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# Comprehensive Bioinformatics Analysis Reveals Kirsten Rat Sarcoma Virus Oncogene (*KRAS*) as the Potential Biomarker of Esophageal Carcinoma

**Muhammad Akram***

Department of Microbiology, King Saud University, Saudi Arabia

***Corresponding author:** Muhammad Akram, Mmakram1234@outlook.com



**Abstract:** In the current study, the expression of the Kirsten rat sarcoma virus oncogene (KRAS) in esophageal carcinoma (ESCA) was examined for its medical and therapeutic relevance. ESCA has a 20% five-year survival rate, placing it seventh in the world in terms of overall rate of mortality. GEPIA2, UALCAN, OncoDB, cBioPortal, STRING, DAVID, and TIMER2 databases are among the bioinformatics tools used to conduct this investigation. According to the analysis, KRAS was significantly ($P < 0.05$) elevated in ESCA samples in contrast to normal tissues, demonstrating that it might play an active role in the proliferation of malignancies. Additionally, the study based on several clinicopathological features showed that KRAS were significantly up-regulated. ESCA patients had a worse overall survival rate (OS) as KRAS was significantly overexpressed. Besides this, the study carried out analyses of drug sensitivity, enrichment, and promoter methylation to inquire about their relationships to KRAS expression in ESCA. The KRAS mutation was demonstrated to have a significant impact on the progression of ESCA via the genetic changes that were observed using cBioPortal. In conclusion, the comprehensive analysis of the findings emphasizes the significance of KRAS up-regulation in the development of ESCA and its potential as a potential biomarker.

**Keywords:** KRAS; ESCA; Therapeutic; Biomarker; Bioinformatics

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## 1. Introduction

Aberrant cellular division constitutes the complex set of illnesses referred to as cancer. Cancer is responsible for around 20 million cases in 2022 alone. Cancer can be classified into different types based on their distinct characteristics and place of origin. Esophageal cancer ranks 7th among fatality rates among all cancers and is the 11th most common malignancy, based on findings from earlier studies [1]. More than 90% of instances of esophageal cancer are esophageal carcinoma (ESCA). There are two distinct subtypes of esophageal carcinoma (ESCA): esophageal squamous cell carcinoma (SCC) and adenocarcinoma. From a gender perspective, there

are differences in the frequency and fatality rates; males account for around 70% of cases, with mortality rates two to five times higher. However, the incidence of ESCA is greater in middle-aged and older populations. Moreover, Asia accounts for over fifty percent of the cases of esophageal cancer (ESCA) globally [2-7]. Key risk factors for ESCA include obesity, poor nutrition, alcohol intake, tobacco use, and gastroesophageal reflux disease (GERD) [8]. In the past few years, the incidence rate of ESCA has generally declined. However, it stands as the cancer with the second lowest 5-year relative survival rate of approximately 20% [9,10]. Scientific analysis, endotherapy, target therapy, staging, and surgery, are some treatments or techniques used for ESCA [11]. The treatments demonstrate minimal therapeutic benefit despite therapeutic progress. Higher mortality rates and an extremely complex malignancy associated with ESCA are identified as treatment challenges. Thus, it is imperative to find significant prognostic, therapeutic, and diagnostic biomarkers for ESCA.

Kirsten rat sarcoma viral oncogene (*KRAS*) encodes the Kirsten rat sarcoma 2viral oncogene homolog protein (*KRAS*) and falls under the RAS family. *KRAS* has a role in numerous pathways that regulate cell division as the PI3K-AKT pathway, Ras-MAPK pathway, Ras-RAF pathway, and Ras-ERK pathway. *KRAS* demonstrates an absence of small molecular binding sites and acts as a regulator facilitating the conversion of GTP to GDP. This results in the activation of *KRAS*. The release of signaling molecules is triggered by *KRAS*, thereby enhancing the process of conveying messages from the cell surface to the nucleus. Thereby, leads to alteration in cell division, maturation, apoptotic processes, and cellular motility [12-15]. Lung and colorectal cancers have worse prognoses when *KRAS* expression is prevalent. Approximately 25 percent of all malignancies are caused by *KRAS* mutations, which are exceedingly prevalent. Pancreatic ductal adenocarcinoma (PDAC), colorectal cancer (CRC), and urogenital cancer are the most prevalent cancers with *KRAS* mutations, with pancreatic tumors exhibiting the highest mutation rates (90%) among these diseases [16-21]. The potential of *KRAS* as a therapeutic, prognostic, and diagnostic biomarker in several malignancies is highlighted by all of these results.

This study planned to use 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. The researchers evaluated *KRAS* expression, mutation, survival, and gene enrichment analysis in ESCA using the bioinformatics method. In this study, the researchers conduct a comprehensive analysis of the *KRAS* gene, 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 ESCA.

In the present study, the objective was to examine *KRAS* as a possible biomarker in ESCA. The study intends to investigate *KRAS* expression, methylation status, mutation, gene enrichment pattern, and prognostic relationships in ESCA. GEPIA2, UALCAN, cBioPortal, OncoDB, KM plotter, Timer2.0, STRING, DAVID, and GSCA databases were among the bioinformatics tools employed.

## 2. Material and method

### 2.1. GEPIA2

A web-based program called GEPIA2 (Gene expression profiling interactive analysis2) is used to thoroughly examine various subtypes of cancer using the GTEx and TCGA databases [22]. GEPIA2 was employed to investigate *KRAS* expression and survival analysis in ESCA based on disease phases and sample types.

### 2.2. Ratification using UALCAN

A web-based tool for thorough cancer OMICS data analysis is called UALCAN [23]. To verify *KRAS* expression

in ESCA based on illness stages and sample types, the researchers utilized the UALCAN database. The study defined statistical significance as $P < 0.05$.

## 2.3. Kaplan-Meier (KM) plotter

The Kaplan-Meier (KM) plotter is an intuitive online tool that utilizes gene expression to assess cancer patients' overall survival (OS) [24]. In the current study, utilizing the GEO and TCGA datasets, the researchers used the KM Plotter with default settings to evaluate the effect of *KRAS* expression on OS in ESCA. A statistical significance threshold of $P < 0.05$ was used.

## 2.4. OncoDB

In the current study, the researchers analyzed the promoter methylation level of the *KRAS* gene in ESCA by using the OncoDB database. OncoDB is an important database that is utilized for the analysis of oncogenic mutation data specifically promoter methylation level [25].

## 2.5. cBioPortal

The cBioPortal is an easily accessible tool designed to do a thorough analysis of multi-omic cancer datasets [26]. The present investigation applied cBioPortal to evaluate the genetic mutational and copy number variation (CNV) trends associated with *KRAS* in ESCA.

## 2.6. Protein-protein interaction network and gene pathway analysis

In the present study, utilizing the STRING (The Search Tool for the Retrieval of Interacting Genes) database, the PPI network of *KRAS*-enriched genes was constructed [27]. The Database for Annotation, Visualization, and Integrated Discovery (DAVID) is an online program to carry out a thorough *KRAS* network gene enrichment study [28]. $P < 0.05$ is the definition of statistical significance.

## 2.7. KRAS expression and immune cell infiltration

A database to evaluate gene expression, tumor purity, and immune cell infiltration is called Tumor Immune Estimation Resource (TIMER) 2.0 [29]. In the present study, the researchers assess the Spearman correlation of the infiltration of $CD8^+$ T immunological cells, B cells, and $CD4^+$ T immunological cells with the *KRAS* expression in ESCA utilizing the TIMER2.0. *P*-value < 0.05 is set as statistically significant.

## 2.8. GSCA database

We employed the GSCA database to examine the relationship between drug sensitivity and *KRAS* mRNA expression. The GSCA database is the most useful resource currently accessible for pharmacological sensitivity evaluation [30].

# 3. Results

## 3.1. KRAS expression analysis ESCA samples and normal samples

In this study, the researchers employed GEPIA2 to evaluate the *KRAS* expression in ESCA samples as compared to normal control samples. The Boxplot evaluation illustrated that *KRAS* was overexpressed in ESCA samples compared to normal samples (**Figure 1**). To determine statistical significance, further study is required, though the boxplot demonstrates a variation in *KRAS* expression among ESCA and normal samples. As observed by

outliers, the *KRAS* expression likewise exhibited expression variability. *KRAS* overexpression suggested it is the positional role in ESCA oncogenesis.

## 3.2. The analysis of KRAS expression in ESCA based on individual cancer stages

Following that, the study investigated *KRAS* expression categorized according to the ESCA individual cancer stage using the GEPIA2 boxplot module. As the cancer stage advances, the violin plot illustrated that the expression of a *KRAS* varied across different stages, suggesting that the variable's values might differ across stages (**Figure 2**). A difference in *KRAS* expression might exist, however, this is not statistically significant (*P*-value < 0.05) because the estimated *P*-value is 0.0667.

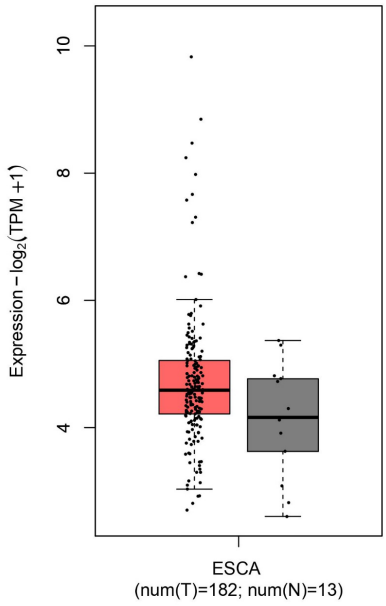


**Figure 1.** The *KRAS* gene expression analysis in ESCA and normal control samples using the boxplot module of GEPIA2

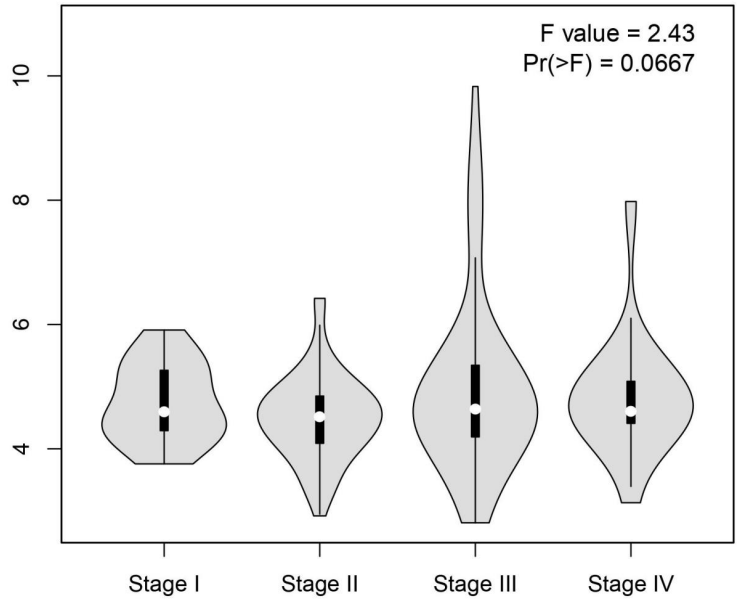


**Figure 2.** The analysis of *KRAS* expression in ESCA based on individual cancer stages using GEPIA2

## 3.3. Survival analysis of KRAS in ESCA

Next, the study investigated the prognostic value of *KRAS* in ESCA patients by using GEPIA2. According to the investigation, ESCA patients with lower *KRAS* expression had better overall survival (OS), whereas those with higher *KRAS* expression had poorer OS (**Figure 3**). However, the derived HR of 1.4 suggested a tendency toward increased risk in high expression of *KRAS*, but the obtained *P*-value of 0.17 demonstrated that the difference was not significant (*P*-value < 0.05).

## 3.4. Corroboration of KRAS expression analysis

Moreover, the study utilized the UALCAN database to corroborate *KRAS* expression analysis on ESCA. First, using data collected from the UALCAN database, the researchers assessed *KRAS* expression in ESCA samples in comparison to a normal control sample (**Figure 4**). The researchers examined that, *KRAS* was overexpressed in ESCA samples compared with normal samples. There was statistical significance among the two variables as the computed *P*-value is $5.604400E^{-4}$.

Further, to corroborate the results, the study analyzed *KRAS* expression in samples of ESCA individual cancer stages. The analysis revealed the distribution of significant overexpression of *KRAS* in ESCA individual cancer stages than normal samples (**Figure 5**). The study analyzed variations between stages 1 and 4 ESCA samples, with *KRAS* being significantly overexpressed in the later sample and vice versa. Altogether, it is validated that *KRAS* is overexpressed in tumor samples and leads to ESCA proliferation.

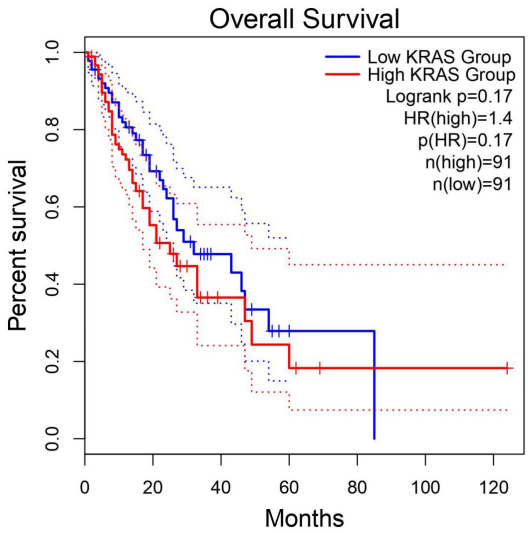


**Figure 3.** Displays *KRAS* expression-based survival map in ESCA patients using GEPIA2

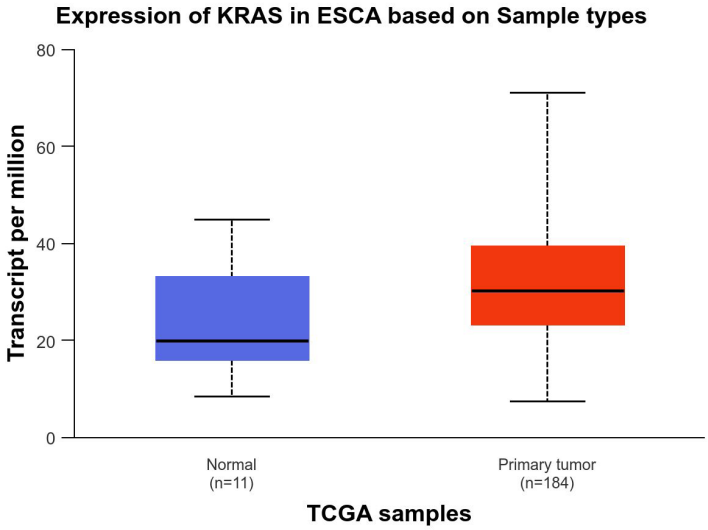


**Figure 4.** The utilization of UALCAN to analyze *KRAS* gene expression in ESCA and normal control sample

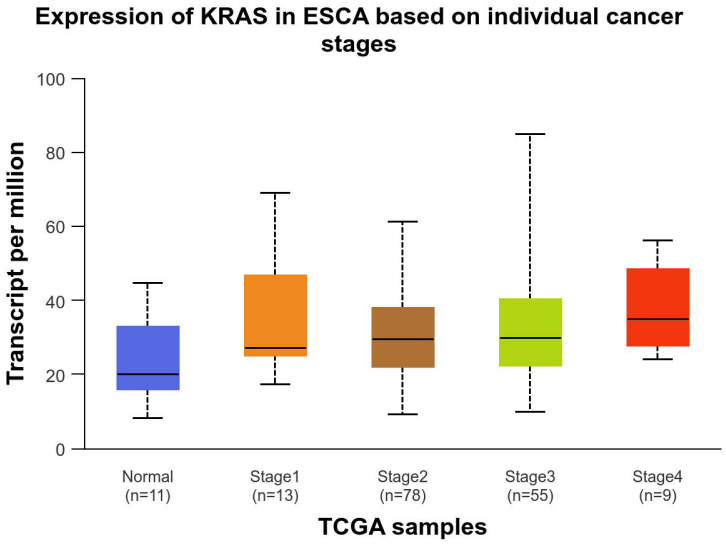


**Figure 5.** The utilization of UALCAN to analyze *KRAS* gene expression in ESCA pathological stages and normal control sample

## 3.5. Validation of survival analysis

Moreover, the study used a KM plotter to perform a survival analysis of *KRAS* in ESCA to validate the findings. The evaluation revealed that in ESCA patients, low expression of *KRAS* was correlated with better OS, whereas overexpression of *KRAS* was correlated with worse OS (**Figure 6**). The calculated hazard ratio, HR = 3.25 (1.56–6.75), indicated that ESCA patients with higher *KRAS* expression had a 3.25-fold greater chance of death

compared to those with lower expression. While the computed *P*-value $9e^{-4}$ indicated a significant ($P < 0.05$) difference among the variables.

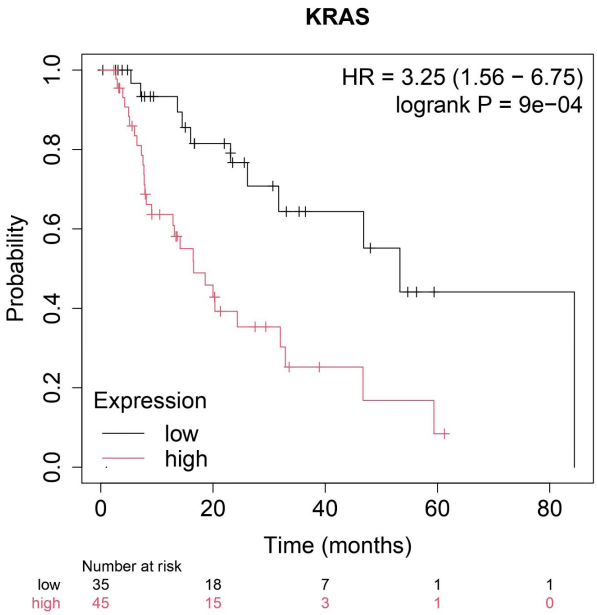


**Figure 6.** Survival analysis of *KRAS* in ESCA by utilizing KM plotter

## 3.6. Promoter methylation of KRAS

Alterations in DNA methylation patterns, including widespread genomic hypomethylation and distinct hypermethylation, are indications for cancer cells [31]. The study analyzed the promoter methylation level of *KRAS* in ESCA utilizing the OncoDB database. The examination indicated that *KRAS* is significantly ($P < 0.05$) hypomethylated in ESCA samples as compared to normal samples (**Figure 7**). This finding suggested a possible explanation for the elevated expression of *KRAS* in ESCA by demonstrating an association between lowered methylation and elevated gene expression.

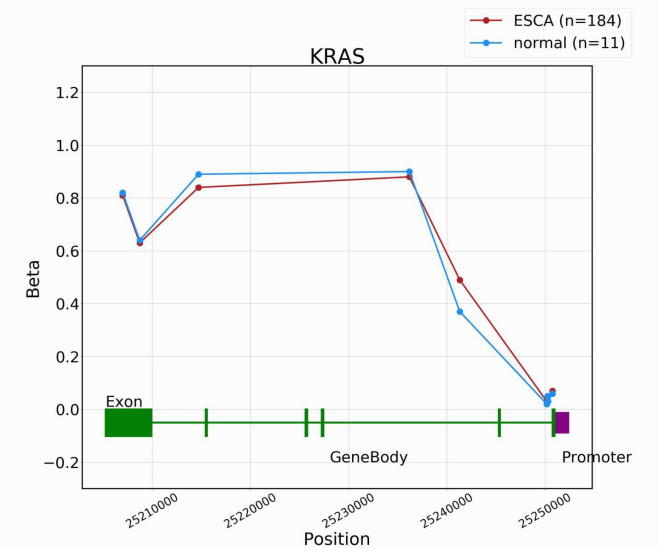


**Figure 7.** Utilizing OncoDB to analyze promoter methylation level of *KRAS* in ESCA

## 3.7. Correlation Analysis of KRAS and immune cell infiltration

It has been stated that immune cell infiltration significantly influences tumor progression [32]. The Pearson association between *KRAS* and immune cell subtype infiltration such as $CD4^+$ T cells, macrophages, and $CD8^+$ T cells, was examined in ESCA. According to the data, $CD8^+$ T cells might show negative correlations, suggesting that when *KRAS* expression grows, their numbers decrease. On the other hand, there may be positive associations between the expression of *KRAS* and certain immune cell types, such as macrophages and $CD4^+$ T cells. This indicates that immunological populations with greater *KRAS* levels are more prevalent within tumors (**Figure 8**). These findings illustrated the substantial involvement of *KRAS* expression in immune cell infiltrations.

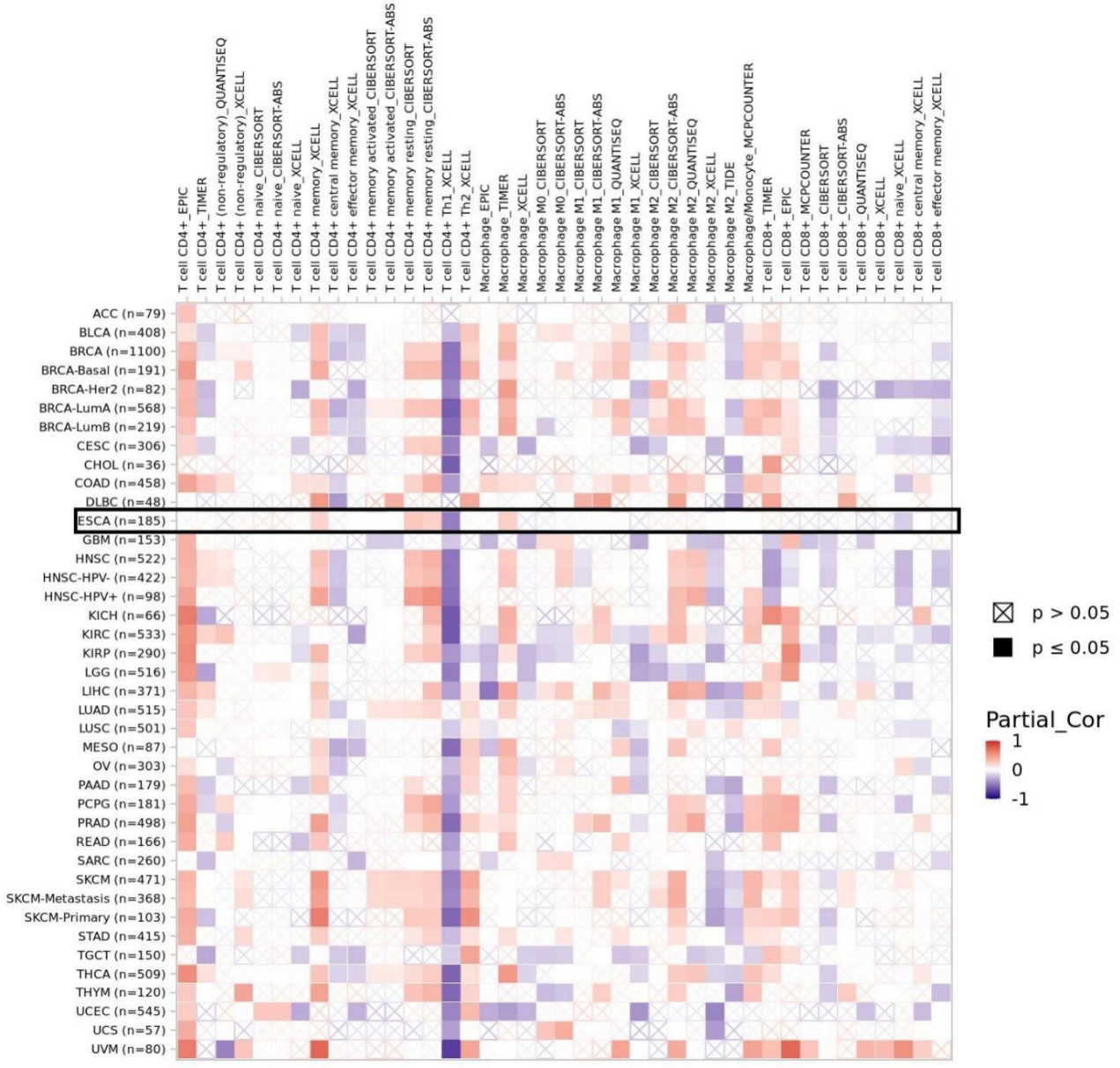


**Figure 8**. Illustration of association between *KRAS* expression and immune infiltrates in ESCA

## 3.8. Genetic alteration of KRAS in ESCA

In the study, the researchers employed cBioPortal to delve into the genetic mutation of *KRAS* in ESCA and identified 15% of genetic mutations. Amplification, missense mutation (putative driver), and deep deletion were assessed mutations (**Figure 9**). This exploration sheds light on how *KRAS* genetic mutation significantly contributes to the progression and development of ESCA.

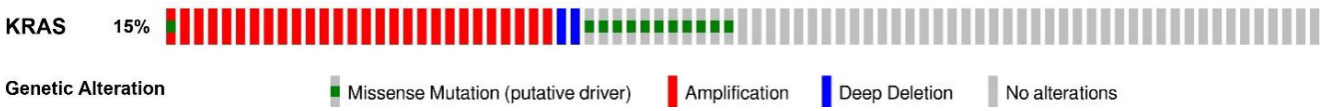


**Figure 9.** Depiction of genetic alteration of *KRAS* in ESCA by utilizing cBioPortal

## 3.9. PPI network and pathway enrichment analysis into the molecular mechanism of HS6ST2

The study performed PPI network analysis to attain a profound understanding of the biological significance of *KRAS*, The STRING database was leveraged initially to construct the protein-protein interaction (PPI) network for *KRAS*, leading to the assessment of ten notable genes associated with this gene (**Figure 10**). This illustrated the diverse associations of the *KRAS* gene and reflects its biological mechanisms. In this instance, the DAVID program was utilized to do GO and KEGG analysis. The researchers documented the initial three terms of biological process (BP), cellular component (CC), molecular function (MF), and KEGG pathways.

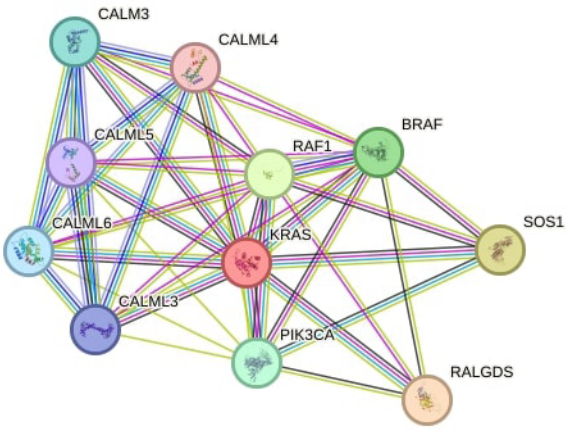


**Figure 10**. The construction of the *KRAS* PPI network using the STRING database

The identified processes of KEGG analysis were Glioma, Neurotrophin signaling pathway, and insulin signaling pathway. In GO analysis, Ras protein signal transduction, epidermal growth factor receptor signaling pathway, insulin-like growth factor receptor signaling pathway, cytoplasm, myosin II complex, plasma membrane, enzyme regulator activity, calcium ion binding, and MAP kinase kinase kinase activity, pathways associated with BP, CC, and MF were observed (**Table 1**).

**Table 1.** Pathways associated with BP, CC, and MF

| 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 |
| 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 |
| 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:0004709~MAP kinase kinase kinase activity | 2 | *BRAF, RAF1* | 0.010827986091960612 |
| 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 |

## 3.10. Drug sensitivity analysis of *KRAS*

The correlation between drug sensitivity and mRNA expression levels for numerous drugs is explored using the GSCA database, with a focus on the *KRAS* gene. A negative relationship was observed between drug sensitivity and *KRAS* expression including belinostat, BI-22536, BRD-K9765114, ciclopirox, compound 23citrate, fluorouracil, linifanib, linsitinib, NSC 19630 and tacedinaline. In contrast, there was a positive correlation and sensitivity of HS6ST2 expression to two minor medications, namely alvocidib and lovastatin (**Figure 11**). These results suggest that *KRAS* might serve as an applicable therapeutic target for the treatment of ESCA.

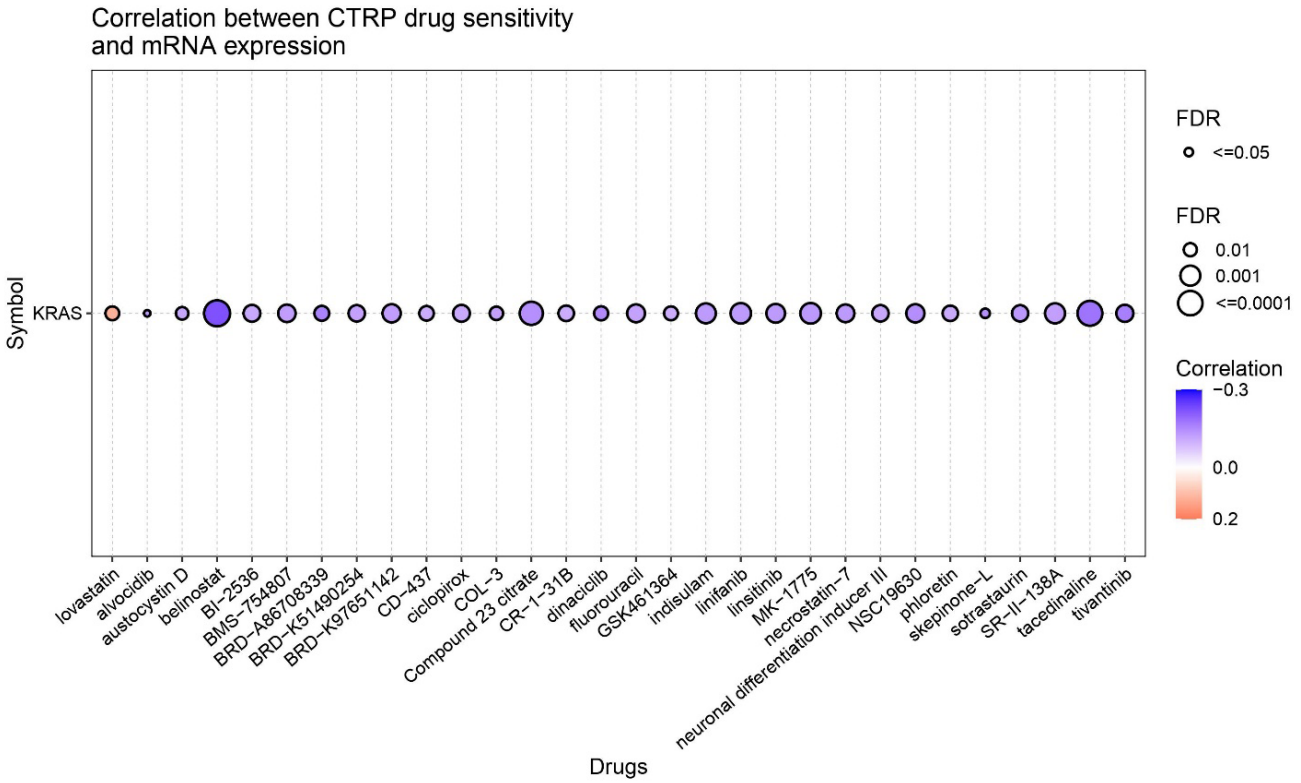


**Figure 11**. Analysis of mRNA expression levels for numerous *KRAS* gene correlations using GSCA

## 4. Discussion

The seventh-highest mortality rate among malignancies, esophageal cancer (EC) is steadily rising in both incidence and prognosis. Over 90% of cases of EC are esophageal carcinoma (ESCA), and there is a 70% greater probability of ESCA in males. It has an extremely high treatment resistance and the second lowest 5-year survival rate (20%). Hence, researchers must identify effective ESCA biomarkers for diagnosis, therapy, and prognosis [6, 33-35]. Kirsten rat sarcoma viral oncogene homolog *KRAS* is an oncogenic gene that encodes a GTPase signaling protein. Numerous malignancies, including pancreatic ductal adenocarcinoma, thyroid, colorectal, pancreatic, and non-small cell lung cancer, have been identified to be associated with the highly mutated *KRAS* gene in tumors [36,37].

In the present study, the researchers initiated the study to apply bioinformatics techniques to shed light on *KRAS*'s potential function as a biomarker. Initially, the researchers utilized the GEPIA2 platform to assess the overexpression of *KRAS* in ESCA compared to normal samples. Additionally, *KRAS* overexpression was evaluated as the researchers analyzed *KRAS* expression across multiple clinical stages of ESCA. Furthermore, GEPIA2 was employed and revealed the overexpression of *KRAS* correlated with an unfavorable prognosis for individuals diagnosed with ESCA. Gene overexpression has been previously shown to be associated with the development of several cancers [38]. Collectively, these results demonstrate *KRAS*'s contribution to the evolution and growth of (ESCA).

Simultaneously, to verify the findings, the researchers further executed survival and expression studies of *KRAS* by using the UALCAN database. The researchers determined that, in comparison to the normal sample,

*KRAS* indicated significant ($P < 0.05$) overexpression in ESCA samples at different stages. This significant ($P < 0.05$) overexpression of *KRAS* contributes to the unfavorable overall survival (OS) in ESCA. Thus, the overexpression of *KRAS* and its association with the proliferation of ESCA have been substantiated. Further, given that these factors influence *KRAS* expression, the researchers investigated genetic alterations and promoter methylation. Any unusual modifications in the methylation levels of the DNA promoter might result in dysregulated gene expression [39]. A negative correlation was identified between *KRAS* overexpression and hypomethylation of the *KRAS* gene in ESCA by utilizing the OncoDB database. Furthermore, cBioPortal was utilized and assessed 15% of *KRAS* genetic mutations in ESCA and suggested *KRAS* expression is stringently regulated by that mutation. Altogether, based on these findings, *KRAS* expression is modulated by genetic alterations and promoter hypomethylation that leads to a progression of ESCA. However, it requires further investigation.

After that, the analysis revealed a mild positive link with $CD4^+$ T cells and macrophages, although a weak negative correlation with $CD8^+$ T cell activation in ESCA was also noted. Further research is essential since this may indicate that $CD8^+$ T cells are not linked to an alternate tumor response, despite the possibility that $CD4^+$ T cells and macrophages contribute to the tumor environment. Research indicated that a wide range of genes control the variety of immune cells inside the tumor microenvironment [40].

Furthermore, the protein-protein interaction (PPI) network about *KRAS* highlighted the relationship between *KRAS* and ten genes. Subsequently, *KRAS* and related genes were enriched in various associated pathways such as Ras protein signal transduction, epidermal growth factor receptor signaling pathway, insulin-like growth factor receptor signaling pathway, cytoplasm, myosin II complex, plasma membrane, enzyme regulator activity, calcium ion binding, and MAP kinase kinase kinase activity. Many biological functions, such as cell cycle regulation, cell proliferation, survival, and immune responses, are linked to these pathways [41–42]. Next, the researchers examine the association between *KRAS* and the susceptibility of several anti-cancer drugs in the research. The findings suggested that elevated *KRAS* expression may play a role in drug resistance as it was linked to decreased sensitivity to a variety of medications. Conversely, higher *KRAS* expression was associated with enhanced responsiveness to lovastatin and alvocidib sensitivity. According to this research, altering *KRAS* expression may be a strategic approach and useful tactic for raising the efficacy of anticancer.

## 5. Conclusion

A thorough analysis identified that ESCA samples demonstrated a *KRAS* up-regulation after employing a variety of bioinformatics methods. Moreover, *KRAS* overexpression in ESCA patients has been correlated with a low overall survival rate and numerous clinicopathological traits. The results demonstrated *KRAS*'s potential as an ESCA prognostic, diagnostic, and therapeutic biomarker. However, before it can be applied in clinical practice, more testing is required.

## Disclosure statement

The author declares no conflict of interest.

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