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Original Article

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

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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".

Conclusions: *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

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-

ceptor 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 (BRCA) (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 beadchip) consisted of 121 ACGEJ tumor samples and 11 non-cancer control 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).

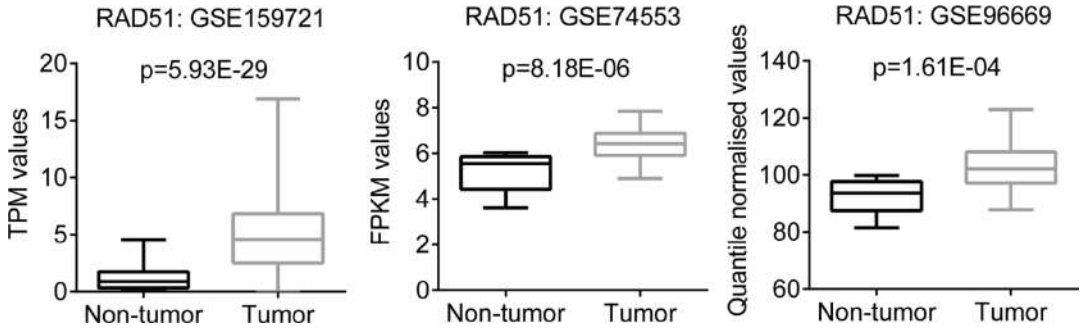

Fig. 1: The expression level of the *RAD51* gene in the GSE159721, GSE74553, and GSE96669 datasets.The differences in the expression levels of the *RAD51* gene between groups were analyzed using the non-parametric Mann-Whitney U test. TPM, transcripts per million. FPKM, fragments per kilobase of transcript per million fragments sequenced

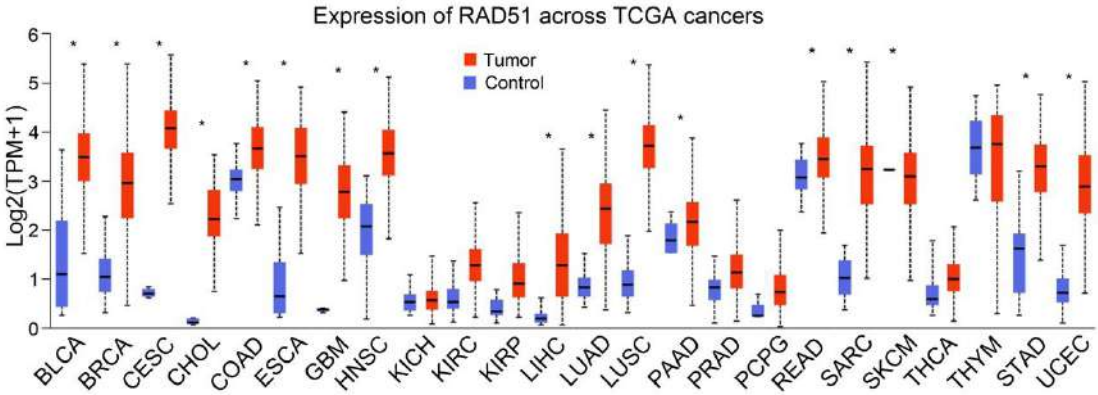

Fig. 2: Bar plot of *RAD51* expression profile across all tumor samples and paired normal tissues in the TCGA platform. TPM, transcripts per million. BLCA, bladder cancer. BRCA, breast cancer. CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma. CHOL, cholangiocarcinoma. COAD, Colon 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 RAD51 gene in human cancers

We assessed the probability of the *RAD51* as a prognostic biomarker in human pan-cancers using the Kaplan-Meier plotter. We found that the *RAD51* 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 *RAD51* had low survival probabilities compared with patients who had low expression levels of *RAD51*. A meta-analysis showed that the high expression level of the *RAD51* gene was a risk factor for the poor prognosis of pan-cancers ($P = 0.03$, Fig. 4).

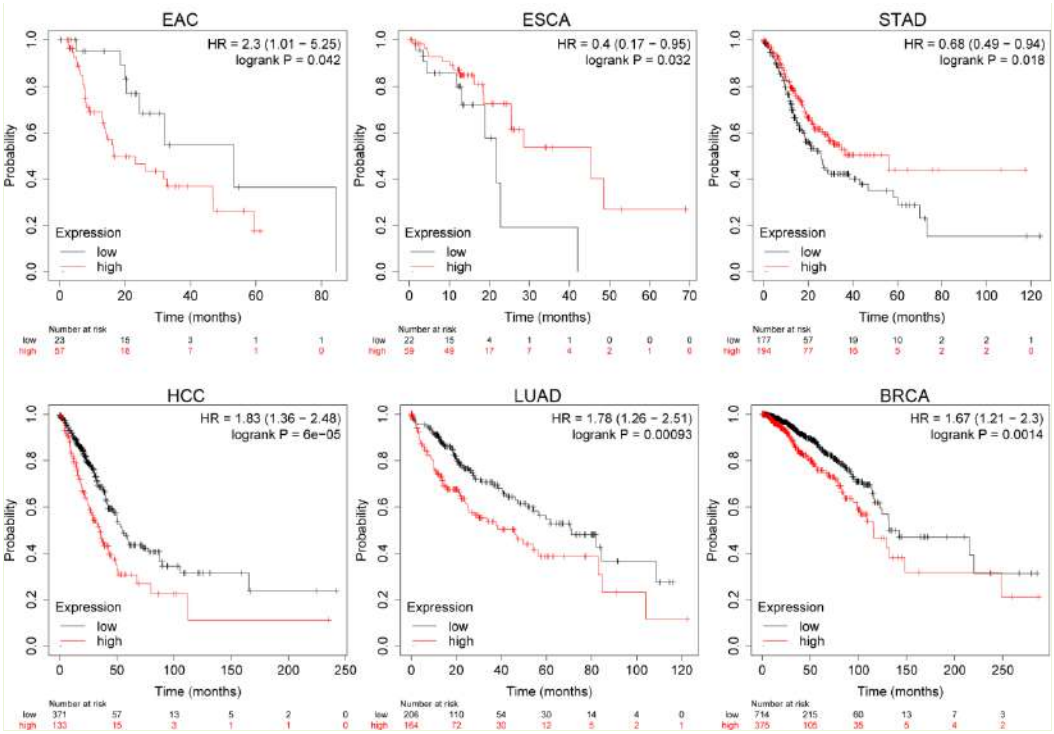

Fig. 3: Kaplan-Meier plotter analysis of the *RAD51* gene. BRCA, breast cancer. EAC, esophageal adenocarcinoma. ESCA, esophageal squamous cell carcinoma. LIHC/HCC, liver hepatocellular carcinoma. LUAD, lung adenocarcinoma. STAD, stomach adenocarcinoma. Differences between groups were analyzed using the 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

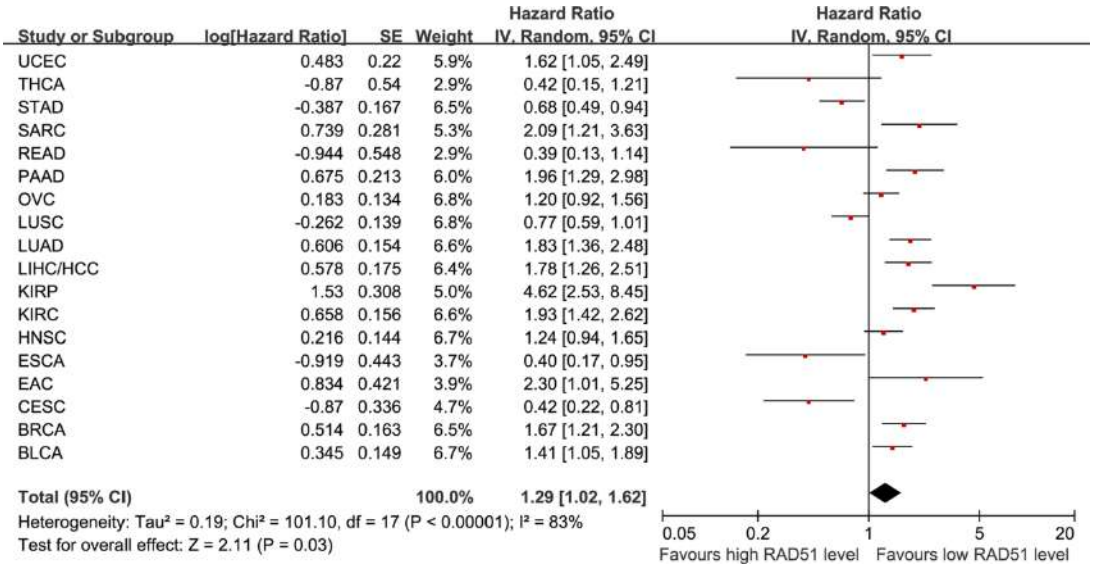

Fig. 4: The meta-analysis for the *RAD51* gene of pan-cancer prognosis. IV, inverse variance. SE, standard error. CI, confident interval. BLCA, bladder cancer. BRCA, breast cancer. CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma. EAC, esophageal adenocarcinoma. ESCA, esophageal 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 ACGEJ 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:0006281: 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).

Table 1: The results of functional enrichment analysis for the *RAD51* gene and related genes

Term	*No*	*P value*	*Genes*
Gene Ontology biological processes (top 20)			
GO:0000724:double-strand break repair via homologous recombination	7	1.23E-12	*RAD51AP1, BLM, RAD51, RPA1, BRCA1, BRCA2, PALB2*
GO:0000732:strand displacement	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:0071479:cellular response to ionizing radiation	3	1.47E-04	*RAD51AP1, BLM, RAD51*
GO:0000722:telomere maintenance via recombination	3	1.57E-04	*RAD51, RPA1, BRCA2*
GO:0036297:interstrand cross-link repair	3	3.70E-04	*RAD51AP1, RAD51, RPA1*

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			
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*

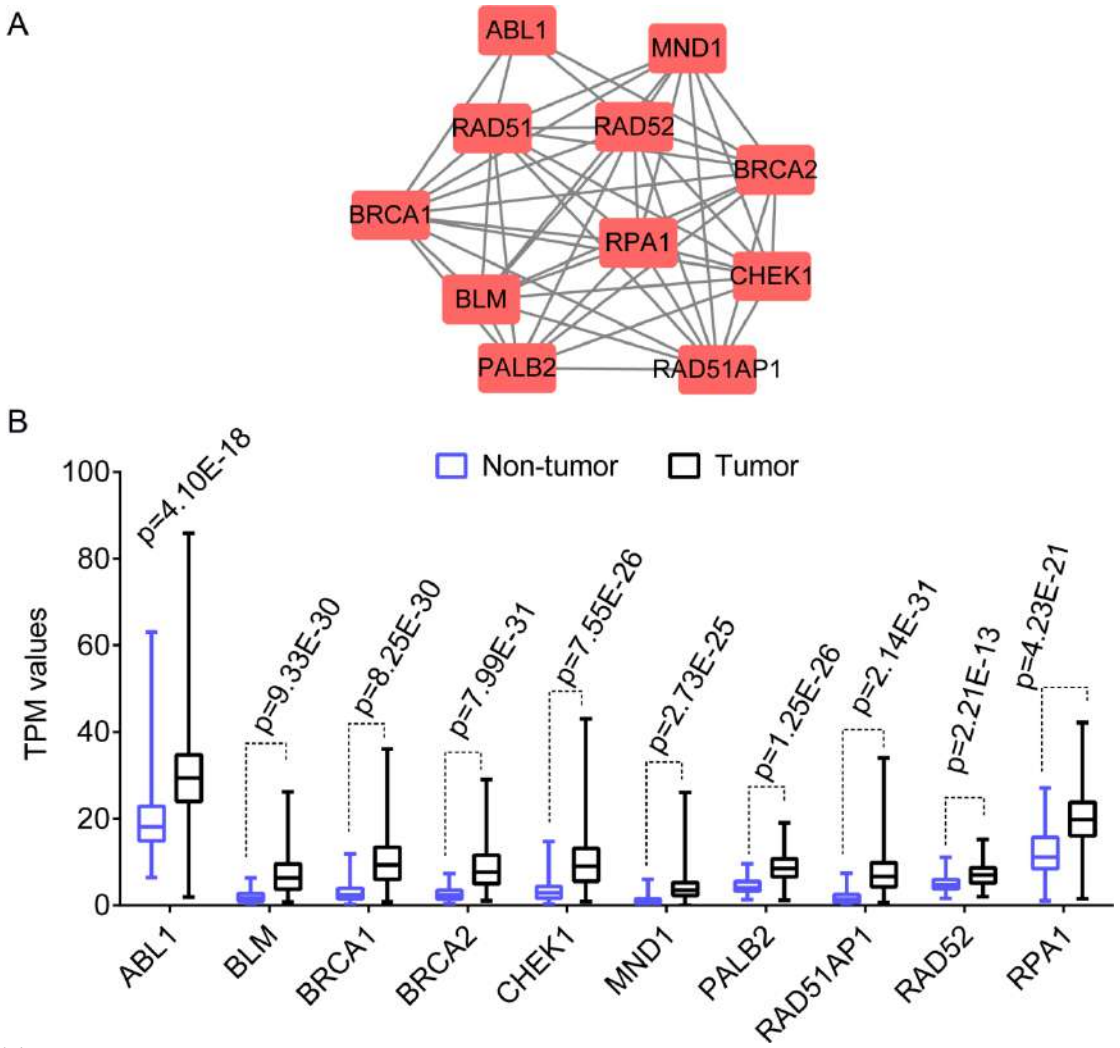

Fig. 5: The protein-protein interaction (PPI) network and expression of *RAD51*-related genes. A, the PPI network of the *RAD51* gene and *RAD51*-related genes. B, the expression profiles of the ten *RAD51*-related genes in the adenocarcinoma at the gastroesophageal junction (ACGEJ) tumor samples and 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 a variety of pathways, including "fatty acid biosynthesis", "viral carcinogenesis", and "proteoglycans in cancer".

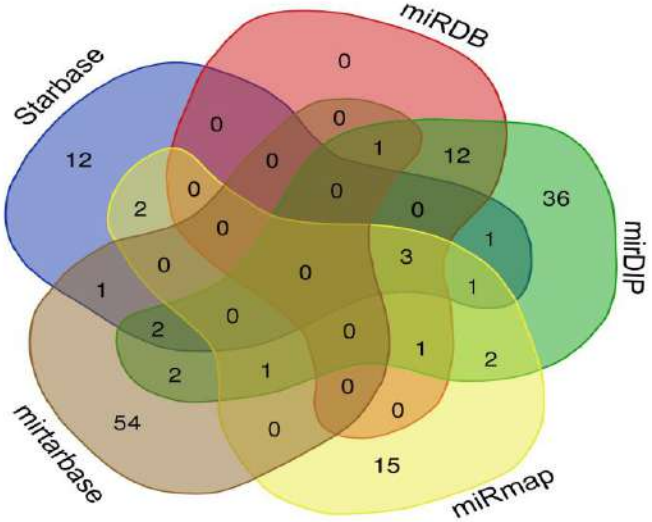

Fig. 6: The Venn diagram showing the key regulatory microRNAs of the *RAD51* gene. The regulatory miRNAs of the *RAD51* gene were identified from five databases, including starbase, TargetScan, miRDB, mirDIP, and miRmap, and miRNA shown in at least three databases, were the key regulatory miRNAs

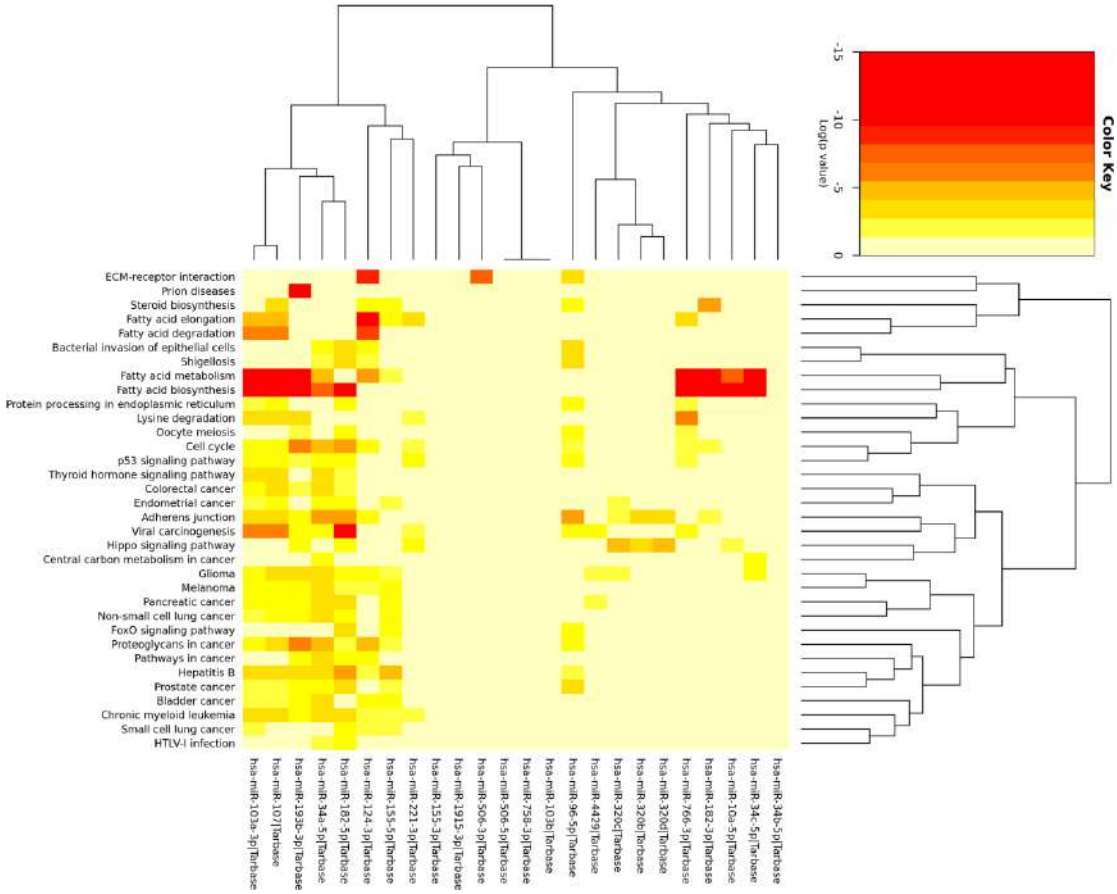

Fig. 7: The functional enrichment analysis result for the microRNAs targeting *RAD51*. The DIANA-miRPath v3.0 (http://www.microrna.gr/miRPathv3) was used to identify the pathways related to the regulatory miRNAs of *RAD51* based on the experimentally validated miRNA interactions derived from DIANA-TarBase

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*.

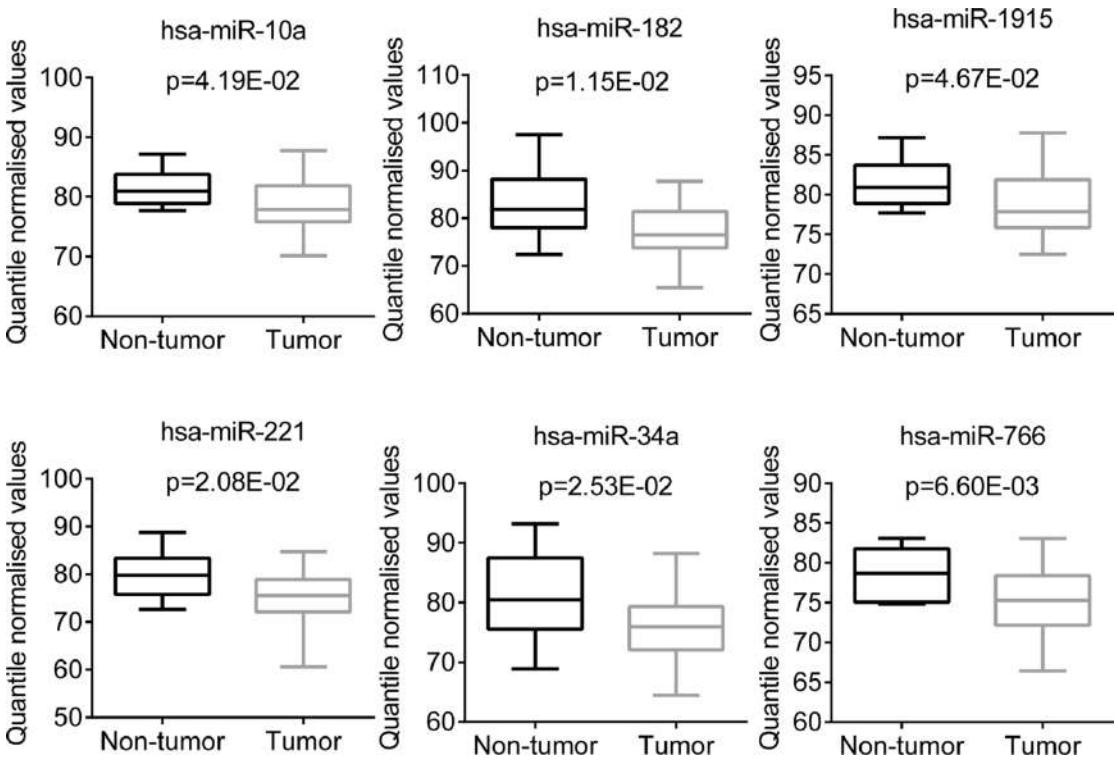

Fig. 8: The expression levels of six microRNAs of the *RAD51* gene in the GSE96669 dataset. The differences in the expression levels of the six miRNAs between groups were analyzed using the non-parametric Mann-Whitney U test

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 chemo-sensitivity 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 upregulated 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.

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Conflict of interest

The authors declare that there is no conflict of interest.

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