SLC39A14 may be a characteristic gene affecting the development of Barrett's esophagus

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Research Article

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Abstract

Ferroptosis is associated with a variety of pathophysiological processes. The inhibition of ferroptosis has been widely concerned in some diseases. However, no study has yet fully elucidated the role of iron death-related genes (FRGS) in Barrett esophagus. The key genes of ferroptosis in Barrett’s esophagus were screened by bioinformatics analysis and verified by experiments. Data were downloaded from the Ferroptosis database (FerrDb) and the Comprehensive Gene Expression Database (GEO) database, and 203 DE-FRGs associated with Barrett's esophagus were obtained, which are associated with immune inflammation, cancer, etc. SLC39A14 was identified as a key gene from these 203 DE-FRGs using SVM-RFE and LASSO algorithms. Functional annotation shows that this gene may have an important impact on Barrett's esophagus through Autophagy animal, HIF-1 signaling pathway, and FoxO signaling pathway and other pathways. Establishing a Barrett's esophagus rat model through “end-to-end anastomosis of esophagus duodenum and preservation of whole stomach”, and detecting the characteristic target SLC39A14 in rat esophageal tissue. And constructing a ceRNA network of characteristic target SLC39A14 related miRNAs and lncRNAs. In summary, this study provides some insights into the pathogenesis of Barrett’s esophagus by combining data mining with experimental verification. On the basis of clinical data mining, animal experiments were conducted to verify the key target SLC39A14. It was revealed that SLC39A14 may be a key gene affecting the occurrence and development of Barrett’s esophageal disease through ferroptosis pathway, and the miRNA and lncRNA bound by SLC39A14 were predicted.

1 Background

Barrett’s esophagus (BE) is a chronic and common disease of the digestive system. It is characterized by mucosal changes at the distal end of the esophagus. The squamous epithelium is covered by columnar epithelium and is a precancerous lesion associated with adenocarcinoma of the esophagus[1]. The prevalence of Barrett’s esophagus in Asian population is around 2%, and the prevalence of Barrett’s esophagus is estimated to be around 5–15% in adults with reflux symptoms[2, 3]. Over time, the progression from chronic esophagitis, manifested by the intestinal metaplasia and dysplasia, to invasive esophageal adenocarcinoma (EAC) can be observed. It is estimated that 10% of patients with BE will progress from metaplasia to dysplasia and finally to EAC. Statistically, a diagnosis of BE is a 30–60-fold risk factor of EAC development, but only a minority of patients with BE will develop EAC[4–6].

Ferroptosis is a type of iron-dependent programmed cell death, which is different from necrosis, apoptosis and autophagy[7]. Ferroptosis plays an important role in inflammation, and treatment with the iron apoptosis inducer RSL-3 in a mouse model of nonalcoholic steatosis hepatitis (NASH) progression has been shown to significantly increase protein levels of proinflammatory cytokines, including TNFα, IL-1β, and IL-6, and to significantly worsen NASH-related biomarkers and histopathology, such as serum chemistry, hepatic osteonecrosis, lobular inflammation, and apoptosis[8]. BE esophageal lesions are chronic inflammatory reactions that occur as a result of exposure of the esophagus to a chronic reflux mixture consisting of gastric acid, bile acids, and bile salts. However, the exact pathogenesis of BE remains unclear. There may be a link between Barrett's esophagus and ferroptosis.
Therefore, this study was designed to explore the potential key genes of ferroptosis in Barrett's esophagus and their potential value as diagnostic markers of the disease.

2 Material and methods

2.1 Bioinformatics analysis

2.1.1 Source of information

In this study, the gene expression profiles of patients with Barrett's esophagus and control samples were obtained from GEO database, and the gene sets with more than 20 standard patients with Barrett's esophagus and control samples were selected. The GSE36223 dataset included 23 Barrett esophageal samples and 23 control samples as a training set for analysis. The GSE39491 dataset included 40 Barrett's Esophagus specimen samples and 40 control samples as the validation set for the analysis. The FRG used in the study (n = 245) was based on FerrDb.

2.1.2 Identification of differentially expressed genes

Collect the expression profiles of 203 FRGs obtained from the GSE36223 database in Barrett's esophagus and healthy samples. Subsequently, Wilcoxon rank-sum test was used to detect the differential expression of FRG (DE-FRG) between the two groups at the level of $P < 0.05$. P values for multiple testing were adjusted by Benjamini-hochberg correction.

2.1.3 Functional Notes

To further analyze the function of DE-FRG, these genes were subjected to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment by the R software “clusterer Profiler” package.

2.1.4 Barrett’s esophagus FERROPTOSIS POTENTIAL key gene

In this manuscript, we employ the majestic least absolute shrinkage and selection operator (LASSO) algorithm, and put into practice the sublime least absolute shrinkage and selection operator (LASSO) algorithm to diminish the dimensionality of our data, utilizing the magnificent glmnet software package(Friedman et al 2010; Yang et al 2019).DE-FRG was employed to safeguard the essence of Barrett's esophagus and healthy specimens, whereas the LASSO algorithm was harnessed to discern the gene biomarkers conjoined with Barrett's esophagus. Meanwhile, the support vector machine recursive feature elimination (SVM-RFE) model was forged via utilization of the support vector machine software package, and the estimable average false positive rate and 10-fold cross-validation were utilized[11]. Furthermore, it was discovered that the paramount genetic biomarker for Barrett's esophagus emerged from the overlap of biomarkers procured by the aforementioned two algorithms.
ROC curves were rendered and the area beneath the curve (AUC-RRB-values, sensitivity, specificity, and accuracy) were ascertained to appraise the diagnostic significance of the designated genetic markers. Furthermore, a Logit model was constructed employing seven marker genes in order to prognosticate the sample type of the GSE36223 dataset, utilizing the R package GLM. Likewise, the ROC curve serves as an appraisal of the diagnostic utility of the aforementioned Logit model we have constructed.

2.1.5 Single-gene gene set enrichment analysis

SsGSEA use that GSEA package of R software. To explore the pathway of marker gene enrichment, the association of marker genes with the rest of the genes was analyzed based on the GSE36223 dataset. Each gene was then ranked based on high-to-low correlations, and the sequenced genes were included in the gene set for analysis. Furthermore, this work considers the KEGG pathway set to be a predefined set for detecting enrichment levels in gene sets.

2.1.6 Validation in the validation set

Expression levels of key genes were verified based on the GSE39491 dataset.

2.2 Animal experiment

2.2.1 Experimental animals

Wistar male rats (8 weeks old), 10 rats, 240g-260g, purchased from Speiford Beijing Biotechnology Co., Ltd., License No. (SCXK (Beijing)2019-0010), bred in Tianjin Institute of Radiology, Animal Ethics No. (NKYY-DWLL-2023-006).

2.2.2 Mode of modeling

The rat model of “esophago-duodenal end-to-side anastomosis + whole stomach preservation” was used in Barrett's esophagus group[12]: Preoperative solid food fasting for 24 hours, water deprivation for 12 hours, anesthesia machine inhalation anesthesia (Isoflurane induction concentration of 4%, maintenance concentration of 2%), abdominal skin incision along the midline, incision length of about 25mm, exposure of abdominal cavity, gastroesophageal junction of the esophagus, closure of gastric stump, distal pylorus 1 cm (duodenal mesentery opposite side) longitudinal incision of 4mm, esophageal stump and duodenal incision anastomosis, intraperitoneal injection of gentamicin sulfate 2U, layer-by-layer suture after closure of abdominal cavity, 6 hours after surgery, eat sugar saline, every other day normal diet. Four weeks after operation, Dextran iron (4 mg KG) was injected intraperitoneally every week. The molding process lasted 22 weeks. The control group was treated with “Open-and-close abdominal pseudo-operation”. After 6 hours, the patients in the control group were fed with sugar-salt solution and normal diet every other day. Five rats in each group.

2.2.3 Principal reagents

SLC39A14 antibody(Affbiotech,DF14224).

2.2.4 Observation indicators and methods
Pathological changes of esophageal tissue were detected by HE staining

The lower esophageal mucosa of the rats was sectioned with paraffin and the changes of esophageal histopathology were observed by HE staining. First, the tissue sections were dehydrated at room temperature, and soaked in alcohol and then xylene to dehydrate the sections. Subsequently, the slices were sequentially soaked in ethanol of each concentration to remove the lipid solution. Then, the sections were soaked in alkaline solution and dye solution in turn to make the nuclei blue or purple and the cytoplasm pink. Finally, the sections were completely dried by washing, dehydrating, and sealing to allow for observation of tissue structure.

Immunohistochemistry was used to detect the expression of potential key genes in Barrett’s esophagus

The immunohistochemical staining method was used to detect key gene expression. It involved sample preparation, fixation, embedding, or tissue preparation. Specific primary antibodies bound to the target protein were used for antigen detection. Secondary antibodies conjugated with fluorescent dyes or enzymes were then applied, forming a complex. A color reaction was initiated to visualize enzymatic activity and create a visible color.

3 Results

3.1 DE-FRGs identification from GSE36223 database

In the GSE36223 dataset, 203 DE FRGs were obtained from 245 DE FRGs, including 121 up-regulated and 82 down-regulated. Figure 1A shows the clustering heatmap of DE-FRGs expression profiles in different samples, and Fig. 1B shows gene connections.

3.2 Functional annotation of DE-FRGs

In order to elucidate the biological functions and pathways related to DE FRGs, GO functional annotation and Reactome pathway analysis were conducted. Therefore, GO molecular function (MF) annotation shows that DE FRGs are significantly enriched as “DNA binding transcription factor binding”, “RNA polymerase II specific DNA binding transcription factor binding”, and “DNA binding transcription activator activity, RNA polymerase II specific” (Fig. 1C). Regarding cellular composition (CC), DE FRG is significantly correlated with “RNA polymerase II transcription regulator complex”, “organelle outer membrane”, and “autophagosome”. In addition, GO biological process (BP) annotations show a close relationship between DE-FRGs and “response to nutrient levels”, “cellular response to chemical stress” and “response to oxidative stress”. GO enrichment analysis (function) is shown in Fig. 1D. Through the analysis of the Reactome pathway, it was found that Human cytomegalovirus infection, Autophagy animal, HIF-1 signaling pathway, and FoxO signaling pathway (Fig. 1E). Based on the above results, DE-FRGs may play an important role in the pathogenesis of Barrett's esophagus by regulating cytokines, immune cells, and autophagy.
3.3 11 DE-FRGs served as genes to diagnose Barrett's esophagus

To consider changes in Barrett's esophagus cases compared with normal subjects, this work focused on predicting whether DE-FRGs could be used in disease diagnosis. Subsequently, 2 different machine learning algorithms LASSO and SVM-RFE were adopted for analysis based on GSE36223 dataset, for the sake of screening DE-FRGs significantly distinguishing Barrett's esophagus from healthy subjects. The penalty parameter was tuned by 10-fold cross-validation in LASSO logistic regression, which selected 3 Barrett's esophagus-related features (Figs. 2A, B). Afterwards, SVM-RFE algorithm was applied in filtering 1 DE-FRGs for identifying the best feature gene combination. At last, this work detected 1 gene (minimal RMSE = 0, maximal accuracy = 1) to be best feature genes (Figs. 2C, D). Thereafter, marker genes acquired based on the above two algorithms were intersected to obtain 11 marker genes (SLC39A14) in subsequent analyses (Fig. 2E). Using R package glm, these 1 marker genes identified were used to build the logistic regression model. According to later ROC curve analysis, the logistic regression model built based on these 11 marker genes well distinguished Barrett's esophagus from healthy samples, and the AUC value was 0.981 (Fig. 2F). Besides, for elucidating whether single genes could be used to differentiate Barrett's esophagus from healthy controls, this work plotted ROC curves for those 1 marker genes. According to Fig. 3G, AUC value was 1 of the 1 gene. Consequently, our logistic regression model was accurate and specific in distinguishing Barrett's esophagus from healthy samples compared with single marker genes.

3.4 Marker genes showed tight relation with various Barrett’s esophagus-related pathways

For better exploring the possible roles of marker genes in distinguishing Barrett's esophagus from healthy samples, the single-gene GSEA-KEGG pathway analysis was carried out. Figure 3 displays those 3 pathways associated with marker genes. Marker genes were comprehensively analyzed, as a result, they were found to be significantly associated with olfactory transduction valine leucine and isoleucine biosynthesis abc transporters.

3.5 Marker gene expression levels in validation set

At last, marker genes expression levels were validated based on GSE39491 dataset. According to our results, SLC39A14 showed similar expression profiles to those in GSE36223 dataset. Typically, SLC39A14(p = 1e − 08) levels elevated among Barrett’s esophagus (Fig. 2H).

3.6 Results of animal experiments

The results of HE staining showed that the epithelial cells of the esophageal mucosa in the control group were well arranged and there was no inflammation such as hyperemia and erosion, while the microvilli and crypts were found in the esophageal tissues of the model group, it is suggested that Barrett’s esophagus’s esophageal model was successfully established in rats (Fig. 4A, B).
Immunohistochemical results showed that the expression of SLC39A14 was increased in the model group compared with the control group (p < 0.05) (Fig. 4C, D, E).

3.7 Construction of characteristic gene-related ceRNA network

Use Randa (https://rnb.cchmc.org/Randa), RDB (http://pridb.gdcb.iastate.edu/RPISeq/), and TargetScan (http://www.targetscan.org/vert_72/) to screen for miR-NAs that bind to SLC39A14. Predict IncRNAs binding to miRNAs using the s-pongeScan database (http://www.lncRNAdatabase.org/spongescan/). Build a ceR-NA network using Cytoscape(Fig. 5).The related miRNAs are HSA-MIR-3145-3P, HSA-MIR-548C-3P, HSA-MIR-4264 and so on. The IncRNAs associated w-ith the related miRNAs are CDR1-AS, RP11-830F9.6, C10orf91 and so on.

4 Discussion

The mechanism of development of Barrett esophagus[13, 14]: Acid reflux and esophageal mucosal injury: Long-term gastric acid reflux is the main cause of the development of Barrett’s esophagus. Gastric acid leads to erosion and injury of esophageal mucosa by acid fluid through relaxation of esophageal sphincter, loss of pumping function of esophageal sphincter and impairment of esophageal mucosal barrier function; Cell ulcer and malignant transformation: During the development of Barrett’s esophagus, mucosal damage caused by long-term acid reflux leads to cell damage and increased inflammatory response. These injuries and inflammatory reactions further lead to the formation of cellular ulcers and even malignant transformation into esophageal adenocarcinoma.

Ferroptosis is a new type of cell death, which is different from the common cell death modes such as apoptosis, necrosis and autophagy. Ferroptosis induces cell death mainly through the accumulation of intracellular iron ions and the production of lipid peroxides[15]. Too much iron in the body triggers the production of oxygen free radicals, which promote inflammation[16]. The chronic injury of esophageal mucosa caused by inflammation may be related to the occurrence of Barrett’s esophagus, but there are few related studies at present. In Barrett’s esophagus, Ferroptosis promotes cellular ulceration and progression of malignant transformation. Recent studies have shown that activation of NRF2 by APE1/REF1 is redox-dependent in Barrett’s related esophageal adenocarcinoma cells[17].And in esophageal adenocarcinoma, targeting NRF2 sensitizes esophageal adenocarcinoma cells to cisplatin through Induction of[18]. Further study of the expression and function of the regulators associated with ferroptosis may lead to new approaches to inhibit the development of Barrett’s esophagus and may be a new therapeutic strategy.

In this study, we found that the occurrence of Ferroptosis in Barrett’s esophagus is closely related to the abnormal expression of regulatory factors such as antioxidant transporters, iron metabolism-related proteins, peroxisomal tumor suppressor proteins, etc. Increased expression of SLC39A14 may be a key target in promoting Ferroptosis in Barrett’s esophagus.
SLC39A14 is an important iron transporter, which mediates the transport of iron and regulates the iron content in cells, thus maintaining the iron homeostasis[19]. Deleting hepatic SLC39A14 expression in Trf-LKO mice significantly reduced hepatic iron accumulation, thereby reducing ferroptosis-mediated liver fibrosis induced by either a high-iron diet or CCL4 injections (Yu et al. 2020). A unique feature of SLC39A14 is its upregulation by proinflammatory conditions, particularly increased interleukin 6 (IL-6) and nitric oxide[21].

This study demonstrates that SLC39A14 is overexpressed in Barrett's esophagus's esophagus, which may be a promising biomarker for early detection and disease surveillance. Targeting SLC39A14 may provide new therapeutic avenues. The relevant miRNAs include HSA-MIR-3145-3P, HSA-MIR-548 C-3P, HSA-MIR-4264, etc. LncRNAs related to miRNAs include CDR 1-AS, RP 11-830F9.6, C10 orf 91, etc. However, further studies are needed to evaluate the clinical utility of SLC39A14 as a diagnostic marker and therapeutic target for BE.

5 Conclusion

Both Ferroptosis regulation and SLC39A14 show promise as potential strategies for the clinical management of Barrett's esophagus. Manipulating Ferroptosis pathways may offer some effective therapeutic options, while SLC39A14 could serve as a valuable diagnostic marker and therapeutic target. Advancements in understanding the clinical applications of these approaches may improve patient outcomes and reduce the burden of Barrett's esophagus and associated complications.

Declarations

Author Contributions

ZG, MLL: analyzing data and writing the original draft. LL, SYL: consult and collect data. LY and YPT: writing the review and editing. YPT: funding acquisition. The authors have read and approved the final manuscript.

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Data availability

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article.

Ethics approval

The study was approved by the Tianjin Institute of Radiology, Animal Ethics No. (NKYY-DWLL-2023-006).

Consent for publication

All authors agree on publishing.
Conflict of interest The authors declare no competing interests.

References


Figures
Figure 1

DE-FRGs expression levels in Barrett’s esophagus and functional analyses for the DE-FRGs. (A) Violin plots show expression patterns of DE-FRGs across samples. (B) The correlation of these genes. (C) GO enrichment analyses indicated that DE-FRGs were significantly related to the function of “DNA binding transcription factor binding”, “RNA polymerase II specific DNA binding transcription factor binding”, and “DNA binding transcription activator activity, RNA polymerase II specific”. (D) GO enrichment analysis
(function) is showed. (E) Reactome pathway analyses indicated that “HIF-1 signaling pathway”, and “FoxO signaling pathway” were enriched.

Figure 2
1 DE-FRG was identified as diagnostic genes for Barrett's esophagus. (A, B) By LASSO logistic regression algorithm, with penalty parameter tuning conducted by 10-fold cross-validation, was used to select 3 psoriasis-related features. (C, D) SVM-RFE algorithm to filter the 1 DE-FRG to identify the optimal combination of feature genes. Finally, 1 gene (maximal accuracy = 1, minimal RMSE = 0) were identified as the optimal feature genes. (E) The marker genes obtained from the LASSO and SVM-RFE models. (F) Logistic regression model to identify the AUC of disease samples. (G) ROC curves for the 1 marker gene. (H) Expression of the marker gene in the validation set. The expression of marker genes in the GSE39491 dataset.
Figure 3

SLC39A14 showed tight relation with various Barrett’s esophagus-related pathways
Figure 4

The expression level of SLC39A14 increased in the esophageal tissue of BE rats. (A) (B) Compared with the control group of rats, the esophageal tissue of BE rats showed intestinal metaplasia pathological changes. (C) (D) (E) Compared with the control group of rats, the expression level of SLC39A14 in the esophageal tissue of BE rats increased.
Figure 5

CeRNA network predicted for characteristic target gene SLC39A14