Intratumoral IL-1R1 expression delineates a distinctive molecular subset with therapeutic resistance in patients with gastric cancer

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ABSTRACT

Background With the essential role of interleukin-1 signaling in cancer-related inflammation, IL-1R1, the main receptor for both IL-1α and IL-1β, demonstrated therapeutic potential in several types of cancer, which has been put into clinical trials. However, the expression profile and critical role of IL-1R1 in gastric cancer (GC) remain obscure. This study aimed to investigate the prognostic significance of IL-1R1 expression and its predictive value for chemotherapy and immunotherapy in GC.

Methods The study enrolled three cohorts, consisting of 409 tumor microarray specimens of GC patients from Zhongshan Hospital, 341 transcriptional data from The Cancer Genome Atlas, and 45 transcriptional data from patients treated with pembrolizumab. IL-1R1 mRNA expression was directly acquired from public datasets, and we also detected IL-1R1 protein expression on tumor microarray by immunohistochemistry. Finally, the associations of IL-1R1 expression with clinical outcomes, immune contexture, and genomic features were analyzed.

Results High IL-1R1 expression predicted poor prognosis and inferior responsiveness to both 5-fluorouracil-based adjuvant chemotherapy (ACT) and immune checkpoint blockade (ICB). IL-1R1 fostered an immunosuppressive microenvironment characterized by upregulated M2 macrophages and exhausted CD8+ T cells infiltration. Moreover, the expression of IL-1R1 was intrinsically linked to genomic alterations associated with targeted therapies in GC.

Conclusions IL-1R1 served as an independent prognosticator and predictive biomarker for ACT and ICB in GC. Furthermore, IL-1R1 antagonists could be a novel agent alone or combined with current therapeutic strategies in GC.

INTRODUCTION

Gastric cancer (GC) ranks the fifth most frequently diagnosed cancer and the fourth leading cause of cancer-associated mortality worldwide.1 Though radical gastrectomy is considered the most effective treatment,2 patients with advanced GC tend to relapse even with surgical interventions. Accordingly, 5-fluorouracil-based adjuvant chemotherapy (ACT) has been widely applied as first-line therapy to reduce postoperative recurrence rate.3 4 However, a significant fraction of patients failed to gain survival benefit due to acquired chemoresistance.5 6 Further investigation of novel therapeutic opportunities is urgently needed to prolong survival and reduce drug resistance in GC.

Fortunately, recent advances in immunotherapy, especially immune checkpoint blockade (ICB), have shed light on new strategies for GC treatment.7 Nevertheless, the current ICB only provides survival benefits for less than 20% of patients with GC. Due to the ineffectiveness of both therapeutic strategies aforementioned in a substantial proportion of GC patients, it is of great clinical significance to investigate emerging biomarkers for further patient stratification and improved treatment tactics.

Prior studies have demonstrated that the efficacy of ACT and ICB is inextricably correlated with the tumor microenvironment (TME), within which the interleukin (IL) family plays dynamic roles in various tumor biological activities.8 9 Our previous studies have demonstrated that IL-9, IL-10 and IL-17 play critical roles in predicting therapeutic effectiveness.10–12 As the earliest discovered member of the IL family, IL-1 has been long known for its pleiotropic effects on inflammation, which promotes progression and metastasis in multiple cancers, primarily through the IL-1R signaling pathway.13–19 IL-1R1, as an essential participant in the IL-1R signaling pathway, is the only receptor that can bind to both agonistic ligands, IL-1α and IL-1β, and subsequently mediates positive signaling transduction via NF-κB and MAP kinase pathways.20–22 Existing literature has elucidated the potential value of IL-1R1 antagonists and anti-IL-1 monoclonal antibodies for inhibiting
primary tumor growth and reversing the acquired resistance to chemotherapy and ICB in multiple models.\textsuperscript{23–27} So far, several clinical trials have been recently carried out for evaluating the therapeutic value of targeting IL-1R1 and the synergistic effect of IL-1R1 antagonists, such as anakinra, with existing therapeutic strategies.\textsuperscript{27–29} In GC, studies showed that both IL-1α and IL-1β were correlated with tumor initiation and progression.\textsuperscript{30, 31} Nevertheless, the prognostic and predictive value of IL-1R1 in GC remains obscure.

Here, we indicated that high IL-1R1 expression predicted poor prognosis and inferior responsiveness to ACT and ICB. Meanwhile, we confirmed that IL-1R1 fostered an immunosuppressive microenvironment featured by upregulated M2 macrophages and exhausted CD8\textsuperscript{+} T cells infiltration. Moreover, the expression of IL-1R1 was intrinsically related to specific molecular subtypes and genomic alterations in GC. In a word, our study has shed light on the clinical and translational significance of IL-1R1 as a stratification biomarker and potential therapeutic target to facilitate personalized therapy in GC.

\section*{PATIENTS AND METHODS}

\subsection*{Patients and gastric tissue samples}

This study enrolled three independent patient cohorts, as illustrated in online supplemental figure S1. The ZSHS cohort consisted of 496 patients recruited from the Zhongshan Hospital, Fudan University (Shanghai, China). However, 87 of them were excluded due to loss of transcriptional data.\textsuperscript{33} The remaining 45 patients with information of drug response were further analyzed, and 43 of whom had accessible clinical information, including OS and progression-free survival (PFS). The clinical data of the ICB cohort were generously provided by the research team of Professor Jeeyun Lee, Division of Hematology-Oncology, Samsung Medical Center.

\subsection*{Immunohistochemistry and evaluation of immunostaining}

Prior to immunohistochemistry (IHC), the tissue microarrays (TMAs) were constructed by Shanghai Outdo Biotech Co, Ltd. The protocol of TMA construction and IHC staining has been described detailedly in our previous study.\textsuperscript{34, 35} The associated antibodies were listed (online supplemental table S1). In our study, all TMA samples were evaluated separately by two independent pathologists (Dr Lingli Chen and Dr Yunyi Kong) who were blinded to the clinicopathological data. Both of them scored independently according to the proportion of stained cells and the cellular staining intensity. Briefly, the proportion of stained cells was defined as the percentage of positive cells, whereas the cellular staining intensity was stratified as 0 (negative staining), 1 (weak staining, light yellow), 2 (moderate staining, yellow-brown), and 3 (strong staining, brown). The mean score of their evaluation was adopted for further analysis. The median value of IL-1R1 IHC score was determined as the cut-off point. The representative images were displayed in online supplemental figure S2. Variations in IL-1R1 IHC score, exceeding 10, were re-evaluated separately by both pathologists to reach a final consensus. The Image-Pro-Plus software V.6.2 was used to further validate the scoring results from two independent pathologists. The processed IHC staining of IL-1R1 with Image-Pro-Plus was displayed in online supplemental figure S3. The IL-1R1 IHC score was closely related to the Image-Pro-Plus mean integrated optical density as demonstrated in online supplemental figure S4.

\subsection*{Statistical analysis}

Pearson’s \(\chi^2\) test and Fisher’s exact test were applied to compare categorical variables. Mann-Whitney U test was applied to compare continuous variables. One-way analysis of variance followed by Tukey multiple comparisons test was applied for the correlation between IL-1R1 expression and TNM stages. OS, DFS, and PFS were analyzed by Kaplan-Meier curves, log-rank test, and multivariate analysis based on Cox regression analysis. The cut-off value for the classification of IL-1R1\textsuperscript{high} and IL-1R1\textsuperscript{low} subgroups was the median value. All analyses were conducted using IBM SPSS Statistics V.20.0, MedCalc 15.6.1, and R 4.0.2 software. The GIBERSORT algorithm was constructed to calculate the relative proportion of 22 immune cell types with the LM22 expression signature. The single sample gene sets enrichment analysis (ssGSEA) implanted in the ‘GSVA’ package was implemented to calculate signature scores. The stromal score, immune score, and ESTIMATE score were directly acquired from https://bioinformatics.
RESULTS

IL-1R1 expression yields a poor prognosis and is involved in tumor progression in GC

To elucidate the clinical significance of IL-1R1 expression in GC, Kaplan-Meier curves and log-rank test were applied to assess OS and DFS between IL-1R1 high/low subgroups in the ZSHS cohort and TCGA cohort, respectively. In both cohorts, patients with high levels of IL-1R1 expression had significantly worse OS (p<0.001 and p=0.030; Figure 1A,B). However, the association between high levels of IL-1R1 expression and worse DFS was only observed in the Zhongshan Hospital (ZSHS) cohort (p=0.001 and p=0.110; Figure 1A,B). Furthermore, multivariate Cox regression analysis showed IL-1R1 was an independent prognosticator for worse OS and DFS in ZSHS cohort after adjustment for confounders (HR: 2.300, 95% CI 1.684 to 3.141, p<0.001; Figure 1A,B). Moreover, since previous studies have demonstrated that IL-1R1 expression was highly correlated with GC formation, we wondered whether the expression of IL-1R1 might differ across TNM stages. Notably, we found that in both the ZSHS cohort and TCGA cohort, TNM stage III patients demonstrated more intensive IL-1R1 expression than TNM stage I patients (online supplemental figure S5A,B). Other clinicopathological characteristics of GC patients with high/low IL-1R1 expression in the ZSHS cohort and TCGA cohort were summarized (online supplemental table S2). Conclusively, these results showed that IL-1R1 serves as an independent adverse prognosticator and might be associated with tumor progression in GC.

IL-1R1 predicts inferior responsiveness to ACT and ICB in GC

Previous studies have demonstrated that the IL-1R signaling pathway was detrimental for 5-fluorouracil-based antitumor efficacy. Considering the limited therapeutic response of GC patients to 5-fluorouracil-based ACT, we wondered if IL-1R1 could be used to select suitable candidates for ACT. In the ZSHS cohort, ACT application predicted significantly better OS, rather than DFS in stage II/III patients (p<0.001 and p=0.230; online supplemental figure S6). However, such overall beneficial effect was only observed in IL-1R1 high subgroup after dividing patients based on IL-1R1 expression (p<0.001 and p=0.180; p=0.001 and p=0.110; figure 2A), which suggested that IL-1R1 expression might have a predictive effect on the responsiveness of GC patients to ACT. A test for interaction between IL-1R1 expression and ACT revealed that the therapeutic effectiveness observed in IL-1R1 high subgroup was significantly inferior to that in IL-1R1 low subgroup (p for interaction=0.001; figure 2A).

Furthermore, we enrolled the ICB cohort consisting of patients treated with pembrolizumab to evaluate the predictive value of IL-1R1 for immunotherapy (table 1). We found that patients in IL-1R1 high subgroup demonstrated a significantly decreased response rate compared with those in IL-1R1 low subgroup (figure 2B). Meanwhile, patients in IL-1R1 high subgroup demonstrated a significantly worse OS and PFS compared with those in IL-1R1 low subgroup (p=0.027 and p=0.010; figure 2C). Since existing research has demonstrated that CD274 (PD-L1) mRNA expression was correlated with the efficacy of pembrolizumab, we further stratified patients based on PD-L1 mRNA expression within IL-1R1 high/low subgroups. Interestingly, the IL-1R1 low/PD-L1 high group showed the highest objective response rate (ORR), while the ORR of IL-1R1 high/PD-L1 high group was the lowest (figure 2D). This result indicated that IL-1R1 could be a crucial factor causing attenuated responsiveness to pembrolizumab, even with high PD-L1 expression. The associations of IL-1R1/PD-L1 expression and molecular parameters were summarized (table 2). Cumulatively, our findings suggested that IL-1R1 could be a potential efficacy predictor for both ACT and ICB in GC.

IL-1R1 fosters an immunosuppressive microenvironment in GC

Prior studies have shown that the IL1-R signaling pathway could mobilize myeloid-derived suppressor cells and tumor-associated macrophages, subsequently fostering an immunosuppressive microenvironment, which was predominantly relevant to prognosis and responsiveness to chemotherapy and immunotherapy. Thus, we used the CIBERSORT algorithm to calculate the relative proportion of 22 human hematopoietic cell phenotypes (LM22) within the TCGA database. We found that memory B cells, monocytes, M0 macrophages, M2 macrophages, and Mast cells resting were significantly enriched in IL-1R1 high subgroup, whereas the enrichment of T cells follicular helper, regulatory T cells, NK cells resting, and Mast cells activated was observed in IL-1R1 low subgroup. We also noticed that the overall immune and stromal content were significantly increased in IL-1R1 high subgroup (figure 3A). To validate the result from the CIBERSORT algorithm, we evaluated the related immune cells infiltration based on IL-1R1 expression in the ZSHS cohort. Notably, only M2 macrophages demonstrated elevated infiltration in IL-1R1 high subgroup, which was consistent with the result from the CIBERSORT algorithm (figure 3B and online supplemental figure S7). Then, to further explore the relationship between IL-1R1 expression and M2 macrophages infiltration, we confirmed that...
both M2 macrophages recruitment and activation associated genes, including CCL2, CSF1R, and IL6ST, and signatures including angiogenesis, hypoxia, EMT, IL6, TGF-β, and IL10 pathway were significantly upregulated in IL-1R1 high subgroup through differential gene expression analysis and ssGSEA (online supplemental figure S8A,C and table S3). However, Kaplan-Meier curves showed that there were no significant differences of OS and DFS based on CD163+ M2 macrophages infiltration within IL-1R1 high/low subgroups (p=0.990 and p=0.170; p=0.500 and p=0.280; online supplemental figure S8B). Furthermore, we explored the expression of immune

Figure 1  IL-1R1 yields a poor prognosis in patients with gastric cancer. (A–B) Kaplan-Meier curves and multivariate Cox regression analysis of overall survival (OS) and disease-free survival (DFS) based on IL-1R1 expression in the Zhongshan Hospital (ZSHS) cohort (n=409) (A) and the Cancer Genome Atlas (TCGA) cohort (n=341) (B) (log-rank test). P<0.05 marked in bold font shows statistical significance. IL, interleukin.

| Variables       | Overall survival | Disease-free survival | HR (95% CI) | P Value |
|-----------------|------------------|-----------------------|-------------|---------|
| Age (≥ 60 vs. < 60) | 1.195 (0.899–1.608) | 1.095 (0.811–1.480) | 0.238   |
| Sex (male vs. female) | 0.842 (0.615–1.153) | 0.754 (0.552–1.030) | 0.842   |
| Location (distal vs. proximal) | 0.977 (0.741–1.337) | 0.996 (0.742–1.336) | 0.976   |
| Lauren (diffuse vs. intestinal) | 1.121 (0.823–1.527) | 1.227 (0.904–1.667) | 0.466   |
| Size (≥ 4cm vs. < 4cm) | 0.990 (0.733–1.337) | 1.135 (0.839–1.535) | 0.948   |
| Grade (per increase in grade) | 1.015 (0.709–1.453) | 0.978 (0.677–1.413) | 0.935   |
| TNM stage (per increase in stage) | 2.568 (1.988–3.318) | 3.488 (2.655–4.583) | <0.001  |
| IL-1R1 (high vs. low) | 2.300 (1.684–3.141) | 2.463 (1.812–3.346) | <0.001  |
| Age (≥ 60 vs. < 60) | 2.142 (1.365–3.361) | 1.586 (0.797–3.156) | 0.001   |
| Sex (male vs. female) | 1.340 (0.904–1.985) | 2.170 (1.084–4.343) | 0.029   |
| Location (distal vs. proximal) | 1.151 (0.794–1.669) | 1.557 (0.838–2.891) | 0.459   |
| Grade (per increase in grade) | 1.349 (0.929–1.958) | 1.692 (0.862–3.319) | 0.116   |
| TNM stage (per increase in stage) | 1.446 (1.092–1.922) | 0.994 (0.651–1.517) | 0.010   |
| IL-1R1 (high vs. low) | 1.398 (0.964–2.029) | 1.542 (0.809–2.940) | 0.078   |

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Figure 2 IL-1R1 expression predicts inferior responsiveness to 5-fluorouracil-based ACT and ICB in gastric cancer. (A) For stage II/III patients in the ZSHS cohort (n=307), Kaplan-Meier curves demonstrated responsiveness to 5-fluorouracil-based ACT in patients stratified by IL-1R1 expression. (B) The waterfall plot and stacked bar plot demonstrated responsiveness to pembrolizumab based on IL-1R1 expression in the ICB cohort (n=45). (C) Kaplan-Meier curves of overall survival (OS) and progression-free survival (PFS) based on IL-1R1 expression in the ICB cohort (n=43). (D) Heatmap demonstrated responsiveness to pembrolizumab and molecular parameters based on PD-L1 mRNA expression within IL-1R1 high/low subgroups in the ICB cohort (n=45). ACT, adjuvant chemotherapy; CIN, chromosomal instability; CR, complete response; EBV, EBV positive; EMT, epithelial-mesenchymal transition; GS, genomically stable; ICB, immune checkpoint blockade; ML, mutation load; MSI, microsatellite instability; ORR, objective response rate; PD, progressive disease; PR, partial response; SD, stable disease; ZSHS, Zhongshan Hospital.
Characterization of IL-1R1 expression in GC

As is known to all, CD8+ T cells are regarded as the primary effector cells in antitumor immunity. However, no significant difference in the quantity of CD8+ T cells was observed between IL-1R1 high/low subgroups. We wondered whether IL-1R1 expression was correlated with the functional status of CD8+ T cells. Through GSEA, we found that exhausted CD8+ T cells signatures were upregulated in IL-1R1 high subgroups (figure 3C). Additionally, Kaplan-Meier curves showed that CD8+ T cells infiltration predicted improved OS and DFS only in IL-1R1 low subgroup, rather than IL-1R1 high subgroup (p=0.005 and p=0.280; p<0.001 and p=0.290; figure 3D). Since CD8+ T cells infiltration failed to serve as a prognosticator in IL-1R1 high subgroup, we further trichotomized patients into various risk subgroups, defined as the low-risk group (IL-1R1low CD8+ T cellslow), intermediate-risk group (IL-1R1low CD8+ T cellslow), and high-risk group (IL-1R1high). Consistent with our hypothesis, the low-risk group demonstrated the most optimal prognosis, whereas the high-risk group demonstrated the worst prognosis regarding OS and DFS (online supplemental figure S9A,C). Furthermore, the results of Cox regression analysis showed that our novel risk stratification model could be used as an independent prognosticator regarding OS and DFS (online supplemental figure S9B,D). Moreover, we sought to evaluate whether various risk subgroups indicated distinct chemotherapeutic responsiveness in stage II/III GC patients. Cox regression analysis was applied,

| Factors                  | IL1R1hi CD274hi | IL1R1lo CD274hi | IL1R1lo CD274lo | P value |
|--------------------------|-----------------|-----------------|-----------------|---------|
| All patients             | 12              | 11              | 11              | 0.001   |
| Immune signature         |                 |                 |                 |         |
| High                     | 9               | 9               | 3               |         |
| Low                      | 3               | 2               | 8               |         |
| Mutation load            |                 |                 |                 | 0.016   |
| High                     | 0               | 5               | 1               |         |
| Moderate                 | 2               | 4               | 5               |         |
| Low                      | 10              | 2               | 5               |         |
| EMT status               |                 |                 |                 | 1.000   |
| Mesenchymal              | 2               | 1               | 2               |         |
| Non-mesenchymal          | 10              | 10              | 9               |         |
| Molecular subtype        |                 |                 |                 | 0.002   |
| CIN                      | 5               | 2               | 3               |         |
| EBV                      | 0               | 4               | 0               |         |
| GS                       | 7               | 1               | 7               |         |
| MSI                      | 0               | 4               | 1               |         |
| MSI status               |                 |                 |                 | 0.020   |
| High                     | 0               | 4               | 1               |         |
| Low                      | 12              | 7               | 10              |         |
| EBV status               |                 |                 |                 | 0.007   |
| Positive                 | 0               | 4               | 0               |         |
| Negative                 | 12              | 7               | 11              |         |
| Response                 |                 |                 |                 | 0.003   |
| PD                       | 7               | 2               | 5               |         |
| SD                       | 4               | 0               | 5               |         |
| PR                       | 1               | 6               | 1               |         |
| CR                       | 0               | 3               | 0               |         |

P<0.05 marked in bold font shows statistical significance.

CIN, chromosomal instability; CR, complete response; EBV, EBV positive; EMT, epithelial-mesenchymal transition; GS, genomically stable; hi, high; lo, low; MSI, microsatellite instability; PD, progressive disease; PR, partial response; SD, stable disease.

Objective patients’ response to pembrolizumab

| All patients (n=45) | IL1R1hi (n=23) | IL1R1lo (n=22) |
|---------------------|----------------|----------------|
| Best overall response | No. | % (95% CI) | No. | % (95% CI) | No. | % (95% CI) |
| Objective response (CR+PR) | 12  | 26.7 (15 to 45) | 2  | 8.7 (1 to 28) | 10  | 45.5 (24 to 68) |
| Disease control     |     |                |     |                |     |                |
| CR                  | 3  | 6.7 (2 to 18) | 0  | 0 (0 to 15) | 3  | 13.6 (3 to 35) |
| PR                  | 9  | 20.0 (9 to 34) | 2  | 8.7 (1 to 28) | 7  | 31.8 (14 to 55) |
| SD                  | 15 | 33.3 (20 to 49) | 9  | 39.1 (20 to 62) | 6  | 27.3 (11 to 50) |
| PD                  | 18 | 40.0 (26 to 56) | 12 | 52.2 (31 to 73) | 6  | 27.3 (11 to 50) |

CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

Table 2: Association between IL1R1/CD274 (PD-L1) expression and molecular parameters

Table 3: Association between IL1R1/CD274 (PD-L1) expression and molecular parameters

| Factors                  | IL1R1hi CD274hi | IL1R1lo CD274hi | IL1R1lo CD274lo | P value |
|--------------------------|-----------------|-----------------|-----------------|---------|
| All patients             | 12              | 11              | 11              | 0.001   |
| Immune signature         |                 |                 |                 |         |
| High                     | 9               | 9               | 3               |         |
| Low                      | 3               | 2               | 8               |         |
| Mutation load            |                 |                 |                 | 0.016   |
| High                     | 0               | 5               | 1               |         |
| Moderate                 | 2               | 4               | 5               |         |
| Low                      | 10              | 2               | 5               |         |
| EMT status               |                 |                 |                 | 1.000   |
| Mesenchymal              | 2               | 1               | 2               |         |
| Non-mesenchymal          | 10              | 10              | 9               |         |
| Molecular subtype        |                 |                 |                 | 0.002   |
| CIN                      | 5               | 2               | 3               |         |
| EBV                      | 0               | 4               | 0               |         |
| GS                       | 7               | 1               | 7               |         |
| MSI                      | 0               | 4               | 1               |         |
| MSI status               |                 |                 |                 | 0.020   |
| High                     | 0               | 4               | 1               |         |
| Low                      | 12              | 7               | 10              |         |
| EBV status               |                 |                 |                 | 0.007   |
| Positive                 | 0               | 4               | 0               |         |
| Negative                 | 12              | 7               | 11              |         |
| Response                 |                 |                 |                 | 0.003   |
| PD                       | 7               | 2               | 5               |         |
| SD                       | 4               | 0               | 5               |         |
| PR                       | 1               | 6               | 1               |         |
| CR                       | 0               | 3               | 0               |         |

P<0.05 marked in bold font shows statistical significance.

CIN, chromosomal instability; CR, complete response; EBV, EBV positive; EMT, epithelial-mesenchymal transition; GS, genomically stable; hi, high; lo, low; MSI, microsatellite instability; PD, progressive disease; PR, partial response; SD, stable disease.
and the results implied that higher risk subgroups might have attenuated responsiveness to 5-fluorouracil-based ACT (online supplemental figure S9E). Conclusively, our findings implied that IL-1R1 might impede the antitumor immunity of CD8⁺ T cells via shaping a dysfunctional phenotype.

Characteristics of IL-1R1 mRNA expression across molecular subtypes and targetable genomic alterations in GC
Since the progressive gene alterations accumulated throughout life are considered a driving force of cancer, we next investigated the differential distributions of somatic gene mutations and GC molecular subtypes.
between IL-1R1 high/low subgroups. To profile a comprehensive landscape of genomic features associated with IL-1R1 expression, we delineated the top 10 gene mutations within GC (figure 4A). Among the top 10 mutated genes, the mutational frequencies of TTN, MUC16, ARID1A, LRP1B, CSMD3, FAT4, FLG, and PCLO were significantly decreased in IL-1R1 high subgroup, along with tumor mutational burden (figure 4A). To further elucidate whether the inferior prognostic merit of IL-1R1 was correlated with certain genomic features among the top 10 mutated genes, we used Cox regression analysis and found that only in TTN, TP53, and CSMD3 mutation subgroup and SYNE1, FLG, and PCLO wildtype subgroup can IL-1R1 expression act as a prognosticator for worse OS (online supplemental figure S10). Since growing evidence has revealed the molecular subtypes of GC as a novel avenue for precision therapy and patient stratification, 39 we subsequently explored the distribution of different molecular subtypes between IL-1R1 high/low subgroups. Notably, within the IL-1R1 high subgroup, the proportion of the genomically stable subtype was significantly higher, while the proportion of the microsatellite instability (MSI) subtype was significantly lower than that of the IL-1R1 low subgroup (figure 4B). Recently, advances in cancer biology have enabled patient selection for targeted precision therapy. 40 Here, based on our findings that certain genomic features demonstrated different patterns between IL-1R1 high/low subgroups, we wondered if IL-1R1 expression was associated with potential therapeutic targets in GC. Thus, we further delineated a comprehensive landscape of genomic features associated with multiple targeted therapies evaluated in clinical trials. 40 First and foremost, we used the COSMIC mutational signatures related to the APOBEC family and DNA damage repair 11 and found that only signature 6, which represented mutational patterns of mismatch repair, demonstrated decreased occurrence in IL-1R1 high subgroup (figure 4C). The gene mutation analysis showed a decreased mutational frequency of PIK3CA and KRAS in IL-1R1 high subgroup (figure 4C). However, no significant differences were observed in CNV between IL-1R1 high/low subgroups (figure 4C). At the transcriptional level, we found that ERBB, EGFR, and VEGF signaling pathways were significantly upregulated in IL-1R1 high subgroup, whereas the homologous recombination repair pathway was significantly downregulated (figure 4C). Conclusively, our findings implied that IL-1R1 might be applied to optimize patient selection and as a potential target to improve the efficacy of current targeted therapies in GC.

**DISCUSSION**

TME represents a pivotal component of cancer, 8 and inflammation is a crucial component of TME. 42 Tumor-promoting inflammation is mainly orchestrated by multiple inflammatory cytokines and chemokines. 43 As a prototypic inflammatory cytokine, IL-1 is involved in a complex cascade that serves an essential role in the initiation and regulation of innate and adaptive immunity. 42 43 Existing literature has elucidated that the IL-1R signaling pathway shapes an immunosuppressive TME primarily through the mobilization and activation of myeloid-derived suppressor cells and tumor-associated macrophages. 16 31 36 In this study, we verified that GC patients with more intensive IL-1R1 expression exhibited inferior OS and DFS. We also found that IL-1R1 expression was positively associated with M2 macrophages and exhausted CD8+ T cells infiltration, highlighting the significance of IL-1R1 as a potent TME modifier in GC. Since IL-1R1 was also expressed in tumor cells (online supplemental figure S2), we believed that the IL-1R signaling pathway might be related to specific biological properties of GC. Herein, we discovered that TNM stage III tumors demonstrated more intensive IL-1R1 expression than stage I tumors. Moreover, tumors with high levels of IL-1R1 expression tended to undergo epithelial-mesenchymal transition. Previous studies have revealed that the IL-1R signaling pathway was involved in the induction of EMT phenotype in an NF-kB/AKT/Wnt-dependent manner. 44 These results indicated that the IL-1R signaling pathway might be associated with the intrinsic aggressiveness of GC. Furthermore, we found that IL-1R1 expression was associated with particular genotypes, especially loss of MSI status and increased genomic stability. This implied that the IL-1R signaling pathway might affect or be affected by specific molecular properties of GC.

Since chemotherapeutic agents also harness the host’s immune system in addition to their direct cytotoxic effects, 35 altered TME via IL-1R1 might blunt its antitumor activity. Prior studies have revealed that 5-fluorouracil triggered activation of inflammasomes in myeloid-derived suppressor cells leading to the production of IL-1β was a crucial mechanism of chemoresistance. 23 24 Consistent with these theories, our study revealed that patients with more intensive IL-1R1 expression exhibited attenuated responsiveness to 5-fluorouracil-based chemotherapy in GC, highlighting the potential value of IL-1R1 for patient stratification. Moreover, since multiple clinical trials have been carried out to evaluate the therapeutic value of IL-1R1 antagonists alone or in combination with existing chemotherapeutic agents in a large variety of cancers, 27 28 IL-1R1 blockade might be available as a novel tactic for GC treatment in the near future.

ICB, which reactsivates tumoricidal T cells via the PD-1/PD-L1 axis, has emerged as a novel and promising therapeutic strategy to eradicate cancer cells. 46 Nevertheless, the ORR remains unsatisfying, especially in GC. 47 Recently, multiple preclinical models have been carried out to evaluate the synergetic effect of anti-IL-1 mAbs with ICB. 25 26 For instance, in triple-negative breast cancer, treatment with anti-IL-1β mAbs significantly potentiated anti-PD-1 therapy. 25 Remarkably, our study revealed that compared with responders to pembrolizumab in GC, non-responders demonstrated more intensive IL-1R1 expression, indicating IL-1R1 expression could be used
Figure 4  Characteristics of IL-1R1 mRNA expression across molecular subtypes and targetable genomic alterations in gastric cancer. (A) The radar chart demonstrated the distribution of the mutational frequencies of the top 10 mutated genes and tumor mutational burden based on IL-1R1 expression (Pearson’s χ² test and Mann-Whitney U test). (B) Chord diagram demonstrated the distribution of different GC molecular subtypes based on IL-1R1 expression (Pearson’s χ² test). (C) Heatmap demonstrated the genomic alterations of potential therapeutic targets in gastric cancer based on IL-1R1 expression (Pearson’s χ² test and Mann-Whitney U test). *P<0.05, **p<0.01, ***p<0.001. CIN, chromosomal instability; EBV, EBV-positive; GS, genomically stable; HRR, homologous recombination repair; MSI, microsatellite instability; NA, not available; TMB, tumor mutational burden.
as a stratification biomarker for anti-PD-1 therapy. Moreover, since the success of anti-IL-1 mAbs with ICB in multiple models aforementioned, IL-1R1 might also be a potential target for evaluation to complement existing ICB strategies in GC.

Advances in sequencing technology have broadened the horizon of understanding the tumor biological properties and therapeutic guidance. The development of emerging therapeutic strategies such as targeted therapy has enabled personalized precision treatment of multiple solid tumors. Nevertheless, only three molecular biomarkers have been identified to predict response to novel therapies in GC. Recently, in a biomarker-guided trial, VIKTORY, patients with GC who were assigned to different groups based on eight biomarkers (RAS aberrations, TP53 mutations, PIK3CA mutations and/or amplification, MET amplification, MET overexpression, alterations, TP53 mutations, PIK3CA mutations and/or amplification) demonstrated significantly prolonged OS and PFS, highlighting the essential role of biomarker-guided targeted therapy for GC patients. In this study, we found that compared with IL-1R1low subgroup, IL-1R1high subgroup demonstrated significantly decreased mutational frequency of several targetable genes, whereas several targetable pathways were significantly upregulated, suggesting the potential possibility of using IL-1R1 as a novel companion stratification biomarker for targeted precision therapy in GC.

Considering the retrospective design of our study, further validation is required to confirm our results within the framework of more extensive, multicentered clinical trials.

In conclusion, our study demonstrated that IL-1R1 was an adverse independent prognosticator and yielded inferior responsiveness to both ACT and ICB in GC. Furthermore, IL-1R1 fostered an immunosuppressive TME and was associated with certain genomic features. Moreover, IL-1R antagonists, such as anakinra, might be applied alone or as complementary therapy to reinvigorate ACT and ICB in GC.

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