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ORIGINAL RESEARCH

Role of Senescence-Associated Biomarkers and Immune Dynamics in Predicting Response to Neoadjuvant Chemoradiotherapy in Rectal Cancer

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Objective: Neoadjuvant chemoradiotherapy (nCRT) is one of the standard treatments for locally advanced rectal cancer (LARC). However, the therapeutic responses to this form of treatment greatly vary from one patient to another. In this work, we focused on changes of serum senescence-associated secretory phenotype (SASP) factors and immune cell infiltration post-nCRT in a search for possible predictors of response to nCRT.

Methods: Twenty rectal cancer patients treated with nCRT were included and underwent assessments before (pre-) and after (post-) the treatment. Inflammatory cytokines such as IL-1α, IL-6, and IL-8; chemokines such as CCL5, CXCL1, and CCL2 in serum; and immune cell infiltrations including CD8+, CD4+, and CD206+ macrophages were assessed by ELISA and IHC, respectively. Tumor regressions were evaluated by MSK three-tier TRG grading system.

Results: Significant post-nCRT upregulation of IL-6, IL-8, IL-1α, CRP, CCL5, and CXCL1 was found, together with increased CD8+ T cell infiltration in tumor regression responders. IL-1α and CCL2 pre-nCRT levels were promised as predictive biomarkers, given that higher pretreatment levels were associated with lower tumor regression. Increased CD8+ cytotoxic T cell infiltration improved treatment outcome, whereas the changes in CD4+ T cells and M2 macrophages did not reach statistical significance.

Conclusion: IL-1α, CCL2, and CD8+ T cells, were identified as candidate markers that might monitor nCRT effectiveness in rectal cancer patients. These findings reinforce insights into the tumor microenvironment modulated by SASP components and immune cells and imply the need for larger studies to validate such associations.

Keywords: rectal cancer, neoadjuvant chemoradiotherapy, nCRT, senescence-associated secretory phenotype, SASP, tumor microenvironment, TME, predictive biomarkers

Introduction

Rectal cancer remains a common and aggressive malignancy, among which locally advanced rectal cancer (LARC) requires intensive multimodal interventions to achieve better outcomes.[1] Neoadjuvant chemoradiotherapy (nCRT), combining radiation with chemotherapy, has been considered a cornerstone in the management of patients with LARC, aiming at tumor downstaging, reducing local recurrence, and improving surgical outcomes.[2] However, response to nCRT has varied among patients, which calls for predictive biomarkers that can predict and estimate the therapeutic efficiency of nCRT.

One cellular mechanism elicited by nCRT is therapy-induced senescence (TIS), a process by which cancer cells are induced to undergo a stable cell cycle arrest due to genotoxic stress.[3] Radiotherapy is a major inducer of therapy-induced senescence (TIS), as ionizing radiation causes DNA damage, oxidative stress, and persistent DNA damage responses (DDR), leading to cell cycle arrest and the development of the Senescence-Associated Secretory Phenotype (SASP).[4] SASP is characterized by the secretion of a wide range of proinflammatory cytokines, chemokines, growth factors, and proteases, which can remodel the tumor microenvironment, influence immune responses, and potentially alter the effectiveness of treatments.[5] In the context of rectal cancer, SASP factors following nCRT may be critical to determine immune cell

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penetration and tumor shrinkage.[6] Although the SASP is recognized as both a key promoter and one of the important consequences of senescence, its role in determining the degree of tumor regression remains poorly understood.

Recent studies have highlighted SASP-related biomarkers as a potential predictor of treatment response, with constituents such as IL-1α, IL-6, IL-8, and GM-CSF associated with immune modulation and tumor regression.[7] Unlike acute inflammation caused by cell death, SASP results in the prolonged secretion of cytokines and chemokines, regulated by NF-κB and C/EBPβ pathways, which remain active in senescent cells. The persistent upregulation of IL-1α, IL-6, and IL-8 post-nCRT aligns with known SASP factors, as IL-1α acts as a master regulator of SASP, while IL-6 and IL-8 drive chronic inflammation and immune recruitment. Additionally, chemokines like CXCL1 and CCL2, which facilitate immune cell infiltration and stromal remodeling, are well-documented SASP components. The difference between SASP-driven inflammation and cell-death-induced inflammation lies in the prolonged nature of cytokine secretion, with senescent cells remaining metabolically active while modulating the tumor microenvironment (TME). This prolonged inflammatory state, rather than a short-lived immune reaction, suggests that the biomarkers assessed in this study predominantly reflect SASP activity rather than mere inflammatory tissue damage.[8]

In the current study, we investigate changes in serum SASP factors as well as the classic inflammatory biomarker, CRP (C-reactive protein), in patients with rectal cancer undergoing nCRT and examine their potential as predictive biomarkers of therapeutic response. By analyzing serum levels of key SASP cytokines, chemokines, and growth factors, in parallel with the characteristics of immune cell infiltration, we aim to elucidate the relationship between SASP and tumor regression and provide knowledge on the use of SASP markers for predicting and monitoring nCRT outcomes in patients with rectal cancer.

Methods and Subjects

Subjects

This study included 20 patients with locally advanced rectal cancer (LARC) who underwent neoadjuvant chemoradiotherapy (nCRT) at The First Affiliated Hospital of Soochow University between July 2020 and July 2022. The inclusion criteria were: (1) histologically confirmed rectal adenocarcinoma staged as T3 or T4; (2) no prior systemic therapy or radiotherapy; (3) availability of pre- and post-nCRT serum samples and tumor tissues. The patients were treated with a standard nCRT regimen of 50.4 Gy in 28 fractions combined with Xeloda/ capecitabine -based chemotherapy.

Enzyme-Linked Immunosorbent Assay (ELISA)

Blood samples were collected from patients one week before nCRT (pre-nCRT) and within one week after completing nCRT (post-nCRT). Serum was separated by centrifugation at 3,000 × g for 10 minutes at 4°C and stored at −80°C until analysis. For each ELISA assay, 50 μL of serum was added to 96-well microplates pre-coated with specific antibodies for the target cytokines or chemokines. Plates were incubated according to the manufacturer's protocol, typically at 37°C for 1–2 hours. After washing with the supplied buffer, biotin-labeled detection antibodies were added, followed by horseradish peroxidase (HRP)-conjugated streptavidin. Tetramethylbenzidine was then added, and the reaction was stopped using 2N sulfuric acid. Absorbance was measured at 450 nm using a microplate reader (Synergy Neo 2, Biotek). Standard curves were generated using recombinant proteins for each analyte, and cytokine concentrations were calculated using curve-fitting software. All assays were performed in duplicate to ensure reliability, and inter- and intra-assay coefficient variations were maintained below 10%.

Immunohistochemistry (IHC) for Immune Cell Infiltration

Tumor tissue samples obtained before and after nCRT were analyzed using IHC to assess immune cell infiltration. Specific antibodies were used to detect CD8+ cytotoxic T cells (CD8, Clone C8/144B, GA62361-2, Dako), CD4+ helper T cells (CD4, Clone 4B12, IR64961-2, Dako), and CD206+ M2 macrophages (CD206/MRC1, #91992, CST). IHC staining was evaluated using a semi-quantitative scoring system based on staining intensity and the percentage of positively stained tumor cells. Scores were assigned on a scale of 0 to 3+: 0 (no protein expression detected), 1+ (weak

expression in <10% of tumor cells), 2+ (moderate expression in >10% of tumor cells with uneven staining), and 3+ (strong expression in >10% of tumor cells with complete and intense staining). Two independent pathologists, blinded to the clinical data, assessed the scores to ensure consistency and minimize observer bias.

Tumor Regression Assessment

Tumor regression grade (TRG) was evaluated using the MSK three-tier grading system based on pathological findings.[9] Patients with TRG1 (complete regression) and TRG2 (partial regression) were classified as responders, while those with TRG3 (minimal or no regression) were considered non-responders.

Statistical Analysis

Statistical analyses were performed using Prism 10. Continuous variables were tested for normality and expressed as mean ± SD or median (interquartile range), depending on distribution. Paired *t*-tests or Wilcoxon signed-rank tests were used for pre- and post-nCRT comparisons. Differences between responders and non-responders were assessed using unpaired *t*-tests or Mann–Whitney *U*-tests. Correlations between biomarkers and TRG were analyzed using Spearman's rank correlation. $P < 0.05$ was considered statistically significant.

Results

Result 1: Clinicopathological Characteristics of Rectal Cancer Patients

The present study analyzed the clinicopathological features of a cohort of 20 rectal cancer patients subjected to nCRT. Among these, 15 were males (75%) and 5 females (25%); 85% of the tumors were staged as T3 and 15% as T4 at the initiation of treatment. The involvement of lymph nodes (N+) was noted in 75% of patients, while the rest were node-negative (N0) in 25% (Table 1).

All patients underwent a standardized nCRT regimen with a total radiation dose of 50.4 Gy in 28 fractions combined with concurrent Xeloda/capecitabine-based chemotherapy. In addition to the efficacy endpoints, treatment-related adverse

Table 1 Clinicopathological Characteristics of Rectal Cancer Patients

Characteristic	Total (n=20)	TRG1-2 (n=14)	TRG3 (n=6)
Sex -no.(%)			
Male	15 (75.0)	11 (78.6)	4 (66.7)
Female	5 (25.0)	3 (21.4)	2 (33.3)
T stage -no.(%)			
T3	17 (85.0)	12 (85.7)	5 (83.3)
T4	3 (15.0)	2 (14.3)	1 (16.7)
N stage -no.(%)			
N0	5 (25.0)	4 (28.6)	1 (16.7)
N+	15 (75.0)	10 (71.4)	5 (83.3)
MSI status -no.(%)			
MSI-high	0 (0.0)	0 (0.0)	0 (0.0)
MSS	18 (90.0)	13 (92.9)	5 (83.3)
NA	2 (10.0)	1 (7.1)	1 (16.7)

events (TRAEs) were prospectively recorded. TRAEs of any grade were observed in 16 of 20 patients (80%). Notably, grade 3 or higher TRAEs occurred in 3 patients (15%). The most frequent grade 3 events included diarrhea (observed in 2 patients, 10%) and neutropenia (observed in 1 patient, 5%). No grade 4 toxicities or treatment-related mortality was recorded. Blood was collected before and after completion of nCRT to evaluate the changes of systemic biomarkers before and after the treatment, focusing particularly on serum SASP factors.

Result 2: Upregulation of Inflammatory Cytokines in Serum After nCRT

We measured serum levels of IL-8, IL-1α, IL-6, and CRP in patients with rectal cancer before (pre-nCRT) and after (post-nCRT) neoadjuvant chemoradiotherapy to determine changes induced by treatment. Levels of IL-8 significantly increased after nCRT with a mean difference of 35.02 (± 27.42 SD, $P < 0.0001$) (Figure 1A). Similarly, levels of IL-1α were increased with a mean difference 2.592 (± 2.296 SD, $P < 0.0001$) (Figure 1C), and CRP levels were also significantly increased ($P < 0.0001$) (Figure 1D) In addition, IL-6 showed a striking increase as its mean levels increased by 37.52 (± 27.72 SD, $P < 0.0001$) (Figure 1B). The obvious elevations in levels of these cytokines suggest a severe inflammatory response after nCRT, which may influence immune cell infiltration and function in the tumor microenvironment. Higher levels of these inflammatory cytokines, especially IL-8, IL-1α, and IL-6, suggest a stronger senescence-associated secretory phenotype (SASP) response, which may be relevant to the processes of both tumor reduction and changes in immune response.

Result 3: Upregulation of Chemokines in Serum Post-nCRT

Next, we measured the level of chemokines, including CCL5, CCL2 and CXCL1 before and after nCRT by ELISA. Our results showed that the levels of these chemokines significantly rose after nCRT. The mean difference for CCL5 levels significantly rose with a mean difference of 15.27 (± 11.54 SD, $P < 0.0001$), as confirmed by the paired *t*-test (Figure 2A). CXCL1 levels also increased, with a mean difference of 17.14 (± 13.26 SD); this change was also statistically significant ($p < 0.0001$) (Figure 2B). Similarly, the levels of CCL2 also showed an increase with a mean difference of 16.59 (± 18.35 SD). It was also found by paired *t*-test to be significant (p=0.0007) (Figure 2C). These data would indicate that nCRT induces significant upregulation in these chemokine markers that might reflect immune response to treatment.

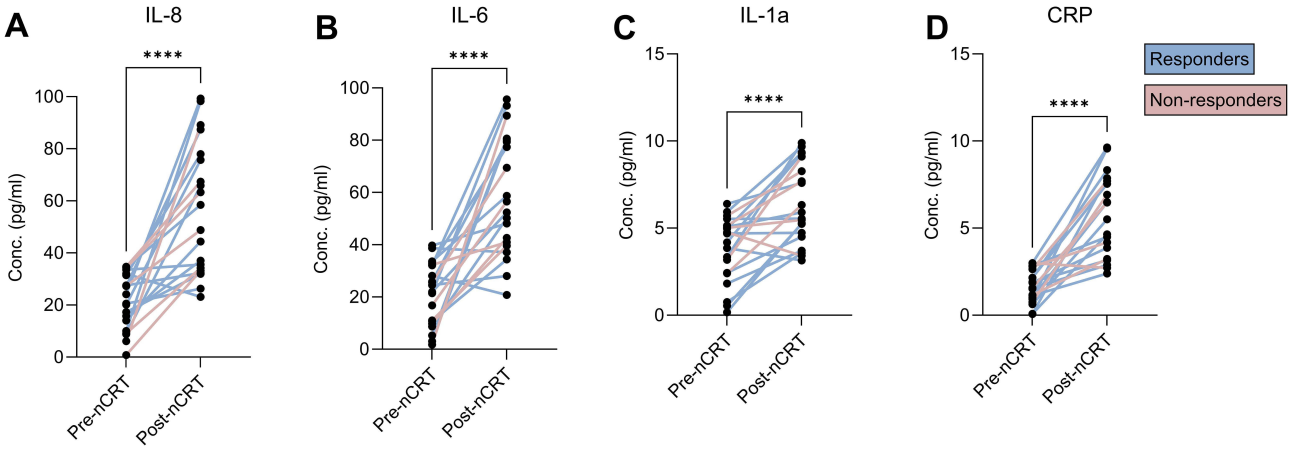

Figure 1 Upregulation of inflammatory cytokines in serum following neoadjuvant chemoradiation therapy (nCRT) in rectal cancer patients. Serum levels of inflammatory cytokines were measured by ELISA before (Pre-nCRT) and after (Post-nCRT) treatment in 20 rectal cancer patients. (**A**) IL-8 levels showed significant increase with mean difference of 35.02 ± 27.42 pg/mL. (**B**) IL-6 levels increased with mean difference of 37.52 ± 27.72 pg/mL. (**C**) IL-1α levels elevated with mean difference of 2.592 ± 2.296 pg/mL. (**D**) C-reactive protein (CRP) levels also showed significant increase after nCRT. Each line represents an individual patient, connecting their pre- and post-treatment values. Statistical significance was determined by paired *t*-test; ****$P < 0.0001$.

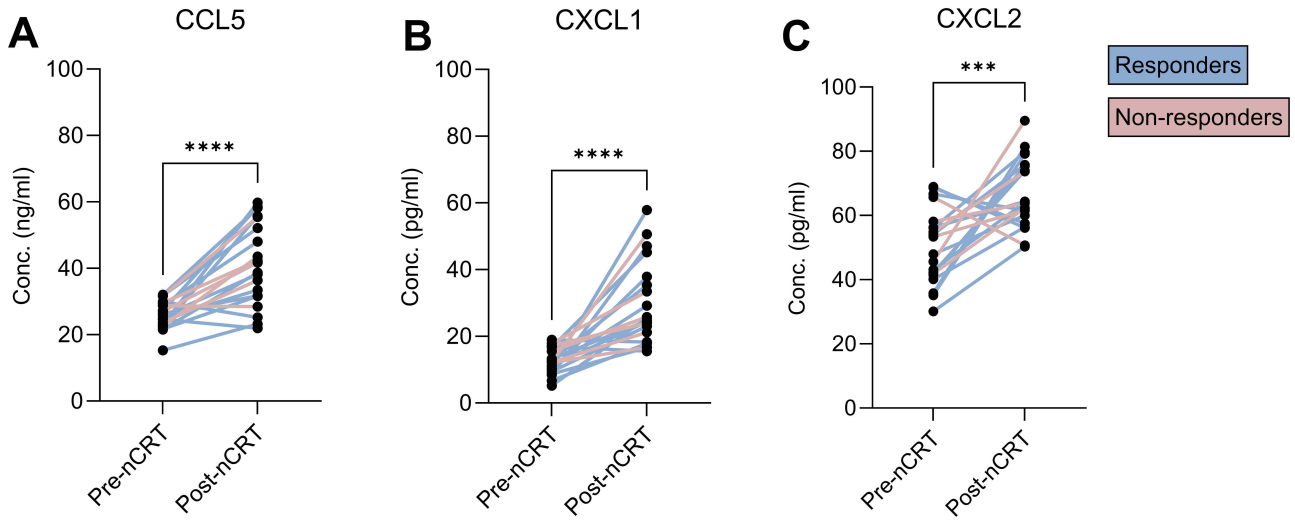

Figure 2 Upregulation of chemokines in serum following neoadjuvant chemoradiation therapy (nCRT) in rectal cancer patients. Serum levels of chemokines were measured by ELISA before (Pre-nCRT) and after (Post-nCRT) treatment in 20 rectal cancer patients. (**A**) CCL5 levels showed significant increase with mean difference of 15.27 ± 11.54 ng/mL ($P < 0.0001$). (**B**) CXCL1 levels increased with mean difference of 17.14 ± 13.26 pg/mL ($P < 0.0001$). (**C**) CCL2 levels elevated with mean difference of 16.59 ± 18.35 pg/mL ($P = 0.0007$). Each line represents an individual patient, connecting their pre- and post-treatment values. Statistical significance was determined by paired *t*-test; ***P < 0.001, ****P < 0.0001.

Result 4: Immune Cell Infiltration Post nCRT

In this section of the project, we used IHC to label different immune cell infiltration, in rectal cancer samples both before nCRT and after nCRT. We labeled CD8+ cytotoxic T cells using an antibody against CD8, CD4+ T help cells using an antibody against CD4, and M2 macrophages using an antibody against CD206. CD8 IHC scores showed significant upregulation following nCRT (t=4.498, df=19, p=0.0002***) (Figure 3A), indicating increased cytotoxic T cell infiltration, while CD4 IHC scores demonstrated mild but significant downregulation (t=2.263, df=19, p=0.0356*) (Figure 3B), suggesting a decrease in T helper cell presence; further, CD206 IHC scores showed significant upregulation following nCRT (t=2.942, df=19, p=0.0084**) (Figure 3C), potentially reflecting the involvement of macrophages in post-treatment inflammatory response and tissue remodeling within the tumor microenvironment (TME). Taken together, these changes propose a complex remodeling of the immune landscape after nCRT, which is characterized by enhanced cytotoxic T-cell response along with shifts in helper T-cell populations and macrophage polarization.

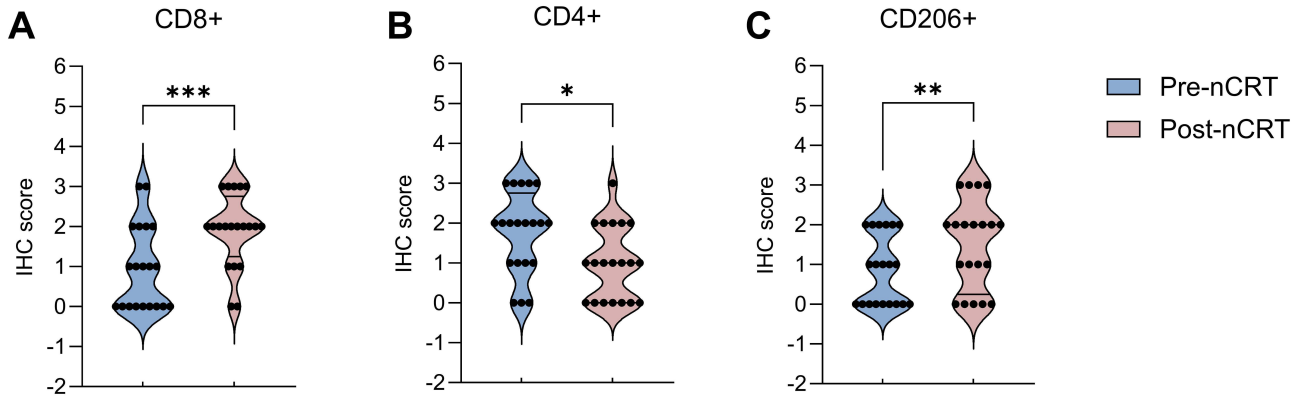

Figure 3 Changes in immune cell infiltration before and after neoadjuvant chemoradiation therapy (nCRT) in rectal cancer patients. Immunohistochemistry (IHC) analysis was performed on rectal cancer tissue samples before (Pre-nCRT, blue) and after (Post-nCRT, pink) treatment. (**A**) CD8+ cytotoxic T cell infiltration showed significant upregulation post-nCRT (t=4.498, p=0.0002). (**B**) CD4+ T helper cell infiltration demonstrated mild but significant downregulation (t=2.263, p=0.0356). (**C**) CD206+ M2 macrophage infiltration showed significant upregulation post-nCRT (t=2.942, p=0.0084). Data are presented as violin plots with individual data points. Statistical significance was determined by paired *t*-test; *P < 0.05, **P < 0.01, ***P < 0.001.

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Result 5: Relationship Between Chemokines and Tumor Regression Post-nCRT

The response of rectal cancer to the neoadjuvant chemoradiation therapy (nCRT) was assessed by the tumor regression grade (TRG) system. Here, we employed the MSK three-tier TRG grade system (TRG=1 means 100% tumor response, TRG=2 means partial tumor regression, and TRG-3 means absence of tumor regression). In this study, we define TRG1 and 2 as responders and TRG3 as non-responders (Figure 4A). To investigate the influence of inflammatory markers on the response of rectal cancer to neoadjuvant chemoradiation therapy (nCRT), we measured the levels of chemokines (CCL5, CXCL1, and CCL2) in patients with divergent treatment responses.

Our analysis showed that there are no differences in pre-nCRT levels of CCl5, CCL2, and CXCL1 between responders and non-responders. CCL5, CXCL1, and CCl2 were elevated after nCRT. However, the CCL2 level was not significantly elevated (Figure 4D), while CCL5 and CXCL1 were markedly upregulated in non-responders (Figure 4B and C). These results suggested that lower basal level of CCL2 and higher elevation after nCRT may indicate the better response of nCRT in rectal cancer patients.

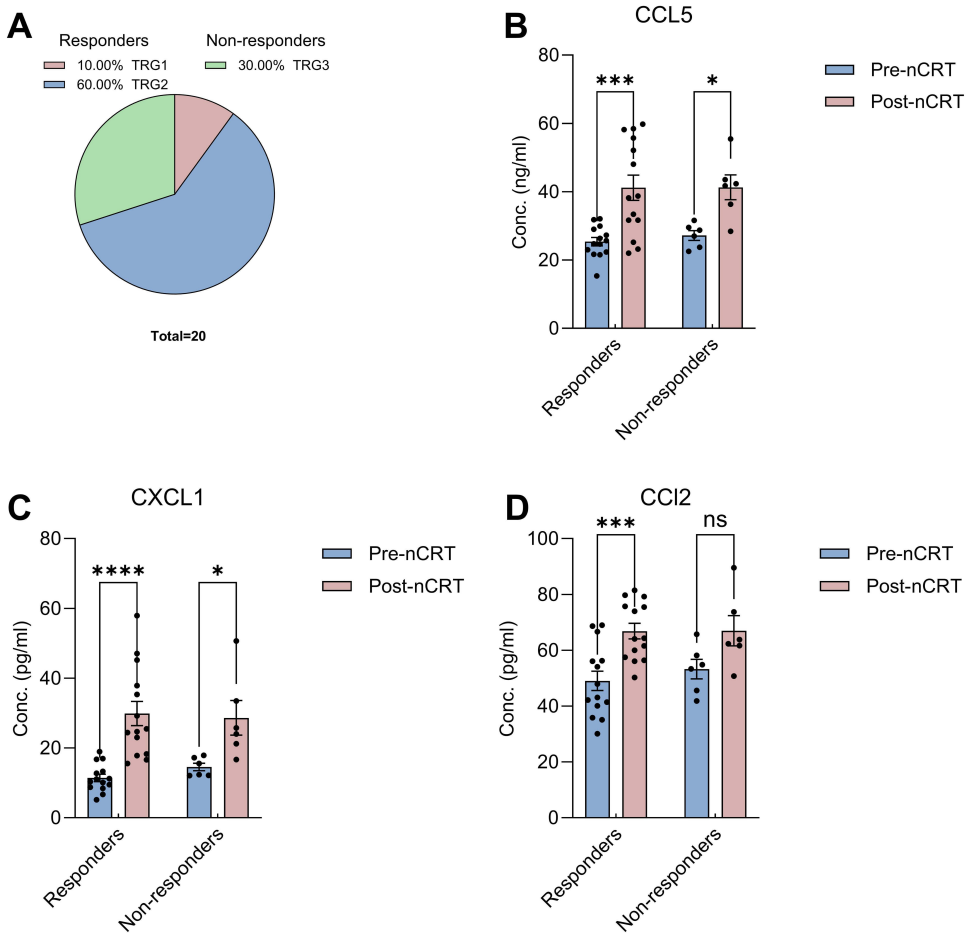

Figure 4 Analysis of chemokine levels in responders and non-responders to neoadjuvant chemoradiation therapy (nCRT). (**A**) Pie chart showing the distribution of tumor regression grades (TRG) among rectal cancer patients (n=20). Responders include patients with TRG1 (10.00%, complete response) and TRG2 (60.00%, partial response), while non-responders comprise patients with TRG3 (30.00%, no response). (**B-D**) Comparison of serum chemokine levels before (Pre-nCRT) and after (Post-nCRT) treatment in responders and non-responders: (**B**) CCL5 levels showed significant elevation after nCRT in both responders (***p < 0.001) and non-responders (*p < 0.05). (**C**) CXCL1 levels demonstrated significant increases in both responders (****p < 0.0001) and non-responders (*p < 0.05). (**D**) CCL2 levels showed significant elevation in responders (***p < 0.001) but not in non-responders (ns, not significant). Data are presented as mean ± SEM with individual data points shown. Statistical significance was determined using paired *t*-tests.

Result 5: Relationship Between Inflammatory Markers and Tumor Regression Post-nCRT

To determine the role of inflammatory markers in the prediction of rectal cancer response to neoadjuvant chemoradiation therapy (nCRT), we assessed the association between levels of major cytokines (IL-1α, IL-6, IL-8) and C-reactive protein (CRP) with tumor regression grade (TRG).

Our analysis showed no differences in pre-nCRT levels of IL-6, IL-8, IL-1α, and CRP when responders and non-responders were compared (Figure 5A, B and D), although there was a tendency for IL-1α to be slightly elevated in non-responders before treatment (Figure 5C). Following nCRT, all four cytokines showed elevated levels, with IL-6, IL-8, and CRP being significantly upregulated, whereas the increase of IL-1α did not reach the level of significance (Figure 5C). These results indicate that increased levels of IL-1α before nCRT and lower upregulation after nCRT might be related to a worse treatment response in patients with rectal cancer.

Result 6: Tumor Regression and Immune Cell Infiltration Post-nCRT

In addition, it explored dynamics of immune cells in the TME in responders versus non-responders to further reveal the association between immune cell infiltration and treatment outcome. After nCRT, responders showed a significant increase in the infiltration of CD8+ cytotoxic T cells in the TME (Figure 6A). For CD4+ T cells, there is a mild decrease; this change is not significant (Figure 6B). Similarly, there is a slight increase in CD206+ M2 macrophage

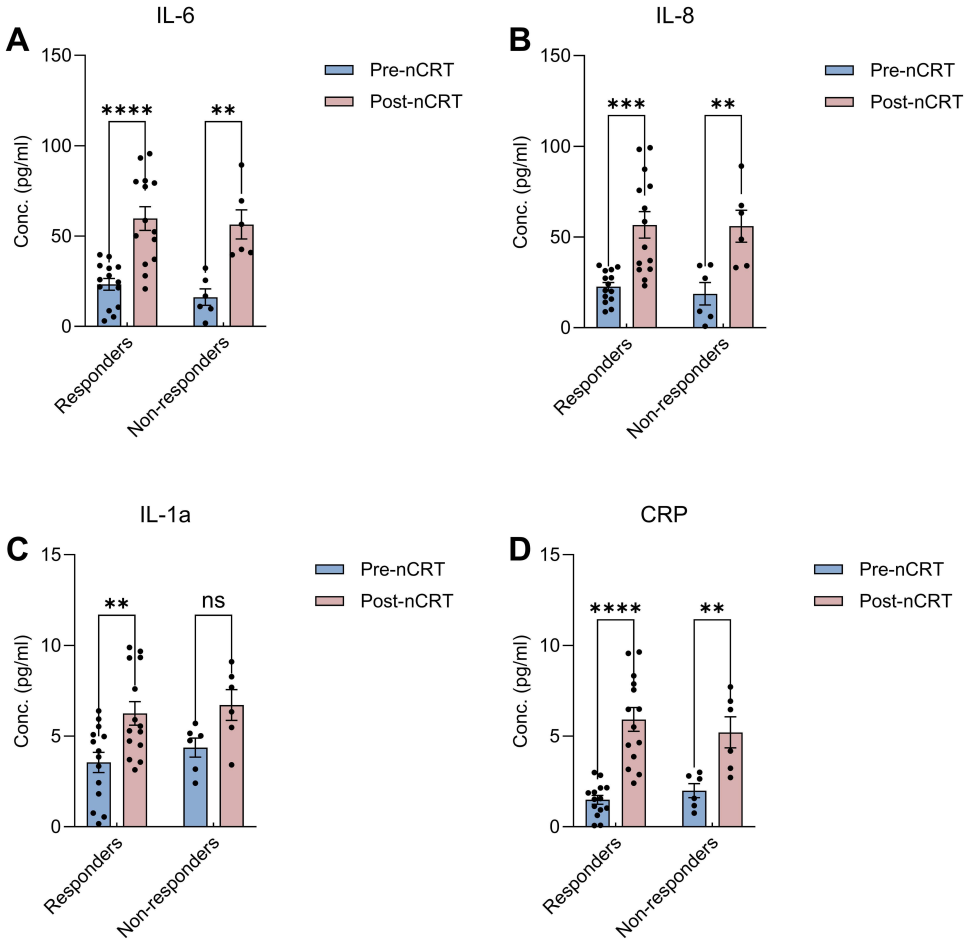

Figure 5 Analysis of inflammatory markers in responders and non-responders to neoadjuvant chemoradiation therapy (nCRT). Analysis of serum inflammatory markers before (Pre-nCRT, blue) and after (Post-nCRT, pink) treatment in responders and non-responders. (**A**) IL-6 levels showed significant elevation after nCRT in both responders (****P < 0.0001) and non-responders (**P < 0.01). (**B**) IL-8 demonstrated significant increases in both responders (***P < 0.001) and non-responders (**P < 0.01). (**C**) IL-1α showed significant elevation in responders (**P < 0.01) but not in non-responders (ns, not significant). (**D**) CRP levels increased significantly in both responders (****P < 0.0001) and non-responders (**P < 0.01). Data are presented as bar graphs with individual data points and mean ± SEM. Statistical significance was determined by paired *t*-test; **P < 0.01, ***P < 0.001, ****P < 0.0001.
Abbreviation: ns, not significant.

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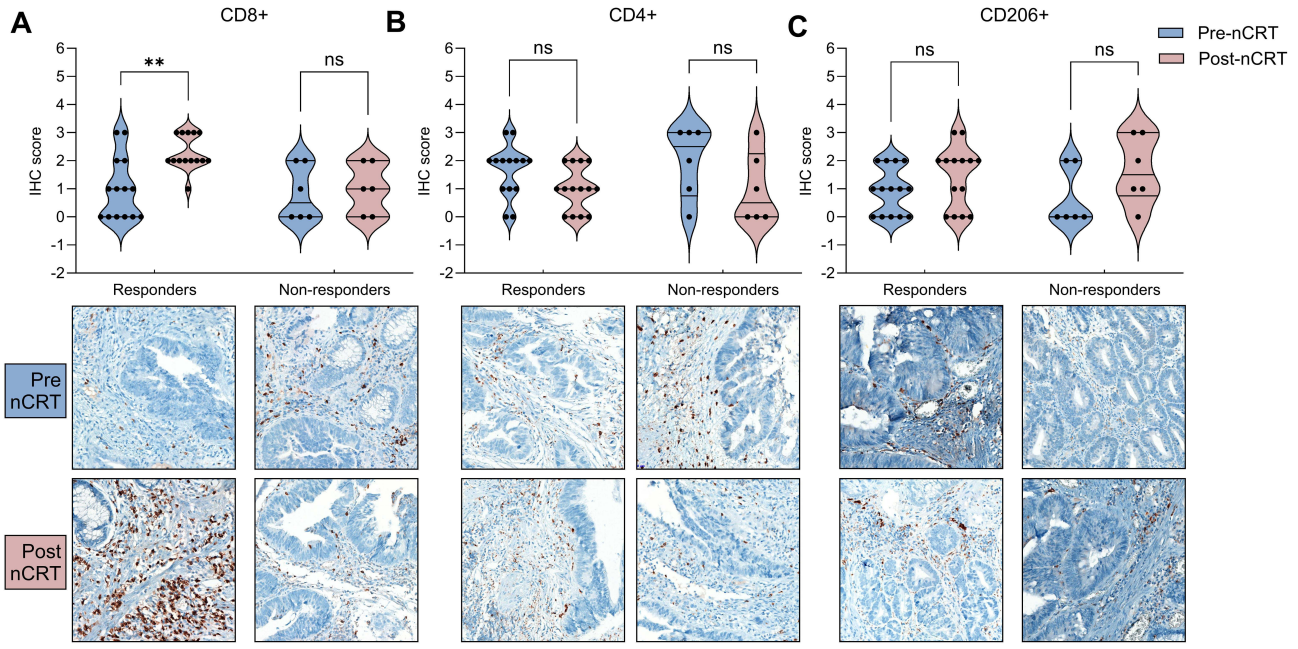

Figure 6 Analysis of immune cell infiltration in responders and non-responders to neoadjuvant chemoradiation therapy (nCRT). Immunohistochemistry (IHC) analysis of immune cell infiltration before (Pre-nCRT) and after (Post-nCRT) treatment in responders and non-responders. IHC images from representative patients were captured at 10× magnification. (**A**) CD8+ cytotoxic T cell infiltration showed significant increase in responders (**P < 0.01) but not in non-responders. (**B**) CD4+ T helper cell infiltration showed no significant changes in either responders or non-responders. (**C**) CD206+ M2 macrophage infiltration showed no significant changes in either responders or non-responders. Data are presented as violin plots with individual data points. Statistical significance was determined by paired *t*-test; **P < 0.01. IHC images from representative patients both before (pre) and after (post) nCRT were captured at 10× magnification.
Abbreviation: ns, not significant.

infiltration after nCRT in both responders and non-responders, but the difference again is not significant (Figure 6C). These results suggest that increased CD8+ cytotoxic T cell infiltration could be associated with better tumor regression after nCRT. Although the expression levels of CD4+ T help cell are downregulated and that of CD206+ M2 macrophage are upregulated after nCRT, the changes do not reach statistical significance, probably due to the smaller number of samples enrolled in the current study. For now, our data did not indicate any correlation of CD4+ T help cell and CD206+ M2 macrophage infiltration with tumor response after nCRT, but we need to increase the number of patients to have a more persuasive conclusion.

Discussion

Rectal cancer is among the most common and challenging malignancies, especially in locally advanced disease stages. Effective management at this stage is important to improve patient outcomes since locally advanced rectal cancer has a higher tendency for metastasis and recurrence. Neoadjuvant chemoradiotherapy became the cornerstone of treatment in LARC. The preoperative strategy tries to shrink the tumor, increasing the possibility of doing a total surgical resection and decreasing the chance of local recurrence, in order to improve both life expectancy and quality of life.

However, responses to nCRT are highly variable among patients. Whereas some patients show a significant regression of their tumors, others hardly derive any benefit from nCRT. Unfortunately, one of the biggest limitations in the clinical setting is still how the treatment outcomes are predicted by good biomarkers. Therefore, nCRT response biomarkers need to be identified urgently.

Our analyses showed significant changes in serum biomarkers after nCRT, including the elevation of inflammatory cytokines such as IL-1α, IL-6, and IL-8, and chemokines like CCL5, CXCL1, and CCL2. These biomarkers are key constituents of SASP, which is often induced by therapy-induced senescence in response to genotoxic stress induced by radiotherapy and chemotherapy and which, in turn, affects the TME. Therapy-induced senescence is a double-edged sword in cancer treatment. On the one hand, it constitutes a mechanism to cease the growth of tumor cells; on the other

hand, SASP released from senescent cells can profoundly influence the TME. Components of SASP, therefore, include pro-inflammatory cytokines and matrix remodeling enzymes that could recruit immune cells to the TME, which may enhance or, in some specific cases, dampen anti-tumor immunity.[8] Compared to C-reactive protein (CRP), which is a general marker of systemic inflammation, the SASP factors analyzed in this study (IL-1α, IL-6, IL-8, CXCL1, and CCL2) offer a more specific and mechanistically relevant indication of therapy-induced senescence (TIS) and tumor microenvironment (TME) remodeling. While elevated CRP levels post-nCRT may indicate inflammation associated with treatment, they do not distinguish between acute immune activation due to tissue damage and chronic immune modulation driven by senescence. In contrast, SASP cytokines and chemokines are persistently secreted by senescent tumor and stromal cells, playing a direct role in shaping the TME by recruiting immune cells, promoting fibrosis, and influencing tumor regression. Markers like IL-1α and CCL2, in particular, are key SASP components that mediate immune cell infiltration and therapy response, making them more promising and biologically relevant biomarkers for predicting treatment efficacy and tumor regression post-nCRT.

One key mechanism may be linked to therapy-induced senescence.[10] The genotoxic stress from nCRT not only halts tumor cell proliferation but also triggers the release of SASP factors.[11] Elevated levels of IL-6, IL-8, and IL-1α can promote a local inflammatory environment that, on one hand, enhances the recruitment of immune cells, and on the other, may activate pro-survival signaling pathways in residual tumor cells.[12] For instance, IL-6 is known to activate the STAT3 pathway, which can contribute to tumor cell survival and even resistance to therapy in some contexts.[13] Similarly, IL-1α may trigger inflammatory cascades that recruit not only effector cells but also potentially immunosuppressive populations, such as myeloid-derived suppressor cells (MDSCs) or regulatory T cells, depending on the balance of signals within the TME.[14]

We demonstrated from our data that high levels of infiltrating CD8+ T cells correlated with better regression of tumors, which showed the cytotoxic function of CD8+ T cells in anti-tumor responses. In contrast, it was found that lower infiltration of CD4+ T cells was associated with improved tumor regression, indicating that high infiltration of CD4 + T cells may counteract CD8+ T cell activity or promote immune suppression within the TME.[15] The present study showed reduced CD4+ T cell infiltration following nCRT, but there is no relation between CD4 and tumor regression. These patterns could give insights into the different roles of CD4+ and CD8+ T cells in response to nCRT and may indicate that a good balance of immune cell types is necessary for the best possible tumor regression.

Our findings have highlighted the potential role of IL-1a and CCL2 in regulating tumor response after nCRT. Lower levels of IL-1α before nCRT and increased levels after nCRT could relate to better therapeutic results in patients with rectal cancer. This is in line with previous publications showing that IL-1α expression was increased in tumors of patients with distant metastases or poor prognosis,[16] and IL-1α supported tumor growth through MDSCs recruitment and inactivation of anti-tumor immune response.[17] Another interesting finding from our investigation is the alteration of CCL2 and tumor regression. Very similar to IL-1a, lower CCL2 levels before nCRT and higher level after nCRT may indicate better tumor regression. Several studies revealed that baseline high expression of CCL2 indicates poor survival in colorectal cancer patients.[18] Also, as a chemokine, CCL2 was found to recruit immune cells during tissue injury and infection. Moreover, CCL2 was shown to induce M2 polarization of macrophages,[19] which was elevated in TME after nCRT in our study.

While this study provides valuable insights into the relationship between SASP components, immune cell infiltration, and tumor regression, it is limited by its sample size and the observational nature of the findings. Additionally, this observatory study did not investigate the role of inflammatory cytokines and chemokines, especially IL-1a and CCL2, in regulating immune cell infiltration. Future research with larger patient cohorts and mechanistic studies will be crucial to confirm these associations and to better understand the signaling pathways through which SASP influences the TME and therapeutic response.

Conclusion

In conclusion, our study highlights the potential of IL-1α and CCL2 as biomarkers for monitoring nCRT effectiveness, as their less elevated levels were linked to reduced tumor regression. In contrast, chemokines such as CCL5 and CXCL1, which were substantially upregulated, may reflect an immunomodulatory response aimed at attracting immune cells to the TME, suggesting a complex interplay of inflammatory signals that influences treatment outcomes. Future studies should focus on validating these biomarkers in larger, multi-center cohorts to confirm their predictive value for nCRT

response in rectal cancer. Additionally, integrating these SASP markers with existing clinical and radiological parameters could improve personalized treatment strategies, enabling better patient stratification for nCRT. Functional studies investigating how IL-1α, IL-6, and CCL2 influence immune cell recruitment and tumor regression could further elucidate mechanistic links between SASP and therapeutic outcomes.

Ethics Approval and Informed Consent

This study was conducted in accordance with the ethical principles outlined in [specific guidelines, such as the Declaration of Helsinki]. Ethical approval was obtained from Ethics Committee of 1st Hospital of Soochow University, with approval reference number 1359486. Informed consent was obtained from all participants prior to inclusion in the study.

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Disclosure

The authors declare that they have no competing interests related to this study.

References

1. Benson AB, Venook AP, Al-Hawary MM, et al. Rectal cancer, Version 2.2022, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Cancer Netw*. 2022;20(10):1139–1167. doi:10.6004/jnccn.2022.0051
2. Hu X, Xue Z, He K, et al. Strategies to optimize treatment for locally advanced rectal cancer. *Cancers*. 2022;15(1):219. doi:10.3390/cancers15010219
3. Saleh T, Bloukh S, Carpenter VJ, et al. Therapy-induced senescence: an "old" friend becomes the enemy. *Cancers*. 2020;12(4):822. doi:10.3390/cancers12040822
4. Coppé JP, Desprez PY, Krtolica A, Campisi J. The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annu Rev Pathol*. 2010;5:99. doi:10.1146/annurev-pathol-121808-102144
5. Campisi J, d'Adda Di Fagagna F. Cellular senescence: when bad things happen to good cells. *Nat Rev mol Cell Biol*. 2007;8(9):729–740. doi:10.1038/nrm2233
6. Schmitt CA, Wang B, Demaria M. Senescence and cancer — role and therapeutic opportunities. *Nat Rev Clin Oncol*. 2022;19(10):619. doi:10.1038/s41571-022-00668-4
7. Faget DV, Ren Q, Stewart SA. Unmasking senescence: context-dependent effects of SASP in cancer. *Nat Rev Cancer*. 2019;19(8):439–453. doi:10.1038/s41568-019-0156-2
8. Chibaya L, Snyder J, Ruscetti M. Senescence and the tumor-immune landscape: implications for cancer immunotherapy. *Semin Cancer Biol*. 2022;86(Pt 3):827–845. doi:10.1016/j.semcancer.2022.02.005
9. Trakarnsanga A, Gönen M, Shia J, et al. Comparison of tumor regression grade systems for locally advanced rectal cancer after multimodality treatment. *J Natl Cancer Inst*. 2014;106(10):dju248. doi:10.1093/jnci/dju248
10. Saleh T, Bloukh S, Hasan M, Al Shboul S. Therapy-induced senescence as a component of tumor biology: evidence from clinical cancer. *Biochim Biophys Acta Rev Cancer*. 2023;1878(6):188994. doi:10.1016/j.bbcan.2023.188994
11. Duro-Sánchez S, Nadal-Serrano M, Lalinde-Gutiérrez M, et al. Therapy-induced senescence enhances the efficacy of HER2-targeted antibody-drug conjugates in breast cancer. *Cancer Res*. 2022;82(24):4670–4679. doi:10.1158/0008-5472.CAN-22-0787
12. D'Ambrosio M, Gil J. Reshaping of the tumor microenvironment by cellular senescence: an opportunity for senotherapies. *Dev Cell*. 2023;58(12):1007–1021. doi:10.1016/j.devcel.2023.05.010
13. Johnson DE, O'Keefe RA, Grandis JR. Targeting the IL-6/JAK/STAT3 signalling axis in cancer. *Nat Rev Clin Oncol*. 2018;15(4):234–248. doi:10.1038/nrclinonc.2018.8
14. Nicolas AM, Pesic M, Engel E, et al. Inflammatory fibroblasts mediate resistance to neoadjuvant therapy in rectal cancer. *Cancer Cell*. 2022;40(2):168–184.e13. doi:10.1016/j.ccell.2022.01.004
15. Kravtsov DS, Erbe AK, Sondel PM, Rakhmilevich AL. Roles of CD4+ T cells as mediators of antitumor immunity. *Front Immunol*. 2022;13. doi:10.3389/fimmu.2022.972021
16. León X, Bothe C, García J, et al. Expression of IL-1α correlates with distant metastasis in patients with head and neck squamous cell carcinoma. *Oncotarget*. 2015;6(35):37398–37409. doi:10.18632/oncotarget.6054
17. Lin D, Mei Y, Lei L, et al. Immune suppressive function of IL-1α release in the tumor microenvironment regulated by calpain 1. *Oncoimmunology*. 2022;11(1):2088467. doi:10.1080/2162402X.2022.2088467

18. Liu Y, Pan J, Pan X, et al. Klotho-mediated targeting of CCL2 suppresses the induction of colorectal cancer progression by stromal cell senescent microenvironments. *Mol Oncol*. 2019;13(11):2460–2475. doi:10.1002/1878-0261.12577
19. Xu R, Li Y, Yan H, et al. CCL2 promotes macrophages-associated chemoresistance via MCPIP1 dual catalytic activities in multiple myeloma. *Cell Death Dis*. 2019;10(10):1–17. doi:10.1038/s41419-019-2012-4