Survival estimation in patients with stomach and esophageal carcinoma using miRNA expression profiles

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\textbf{A B S T R A C T}

Identifying a miRNA signature associated with survival will open a new window for developing miRNA-targeted treatment strategies in stomach and esophageal cancers (STEC). Here, using data from The Cancer Genome Atlas on 516 patients with STEC, we developed a Genetic Algorithm-based Survival Estimation method, GASE, to identify a miRNA signature that could estimate survival in patients with STEC. GASE identified 27 miRNAs as a survival miRNA signature and estimated the survival time with a mean squared correlation coefficient of 0.80 ± 0.01 and a mean absolute error of 0.44 ± 0.25 years between actual and estimated survival times, and showed a good estimation capability on an independent test cohort. The miRNAs of the signature were prioritized and analyzed to explore their roles in STEC. The diagnostic ability of the identified miRNA signature was analyzed, and identified some critical miRNAs in STEC. Further, miRNA-gene target enrichment analysis revealed the involvement of these miRNAs in various pathways, including the somatotrophic axis in mammals that involves the growth hormone and transforming growth factor beta signaling pathways, and gene ontology annotations. The identified miRNA signature provides evidence for survival-related miRNAs and their involvement in STEC, which would aid in developing miRNA-target based therapeutics.

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1. Introduction

Stomach and esophageal carcinomas (STEC) are among the most prevalent malignant diseases causing thousands of deaths globally. Worldwide, stomach cancer ranks sixth in cancer incidence, with 1,089,103 new cases, and third in cancer mortality, with 768,793 deaths, while esophageal cancer ranks tenth in cancer incidence, with 1,089,103 new cases, and third in cancer mortality, with 46.7 %, 25.1 %, and 4.8 %, respectively, of esophageal cancer cases [2,3]. For localized, regional, and metastatic disease, five-year survival is 64 %, 28.2 %, and 5.3 %, respectively, for stomach cancer, and 46.7 %, 25.1 %, and 4.8 %, respectively, for esophageal cancer [2,3]. Treatment for STEC is selected based on disease stage [4,5]. Surgery can be curative but is offered mainly in early disease stages. Chemotherapy and chemoradiotherapy provide an added survival benefit to surgery in early-stage disease and are offered without surgery in later disease stages. Targeted therapies (e.g., Trastuzumab, an inhibitor of human epidermal growth factor receptor 2) improve survival in STEC and are increasingly being used in STEC treatment [6], and immunotherapy and other emerging therapies continue to be evaluated for improvement in STEC survival [4,5].

Biomarkers associated with STEC survival are potential targets for designing new STEC treatments to improve patient survival
MicroRNAs (miRNAs) function as oncogenes or tumor suppressor genes in STEC [6,7] and have been investigated as biomarkers of STEC diagnosis and prognosis [9,10]. Roles for miRNAs in STEC progression and survival have been described in several reports. For example, low levels of mir148a, a miRNA that suppresses cell invasion and migration, are associated with advanced clinical stage and poor prognosis in stomach cancer [11]. Mir-616-3p promotes angiogenesis and metastasis and is correlated with poor prognosis in stomach cancer [12]. Elevated miR-21 expression is linked to lymph node metastasis [13] and poor prognosis [14] in esophageal cancer. Mir-375 targets proteins involved in cancer cell proliferation and invasion [15], and its downregulation is associated with advanced cancer staging and poor prognosis in esophageal squamous cell carcinoma [16]. Aberrant miRNA expression has also been identified in STEC. Hwang et al. identified miRNAs, including mir-601, mir-107, mir-18a, mir-370, mir-300 and mir-96 that were significantly expressed in early gastric cancers when compared to normal samples [17]. A serum biomarker miRNA panel consisting of 12 miRNAs was developed for risk assessment in patients with gastric cancer [18]. Furthermore, several dysregulated miRNAs have been found in esophageal tumors that regulate carcinogenesis [19,20]. A quantitative RT-qPCR study on patients with esophageal carcinoma revealed three miRNAs, including mir-34a-5p, mir-148a-3p and mir-181a-5p that were associated with the cancer progression [21].

In most studies, associations between miRNAs and STEC survival have been based on results from a single study sample assessed using the log-rank test to compare Kaplan-Meier survival curves or Cox proportional hazards regression analysis [22–25]. A few other studies have employed discovery and validation stages in their design to increase the strength of the evidence supporting associations between miRNAs and STEC survival. These include studies that have identified differentially expressed miRNAs in STEC in the discovery stage and tested for association between the miRNAs and survival in an independent STEC study sample in the validation stage [26–31]. Machine learning methods are also being applied to identify miRNAs associated with STEC survival. In a study of esophageal squamous cell carcinoma, a recursive feature elimination-support vector machine algorithm along with LASSO Cox proportional hazards regression was used to identify miRNAs associated with survival and build a prognostic model in a training sample, and the prognostic model was shown to correlate with survival in an independent, test sample [32]. While these previous reports indicate that miRNAs have potential clinical value as biomarkers of prognosis in STEC, they have not addressed whether miRNAs can predict STEC survival time in individual patients.

To design a personalized survival prediction model, it is necessary to identify biomarkers that show a robust association with survival in STEC patients. Accordingly, this study aimed to develop a genetic algorithm (GA)-based survival estimation method (GASE) to identify a survival-associated miRNA signature and estimate survival time in patients with STEC. A genetic algorithm (GA)-based survival estimation method (GASE) is proposed for estimating the survival time in STEC patients using miRNA expression profiles. GASE was developed using support vector regression (SVR) that incorporates an optimal feature selection algorithm inheritable bi-objective combinatorial genetic algorithm (IBCGA) [33]. The identified miRNA signature was analyzed further to explore miRNA association with STEC. The system overview of GASE is shown in the graphical abstract.

2. Material and methods

The miRNA expression profiles of patients with STEC were retrieved from The Cancer Genome Atlas (TCGA) database. These data were generated using an Illumina Hiseq 2000 sequencing platform. The number of patients with STEC in the initial dataset was 628. After excluding the patients without survival information and those whose survival time was less than 30 days, the final dataset consisted of 123 patients with miRNA expression profiles and clinical data, including days to death. Each miRNA expression profile consisting of 500 miRNAs was used for the survival estimation procedure. For the independent validation, we used a cohort of 393 patients who were alive with STEC at last follow-up in the TCGA.

2.1. Survival estimation method GASE

The GASE’s two primary objectives were to estimate the survival time and simultaneously identify the miRNA signature associated with survival in patients with STEC. GASE was developed using SVR and an optimal feature selection algorithm IBCGA. The optimization technique implemented in GASE was adopted from previous studies [34–36]. SVM is a supervised machine learning method, which has demonstrated good prediction capability in solving classification and regression problems in various biomedical fields, especially in cancer genomics [37]. SVM uses a nonlinear transformation to find the relation between input and output variables by generating a hyperplane that optimally fits in the high dimensional space and carries out the regression function [38]. The tuning of the parameters C, γ, and ν determine the performance of SVR; hence parameter tuning plays a vital role in the SVM modeling process. The minimization of the loss function can be optimized using the following objective function for the given input data points.

$$\min \frac{1}{2} ||w||^2 + C \sum_{i=1}^{N} \xi_i + \xi_i^*$$

(1)

where ||w|| is the magnitude of the vector to the surface, C is a regularization parameter, $\xi_i$ and $\xi_i^*$ are slack variables, $\xi_i \geq 0$, $\xi_i^* \geq 0$, and $i = 1, 2, ..., N$.

The optimal parameters of GASE were tuned based on an intelligent evolutionary algorithm (IEA) [39]. In the optimization process, IBCGA [33] was used to identify a small set of miRNAs while maximizing the fitness function in terms of squared correlation coefficient. GASE prediction performance was evaluated using two metrics, squared correlation coefficient and mean absolute error. IBCGA effectively solves bi-objective combinatorial problems where a small set of informative features will be selected from a large number of candidate features. The applications of IBCGA in identifying biomarkers in cancer research have been demonstrated in previous studies [34–36,40,41]. In the optimal feature selection process, all the candidate features were encoded into binary variables, including the parameters C, γ, and ν of the SVR. The detailed steps involved in IBCGA can be found in the supplementary methods. After identifying the miRNA signature, main effect difference (MED) [42] analysis was used to prioritize the miRNAs of the signature based on their contribution to the prediction performance.

2.2. Feature appearance score

To ensure robustness, we performed 50 independent runs of GASE and selected one feature set with the highest appearance score for the analysis. The feature appearance score (FAS) indicates the frequency of the features that appeared in the 50 independent runs. A feature set with a more significant appearance score suggests that the feature frequency in that particular set is higher when compared to other features across the independent runs. There are $S_i$ features in the t-th signature. The frequency score for each feature $m$ presented in the miRNA signatures can be calculated as follows.
Feature appearance score is defined as:

$$Feature\ appearance\ score = \sum_{i=1}^{S_{t}} f(m_{i})/S_{t} \quad (2)$$

where $m$ is the miRNA of the $t$-th signature.

2.3. LASSO and elastic net

To evaluate the estimation ability of GASE, we compared the prediction performance with some standard regression methods, including ridge [43], Lasso [44] and elastic net [45]. We used the miRNA expression profiles and survival time of 123 patients with STEC as input. The minimum $\lambda$ was selected after 100 independent runs of LASSO and elastic net using 10-CV.

2.4. Strong evidence on miRNA-gene target interaction

To identify the target genes of the selected miRNAs, we used the miRTarBase (9.0 beta) database [46] to extract the experimentally verified microRNA-target interactions (MTIs) with strong evidence, which are validated by reporter assay, Western blot, and qPCR.

2.5. Gene set enrichment test

Gene-set libraries are used to organize accumulated knowledge about the function of groups of genes. We used Enrichr [47,48], which is a web-based application that includes the latest gene-set libraries, to perform gene-set enrichment analysis. We evaluated the ability of Enrichr to rank terms from gene-set libraries by combining the $p$-value computed using Fisher’s exact test with the z-score of the deviation from the expected rank by multiplying these two numbers as follows:

$$c = \log(p) \cdot z$$

where $z = z$-score and $p = p$-value.

This study used six Gene-set libraries, including 1) WikiPathway Human 2021 [49], 2) Kyoto Encyclopedia of Genes and Genomes (KEGG), 3) MSigDB Hallmark [50], 4) Gene Ontology Molecular Function 2021 [51], 5) Gene Ontology Biological Process, and 6) Gene Ontology Cellular Component.

3. Results

3.1. GASE prediction performance

We used a survival estimation method, GASE, to identify a miRNA signature and estimate the survival time in patients with STEC. One hundred and twenty-three patients with miRNA expression profiles were retrieved from the TCGA database. GASE identified 27 miRNAs as a survival miRNA signature and estimated the survival time with a mean squared correlation coefficient ($R^2$) of 0.80 ± 0.01 and a mean absolute error (MAE) of 0.44 ± 0.25 years between actual and estimated survival times.

A robust miRNA signature was selected by measuring the frequency appearance score (FAS) using 50 independent runs of GASE. A miRNA signature with the highest FAS indicates higher frequencies of miRNAs in the signature across the independent runs of GASE. The mean FAS obtained for the independent runs was 15.5 ± 1.45, while the highest FAS was 18.85 (shown in Supplementary Fig. S1 and Supplementary Table S1). The feature set with the highest FAS was selected for the analysis. This feature set obtained a $R^2$ of 0.80 and a MAE of 0.43 years between actual and estimated survival times, and selected 27 miRNAs as a signature to estimate survival time in patients with STEC.

3.2. Prediction performance comparison and validation

Next, we compared GASE with some standard machine learning methods on their performance to predict survival times. The machine learning methods used in the comparison included ridge regression, least absolute shrinkage and selection operator (Lasso) and elastic net. Ridge regression obtained a $R^2$ of 0.77 and a MAE of 0.54 years between actual and estimated survival times. Lasso obtained a $R^2$ of 0.51 and a MAE of 0.69 years between actual and estimated survival times, and elastic net obtained a $R^2$ of 0.50 and a MAE of 0.71 years between actual and estimated survival times, respectively. In comparison, GASE obtained a highest $R^2$ of 0.83 and a MAE of 0.41 years between actual and estimated survival times (Table 1). The results indicated that the performance of GASE was better than that of the standard machine learning methods. The correlation plots of GASE and the other machine learning methods are shown in Supplementary Fig. S2A-D.

Next, the estimation ability of GASE was validated using a validation dataset consisting of 393 patients with STEC along with their follow-up times. The follow-up times of these patients were in the range of 0.3–56 months. We attempted to estimate the survival times of these patients using the GASE prediction model. The mean follow-up times observed in patients with STEC was 8.09 ± 1.29 months. The mean predicted survival time of these patients was 17.74 ± 10.50 months. GASE achieved an accuracy of 80.41% for estimating the survival times of patients whose estimated survival times were lower than the follow-up times (mean follow-up time 4.0 ± 5.9 months). The mean estimated survival time of the 316 patients was 19.10 ± 10.28 months, and a mean prediction error of 12.15 months was obtained for the remaining patients. The results could be interpreted as follows: an estimated survival time that was higher than the patient’s follow-up time was considered a correct prediction, whereas an estimated survival time that was lower than the follow-up time was considered a prediction error. The follow-up and estimated survival times of these patients are shown in Fig. 1.

3.3. Ranking of miRNA signature

The miRNAs of the identified miRNA signature were ranked based on their contribution towards estimating the survival time using main effect difference (MED) [42] analysis. A higher MED score represents greater contribution towards the prediction of survival time. A miRNA with a higher MED score indicates superior prediction ability towards the survival time estimation, whereas a lower-scoring miRNA indicates a smaller contribution to survival time estimation. The top 10 ranked miRNAs are shown in Table 1. The prioritization of miRNAs based on their contribution to the survival estimation is shown in Fig. 2.
3.4 Diagnosis prediction

The diagnostic ability of the identified miRNA signature was measured by distinguishing healthy and STEC patients using CancerMiRNome database [52]. The individual miRNAs that compose the miRNA signature had AUCs in a range of 0.49–0.94 for distinguishing healthy from STEC patients, as shown in Table 3. Among the signature miRNAs, 13 miRNAs, including hsa-miR-93-5p, hsa-miR-1 81b-5p, hsa-miR-125a-5p, hsa-miR-1301-3p, hsa-miR-30e-5p, hsa-miR-767-5p, hsa-miR-16-5p, hsa-miR-675-3p, hsa-miR-326, hsa-miR-760, hsa-miR-20a-3p, hsa-miR-664a-5p, and hsa-miR-130a-5p were good diagnostic predictors of esophageal carcinoma (ESCA) (AUC > 0.70), as shown in Fig. 3. Ten miRNAs, including hsa-miR-30e-5p, hsa-miR-1301-3p, hsa-miR-125a-5p, hsa-miR-93-5p, hsa-miR-326, hsa-miR-532-5p, hsa-miR-9-5p, hsa-miR-181b-5p, hsa-miR-193a-5p, and hsa-let-7 g-5p were good diagnostic predictors of stomach adenocarcinoma (STAD) (AUC > 0.7), as shown in Fig. 4.

3.5 Expression differences of the miRNA signature

Expression difference analysis was performed to measure the significance in the expression levels of the identified miRNA signature between normal and tumor tissues of ESCA and STAD patients using the CancerMiRNome database [52]. There were 14 miRNAs, including hsa-miR-625-3p, hsa-miR-664a-5p, hsa-miR-326, hsa-miR-130a-5p, hsa-miR-20a-3p, hsa-miR-675-3p, hsa-miR-760, hsa-miR-1301-3p, hsa-miR-16-5p, hsa-miR-767-5p, hsa-miR-130a-5p, hsa-miR-181b-5p, hsa-miR-93-5p, hsa-miR-193a-5p, and hsa-miR-193b-5p, which showed a significant difference (p < 0.05) between normal and ESCA samples (Table 4). There were 19 miRNAs, including hsa-miR-664a-5p, hsa-miR-767-5p, hsa-miR-9-5p, hsa-miR-93-5p, hsa-miR-193a-5p, and hsa-miR-30e-5p, which showed a significant difference (p < 0.05) between normal and STAD patients (Table 4). The top 10 ranked miRNAs and their expression differences between healthy and ESCA and STAD patients are shown in Figs. 5 and 6, respectively.

Table 2: Ranking of miRNA signature and corresponding MED scores.

| Rank | miRNA      | MIMAT-ID   | MED       |
|------|------------|------------|-----------|
| 1    | hsa-miR-760| MIMAT0004957| 1.728135  |
| 2    | hsa-miR-767-5p | MIMAT0003882 | 1.480966  |
| 3    | hsa-miR-1301-3p | MIMAT0002797 | 1.344602  |
| 4    | hsa-miR-891a-5p | MIMAT0004902 | 1.14225   |
| 5    | hsa-miR-532-5p | MIMAT0002888 | 1.139153  |
| 6    | hsa-miR-29a-5p | MIMAT0004503 | 0.887408  |
| 7    | hsa-miR-125a-5p | MIMAT0000609 | 0.88658   |
| 8    | hsa-miR-130a-5p | MIMAT0004593 | 0.863724  |
| 9    | hsa-miR-329-3p | MIMAT0001629 | 0.844311  |
| 10   | hsa-miR-496 | MIMAT0002818 | 0.818043  |
| 11   | hsa-miR-20a-3p | MIMAT0004493 | 0.724058  |
| 12   | hsa-miR-125a-5p | MIMAT0000443 | 0.63757   |
| 13   | hsa-miR-181b-5p | MIMAT0000257 | 0.590379  |
| 14   | hsa-miR-675-3p | MIMAT0006790 | 0.578151  |
| 15   | hsa-miR-9-5p  | MIMAT0000441 | 0.484588  |
| 16   | hsa-miR-664a-5p | MIMAT0005948 | 0.425219  |
| 17   | hsa-miR-93-5p  | MIMAT000093 | 0.364274  |
| 18   | hsa-miR-30e-5p  | MIMAT000092  | 0.335408  |
| 19   | hsa-miR-376c-3p  | MIMAT000720 | 0.345478  |
| 20   | hsa-miR-326  | MIMAT000756 | 0.312151  |
| 21   | hsa-miR-193a-5p  | MIMAT000614 | 0.275742  |
| 22   | hsa-miR-532-3p  | MIMAT0004780 | 0.268942  |
| 23   | hsa-miR-625-3p  | MIMAT0004808 | 0.259763  |
| 24   | hsa-miR-106a-5p  | MIMAT000103 | 0.213422  |
| 25   | hsa-let-7 g-5p  | MIMAT000414 | 0.152833  |
| 26   | hsa-let-7f-5p  | MIMAT000067 | 0.04358   |
| 27   | hsa-miR-193b-5p | MIMAT0004767 | 0.010963  |
3.6. MiRNA-gene target enrichment analysis

There were 558 miRNA target interactions (MTI) with strong evidence, which included 32 miRNAs and 352 target genes from miRTarBase (Supplementary Table S2). We performed gene-set enrichment analysis using three pathway libraries: WikiPathway, KEGG, and MSigDB Hallmark, shown in Fig. 7. The highly enriched pathways in WikiPathway, KEGG, and MSigDB Hallmark were the somatotrophic axis and its relationship to dietary restriction and aging (WP4186) (adjusted p-value: 1.34E-10, Odds ratio: 117888, combined score: 2,862,498), pancreatic cancer (adjusted p-value: 1.54E-34, Odds ratio: 44.55, combined score: 3655.72), and apoptosis (adjusted p-value: 5.17E-20, Odds ratio: 12.68, combined score: 563.12), respectively, shown in Supplementary Tables S3-S5.

Additionally, the miRNA signature-gene interaction network was built using miRTarBase [46], TarBase V8. [53] and miRecords [54]. There were 28,057 edges associated with 10,525 genes. We reduced the low priority edges using the shortest path network measures [55]. The final network, consisting of 832 edges 93 targeted genes, is shown in Supplementary Fig. S3.

The Gene Ontology (GO) annotations of the target genes were in three categories: biological process, molecular function, and cellular component. The highly enriched pathways for biological process, molecular function, and cellular component were positive regulation of smooth muscle cell apoptosis process (GO:0034393), I-SMAD binding (GO:0070411), and serine/threonine protein kinase complex (GO:1902554), respectively, as shown in Supplementary Figs. S4-S6 and Supplementary Table S6.
Fig. 3. Diagnosis prediction ability of miRNAs was evaluated in ESCA using ROC curves.

Fig. 4. Diagnosis prediction ability of miRNAs was evaluated in STAD using ROC curves.
3.7. MicroRNAs in cancers

The roles of the top 10 ranked miRNAs in various diseases and cancers were examined using the Human microRNA Disease Database (HMDD v3.2) [56], miRTarbase, and by reviewing the scientific literature. The information from these resources indicates that the top 10 ranked miRNAs are involved in STEC. A quantitative real-time PCR analysis reported that hsa-miR-760 is significantly downregulated in ESCA tissues and cell lines, suggesting that this miRNA could be used as a prognostic indicator [57]. Significant differential expression of hsa-miR-1301-3p in ESCA tissues indicates that this miRNA could be used as a prognostic biomarker for ESCA [60]. Zeng and colleagues reported the downregulation of hsa-miR-532-5p in gastric cancer cells, and its expression is associated with poorer survival in patients with gastric cancer [61]. Tokumaru and colleagues demonstrated the association of hsa-miR-29a with overall survival in patients with gastric cancer, and lower expression of hsa-miR-29a worsens the overall survival in patients with gastric cancer [62]. Hsa-miR-16-5p has been used as a prospective biomarker for prognosis prediction in patients with gastric cancer and ESCA [63,64]. Hsa-miR-130a-5p affects cell growth, migration and invasion by targeting cannabinoid receptor 1 in gastric cancer cells [65], and it also deregulates PTEN and controls malignant cell survival and tumor growth in multiple cancers [66]. Hsa-miR-329-3p acts as a tumor suppressor by targeting T lymphoma invasion and metastasis in gastric cancer cells and could be utilized as potential therapeutic target [67]. Hsa-miR-496 is downregulated in gastric cancer cell lines, and it inhibits cell proliferation via targeting Lyn kinase in gastric cancer cell lines [68]. Among the top 10 ranked miRNAs, the roles of two miRNAs, hsa-miR-769-5p and hsa-miR-891a-5p, have not been reported previously in either STAD or ESCA.

Additionally, a miRNA-disease network was constructed for the miRNA signature using miRNet 2.0 [55]. The miRNAs of the signature were observed to be involved in several diseases. In the miRNA-disease association network, there were 12 nodes (miRNAs) with 132 edges associated with 85 diseases, shown in Supplementary Fig. S7.

### 4. Discussion

miRNAs provide a way to explore disease mechanisms in various cancers, including STEC. The clinical applications of miRNAs in cancer rely on identifying miRNA signatures as potential biomarkers and developing miRNA-target based therapeutics. Accordingly, we developed a survival time estimation method, GASE, to identify a miRNA signature that was correlated with STEC patient survival. Computational methods for feature selection often suffer from issues related to data quality and high dimensionality, especially when dealing with biomedical data. To address the challenges of identifying the right biomarker, we used an optimal feature selection algorithm, IBCGA, which is good at identifying an optimal subset of features while considering the trade-off between accuracy and complexity. This approach is particularly useful for identifying miRNA signatures that can be used to develop personalized therapeutic strategies for STEC patients.

### Table 4

| miRNA signature | Normal vs ESCA p-value | Normal vs STAD p-value |
|-----------------|------------------------|------------------------|
| hsa-miR-760     | 0.003                  | 0.0297                 |
| hsa-miR-767-5p  | 0.0004                 | 0.0003                 |
| hsa-miR-1301-3p | 0.0001                 | <0.0001                |
| hsa-miR-891a-5p | 0.873                  | 0.673                  |
| hsa-miR-532-5p  | 0.3987                 | <0.0001                |
| hsa-miR-29a-5p  | 0.3481                 | 0.008                  |
| hsa-miR-16-5p   | 0.0005                 | 0.0911                 |
| hsa-miR-130a-5p | 0.0115                 | 0.1496                 |
| hsa-miR-329-3p  | 0.4576                 | 0.6845                 |
| hsa-miR-496     | 0.1857                 | 0.1031                 |
| hsa-miR-20a-3p  | 0.005                  | <0.0001                |
| hsa-miR-125a-5p | <0.0001                | <0.0001                |
| hsa-miR-181b-5p | <0.0001                | <0.0001                |
| hsa-miR-679-3p  | 0.0033                 | 0.1292                 |
| hsa-miR-9-5p    | 0.9717                 | <0.0001                |
| hsa-miR-664a-5p | 0.0177                 | 0.0002                 |
| hsa-miR-93-5p   | <0.0001                | <0.0001                |
| hsa-miR-30e-5p  | <0.0001                | <0.0001                |
| hsa-miR-376c-3p | 0.5339                 | 0.0033                 |
| hsa-miR-326     | 0.0117                 | <0.0001                |
| hsa-miR-193a-5p | 0.3494                 | <0.0001                |
| hsa-miR-532-3p  | 0.8464                 | <0.0001                |
| hsa-miR-625-3p  | 0.023                  | 0.9569                 |
| hsa-miR-106a-5p | 0.1783                 | 0.5017                 |
| hsa-let-7 g-5p  | 0.3872                 | 0.0003                 |
| hsa-let-7f-5p   | 0.7968                 | 0.0026                 |
| hsa-miR-193b-5p | 0.343                  | <0.0001                |

**Abbreviation**: ESCA—Esophageal carcinoma, STAD—Stomach adenocarcinoma.
Fig. 6. Comparison of expression of the top 10 ranked miRNAs between normal and STAD samples using boxplot representation. (*) indicates p < 0.05.

Fig. 7. The pathways enrichment analysis of miRNA signature targeted genes in three categories, (A) wiki pathways, (B) KEGG pathways, and (C) MSigDB hallmark.
The miRNA-gene target interaction analysis showed that the target genes were highly enriched in the somatotrophic axis and its relationship to dietary restriction and the aging (WP4186) pathway. The somatotrophic axis in mammals involves signaling by growth hormone (GH), which is produced by the anterior pituitary, and its secondary mediator, insulin-like growth factor 1 (IGF-1). In a previous study, growth hormone–releasing hormone and its receptor (GHRH-R) were found primarily in the anterior pituitary gland, gastric cancers, other solid tumors, and lymphomas. Increased levels of GHRH-R in tumor samples from patients with gastric cancer are associated with poor outcomes [69]. Another important enriched pathway of the miRNA signature was transforming growth factor beta (TGF-β) signaling pathway. TGF-β is a cytokine that participates in both physiological and pathological processes including tumorigenesis [70]. During tumor progression, TGF-β signaling regulates the immune/inflammatory response and the tumor microenvironment. It also regulates tumor growth, epithelial-mesenchymal transition (EMT), and cancer cell stemness depending on tumor stage and cellular context [71]. EMT is also an enriched pathway from MSigDB Hallmark (adjusted p-value: 8.24E-19, Odds ratio: 11.13, combined score: 506.81), which is consistent with this biological mechanism. Abnormal TGF-β signaling has been associated with progression of gastrointestinal cancer [72], which includes esophageal, gastric, liver, colorectal, and pancreatic carcinomas that, collectively, are major causes of cancer-related deaths worldwide [73]. Several TGF-β-based therapies have been developed for the treatment of gastrointestinal cancers and have displayed efficacy in clinical trials [74,75]. Additional support for the role of TGF-β signaling in STEC was obtained from the GO annotation analysis, which showed that I-STAT binding (GO:0070411) was enriched in the GO molecular function category (adjusted p-value: 1.03E-06). Nuclear accumulation of active SMAD complexes is crucial for the transduction of TGF-β superfamily signals from transmembrane receptors to the nucleus.

The top hits for gene target enrichment analysis also indicated that the miRNA signature was related to miRNAs involved in DNA damage response, epidermal growth factor receptor tyrosine kinase inhibitor resistance, apoptosis, Wnt/beta-catenin signaling, and angiogenesis. DNA damage response pathways are known to be related to therapy resistance in STEC [76,77], and resistance to epidermal growth factor receptor tyrosine kinase inhibitors are relevant to survival in STEC, consistent with the use of epidermal growth factor receptor tyrosine kinase inhibitors as targeted therapy in STEC [78,79]. The Wnt/beta-catenin signaling pathway has been implicated in cancer progression in STEC [80], and the dysregulation of apoptosis and angiogenesis are known to promote tumor growth [81,82]. This suggests the miRNAs in the signature and the putative gene targets of these miRNAs are possible molecular targets for exploitation in the pursuit to create new therapies for STEC.

In addition to being associated with survival, the miRNAs in the signature could discriminate between healthy and STEC patients, and were differentially expressed between the healthy and tumor tissues of patients with STEC. This suggests that the capability of these miRNAs to function as prognostic or diagnostic biomarkers. Further investigation is needed to determine the utility of the miRNA signature as a prognostic biomarker for monitoring response to therapy or predicting survival after therapy in STEC patients and as a biomarker for early STEC diagnosis. Other questions for study are whether the miRNA signature can perform as a biomarker in STEC of different types and stages, and whether the miRNA signature can be detected in blood at a level of accuracy comparable to that in tumor tissue (to allow for the possibility of performing liquid biopsies for biomarker detection).

In conclusion, a better understanding of the miRNA signature in survival predictions will aid in developing treatment strategies for STEC. We anticipate that the miRNA signature identified here could help in understanding the roles of miRNAs in STEC and developing miRNA-based cancer therapeutics.

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Author contributions
S.Y.S. designed the system, carried out the detail study and supervised the study. S.Y.S, M.T, T.C, P.A, S.K.S, A.B and S.Y.H, participated in data analysis, manuscript preparation and discussed the results. All authors have read and approved the final manuscript.

Availability of data and materials
All the data used in this analysis can be found on the TCGA data portal [https://portal.gdc.cancer.gov/].

Ethics approval and consent to participate
Not applicable.

Consent to publish
Not applicable.

CRediT authorship contribution statement
Srinivasulu Yerukala Sathipati: Conceptualization, Data curation, Writing – original draft, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision. Ming-Ju Tsai: Validation, Visualization, Formal analysis. Tonia Carter: Formal analysis, Data curation, Writing - review & editing. Patrick Allaire: Formal analysis. Sanjay K Shukla: Formal analysis. Afsin Beheshiti: Formal analysis, Writing - review & editing. Shinn-Ying Ho: Formal analysis.

Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.csbj.2022.08.025.

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