neutrophil in ESCA; however, weak negative correlation was observed with CD8+ T cells (**Figure 9).** These results demonstrated KRAS's function as biomarker in immune cell infiltrations.

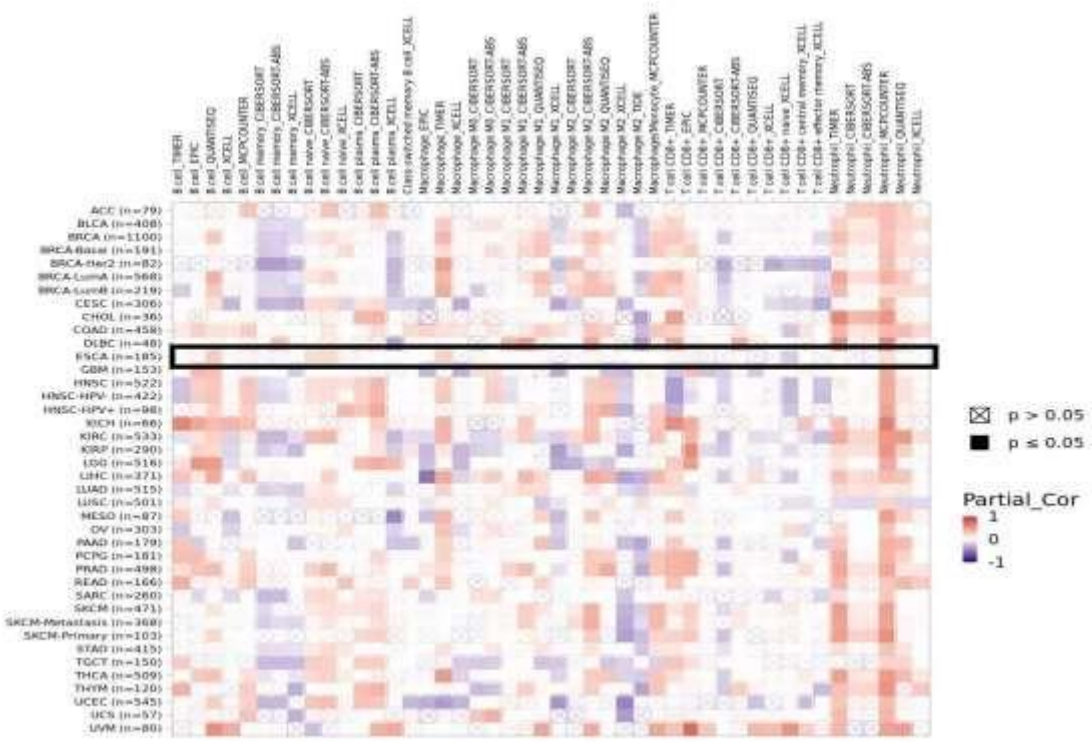

Figure 9: Analysis of association between KRAS and immune cell infiltration using TIMER2.0

Discussion

Kirsten rat sarcoma viral oncogene homologue (KRAS) an important part of RAS family and involved in various cellular function as cell division and cell development. KRAS is a highly mutated gene which contributes to the development and progression of several cancers, including thyroid, colorectal, pancreatic, and non-small cell lung cancer. (41, 42). However, there hasn't been adequate study conducted regarding KRAS function in esophageal carcinoma (ESCA). KRAS expression in ESCA was thoroughly investigated using the bioinformatics analysis techniques with the intent to identify its potential significance as a biomarker.

In present study, our investigation indicated that, in comparison to normal samples, KRAS was significantly ($p < 0.05$) up-regulated in ESCA. However, best to our knowledge, patients with ESCA with various clinicopathological features have not received adequate treatments with biomarker-based methods. Thus based on this, we assessed KRAS expression based on several ESCA clinicopathological characteristics and investigated KRAS overexpression. As per previous studies gene overexpression have association with in cancers progression (43, 44). Thus, our results suggested a function for KRAS in the beginning and progression of ESCA. Following this, investigation determined that, KRAS overexpression was associated to low OS of ESCA patient. Simultaneously using GEPIA2, we also carried out survival analysis and KRAS expression. Our analysis revealed that KRAS was overexpressed, which leads to poor OS in ESCA. This investigation suggests that ESCA progresses as a result of KRAS overexpression.

Additionally, we also analyzed genetic alterations and promoter methylation as these variables affect KRAS expression(45, 46). Gene expression can get aberrant as a result to any abnormal alteration in the DNA promoter methylation level (47). In our study, we noted that KRAS was hypomethylated in ESCA samples as compared to normal samples as per expectations. These results indicates that promoter methylation regulates KRAS expression in ESCA. Moreover, our analysis using cBioPortal illustrated that 15% of esophageal cancer (ESCA) cases exhibit mutations in the KRAS gene. These findings suggested that KRAS expression is notably affected by these mutations and plays a critical role in the progression and development of ESCA. However, it requires further investigation.

Further, according to recent findings, numerous genes regulate the diversity of immune cells in the tumor microenvironment (48). Our investigation highlighted weak positive correlation with B cells, macrophages and neutrophil; however, observed weak negative correlation with CD8+ T cells in ESCA. This may suggest that CD8+ T cells may not associated with an alternative tumor response, even though other immune cells may contribute to the tumor environment, further studies are crucial. Moreover, in our study PPI network of KRAS was constructed and illustrated that KRAS is associated with 10 genes. Subsequently, the associated pathways of KRAS and related genes were identified by enrichment analysis. Many biological functions, such as cell cycle regulation, cell proliferation, survival, and immune responses, are linked to signal transduction pathways involving Ras proteins, epidermal growth factor receptors, insulin-like growth factor receptors, neurotrophin

receptors, and insulin receptors(49-51). The KRAS associated genes are enriched with these pathways and development of cancer is significantly aided by the dysregulation of these pathways.

Conclusion:

In conclusion, In comparison to normal samples, we found that ESCA samples had an overexpression of KRAS. Furthermore, various clinicopathological characteristics and a poor overall survival rate in patients with ESCA have been associated to this overexpression of KRAS. Our study is a preliminary investigation that reports on the diagnostic and prognostic potential of KRAS in ESCA based on widespread clinicopathological attributes. However, further research needed to be conducted before any therapeutic implications.

Disclosure statement

The author declares no conflict of interest.

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