Expression and Prognostic Value of RAD51 in Adenocarcinoma at the Gastroesophageal Junction

Darebai Redati, Xinhui Yang, Cheng Lei, Lin Liu, Lei Ge, *Haijiang Wang

Department of Gastrointestinal Surgery, The Affiliated Cancer Hospital of Xinjiang Medical University, Urumqi 830011, Xinjiang, China

*Corresponding Author: Email: frank80828@163.com

(Received 09 Feb 2022; accepted 22 Apr 2022)

Abstract
Background: The RAD51 recombinase is involved in homologous recombination and DNA repair. However, the association of RAD51 with the prognosis of adenocarcinoma at the gastroesophageal junction (ACGEJ) is not clear. We aimed to investigate the association of RAD51 with ACGEJ prognosis.

Methods: The difference in the expression level of RAD51 between ACGEJ tumors and control tissues in the microarray datasets (GSE159721, GSE74553, and GSE96669) were compared. The online Kaplan-Meier plotter survival analysis and meta-analysis were used to analyze the association of RAD51 with overall survival in pan-cancers. MiRNAs targeting RAD51 were identified and their expression profiles in ACGEJ tumors were analyzed. Functional enrichment analysis was performed for miRNAs of RAD51.

Results: RAD51 was upregulated in ACGEJ tumors compared with control tissues (P < 0.05). High RAD51 level was correlated with a poor prognosis in stomach adenocarcinoma and esophageal cancer. The meta-analysis showed that high RAD51 level was correlated with a poor prognosis in TCGA pan-cancers (P = 0.03). Six regulatory miRNAs of RAD51, including hsa-miR-182, hsa-miR-221, and hsa-miR-34a, were downregulated in ACGEJ tumor tissues and were associated with pathways including “fatty acid biosynthesis” and “viral carcinogenesis”.

Conclusion: RAD51 is a potent prognostic biomarker in ACGEJ. MiRNAs including hsa-miR-182, hsa-miR-221, and hsa-miR-34a might play crucial roles in ACGEJ by regulating the RAD51 gene.

Keywords: Gastroesophageal junction adenocarcinoma; RAD51 recombinase; MicroRNAs; The cancer genome atlas

Introduction

Gastric cancer is one of the most commonly diagnosed cancer worldwide (1,2). Also, the incidence of adenocarcinoma at the gastroesophageal junction (ACGEJ) has increased rapidly over the past few decades (3). The reason why ACGEJ remains a malignancy of great interest because there are many risk factors, such as smoking, obesity, and gastroesophageal reflux (3). ACGEJ is divided into three subtypes (Siewert type I, II, and III) according to the Siewert classification and proximal/distal of the anatomic gastric cardia and the overall survival of the three subtypes were different. The prognosis of ACGEJ is related to several factors, including the extent of nodal involvement (4,5), human epidermal growth factor re-
receptor 2 (HER2) status (6), neoadjuvant chemotherapy, and surgical strategy (7,8). More numbers of resected lymph nodes were associated with better survival in Siewert type II ACGEJ patients (5). Also, there is a controversy on the prognostic role of HER2 status in ACGEJ patients (6,9). Genetic factors including microRNAs and genes are biomarkers associated with the diagnosis or prognosis of ACGEJ (10,11). The discovery of new biomarkers plays an important role in an early, rapid, and accurate determination of disease occurrence and prognosis of human cancers.

The RAD51 recombinase is a RecA-like recombination and DNA repair protein involved in homologous recombination and DNA repair (12,13). The RAD51 protein interacts with the ssDNA-binding protein RPA and the RAD52 protein for the homologous pairing and strand transfer of DNA (12,13); and interacts with proteins BRCA1 and BRCA2 to play roles in responses to DNA damage (14,15). The stable recruitment of RAD51 to double-strand breaks is dependent on proteins including the RAD51 paralogs and BRCA1/2 (16). Cediranib-induced tumor hypoxia suppressed the expression of the homology-directed DNA repair factors including BRCA1/2 and RAD51, and then conferred sensitivity to olaparib in tumor cells (17). Recent studies showed that the RAD51 gene is a potential prognostic marker for multiple tumors, including colorectal adenocarcinoma (COAD) (18), hepatocellular carcinoma (HCC) (19), breast cancer (BCRA) (20), and colorectal cancer (CRC) (21). However, evidence showing the association of the RAD51 gene with ACGEJ prognosis is lacking.

Advances in microarray dataset, sequencing technology, and The Cancer Genome Atlas (TCGA) program promote the discovery of diagnostic and prognostic biomarkers contributing to the early, rapid, and accurate determination of tumor development and prognosis. We aimed to evaluate the association of the RAD51 gene with ACGEJ prognosis using microarray datasets and TCGA program. The regulatory microRNAs (miRNAs) of RAD51 were also identified to detect the potential miRNA-RAD51 regulatory axes related to ACGEJ prognosis.

Materials and Methods

Microarray datasets

This is a bioinformatics analysis based on gene expression microarray datasets conducted in 2021. Gene expression microarray datasets (GSE159721, GSE74553, and GSE96669) of ACGEJ were downloaded from the National Center of Biotechnology Information Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) on October 10, 2021. The datasets were selected because they included ≥50 ACGEJ tumor samples and the adjacent non-tumor tissue samples. The GSE159721 dataset (GPL20795, HiSeq X Ten [Homo sapiens]) included 123 ACGEJ tumor samples and 123 paired adjacent non-tumor tissue samples. The GSE96669 dataset (GPL10558, Illumina HumanHT-12 V4.0 expression bead-chip) consisted of 121 ACGEJ tumor samples and 123 paired adjacent non-tumor tissue samples. The GSE74553 dataset (GPL17692, [HuGene-2_1-st] Affymetrix Human Gene 2.1 ST Array [transcript (gene) version]) included 70 ACGEJ tumor samples and 13 normal esophageal squamous and gastric mucosa sections.

Data processing and RAD51 expression

The expression level of the RAD51 gene in the three datasets were downloaded and extracted from the GSE159721, GSE74553, and GSE96669 datasets. The difference in the expression level of RAD51 between the tumor and control samples was compared. Moreover, the expression profiles of the RAD51 gene across TCGA pan-cancers were determined in the UALCAN web resource (http://ualcan.path.uab.edu/index.html).

RAD51-related genes and protein-protein interaction (PPI) network

The PPI pairs related to the RAD51 gene were screened in the STRING database (Version 10.0;
http://www.string-db.org/) with the cutoff value of a score > 0.4. Genes related to the RAD51 gene were identified and used for the functional enrichment analysis. The Cytoscape software (version: 3.6.0, http://www.cytoscape.org/) was used to construct the PPI network.

**Functional enrichment analysis for RAD51**

Functional enrichment analysis of the Gene Ontology (GO) biological process and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways related to the genes related to the RAD51 gene was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID, version 6.8; https://david.ncifcrf.gov/). The items significantly associated with RAD51-related genes were selected using the criteria of p < 0.05 and count ≥ 2.

**Overall survival analysis for the RAD51 gene**

The Kaplan-Meier plotter (https://kmplot.com/analysis/index.php?p=service) is a meta-analysis-based discovery and validation of survival biomarkers based on the GEO, European Genome-phenome Archive (EGA), and TCGA databases. The probabilities of RAD51 with overall survival in 21 cancers, including breast, ovarian, lung, and gastric cancer, were assessed using the Kaplan-Meier plotter with automatically selected best cutoffs.

**Identification of miRNAs targeting RAD51**

The regulatory miRNAs of the RAD51 gene were identified from five databases, including starbase (pancancerNum ≥ 10; http://starbase.sysu.edu.cn/), TargetScan (context++ score percentile ≥ 99; http://www.targetscan.org/vert_71/), miRDB (Target Score ≥ 75; http://www.mirdb.org/), mirDIP (Score Class = High; http://ophid.utoronto.ca/mirDIP/), and miRmap (score > 75; https://mirmap.ezlab.org/app/). MiRNAs in at least three databases were obtained using the Venn map (http://bioinformatics.psb.ugent.be/webtools/Venn/) and were the key regulatory miRNAs of RAD51. Also, the experimentally validated miRNAs targeting RAD51 were identified from the published studies in PubMed, Medline, and Web of Science. The differences in the expression levels of the key regulatory miRNAs between the ACGEJ tumor samples and non-tumor samples in the microarray datasets were analyzed.

**Functional enrichment analysis for miRNAs**

The DIANA-miRPath v3.0 (http://www.microrna.gr/miRPathv3) is an online miRNA pathway analysis web-server dedicated to the assessment of miRNA regulatory roles and the identification of controlled pathways. We used the DIANA-miRPath to identify the pathways related to the regulatory miRNAs of RAD51 based on the predicted miRNA targets provided by the experimentally validated miRNA interactions derived from DIANA-TarBase.

**Statistical analysis**

The statistical analysis was performed in the SPSS 22.0 software (IBM SPSS, IBM, Armonk, NY, USA) and the Review Manager (RevMan version 5.0; Cochrane Collaboration, Oxford, UK). The differences in the expression levels of the RAD51 gene and related miRNAs between groups were compared using the non-parametric Mann-Whitney U test. A meta-analysis was performed to evaluate the probability of the RAD51 gene for predicting the prognosis of cancers. The cutoff value for the significant difference was set at P < 0.05.

**Results**

**RAD51 is upregulated in ACGEJ tumors**

The RAD51 gene is upregulated in ACGEJ tumor samples compared with the non-tumor control samples in the datasets GSE159721 (P = 5.93E-29), GSE74553 (P = 8.18E-06), and GSE96669 (P = 1.61E-04; Fig. 1). We found the expression levels of the RAD51 gene were upregulated in several other human cancer tissues.
compared with control, including BRCA, esophageal cancer/esophageal squamous cell carcinoma (ESCA), HCC, and stomach adenocarcinoma (STAD, \( P < 0.05 \); Fig. 2).

**Fig. 1:** The expression level of the \( R A D 51 \) gene in the GSE159721, GSE74553, and GSE96669 datasets. The differences in the expression levels of the \( R A D 51 \) gene between groups were analyzed using the non-parametric Mann-Whitney U test. TPM, transcripts per million. FPKM, fragments per kilobase of transcript per million fragments sequenced.

**Fig. 2:** Bar plot of \( R A D 51 \) expression profile across all tumor samples and paired normal tissues in the TCGA platform. TPM, transcripts per million. BLCA, bladder cancer. BRCA, breast cancer. CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma. CHOL, cholangiocarcinoma. COAD, colon adenocarcinoma. ESCA, esophageal squamous cell carcinoma. GBM, glioblastoma multiforme. HNSC, head and neck squamous cell carcinoma. KICH, kidney chromophobe. KIRC, kidney renal clear cell carcinoma. KIRP, kidney renal papillary cell carcinoma. LIHC/HCC, liver hepatocellular carcinoma. LUAD, lung adenocarcinoma. LUSC, lung squamous cell carcinoma. PAAD, pancreatic adenocarcinoma. PCPG, pheochromocytoma and paraganglioma. PRAD, prostate adenocarcinoma. READ, rectum adenocarcinoma. SARC, sarcoma. SKCM, skin cutaneous melanoma. STAD, stomach adenocarcinoma. THCA, thyroid carcinoma. THYM, thymoma. UCEC, uterine corpus endometrial carcinoma.

**Survival analysis for the \( R A D 51 \) gene in human cancers**

We assessed the probability of the \( R A D 51 \) as a prognostic biomarker in human pan-cancers using the Kaplan-Meier plotter. We found that the \( R A D 51 \) gene might be a prognostic biomarker in multiple types of human cancers, including esophageal adenocarcinoma (EAC; hazard ratio, HR=2.30, logrank \( P = 0.042 \)), ESCA (HR=0.40, logrank \( P = 0.032 \)), and STAD (HR=0.68, logrank \( P = 0.018 \); Fig. 3). For instance, EAC, HCC, and BRCA patients who had high expression levels of \( R A D 51 \) had low survival probabilities compared with patients who had low expression levels of \( R A D 51 \). A meta-analysis showed that the high expression level of the \( R A D 51 \) gene was a risk factor for the poor prognosis of pan-cancers (\( P = 0.03 \), Fig. 4).
Fig. 3: Kaplan-Meier plotter analysis of the RAD51 gene. BRCA, breast cancer. EAC, esophageal adenocarcinoma. ESCA, esophageal squamous cell carcinoma. LIHC/HCC, liver hepatocellular carcinoma. LUAD, lung adenocarcinoma. STAD, stomach adenocarcinoma. Differences between groups were analyzed using the logrank test. Samples are divided into high and low expression group according to the median expression levels of the RAD51 gene in the corresponding cancer.

Fig. 4: The meta-analysis for the RAD51 gene of pan-cancer prognosis. IV, inverse variance. SE, standard error. CI, confidence interval. BLCA, bladder cancer. BRCA, breast cancer. CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma. EAC, esophageal adenocarcinoma. ESCA, esophageal squamous cell carcinoma. HNSC, head and neck squamous cell carcinoma. KIRC, kidney renal clear cell carcinoma. KIRP, kidney renal papillary cell carcinoma. LIHC/HCC, liver hepatocellular carcinoma. LUAD, lung adenocarcinoma. LUSC, lung squamous cell carcinoma. OVC, ovarian cancer. PAAD, pancreatic adenocarcinoma. READ, rectum adenocarcinoma. SARC, sarcoma. STAD, stomach adenocarcinoma. THCA, thyroid carcinoma. UCEC, uterine corpus endometrial carcinoma.
**RAD51-related genes and the PPI network**

Ten genes related to the RAD51 gene were identified from the STRING database (Fig. 5A). All the ten genes were upregulated in ACCEJ tumor samples compared with the non-tumor control samples in the GSE159721 dataset (P < 0.05, Fig. 5B). Functional enrichment analysis showed that the gene cluster was associated with 35 biological processes, including “GO:00006281: DNA repair”, “GO:0042127: regulation of cell proliferation”, and “GO:0034599: cellular response to oxidative stress”, and four KEGG pathways, including “hsa03460: Fanconi anemia pathway”, “hsa03440: Homologous recombination”, “hsa05200: Pathways in cancer”, and “hsa05212: Pancreatic cancer” (Table 1).

| Term | No | P value     | Genes                                      |
|------|----|-------------|--------------------------------------------|
| GO:0000724:double-strand break repair via homologous recombination | 7  | 1.23E-12    | RAD51AP1, BLM, RAD51, RPA1, BRCA1,         |
|      |    |             | BRCA2, PALB2                               |
| GO:0000732:strand displacement in DNA repair | 6  | 1.48E-12    | RAD51AP1, BLM, RAD51, BRCA1,               |
|      |    |             | BRCA2, PALB2                               |
| GO:0000731:DNA synthesis involved in DNA repair | 6  | 7.30E-12    | RAD51AP1, BLM, RAD51, BRCA1,               |
|      |    |             | BRCA2, PALB2                               |
| GO:0006281:DNA repair | 7  | 1.41E-09    | RAD52, RAD51AP1, BLM, RAD51, CHEK1, RPA1,  |
|      |    |             | BRCA1                                      |
| GO:0006974:cellular response to DNA damage stimulus | 6  | 6.66E-08    | RAD52, BLM, RAD51, CHEK1, ABL1,            |
|      |    |             | BRCA1                                      |
| GO:0006310:DNA recombination | 5  | 1.14E-07    | RAD52, BLM, RAD51, RPA1, BRCA1             |
| GO:0031052:chromosome breakage | 3  | 3.19E-06    | BRCA1, BRCA2, PALB2                        |
| GO:0006302:double-strand break repair | 4  | 6.82E-06    | RAD52, MND1, BRCA1, BRCA2                  |
| GO:0001833:inner cell mass cell proliferation | 3  | 2.10E-05    | CHEK1, BRCA2, PALB2                        |
| GO:0010569:regulation of double-strand break repair via homologous recombination | 3  | 3.81E-05    | RAD51AP1, RAD51, CHEK1                     |
| GO:0016925:protein sumoylation | 4  | 3.82E-05    | RAD52, BLM, RPA1, BRCA1                    |
| GO:1901796:regulation of signal transduction by p53 class mediator | 4  | 4.54E-05    | BLM, CHEK1, RPA1, BRCA1                    |
| GO:0010165:response to X-ray | 3  | 7.33E-05    | BLM, RAD51, BRCA2                          |
| GO:0006260:DNA replication | 4  | 8.83E-05    | BLM, CHEK1, RPA1, BRCA1                    |
| GO:0004800:cellular response to ionizing radiation | 3  | 1.47E-04    | RAD51AP1, BLM, RAD51                      |
| GO:000722:telomere maintenance via recombination | 3  | 1.57E-04    | RAD51, RPA1, BRCA2                        |
| GO:0036297:interstrand cross-link repair | 3  | 3.70E-04    | RAD51AP1, RAD51, RPA1                     |

Table 1: The results of functional enrichment analysis for the RAD51 gene and related genes
| GO:1990426:mitotic recombination-dependent replication fork processing | 2 | 1.19E-03 | RAD51, BRCA2 |
| GO:0072757:cellular response to camptothecin | 2 | 2.38E-03 | BLM, RAD51 |
| GO:0000730:DNA recombinase assembly | 2 | 2.97E-03 | RAD52, RAD51 |
| GO:0048478:replication fork protection | 2 | 3.57E-03 | BLM, BRCA2 |
| GO:0072711:cellular response to hydroxyurea | 2 | 4.16E-03 | BLM, RAD51 |
| GO:0006975:DNA damage induced protein phosphorylation | 2 | 4.76E-03 | CHEK1, ABL1 |
| GO:0042127:regulation of cell proliferation | 3 | 5.13E-03 | CHEK1, ABL1, BRCA1 |
| GO:0000729:DNA double-strand break processing | 2 | 8.90E-03 | BLM, BRCA1 |
| GO:0006978:DNA damage response, signal transduction by p53 class mediator resulting in transcription of p21 class mediator | 2 | 9.49E-03 | BRCA1, BRCA2 |
| GO:0031572:G2 DNA damage checkpoint | 2 | 1.18E-02 | CHEK1, BRCA1 |
| GO:0031297:replication fork processing | 2 | 1.60E-02 | BLM, RAD51 |
| GO:0045931:positive regulation of mitotic cell cycle | 2 | 1.66E-02 | ABL1, BRCA2 |
| GO:0007131:reciprocal meiotic recombination | 2 | 1.77E-02 | RAD51, MND1 |
| GO:0006298:mismatch repair | 2 | 2.07E-02 | RPA1, ABL1 |
| GO:0006289:nucleotide-excision repair | 2 | 2.42E-02 | RPA1, BRCA2 |
| GO:0008630:intrinsic apoptotic signaling pathway in response to DNA damage | 2 | 2.76E-02 | ABL1, BRCA1 |
| GO:0045893:positive regulation of transcription, DNA-templated | 3 | 3.59E-02 | BLM, BRCA1, BRCA2 |
| GO:0034599:cellular response to oxidative stress | 2 | 3.75E-02 | RAD52, ABL1 |

**KEGG pathways**

| Pathway | ID | Significance | Genes |
|---------|----|--------------|-------|
| hsa03460:Fanconi anemia pathway | 6 | 1.23E-09 | BLM, RAD51, RPA1, BRCA1, BRCA2, PALB2 |
| hsa03440:Homologous recombination | 5 | 1.76E-08 | RAD52, BLM, RAD51, RPA1, BRCA2 |
| hsa05200:Pathways in cancer | 3 | 7.25E-02 | RAD51, ABL1, BRCA2 |
| hsa05212:Pancreatic cancer | 2 | 7.32E-02 | RAD51, BRCA2 |
**Fig. 5:** The protein-protein interaction (PPI) network and expression of **RAD51**-related genes. A, the PPI network of the **RAD51** gene and **RAD51**-related genes. B, the expression profiles of the ten **RAD51**-related genes in the adenocarcinoma at the gastroesophageal junction (ACGEJ) tumor samples and the non-tumor control samples in the GSE159721 dataset. The differences in the expression levels of the ten genes between groups were analyzed using the non-parametric Mann-Whitney U test.

**Key regulatory miRNAs of the **RAD51** gene**

Nine key regulatory miRNAs of the **RAD51** gene were identified from databases (Fig. 6), and other 15 experimentally validated miRNAs targeting **RAD51** were identified from online searching. Functional enrichment analysis showed that these miRNAs were related to multiple pathways (Fig. 7). For instance, hsa-miR-34a-5p, hsa-miR-193b-3p, and hsa-miR-103a-3p were associated with a variety of pathways, including “fatty acid biosynthesis”, “viral carcinogenesis”, and “proteoglycans in cancer”.
Fig. 6: The Venn diagram showing the key regulatory microRNAs of the *RAD51* gene. The regulatory miRNAs of the *RAD51* gene were identified from five databases, including starbase, TargetScan, miRDB, mirDIP, and mirmap, and miRNA shown in at least three databases, were the key regulatory miRNAs.

Fig. 7: The functional enrichment analysis result for the microRNAs targeting *RAD51*. The DIANA-miRPath v3.0 (http://www.microrna.gr/miRPathv3) was used to identify the pathways related to the regulatory miRNAs of *RAD51* based on the experimentally validated miRNA interactions derived from DIANA-TarBase.
Expression validation of the key regulatory miRNAs
Validation of the key regulatory miRNAs of RAD51 in microarray datasets showed that six miRNAs, including hsa-miR-10a, hsa-miR-182, hsa-miR-1915, hsa-miR-221, hsa-miR-34a, and hsa-miR-766, were downregulated in ACGEJ tumor samples compared with the non-tumor control samples in the GSE96669 dataset (Fig. 8). These results indicated that the regulatory miRNAs might play important roles in ACGEJ prognosis and development by regulating RAD51.

![Fig. 8: The expression levels of six microRNAs of the RAD51 gene in the GSE96669 dataset. The differences in the expression levels of the six miRNAs between groups were analyzed using the non-parametric Mann-Whitney U test](image)

Discussion
This study showed that the RAD51 gene was upregulated in ACGEJ tumor tissues compared with the adjacent non-tumor tissues. The overexpression of RAD51 was correlated with a poor prognosis in ACGEJ patients. Six miRNAs targeting RAD51, including hsa-miR-10a, hsa-miR-182, hsa-miR-1915, hsa-miR-221, hsa-miR-34a, and hsa-miR-766, were downregulated in ACGEJ tumor samples compared with the non-tumor control samples. Also, the regulatory miRNAs of RAD51 were associated with multiple pathways related to cancers and the metabolism of fatty acids. These results showed that the RAD51 gene and miRNAs of RAD51 might have important roles in the prognosis of ACGEJ.

The RAD51 recombinase is a DNA repair protein that regulates homologous recombination by interacting with ssDNA-binding protein RPA and RAD52 (12,13). Elevated expression of RAD51 is related to a decreased chemosensitivity in tumor cells (22-24). The overexpression of RAD51 was associated with a poor prognosis in neuroblastoma patients and the silencing of RAD51 increased the chemosensitivity to doxorubicin in human neuroblastoma cells (22). Silencing of the RPA1 gene reduced the RAD51
recruitment at the DNA lesion site, suppressed DNA repair, and increased the radio-sensitivity in CNE-2R cells (25). Moreover, miR-506 is a regulator of chemo-sensitivity through suppressing RAD51-mediated homologous recombination (24). Our present study showed that the RAD51 gene and RAD51-related genes, including RPA1, BRCA1/2, RAD52, and RAD51AP1, were up-regulated in ACGEJ tumor tissues compared with the adjacent non-tumor tissues. Moreover, the high expression level of the RAD51 gene was related to poor prognoses in ACGEJ patients and in TCGA pan-cancers. These results revealed that the RAD51 gene might be used as a prognostic biomarker in ACGEJ.

Among the downregulated regulatory miRNAs of the RAD51 gene, hsa-miR-182, hsa-miR-221, hsa-miR-34a, and hsa-miR-766 were associated with the prognosis of a variety of human cancers (26-29). The prognostic values of miRNAs in human cancers are tumor type-dependent (29-35). For instance, miRNA-182 overexpression resulted in low survival ratios in CRC (29,31) and papillary thyroid carcinoma (30), and a good prognosis in non-small cell lung cancer (32). The upregulation of miRNA-221 was related to the poor prognosis in glioblastoma (33) and a good prognosis in CRC (34). Chen et al. (35) indicated that the downregulation of miR-34a was strongly related to shorter overall survival in patients with cervical cancer. miR-34a overexpression resulted in a poor prognosis in COAD (36). These results showed that the regulatory miRNAs of the RAD51 gene might have crucial roles in the development of human cancers including ACGEJ.

Conclusion

The RAD51 gene was upregulated in the ACGEJ tumor tissues compared with the adjacent non-tumor tissues, and its overexpression was correlated with a poor prognosis in ACGEJ patients. The high expression level of RAD51 was correlated with a poor prognosis in the TCGA pan-cancers. The regulatory miRNAs of RAD51, including hsa-miR-10a, hsa-miR-182, hsa-miR-1915, hsa-miR-221, hsa-miR-34a, and hsa-miR-766, might play crucial roles in the development and prognosis of ACGEJ by regulating the RAD51 gene. However, the important roles of these miRNAs in the development and prognosis of ACGEJ should be validated using preclinical experiments and clinical cohort studies.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgements

Fund program: Scientific research project of Special training program for Scientific and technological talents of Xinjiang ethnic minorities Number: 2020D03008.

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Joshi SS, Badgwell BD (2021). Current treatment and recent progress in gastric cancer. CA Cancer J Clin, 71(3):264-279.
2. Eusebi LH, Telese A, Marasco G, Bazzoli F, Zagari RM (2020). Gastric cancer prevention strategies: a global perspective. J Gastroenterol Hepatol, 35(9):1495-1502.
3. Buas MF, Vaughan TL (2013). Epidemiology and risk factors for gastroesophageal junction tumors: understanding the rising incidence of this disease. Semin Radiat Oncol, 23(1):3-9.
4. Pedrazzani C, de Manzoni G, Marrelli D, et al (2007). Lymph node involvement in advanced gastroesophageal junction adenocarcinoma. J Thorac Cardiovasc Surg, 134(2):378-385.

Available at: http://ijph.tums.ac.ir
5. Lai S, Su T, He X, Lin Z, Chen S (2018). Prognostic value of resected lymph nodes numbers for Siewert II gastroesophageal junction cancer. *Onco Targets Ther.*, 9(2):2797-2809.

6. Janjigian Y, Werner D, Pauligk C, et al (2012). Prognosis of metastatic gastric and gastroesophageal junction cancer by HER2 status: a European and USA International collaborative analysis. *Ann Oncol.*, 23(10):2656-2662.

7. Al-Batran S-E, Homann N, Pauligk C, et al (2017). Effect of adjuvant chemotherapy followed by surgical resection on survival in patients with limited metastatic gastric or gastroesophageal junction cancer: the AIO-FLOT3 trial. *JAMA Oncol.*, 3(9):1237-1244.

8. Kamarajah SK, Phillips AW, Griffiths EA, Ferri L, Hofstetter WL, Markar SR (2021). Esophagectomy or total gastrectomy for Siewert 2 gastroesophageal junction (GEJ) adenocarcinoma? A registry-based analysis. *Ann Surg Oncol.*, 28(13):8485-8494.

9. Roy PS, Nyodu T, Hazarika M, et al (2019). Prevalence of HER2 expression and its correlation with clinicopathological parameters in gastric or gastroesophageal junction adenocarcinoma in North-East Indian population. *APJCP*, 20(4):1139-1145.

10. Song D, Tian J, Hu Y, et al (2020). Identification of biomarkers associated with diagnosis and prognosis of gastroesophageal junction adenocarcinoma—a study based on integrated bioinformatics analysis in GEO and TCGA database. *Medicina*, 99(51):e23605.

11. Odenthal M, Hee J, Gockel I, et al (2015). Serum micro RNA profiles as prognostic/predictive markers in the multimodality therapy of locally advanced adenocarcinomas of the gastroesophageal junction. *Int J Cancer*, 137(1):230-237.

12. Laurin E, Marson D, Ferrmeglia A, Aulie S, Ferrmeglia M, Pricl S (2020). Role of RAD51 and DNA repair in cancer: a molecular perspective. *Pharmacol Ther.*, 208:107492.

13. Demeyer A, Benhelli-Mokrani H, Chenais B, Weigel P, Fleury F (2021). Inhibiting homologous recombination by targeting RAD51 protein. *Biochim Biophys Acta Rev Cancer*, 1876(2):188597.

14. Chen JJ, Silver D, Cantor S, Livingston DM, Scully R (1999). BRCA1, BRCA2, and RAD51 operate in a common DNA damage response pathway. *Cancer Res.*, 59(7 Supplement):1752s-1756s.

15. Bishara LA, Machour FE, Awad SW, Ayoub N (2021). NELF complex fosters BRCA1 and RAD51 recruitment to DNA damage sites and modulates sensitivity to PARP inhibition. *DNA Repair,* 97:103025.

16. Kolinjivadi AM, Sannino V, de Antoni A, Técher H, Baldi G, Costanzo V (2017). Moonlighting at replication forks—a new life for homologous recombination proteins BRCA1, BRCA2 and RAD51. *FEBS Lett.*, 591(8):1083-1100.

17. Kaplan AR, Guehle SE, Liu Y, et al (2019). Cediranib suppresses homology-directed DNA repair through down-regulation of BRCA1/2 and RAD51. *Sci Transl Med*, 11(492):eaav4508.

18. Tennstedt P, Fresow R, Simon R, et al (2013). RAD51 overexpression is a negative prognostic marker for colorectal adenocarcinoma. *Int J Cancer*, 132(9):2118-2126.

19. Xu H, Xiong C, Chen Y, Zhang C, Bai D (2021). Identification of RAD51 as a prognostic biomarker correlated with immune infiltration in hepatocellular carcinoma. *Bioengineered*, 12(1):2664-2675.

20. Söderlund K, Skoog L, Fornderner T, Askmalm MS (2007). The BRCA1/BRCA2/RAD51 complex is a prognostic and predictive factor in early breast cancer. *Radiother Oncol*, 84(3):242-251.

21. Li Y, Wang W-y, Xiao J-h, et al (2017). Overexpression of RAD51 predicts poor prognosis in colorectal cancer: our experience with 54 patients. *Plos One*, 12(1):e0167868.

22. Xu Y, Chen K, Cai Y, Cheng C, Zhang Z, Xu G (2019). Overexpression of RAD51 predicts poor prognosis and silencing of RAD51 increases chemo-sensitivity to doxorubicin in neuroblastoma. *Am J Transl Res*, 11(9):5788.

23. Du L-Q, Wang Y, Wang H, Cao J, Liu Q, Fan F-Y (2011). Knockdown of RAD51 expression induces radiation-and chemo-sensitivity in osteosarcoma cells. *Med Oncol*, 28(4):1481-1487.

24. Liu G, Xue F, Zhang W (2015). miR-506: a regulator of chemo-sensitivity through suppression of the RAD51-homologous recombination axis. *Chin J Cancer*, 34(3):1-3.

25. Zhang Z, Huo H, Liao K, et al (2018). RPA1
downregulation enhances nasopharyngeal cancer radiosensitivity via blocking RAD51 to the DNA damage site. Exp Cell Res, 371(2):330-341.

26. Liu S, Lin Z, Zheng Z, et al (2020). Serum exosomal microRNA-766-3p expression is associated with poor prognosis of esophageal squamous cell carcinoma. Cancer Sci, 111(10):3881-3892.

27. Zhang R, Pang B, Xin T, et al (2016). Plasma miR-221/222 family as novel descriptive and prognostic biomarkers for glioma. Mol Neurobiol, 53(3):1452-1460.

28. Guo J, Liu C, Wang W, et al (2018). Identification of serum miR-1915-3p and miR-455-3p as biomarkers for breast cancer. PloS One, 13(7):e0200716.

29. Liu H, Du L, Wen Z, et al (2013). Up-regulation of miR-182 expression in colorectal cancer tissues and its prognostic value. Int J Colorectal Dis, 28(5):697-703.

30. Yao X-G, Tan Q, Liu P-P, Feng L-J (2019). Tissue microRNA-182 expression level and its potential prognostic value for papillary thyroid carcinoma. Int J Clin Exp Pathol, 12(8):3128-3133.

31. Rapti S-M, Kontos CK, Papadopoulos IN, Scorilas A (2014). Enhanced miR-182 transcription is a predictor of poor overall survival in colorectal adenocarcinoma patients. Clin Chem Lab Med, 52(8):1217-1227.

32. Stenvold H, Donnem T, Andersen S, Al-Saad S, Busund I-T, Bremnes RM (2014). Stage and tissue-specific prognostic impact of miR-182 in NSCLC. BMC Cancer, 14(1):1-10.

33. Chen Y-Y, Ho H-L, Lin S-C, Ho TD-H, Hsu C-Y (2018). Upregulation of miR-125b, miR-181d, and miR-221 predicts poor prognosis in MGMT promoter-unmethylated glioblastoma patients. Am J Forensic Med Pathol, 149(5):412-417.

34. Yuan K, Xie K, Fox J, et al (2013). Decreased levels of miR-224 and the passenger strand of miR-221 increase MBD2, suppressing maspin and promoting colorectal tumor growth and metastasis in mice. Gastroenterology, 145(4):853-864. e859.

35. Chen A-H, Qin Y-E, Tang W-F, Tao J, Song H-m, Zuo M (2017). MiR-34a and miR-206 act as novel prognostic and therapy biomarkers in cervical cancer. Cancer Cell Int, 17(1):63.

36. Rapti S-M, Kontos CK, Christodoulou S, Papadopoulos IN, Scorilas A (2017). miR-34a overexpression predicts poor prognostic outcome in colorectal adenocarcinoma, independently of clinicopathological factors with established prognostic value. Clin Biochem, 50(16-17):918-924.