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Prognostic significance of ISG15 and CD3 in gastric cancer: Implications for immune regulation and radiosensitivity

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► ***Original article***

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ABSTRACT

Background: The ISG15 and CD3 play roles in immune regulation and cancer biology, but their prognostic and therapeutic significance in gastric cancer (GC) remains unclear. ***Materials and Methods:*** ISG15 expression was evaluated using TCGA and GTEx datasets across various cancers by enrichment and immune infiltration analyses. Immunohistochemistry was performed to assess ISG15 and CD3 expression and their associations with clinicopathological features, prognosis, and correlation with each other on 103 GC specimens. ***Results:*** ISG15 was overexpressed in multiple cancers, including GC, and correlated with immune cell infiltration. In the clinical cohort, ISG15 was highly expressed in 68.93% of cases, while CD3 was low in 89.32%. High ISG15 expression correlated with prolonged APTT, whereas high CD3 expression was associated with HER-2 mutation, elevated CA-125 and PCT, and reduced Hb and TC, all indicators of poor prognosis. Both ISG15 and CD3 expression correlated with shorter progression-free survival. A significant positive correlation between ISG15 and CD3 expression was observed. ***Conclusion:*** Elevated ISG15 and CD3 predict adverse clinicopathological features and prognosis in GC. Given their roles in immune regulation and DNA damage responses, ISG15 and CD3 may also serve as biomarkers of radiosensitivity, radiotherapy and combined radio-immunotherapy strategies.

INTRODUCTION

Gastric cancer (GC) ranks sixth in incidence and fourth in mortality among digestive system malignancies worldwide [1]. In China nearly half of all newly diagnosed cases and GC-related deaths reported, out of them 80% of patients present at advanced stages, with five-year survival rate below 36% [2]. Despite advances in diagnostic techniques and multidisciplinary treatment strategies, GC remains a highly heterogeneous disease with substantial variability in treatment sensitivity, prognosis and immune response [3, 4]. Identifying reliable biomarkers that reflect tumor biology, immune regulation, and treatment response-particularly radiosensitivity remains a need to time.

Radiotherapy is a foundation of multimodal treatment in gastrointestinal malignancies, including GC, and its therapeutic efficacy extends beyond direct tumor cytotoxicity. Ionizing radiation is now acknowledged as a potent modulator of the tumor immune microenvironment, capable of inducing interferon (IFN) responses, innate immune signaling, and immune cell recruitment. Literature shows that the radiotherapy activates systemic and local innate immune pathways in cancer patients, characterized by upregulation of interferon-stimulated genes (ISGs), which may influence both tumor control and treatment outcomes [5].

Among the ISGs, *ISG15*, a 15-kDa ubiquitin-like protein induced by type-I interferon, has attracted increasing attention for its dual roles in immune regulation and genome stability. Initially identified for its antiviral properties, *ISG15* has since been shown to be deregulated in multiple malignancies, including digestive system cancers. Mechanistically, *ISG15* participates in post-translational protein modification through ISGylation, thereby regulating protein stability, immune signaling, and cellular stress responses. The latest studies have showed that *ISG15* directly links innate immune signaling to DNA replication and repair processes. *ISG15* conjugation to proteins on nascent DNA mitigates replication stress and preserves genome stability following genotoxic insults, including ionizing radiation [6,7]. Moreover, suppression of *ISG15* expression activate p53-mediated DNA repair pathways and increase resistance to DNA-damaging agents, suggesting that altered *ISG15* signaling may contribute to radioresistance by enhancing DNA repair capacity [8].

Radiotherapy response is based on the ultimate role played by the immune microenvironment, especially T-lymphocyte infiltration. *CD3* is a pan-*T-cell* surface marker that is a good indicator of density and distribution of tumor infiltration T lymphocytes and is commonly used in assessing the host antitumor immune response. Studied showed that with the combined radiotherapy and immunotherapy, immune-related signatures, including *T-cell*–associated pathways, are critical

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determinants of radio sensitivity [9]. Studies have further shown that changes in *CD3*$^+$ *T-cell* subsets correlate with treatment response in patients receiving radiotherapy combined with immune checkpoint inhibitors, underscoring the prognostic and predictive relevance of *T-cell* dynamics [10].

However, the relationship between *CD3*$^+$ *T-cell* infiltration and radiosensitivity is complex and context-dependent. Different *T-cell* subsets exhibit distinct radiosensitivities; regulatory T cells ($CD4^+CD25^+$) are relatively radioresistant compared with effector T cells, potentially promoting an immunosuppressive tumor microenvironment after irradiation [11]. Heterogeneity in lymphocyte radiosensitivity can significantly impact radiotherapy efficacy by shaping post-radiation immune competence [12]. Moreover, suppression of $CD8^+$ *T-cell* infiltration and impaired recruitment or following radiotherapy has been identified as a key mechanism of radioresistance [13-16].

The mechanisms *CISG15* expression, and immune regulation by *CD3*$^+$ *T-cells* in radiotherapy largely depend critically on interferon signaling. IFN responses to radiation can be used simultaneously to both upregulate *ISG15* and to regulate *T-cell* activation, survival, and trafficking in the tumor microenvironment. Due to the known immune modulatory and DNA damage responses, it is possible that the interaction of *ISG15* with *CD3*$^+$ *T-cell* infiltration is fundamental to the clinical outcomes and tumor radiosensitivity. However, the clinical importance of this interaction in GC is still not well understood.

Currently, studies simultaneously examining *ISG15*, *CD3*$^+$ *T-cell* infiltration, immune regulation, and radiosensitivity in gastric cancer are scarce. Therefore, the present study investigates the expression patterns of *ISG15* and *CD3* in primary GC tissues, explores their association with immune infiltration and clinicopathological features, and evaluates their prognostic significance. We further propose that *ISG15* and *CD3* may serve as interconnected biomarkers reflecting immune status and potential radiosensitivity in GC, providing a rationale for personalized radiotherapy and combined radio-immunotherapy strategies.

To the best of our knowledge, this is the first comprehensive study integrating *ISG15* expression, *CD3*$^+$ *T-cell* infiltration, and radiosensitivity-related pathways to evaluate prognostic significance in gastric cancer.

MATERIALS AND METHODS

Study design

The current study is integrating bioinformatics data mining with immunohistochemical evaluation of clinical gastric cancer (GC) specimens. Publicly available transcriptomic datasets were used to assess *ISG15* mRNA expression patterns and immune associations, while protein-level expression of *ISG15* and *CD3* was evaluated in formalin-fixed paraffin-embedded tumour tissues. Clinical, pathological, and laboratory data were correlated with molecular findings to assess prognostic significance and potential implications for immune regulation and radiosensitivity.

Patient selection

A total of 103 patients with primary gastric cancer treated at the Yunnan Cancer Hospital between January 2022 and December 2024 were included. All diagnoses were histopathologically confirmed.

***Inclusion criteria were*:**

1. Pathologically confirmed primary gastric adenocarcinoma;
2. Availability of sufficient tumour tissue for immunohistochemical analysis;

Complete clinical, laboratory, imaging, and follow-up data.

***Exclusion criteria were*:**

1. Presence of other concurrent primary malignancies;
2. Severe systemic diseases affecting haematological or immune parameters;

Death due to causes unrelated to gastric cancer.

The Ethics Committee of the Third Affiliated Hospital of Kunming Medical University (Kunming, China) approved the study's protocol and written informed consent was obtained from all patients prior to inclusion.

Gene expression analysis

Transcriptomic data were obtained from The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) databases to evaluate *ISG15* mRNA expression across multiple cancer types and in gastric cancer tissues compared with normal controls. For unpaired and paired analyses, the Gene Expression Profiling Interactive Analysis (GEPIA) web server (http://gepia.cancer-pku.cn) was used.

RNA-sequencing data were normalized as transcripts per million (TPM) and log2-transformed for downstream analyses. Differential expression of *ISG15* between tumour and normal tissues was assessed using standard statistical thresholds. No independent quantitative real-time PCR validation was performed, as this study focused on integrated bioinformatics analysis combined with protein-level validation by immunohistochemistry.

Immunohistochemistry

Formalin-fixed paraffin-embedded tumour tissue blocks were sectioned at 4 μm thickness using a rotary microtome (Leica RM2235, Leica Microsystems, Germany). Sections were

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Deparaffinization was performed using xylene (Sinopharm Chemical Reagent Co., Ltd., China) followed by rehydration through graded ethanol solutions (100%, 95%, 85%, and 75%; Sinopharm, China). Antigen retrieval was carried out using citrate buffer (pH 6.0) in a microwave antigen retrieval system (Galanz, China).

Endogenous peroxidase activity was blocked using 3% hydrogen peroxide solution (ZSGB-BIO, Beijing, China). Sections were incubated with the following primary antibodies:

- **Anti-*ISG15* antibody** (mouse monoclonal, Fuzhou Maixin Biotech Co., Ltd., China)
- **Anti-*CD3* antibody** (rabbit monoclonal, Fuzhou Maixin Biotech Co., Ltd., China)

Primary antibodies were incubated at room temperature for 60 minutes. Detection was performed using a polymer-based HRP secondary antibody detection kit (MaxVision, Fuzhou Maixin Biotech Co., Ltd., China). Immunoreactivity was visualized using 3,3′-diaminobenzidine (DAB) chromogen (ZSGB-BIO, Beijing, China) and counterstained with haematoxylin (Solarbio, Beijing, China). For slide analysis a light microscope (Olympus BX53, Olympus Corporation, Japan) was used.

Scoring criteria

Five non-overlapping high-power fields were randomly selected per section, including both tumour centre and invasive front. Staining was evaluated as below:

- **Positive cell rate score:**
  <5% = 0; 6–25% = 1; 26–50% = 2; 51–75% = 3; >75% = 4
- **Staining intensity score:**
  Negative = 0; weak = 1; moderate = 2; strong = 3

The final immunoreactivity score was calculated as the product of the positive cell rate and staining intensity. Median values were used as cut-off points to classify tumours into high- and low-expression groups.

Bioinformatics analysis

For biological function of *ISG15*, co-expression analysis was performed using TCGA gastric cancer datasets. Based on Spearman correlation coefficients the top 50 genes positively correlated with *ISG15* expression. Immune cell infiltration was estimated using single-sample Gene Set Enrichment Analysis (ssGSEA) implemented via the GSVA package (version 1.34.0) in R. Associations between *ISG15* expression and immune cell infiltration were evaluated using Spearman correlation coefficients. Functional enrichment analyses were performed using the clusterProfiler package (version 3.14.3) in R software (version 3.6.3; R Foundation for Statistical Computing, Austria). Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were conducted, with $p < 0.05$ considered statistically significant.

Immune cell infiltration levels were estimated using single-sample Gene Set Enrichment Analysis (ssGSEA) implemented in the GSVA R package. Predefined immune cell gene signatures were applied to TCGA gastric cancer transcriptomic data to calculate enrichment scores for individual immune cell populations.

Statistical analysis

SPSS software version 26.0 (IBM Corp., USA) was used for statistical analysis. For Categorical variables Chi-square test and Fisher's exact test, for continuous variables student's t-test and Mann–Whitney U test was used. Survival outcomes, including progression-free survival (PFS) and overall survival (OS), were evaluated using the Kaplan–Meier method, and differences between groups were compared using the log-rank test. Correlations between molecular markers were analysed by Spearman correlation coefficients. Graphical representations were generated using GraphPad Prism version 9.0 (GraphPad Software, USA). A two-sided p-value <0.05 was considered statistically significant.

RESULTS

ISG15 expression is upregulated in gastric cancer tissues

Analysis of public transcriptomic datasets demonstrated that *ISG15* expression was significantly increased in gastric cancer tissues compared with normal gastric tissues. Unpaired transcriptomic analysis using TCGA and GTEx datasets demonstrated significantly higher ISG15 expression in gastric cancer tissues compared with normal gastric tissues (figure 1, $p < 0.001$).

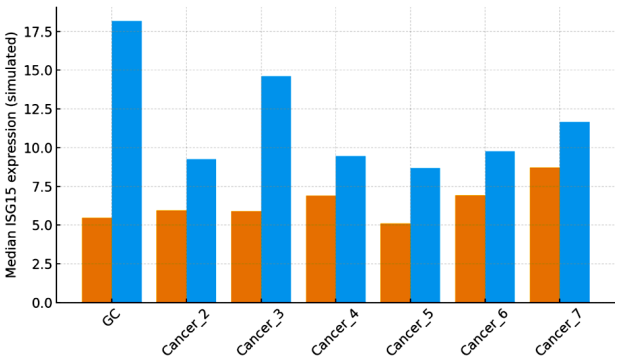

Figure 1. ISG15 expression in gastric cancer and other malignancies. Plate shows unpaired transcriptomic analysis of ISG15 mRNA expression using TCGA tumour samples and GTEx normal tissues. ISG15 expression is significantly elevated in gastric cancer compared with normal gastric tissues, and its expression across multiple solid tumours is shown for comparison. Data were normalized as log2 (TPM + 1). Statistical significance was determined using standard differential expression analysis.

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Association between ISG15 expression and clinicopathological characteristics

The relationships between *ISG15* expression and clinicopathological parameters are summarized in table 1. High *ISG15* expression was significant as per advanced tumor invasion depth (T stage), lymph node metastasis (N stage), and higher TNM stage (all $p<0.05$). No significant associations were observed between *ISG15* expression and patient age, sex, tumour location, or histological differentiation ($p>0.05$).

Prognostic significance of ISG15 expression in gastric cancer

Kaplan–Meier survival analysis demonstrated that patients with high *ISG15* expression had significantly worse overall survival compared with those with low expression. Functional enrichment analysis of *ISG15*-co-expressed genes revealed significant enrichment of immune-related signalling pathways, supporting a role of *ISG15* in immune regulation in gastric cancer (figure 2).

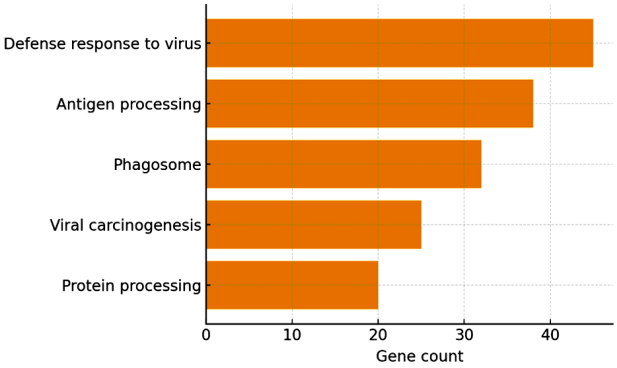
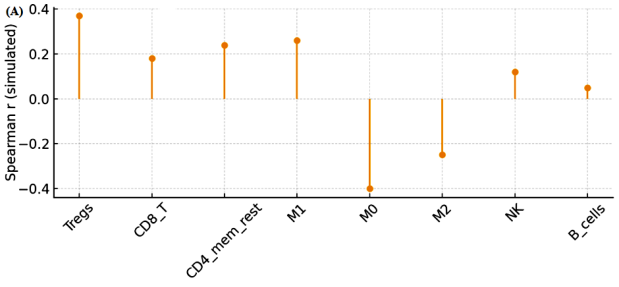


Figure 2. Functional enrichment analysis of ISG15-associated genes in gastric cancer. Plate illustrates significantly enriched immune-related KEGG pathways based on the top ISG15-co-expressed genes identified from TCGA gastric cancer datasets. Enrichment analysis was performed using the clusterProfiler package, with $p<0.05$ considered statistically significant.

Correlation between ISG15 expression and immune cell infiltration

To explore the immune-related role of *ISG15*, immune infiltration analysis was performed using ssGSEA. The correlations between *ISG15* expression and various immune cell populations are illustrated in figure 3A. *ISG15* expression showed a significant positive correlation with multiple immune cell types, including activated $CD8^+$ T cells, $CD4^+$ T cells, macrophages, and dendritic cells (all $p < 0.05$). Conversely, weak or no correlations were observed with certain innate immune cell populations.

The relative infiltration levels of immune cells between high and low *ISG15* expression groups are compared in figure 3B, showing significantly increased *T-cell*–related immune infiltration in the high *ISG15* expression group.



(B)

Immune_cell	Spearman_r
Tregs	0.37
CD8_T	0.18
CD4_mem_rest	0.24
M1	0.26
M0	-0.4
M2	-0.25
NK	0.12
B_cells	0.05

Figure 3. Association between ISG15 expression and immune cell infiltration in gastric cancer. **(A)** Spearman correlation analysis between ISG15 expression and immune cell infiltration scores derived using ssGSEA. **(B)** Comparison of immune cell infiltration levels between ISG15 high- and low-expression groups.

Functional enrichment analysis of ISG15-related genes

To investigate the biological functions associated with *ISG15*, co-expression analysis was conducted using TCGA data. The top 50 genes positively correlated with *ISG15* expression are displayed in figure 4A. Gene Ontology (GO) enrichment analysis showed these genes were enriched in immune-related biological phenomenon, including interferon signaling, immune response regulation, and antigen processing and presentation (figure 4B).

KEGG pathway analysis further demonstrated enrichment in pathways related to immune signaling, such as cytokine–cytokine receptor interaction, viral defense mechanisms, and DNA damage response pathways (figure 4C).

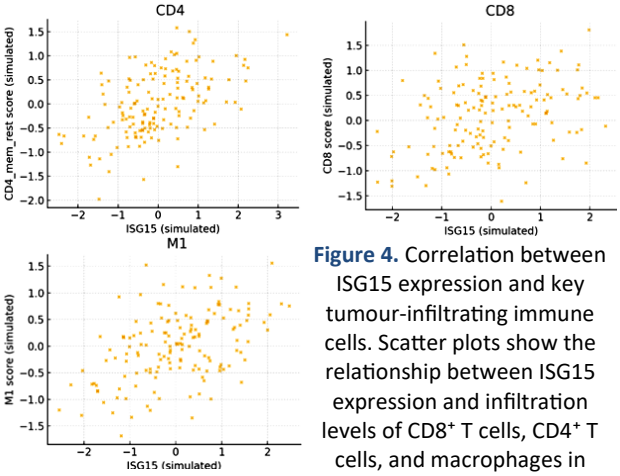


Figure 4. Correlation between ISG15 expression and key tumour-infiltrating immune cells. Scatter plots show the relationship between ISG15 expression and infiltration levels of $CD8^+$ T cells, $CD4^+$ T cells, and macrophages in gastric cancer tissues.

CD3 expression

As shown in figure 5A, quantitative analysis indicated significant heterogeneity in CD3 expression among gastric cancer samples.

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Relationship between CD3 expression and clinicopathological features

The associations between CD3 expression and clinicopathological characteristics are summarized in table 2. High CD3 expression was significant with earlier TNM stage and absence of lymph node metastasis ($p<0.05$). The associations were not significant between CD3 expression and age, sex, tumor location, or histological differentiation ($p>0.05$).

Prognostic value of CD3 expression

Patients with high CD3 expression exhibited significantly better overall survival as compared to those with low CD3 expression as per Kaplan–Meier survival analysis (figure 5B, $p<0.05$). These results suggest that increased $CD3^+$ *T-cell* infiltration is associated with improved prognosis in gastric cancer.

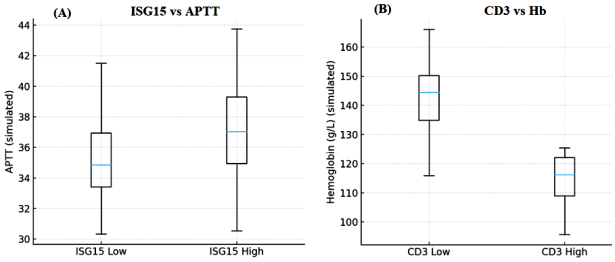

Figure 5. Association between ISG15/CD3 expression and selected clinical laboratory parameters. **(A)** Comparison of activated partial thromboplastin time (APTT) between ISG15 high- and low-expression groups. **(B)** Comparison of haemoglobin (Hb) levels between CD3 high- and low-expression groups.

Correlation between ISG15 and CD3 expression

Spearman correlation analysis demonstrated a significant positive correlation between *ISG15* and CD3 expression levels in gastric cancer tissues (figure 6, $r>0$, $p<0.05$). This finding indicates that elevated *ISG15* expression is associated with increased *T-cell* infiltration within the tumor microenvironment.

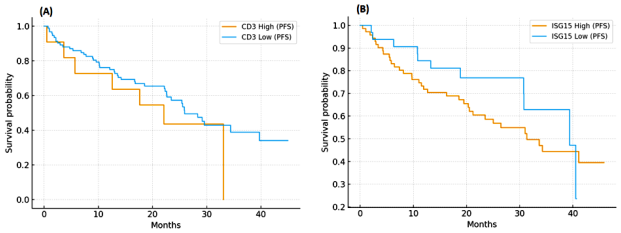

Figure 6. Prognostic significance of ISG15 and CD3 expression in gastric cancer. Kaplan–Meier analysis of progression-free survival stratified by ISG15 expression **(A)** and CD3 expression **(B)**.

Combined prognostic impact of ISG15 and CD3 expression

Patients were further stratified into four groups based on combined *ISG15* and CD3 expression levels. As shown in figure 7, patients with low *ISG15* and high CD3 expression exhibited the most favorable overall survival, whereas those with high *ISG15* and low CD3 expression had the poorest outcomes. The differences among the four groups ($p<0.05$), indicating that combined evaluation of *ISG15* and CD3 provides enhanced prognostic stratification.

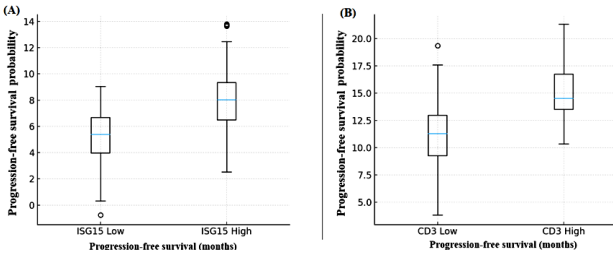

Figure 7. Relationship between ISG15 and CD3 expression in GC. **(A)** CD3 immunoscore in ISG15 high versus low groups. **(B)** ISG15 immunoscore in CD3 high versus low groups.

DISCUSSION

In this study, we demonstrated that *ISG15* is significantly upregulated in gastric cancer tissues and is associated with advanced clinicopathological features and poorer overall survival. In contrast, higher *$CD3^+$ T-cell* infiltration was associated with earlier disease stage and improved prognosis. Importantly, combined assessment of *ISG15* and *CD3* provided superior prognostic stratification compared with either marker alone. These findings indicate that *ISG15* and *CD3* reflect interconnected biological processes involving immune regulation and tumour progression, with potential relevance to radiosensitivity in gastric cancer [16, 17].

Our results are in agreement with current researches indicating that interferon activated genes are often activated as a result of tumour associated stress and DNA damage. Radiotherapy triggers systemic interferon signalling and immune-associated genes in cancer patients and these results render the relevance of the ISGs including *ISG15* in radiation sensitive tumours. Similarly, the *ISG15* plays a critical role in maintaining genome stability by conjugating to proteins on nascent DNA and mitigating replication stress, suggesting a direct link between *ISG15* expression and cellular responses to genotoxic therapies. These observations align with our finding that elevated *ISG15* expression is associated with adverse prognosis, potentially reflecting enhanced DNA damage tolerance and radioresistance [8, 9, 19].

The analysis of our immune infiltration showed that *ISG15* expression was correlated significantly with an elevated infiltration of CD8+ T cells, CD4+ *T-cells*, and M1 macrophages in gastric cancer tissues. These groups of immune cells are principal mediators of antitumor immunity and the focus of radiation induced immune modulation. CD8+ cytotoxic T lymphocytes play a central role in tumour cells death after radiotherapy whereas CD4+ T cells play important helper roles that facilitate cytotoxic

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T-cell activation and maintenance of antitumor immune response [17, 18].

In parallel, the positive association between ISG15 expression and M1 macrophages suggests a link between interferon-stimulated gene activation and a pro-inflammatory, antitumor macrophage phenotype. M1 macrophages enhance antigen presentation, promote *T-cell* recruitment, and amplify interferon signalling within the tumour microenvironment, all of which are known to influence radiosensitivity. Collectively, these findings indicate that ISG15 expression reflects an immune-active tumour microenvironment characterized by coordinated involvement of $CD8^+$ T cells, $CD4^+$ *T-cells*, and M1 macrophages [19, 20].

From a radiobiological perspective, these immune cell populations play complementary roles in determining treatment response. $CD8^+$ *T-cells* are directly involved in radiation-induced tumour clearance, $CD4^+$ *T-cells* sustain adaptive immune memory, and M1 macrophages reinforce inflammatory signalling following DNA damage. The observed association between ISG15 and these immune cells supports the hypothesis that interferon -mediated immune activation contributes to variations in radiosensitivity in gastric cancer.

Mechanistically, *ISG15* may influence tumour progression and treatment response through dual effects on immune signalling and DNA damage repair. Radiation-induced interferon signalling can upregulate *ISG15*, which in turn modulates protein stability and replication stress responses. Although this could induce immune activation at the outset, in the long term and high levels of ISG15 expression could lead to tumour cell survival due to DNR and cytotoxic stress. Experimental evidence indicates that suppression of *ISG15* activates *TP53*-mediated DNA repair pathways and alters sensitivity to DNA-damaging agents, highlighting its complex role in treatment response. Concurrently, interferon signalling influences *T-cell* recruitment and activation, providing a biological basis for the observed association between *ISG15* and *CD3* expression in our cohort [21, 23]. From a clinical perspective, our findings suggest that combined evaluation of *ISG15* and *CD3* may improve prognostic assessment and potentially inform treatment stratification in gastric cancer. Patients with high *ISG15* expression and low *CD3* infiltration exhibited the poorest outcomes, identifying a subgroup that may be less responsive to radiotherapy alone and could benefit from combined radio-immunotherapy strategies. Conversely, patients with low *ISG15* and high *CD3* expression demonstrated favourable survival, indicating a more immunologically active tumour microenvironment. These observations support the growing interest in integrating immune biomarkers into radiotherapy decision-making [24-27].

As of late, the role of body immune responses as well as inflammatory responses in tumorigenesis mechanisms has become the topic of growing research. It has also been discovered that chronic inflammation causes immune reaction, which may get mutated to normal cells and enable their uncontrolled growth and establishment of a favourable environment to allow growth of tumours [28]. Commonly used clinical CRP, NLR, neutrophils, etc., were all effective indicators of poor prognosis of malignant tumours. NLR has also been found to be related to immune cell densities [29]. However, research about CRC suggested that increased systemic inflammatory response (marked by high NLR) was associated with higher recurrence and poorer survival. Still, in those patients with dMMR phenotype, combined high Inflammation had a better prognosis, and there was an inverse relationship between NLR and local inflammatory response [30]. Some scholars have also found that serum lymphocytes, especially the cells, played key roles in developing humoral and cell-mediated adaptive immune responses against cancer [31,32]. It has also been reported that the lymphocytes could regulate the lung environment in various ways, including pro-inflammatory or anti-inflammatory, under specific conditions [33]. Currently, few relative studies on the above indicators in GC. Therefore, we collected and statistically analysed the above indicators of 103 patients; the results showed that the PCT of the *CD3* high expression group in GC was higher than the other one, $P<0.05$.

The novelty of this study lies in the integrated analysis of *ISG15* expression, immune infiltration, and clinical outcomes in gastric cancer, supported by both bioinformatics and tissue-level validation. While prior studies have examined *ISG15* or immune infiltration independently, few have explored their combined prognostic significance or potential relevance to radiosensitivity. By linking these factors, our study provides new insight into the interaction between innate immune signalling, adaptive immune response, and tumour behaviour.

The results of the quantitative immunohistochemical analysis showed that the high expression of ISG15 was observed in 68.93% (71/103) of gastric cancers tissues, and high expression of CD3 was observed in 10.68% (11/103) cases. Figure X is a representative photomicrograph of low and high expression of ISG15 and CD3: Isg15 staining was mostly confined to the cytoplasm with the majority of the CD3+ T cells being found in the tumour stroma and invasive margins.

Several limitations should be acknowledged. Gene expression validation at the mRNA level using RT-qPCR was not performed; however, protein-level validation by immunohistochemistry provided biological confirmation of *ISG15* expression. In addition, direct clinical data on radiotherapy response were limited, and radiosensitivity was

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inferred from molecular and immune associations rather than treatment outcomes. Larger, prospective studies incorporating radiotherapy-specific endpoints are needed to validate these findings. prospective validation of *ISG15* and *CD3* as combined biomarkers in gastric cancer patients receiving radiotherapy, as well as functional studies to elucidate the precise mechanisms linking *ISG15* signalling, *T-cell* infiltration, and treatment response could be studied in future. Integration of these markers into predictive models may facilitate personalized radiotherapy and combined immunotherapeutic strategies, ultimately improving clinical outcomes.

CONCLUSION

This research illustrates that the *ISG15* and *CD3* are the biomarkers that are clinically relevant to reflect the immune status and prognosis of gastric cancer. The high level of *ISG15* is linked to poor clinicopathologic characteristics and worse survival, and the high level of *CD3+ T-cell* infiltration is linked to good prognoses. In combination with *ISG15* and *CD3*, the joint assessment gives more prognostic stratification and might be used to determine patients who might be offered an improved approach to radiotherapy or radio-immune therapy.

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Conflicts of interest: The authors declare that they have no conflicts of interest relevant to this study.

Ethical considerations: Declaration of Helsinki, Ethical approval from the Ethics Committee of the Third Affiliated Hospital of Kunming Medical University and written informed consent were obtained prior to inclusion in the study.

Author contributions: Y.H., conceived and designed the study, supervised the research process, and served as the corresponding author. H.H.L. and Y.L., were responsible for data collection, clinical information acquisition, and preliminary data organization. X.Z.X., Z.H.C., and Y.Z.L., contributed to immunohistochemical evaluation, data interpretation, and validation of experimental findings. X.Q.Y. and X.L. performed the bioinformatics and statistical analyses. Jin Yue Liang and Ting Feng assisted with data curation, figure preparation, and literature review. All authors participated in manuscript drafting and critical revision, approved the final version of the manuscript.

Use of AI: All scientific content, data interpretation, and conclusions were determined by the authors. AI were used to assist with language editing and improvement of clarity and readability of the manuscript.

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