C3aR1 Correlates With Immune Cell Infiltration and Serves as Prognostic and Therapeutic Biomarker in Gastric Cancer

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Abstract

As the fourth most common malignancy worldwide, gastric cancer can lead more than 720 000 patient death every year. Precisely therapeutic intervention can significantly improve patients' survival status underlying the precise clarification by molecular indexes. Identifying the biomarkers highly associated with disease prognosis will be helpful to guide the clinical therapy. C3ar1 is an essential receptor in the complement system, and participates in various biological processes associated with immunological responses. To identify the crucial roles of C3AR1 in gastric cancer tumorigenesis, we determined the mRNA profile, protein expression levels and the clinicopathological indexes using cBioportal, Kaplan-Meier plotter and the Human Protein Atlas databases. To identify the molecular network in C3AR1-expressed gastric cancer, we obtained the differentially expressed genes using the GEPIA database compared with normal stomach tissues. Furthermore, we analyzed the biological impact of these differentially expressed genes using protein-protein interaction network and gene set enrichment analysis, in which we identified the hub genes and critical pathways influenced by over-expressed *C3AR1* in gastric cancer. Finally, we evaluated the correlation between the *C3AR1* expression levels and immune cell infiltration levels utilizing the Tumor Immunoassay Resource database. Our results revealed that the higher expression level of *C3AR1* can lead higher infiltration of T cell CD8+, T cell CD4+, macrophage, neutrophil, B cell and myeloid dendritic cells into tumor tissue. Moreover, we also found that higher infiltration of macrophage cells into tumor tissue can worsen the survival of patients with gastric cancer, which may be highly associated with the polarization states of macrophages (TAM and M2 status). Our investigation suggest that *C3AR1* can be as an efficient diagnostic biomarkers for gastric cancer therapy.

Introduction

Gastric cancer is an important public health problem leading to cancer death, which is the fourth most common cancer worldwide. According to clinical estimation, more than 950 000 cases were newly diagnosed as gastric cancer, and it can cause almost 720 000 death every year¹⁻². Owing to the high disease incidence and mortality rates, the burden to well investigate the epidemiology, pathology, molecular mechanism and therapeutic strategies still remains high³. The Cancer Genome Atlas research results have systematically explored the full genomic profiles of primary gastric cancer⁴, in which gastric cancer contains four sub-types. In order to provide emerging and canonically therapeutic strategies, previous literature elicited that the higher correlation between MLH1 promotor⁵ and HER2⁶ with hypermethylation levels corroborated the effects of overall survival on gastric cancer patients. Indeed, although the molecular networks to regulate the tumorigenesis of different-stage gastric cancer are complex, specific gene levels in gastric cancer compared with normal tissue will be helpful to determine disease prognosis and provide customize treatment.

Tumor microenvironment has been elucidated as an essential factor to influence the therapeutic benefit with chemotherapeutic interventions⁷⁻⁸. Among the various immune systems, complement system has been proved that microenvironment can strongly affect the proliferation and metastasis of cancer cells⁹.

Among these components in complement system, e.g. C1-9 proteins, C3aR and C5aR, the present investigation indicated that complement also plays a critical role in regulating adaptive immunity to recruit T and B cells into tumor tissue¹⁰, modulating the microenvironment of tumor tissues. Complement system contains three subtypes of pathways: alternative pathway, classical pathway, and lectin pathway¹¹. In the alternative pathways, C3a and C5a can anchor to relative receptor, named as C3aR, and further activate the downstream pathways. Except the crucial roles in the complement system, C3aR also involved into other biological processes, for example as an immune checkpoint receptor in cancer immunotherapy¹², leading the neurodevelopmental damage¹³⁻¹⁴, participating in white matter injury after hyperperfusion¹⁵⁻¹⁶. For proliferation of cancer cells, Chen B et al. demonstrated that down-regulation of C3aR can inhibit the cell proliferation in hepatocellular carcinoma affecting the expression levels of extracellular matrix¹⁷⁻¹⁸; over-activation of complement C3 can lead PD-L1 treatment resistance by modulating infiltration levels of tumor-associated macrophages through regulating the C3a-C3aR-PI3Kgamma signaling pathway¹⁸; Mueller-Ortiz SL et al. revealed that overexpression of C3aR can provides the host protection against listeria monocytogenes-induced apoptosis¹⁹; Nabizadeh JA et al. showed that higher levels of C3aR in tumor tissues can promote the melanoma tumorigenesis by inhibiting neutrophil and T cell CD4+ response²⁰. As a consequence, the role of C3aR in gastric cancer remains to be determined in the future.

Here, we comprehensively analyzed the role of C3AR1 in gastric cancer tumorigenesis and immune cell infiltration. The expression profile of C3AR1 mRNA and survival time of gastric patients were analyzed using cBioportal and Kaplan-Meier plotter. To further identify the impact of differentially expressed genes in gastric cancer, the protein-protein interaction network analysis, gene ontology, and gene set enrichment analysis were employed. Finally, the correlation between the C3AR1 profile and immune cell infiltration was further analyzed to identify how overexpressed C3AR1 affected the tumor immunological microenvironment using the TIMER database. We believed that our investigation will provide a novel glance at the role of C3AR1 in gastric cancer tumorigenesis and highlight the potential application in gastric cancer diagnosis.

Materials And Methods

Database and clinical information analysis for gastric patients

In this study, the mRNA expression level of C3AR1 in gastric patients was collected in cBioportal database (<http://www.cbioportal.org/>) and TCGA database (<https://portal.gdc.cancer.gov/>; Data Release 29.0 - March 31, 2021). The key words utilized in this website is "C3AR1", and then the gastric cancer subtype was selected for further analysis. The survival analysis of gastric patients were employed using Kaplan-Meier plotter (<http://kmplot.com/analysis/index.php?p=service&cancer=gastric>), in which threshold value of C3AR1 mRNA was using auto-select best cutoff method. The correlation between C3AR1 and clinical indexes were obtained from UALCAN database (<http://ualcan.path.uab.edu/index.html>), in which the key word is "C3AR1". IHC staining of normal

stomach and tumor tissues were obtained from the Human Protein Atlas database (<https://www.proteinatlas.org/>). The subtypes of investigation is normal tissue-stomach and pathology-stomach cancer, and the images were selected without any especially choices.

Profile of C3AR1 expression by GEPIA

The C3AR1 expression levels between TCGA tumor and normal tissue were obtained from GEPIA 2.0 (<http://gepia2.cancer-pku.cn/#index>), in which expression level of C3AR1 in normal tissue was obtained from GTEx database (<https://xenabrowser.net/datapages/>). The key word used in this website is “C3AR1”. The differentially expressed genes were obtained from GEPIA2.0 database with $|\log_2(\text{FoldChange})| > 1.5$ & $p\text{-value} < 0.01$.

Protein-protein interaction (PPI) network and Gene Ontology analysis

The differentially expression gene clusters were obtained from GEPIA2.0 database, and prepared as TXT format. The PPI analysis was analyzed using STRING website (<https://string-db.org/>), in which the minimum required interaction score was set as 0.9 and number of kMean is 5. The database was uploaded to STRING website in Multiple sequences section. The interaction files were downloaded as TSV format. The interaction images of PPI presented in investigation were obtained using Cytoscape version 3.6.0, and running parameters were set as default. All the genetic functions are confirmed by GENE module of NCBI database. The GO analysis was performed in the go:profiler website (<https://biit.cs.ut.ee/gprofiler/gost>), and all the parameters are set as default except marked the ordered query. The differentially expressed genes utilized in GO analysis were obtained from GEPIA2.0 database as above mentioned.

Gene set enrichment analysis (GSEA)

Before GSEA process, running files of DEGs containing fold change and p-value was prepared as .rnk files. These data were obtained from GEPIA2.0 database as above mentioned, including p-value and fold change. The final .rnk data only contains the gene name and fold change in separated rows. Then, GSEAPreranked analysis was undertook using GSEA v4.1.0 software (<https://www.gsea-msigdb.org/gsea/msigdb/search.jsp>). Number of permutations was set as 2000 without gene collapse. The threshold value is set as NOM p-value < 0.05 & FDR q-value < 0.05 & |NES| > 1.0. All the gene sets were obtained from the GSEA Molecular Signatures Database (MSigDB v7.4) (<https://www.gsea-msigdb.org/gsea/msigdb/index.jsp>), which is the Hallmark gene set (<http://www.gsea-msigdb.org/gsea/msigdb/collections.jsp#H>).

Immune cell infiltration analysis

To explore the correlation between C3AR1 gene expression and immune cell infiltration, TIMER 2.0 (<http://timer.cistrome.org/>) was employed to identify infiltration status. The immune infiltration was performed in the “Immune-Gene” module, in which the key word of Gene expression is “C3AR1” and key word of “immune Infiltrates” are listed: T cell CD8+, T cell CD4+, B cell, Macrophages, DC, Neutrophil, NK

cell. The threshold value of Spearman's p value for further analysis is less than 0.05. Based on the value of Spearman's correlation coefficient, immune cell infiltration can be divided into three types: none ($|Cor|=0$), medium ($0<|Cor|<0.15$) and high ($|Cor|\geq 0.15$).

Statistical analysis

The clinical information of gastric patients includes the tumor grade, stage, *TP53* status. The clinical endpoints were overall survival and progression-free survival. All the results generated from the relative website with the HR and p -value data. P value < 0.05 is the cutoff value to identify whether significance. The GO analysis and GSEA results were presented by using Graphpad_prism version 5.0.

Results

High expression level of C3AR1 in gastric tumor tissue

C3AR1 plays an important role in the complement system for downstream signaling transduction, for example recruiting C3a/b and C5 to activate the local immune response. Here, we firstly analyzed the genetic status of C3AR1 in gastric cancer patients, obtained from the TCGA database ($n=440$). As shown in Figure 1A, most of the C3AR1 gene did not occur mutation, and there are only 3% percentage (15/440) of gastric cancer patients harboring with C3AR1 mutation, including amplification, missense mutation, deep deletion, and truncating mutation. Moreover, we also further analyzed the mutation frequency of C3AR1 dependence on subtypes of gastric cancer, as shown in Figure 1B. Here, we found that only three types of stomach adenocarcinoma contained the site mutation of C3AR1, i.e. diffuse-type stomach adenocarcinoma (4/72), mucinous stomach adenocarcinoma (4/72), stomach adenocarcinoma (7/165), and tubular stomach adenocarcinoma (3/79). Finally, the mutation status of C3AR1 proteins in gastric cancer patients also displayed that few sense mutations can be identified in TCGA database. These results indicated that sense mutation of the C3AR1 gene in gastric cancer is not the major, and expression level may be the major factor to affect tumorigenesis of gastric cancer.

To explore the correlation between C3AR1 and tumor status, we firstly examined the expression levels of C3AR1 in various tumor tissues and identified the difference compared to normal tissue. As shown in Figure 1D, we can find that expression levels of C3AR1 in most tumor tissues are higher than in normal tissue, especially for gastric tumor tissue ($p<0.001$). Then, we explored the expression levels of C3AR1 protein in gastric tissue using the Human Protein Atlas database. As shown in Figure 1E, we can find that C3AR1 was mainly expressed in tumor tissues, meanwhile C3AR1 only was observed in vasculature. Together, these results demonstrated that the C3AR1 may be associated with tumorigenesis of gastric cancer.

Gastric cancer patients benefits from higher expression level of C3AR1

Here, we utilized the Kaplan-Meier plot to evaluate the impact of C3AR1 expression levels on patient survival. For K-M analysis, all the gastric cancer patients were divided into two groups and the threshold value of C3AR1 was set as 320. As shown in Figure 2A-C, we can observe that the survival curve of gastric cancer patients with the higher expression level of C3AR1 is favorable than low expression group, including overall survival, first progression, and post progression survival. Meanwhile, we also found that median survival times of gastric cancer patients with highly expressed C3AR1 is almost 2-3 times than low expression group. For further analyzing the survival period depended on tumor grade, we found that only overall survival of gastric cancer patients at Stage I was significantly favorable than the low expression group ($p=0.028$), and the median survival time is almost 3 times than the low expression group (113.2/35.77). Together, these results revealed that C3AR1 expression levels can be treated as the prognostic biomarker for gastric cancer.

Correlation of C3AR1 and major clinicopathological indexes

To determine the correlation between C3AR1 expression levels and clinicopathological indexes, the UALCAN database was employed to examine the role of C3AR1 in gastric cancer patients. The methylation level of C3AR1 gene promoters is highly associated with expression level of C3AR1. However, we did not observe the difference between normal tissue and primary tumor tissue ($p=0.258$). For other indexes, e.g. *TP53* mutation status, *H.pylori* infection status, individual cancer stage, tumor grade, and nodal metastasis status, most of these indexes showed a positive correlation with higher progression of gastric cancer, implying the critical role of *C3AR1* in tumorigenesis. Finally, we also characterized the expression level of *C3AR1* dependence on the individual patients. As shown in Figure 4, the top-ranked genes with down-regulation and up-regulated were list. Together, these results indicated that higher expression of *C3AR1* may be highly associated with tumor progression.

Protein-protein interaction (PPI) network analysis

To further analyze the biological impact of overexpressed C3AR1 in gastric cancer, protein-protein interaction network analysis was employed. Firstly, we obtained the differentially expressed genes from GEPIA2 database, which contained 1244 genes ($|\log_2\text{FoldChange}|>1.5$ & FDR q-value <0.01). To utilize the STRING database, PPI network of these differentially expressed genes was built, and the network was presented as in Figure 5A. The top-ranked 9 hub genes by count of interaction nodes are *CDK1*, *ITGAM*, *UBE2C*, *CCNB1*, *THBS1*, *CXCL12*, *POMC*, *CCNB2* and *ADCY4*. The relative biological function of these hub genes is listed in Table 2, and most of these genes are highly associated with molecular regulation of cell cycle process. The independent interaction networks of these hub genes are listed in Figure 5B, and they can be attributed to three annotations, i.e. cellular component organization, regulation of DNA metabolic process, and regulation of DNA-dependent DNA replication. Moreover, the PPI network of the top 5 hub genes is list in Figure 6, which clearly showed the correlation between cell cycle regulation and cellular matric organization. The correlation between hub genes and *C3AR1* was displayed in Figure 7, and these

results indicated that *CCNB1/2*, *UBE2C* and *CDK1* displayed the negative correlation. Together, these results indicated that these hub genes may participate into regulation of gastric cancer progression.

Gene ontology and gene set enrichment analysis of DEGs

To further analyze the effect of overexpressed *C3AR1* in gastric cancer, gene ontology (GO) analysis was employed depended on different working clusters: up-regulated and down-regulated genes. For up-regulated genes, only cellular components and molecular function-related annotations have been identified as shown in Figure 8A-B. For cellular components, these annotations can be attributed to the regulators of cell cycle and chromosome, i.e. cyclin B1-CDK1 complex, chromosome passenger complex, cyclin-dependent protein kinase holoenzyme complex, condensed chromosome, chromosomal region, chromosome & centromeric region and kinetochore. For biological processes, these annotations are associated with a mitotic process, e.g. regulation of mitotic sister chromatid separation, mitotic spindle organization, and nuclear division. These results suggested that higher expression of *C3AR1* in gastric cancer may participate into regulation of cell cycle processes

For down-regulated genes, cellular components, biological process and molecular function-related annotations are enriched as shown in Figure 8C-E. For molecular function, these annotations are attributed to extracellular regulation, e.g. extracellular organelle and extracellular exosome. Among these biological process-related annotations, we can find that the extracellular processes and inflammation response were enriched, i.e. extracellular structure organization and inflammatory response. Moreover, molecular function-related annotations are attributed to extracellular processes, including RAGE receptor binding, signaling receptor binding, lipid transporter activity, fibronectin binding, glycosaminoglycan binding, structural molecule activity, and extracellular matrix structural constituent. Together, these results implied that overexpressed *C3AR1* in gastric cancer can affect the cell cycle and extracellular organization.

To further analyze the impact of overexpressed *C3AR1* in gastric cancer on the pathway, gene set enrichment analysis (GSEA) was employed as shown in Figure 9, in which we utilized Hallmark gene set. For GSEA results, we identified 4 up-regulated pathways and three down-regulated pathways, i.e. Hallmark_G2M_checkpoint, Hallmark_E2F_targets, Hallmark_mitotic_spindle, Hallmark_spermatogenesis, Hallmark_xenobiotic_metabolism, Hallmark_apoptosis and Hallmark_epithelial_mesenchymal_transition. These annotations are highly associated with cell cycle regulation and anti-apoptosis process, consistent with PPI analysis results.

Overexpressed *C3AR1* affects immune cell infiltration in gastric cancer

According to the above analysis, we found that overexpressed *C3AR1* can affect the extracellular process, which may be associated with tumor microenvironment. Here, we analyze the immune cell infiltration

level in gastric tissues using the TIMER database, compared to normal tissue. As shown in Figure 10A-F and Table 3, we systematically explored the correlation between *C3AR1* expression level and immune cell infiltration levels. Although infiltration levels of these common immune cells, i.e. T cell CD8+, T cell CD4+, macrophage, B cell, neutrophil, and myeloid dendritic cells, are positively associated with C3AR1 in gastric tissues, only the infiltration level of macrophages in gastric tissue is significantly correlated with survival time ($p=0.0108$ & 0.0226). As shown in Figure 10C, higher infiltration of macrophages in tumor tissues can worsen the survival time of gastric patients, implying the critical role of the macrophages in the regulation of tumor microenvironment.

To further identify the critical role of macrophage, we further analyzed the correlation between *C3AR1* and macrophage biomarkers dependence on macrophage polarization status. As shown in Figure 10, we can observe that these biomarkers of tumor-associated macrophage and M2 macrophage are highly positively with *C3AR1* expression, in which coefficient of these biomarkers with *C3AR1* is more than 0.5, demonstrating that higher *C3AR1* expression can lead the polarization of macrophage to TAM and M2 status.

Discussion

The complement system is a critical system for immune response in the vasculature and also involves into many biological processes to regulate the local microenvironment. *C3AR1*, coding Complement C3a Receptor 1, plays an essential role in signaling transduction of the complement system. Here, we systematically investigated the expression levels of *C3AR1* in gastric cancer using the cBioportal, HPA, Oncomine, TIMER, PPI, and GSEA database, and then identified the correlation among C3AR1, survival time, and immune cell infiltration. To perform this analysis, we explore the roles of C3AR1 in tumorigenesis and immune cell infiltration and identified C3AR1 can be as the prognostic biomarkers in gastric cancer.

Firstly, we identified the genetic status and mRNA expression levels of *C3AR1* in gastric patients, and these results confirmed the higher expression level of *C3AR1* in gastric tissues (Figure 1). Then, the K-M plot results clearly showed that higher expression levels of *C3AR1* can improve the survival of gastric patients, implying the essential role of *C3AR1* in affecting the molecular pathologies (Figure 2). Using the UCLAN database, we found that the expression levels of *C3AR1* are positively correlated with *TP53* mutation status, H.pylori infection status, individual cancer stage, tumor grade, and nodal metastasis status (Figure 3). Especially for H.pylori, the infection can increase the expression level of *C3AR1* in gastric cancer, implying that infection may activate the complement system.

Second, we further analyzed the biological impact of overexpressed C3AR1 using PPI and GO analysis. To perform the PPI analysis of differentially expressed genes, top-ranked 9 genes, i.e. *CDK1*, *ITGAM*, *UBE2C*, *CCNB1*, *THBS1*, *CXCL12*, *POMC*, *CCNB2* and *ADCY4*, were identified, which are associated with regulation of cell cycle (Figure 5-7). The further GO analysis dependence on up-regulated and down-regulated genes clearly showed that these differentially expressed genes are related to cell cycle

regulation and extracellular organization (Figure 8). The GSEA analysis results confirmed the up-regulation of the cell cycle pathway and anti-apoptosis pathways. These enriched results confirmed that gastric patients can benefit from expression levels of *C3AR1*.

Finally, we performed the immune cell infiltration analysis using the TIMER database to identify the biological impact of infiltrated immune cells on the survival of gastric patients. The immune cell infiltration analysis results clearly showed that infiltration levels of macrophages are positively associated with the survival time of gastric patients. The polarization of macrophages also plays different roles to affect the tumor microenvironment. Here, only tumor-associated macrophage and M2 were positively correlated with *C3AR1* expression levels. These results implied that higher *C3AR1* expression levels can recruit more macrophages to infiltrate into tumor tissue, and it can further improve the microenvironment of gastric cancer, which can improve the therapeutic effect of drugs.

Conclusions

In summary, we found that *C3AR1* is highly associated with gastric cancer and affects patient survival. The PPI and GSEA analysis revealed that over-expressed *C3AR1* may promote the cell cycle regulation process and inhibit cell apoptosis, suggesting continuous cell proliferation. TIMER analysis revealed that higher *C3AR1* can promote immune cell infiltration and display a higher association between macrophage infiltration and *C3AR1* expression, implying the critical role of the macrophage in promoting gastric cancer tumorigenesis. Our findings further explore the roles of *C3AR1* in gastric cancer and suggests the development of *C3AR1* diagnosis and therapy in the future.

Declarations

Acknowledge

None. All authors claim no competing interest in this study.

Authors' contributions

Weifeng Liu together performed the analysis and generated all the figures in this manuscript; Zhijie Chu, Cheng Yang, Tianbao Yang, Yanhui Yang, Junjun Sun participated into the preparation of manuscript and revised the manuscript; Weifeng Liu designed and supervised this assays, writing this manuscript and further revision.

References

1. Smyth, E. C.; Nilsson, M.; Grabsch, H. I.; van Grieken, N. C.; Lordick, F., Gastric cancer. *Lancet (London, England)* **2020**, *396* (10251), 635-648.

2. Van Cutsem, E.; Sagaert, X.; Topal, B.; Haustermans, K.; Prenen, H., Gastric cancer. *The Lancet* **2016**, *388* (10060), 2654-2664.
3. Soerjomataram, I.; Lortet-Tieulent, J.; Parkin, D. M.; Ferlay, J.; Mathers, C.; Forman, D.; Bray, F., Global burden of cancer in 2008: a systematic analysis of disability-adjusted life-years in 12 world regions. *The Lancet* **2012**, *380* (9856), 1840-1850.
4. Network, C. G. A. R., Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* **2014**, *513* (7517), 202-9.
5. Sakata, K.; Tamura, G.; Endoh, Y.; Ohmura, K.; Ogata, S.; Motoyama, T., Hypermethylation of the hMLH1 gene promoter in solitary and multiple gastric cancers with microsatellite instability. *British journal of cancer* **2002**, *86* (4), 564-7.
6. Van Cutsem, E.; Bang, Y. J.; Feng-Yi, F.; Xu, J. M.; Lee, K. W.; Jiao, S. C.; Chong, J. L.; López-Sanchez, R. I.; Price, T.; Gladkov, O.; Stoss, O.; Hill, J.; Ng, V.; Lehle, M.; Thomas, M.; Kiermaier, A.; Rüschhoff, J., HER2 screening data from ToGA: targeting HER2 in gastric and gastroesophageal junction cancer. *Gastric cancer : official journal of the International Gastric Cancer Association and the Japanese Gastric Cancer Association* **2015**, *18* (3), 476-84.
7. Cho, M. S.; Vasquez, H. G.; Rupaimoole, R.; Pradeep, S.; Wu, S.; Zand, B.; Han, H. D.; Rodriguez-Aguayo, C.; Bottsford-Miller, J.; Huang, J.; Miyake, T.; Choi, H. J.; Dalton, H. J.; Ivan, C.; Baggerly, K.; Lopez-Berestein, G.; Sood, A. K.; Afshar-Kharghan, V., Autocrine effects of tumor-derived complement. *Cell reports* **2014**, *6* (6), 1085-1095.
8. Hanahan, D.; Weinberg, R. A., Hallmarks of cancer: the next generation. *Cell* **2011**, *144* (5), 646-74.
9. Zhao, L.; Zhang, Z.; Lin, J.; Cao, L.; He, B.; Han, S.; Zhang, X., Complement receptor 1 genetic variants contribute to the susceptibility to gastric cancer in chinese population. *Journal of Cancer* **2015**, *6* (6), 525-30.
10. Sarma, J. V.; Ward, P. A., The complement system. *Cell and tissue research* **2011**, *343* (1), 227-35.
11. Afshar-Kharghan, V., The role of the complement system in cancer. *The Journal of clinical investigation* **2017**, *127* (3), 780-789.
12. Wang, Y.; Zhang, H.; He, Y. W., The Complement Receptors C3aR and C5aR Are a New Class of Immune Checkpoint Receptor in Cancer Immunotherapy. *Frontiers in immunology* **2019**, *10*, 1574.
13. Young, K. G.; Yan, K.; Picketts, D. J., C3aR signaling and gliosis in response to neurodevelopmental damage in the cerebellum. *Journal of neuroinflammation* **2019**, *16* (1), 135.

14. Litvinchuk, A.; Wan, Y. W.; Swartzlander, D. B.; Chen, F.; Cole, A.; Propson, N. E.; Wang, Q.; Zhang, B.; Liu, Z.; Zheng, H., Complement C3aR Inactivation Attenuates Tau Pathology and Reverses an Immune Network Deregulated in Tauopathy Models and Alzheimer's Disease. *Neuron* **2018**, *100* (6), 1337-1353.e5.
15. Zhang, L. Y.; Pan, J.; Mamtilahun, M.; Zhu, Y.; Wang, L.; Venkatesh, A.; Shi, R.; Tu, X.; Jin, K.; Wang, Y.; Zhang, Z.; Yang, G. Y., Microglia exacerbate white matter injury via complement C3/C3aR pathway after hypoperfusion. *Theranostics* **2020**, *10* (1), 74-90.
16. Zheng, J. M.; Wang, S. S.; Tian, X.; Che, D. J., Sustained activation of C3aR in a human podocyte line impairs the morphological maturation of the cells. *Molecular medicine reports* **2020**, *22* (6), 5326-5338.
17. Chen, B.; Zhou, W.; Tang, C.; Wang, G.; Yuan, P.; Zhang, Y.; Bhushan, S. C.; Ma, J.; Leng, J., Down-Regulation of C3aR/C5aR Inhibits Cell Proliferation and EMT in Hepatocellular Carcinoma. *Technology in cancer research & treatment* **2020**, *19*, 1533033820970668.
18. Li, Y.; Zhu, S.; Xue, M.; Jing, Y.; Liu, X.; Cai, D.; Zhao, Y.; Bian, Y.; Zhang, Z.; Zhang, L., Aristolochic acid I promotes the invasion and migration of hepatocellular carcinoma cells by activating the C3a/C3aR complement system. *Toxicology letters* **2020**.
19. Mueller-Ortiz, S. L.; Morales, J. E.; Wetsel, R. A., The receptor for the complement C3a anaphylatoxin (C3aR) provides host protection against *Listeria monocytogenes*-induced apoptosis. *Journal of immunology (Baltimore, Md. : 1950)* **2014**, *193* (3), 1278-89.
20. Nabizadeh, J. A.; Manthey, H. D.; Steyn, F. J.; Chen, W.; Widiapradja, A.; Md Akhir, F. N.; Boyle, G. M.; Taylor, S. M.; Woodruff, T. M.; Rolfe, B. E., The Complement C3a Receptor Contributes to Melanoma Tumorigenesis by Inhibiting Neutrophil and CD4+ T Cell Responses. *Journal of immunology (Baltimore, Md. : 1950)* **2016**, *196* (11), 4783-92.

Tables

Table 1. Kaplan-Meier plotter to determine the effect of different clinicopathological factors on the expression of C3AR1 in gastric cancer

Clinicopathological characteristics	Overall survival (n=557)			Progression-free survival (n=522)		
	N	Hazard ratio	p-value	N	Hazard ratio	p-value
Stage						
1	69	0.25(0.09-0.72)	0.0057	69	0.26(0.09-0.81)	0.012
2	145	1.68(0.89-3.15)	0.017	145	1.55(0.82-2.95)	0.18
3	319	0.69(0.5-0.94)	0.017	319	0.71(0.49-1.04)	0.074
4	152	1.2(0.82-1.76)	0.34	152	1.32(0.83-2.1)	0.24

Table 2. Biological functions of top 10 hub genes.

Gene	Biological functions	Gene	Biological functions
CDK1	an anaphylatoxin released during activation of the complement system.	CXCL12	Playing a role in many diverse cellular functions, including embryogenesis, immune surveillance, inflammation response, tissue homeostasis, and tumor growth and metastasis.
ITGAM	leukocyte-specific integrin referred to as macrophage receptor 1 ('Mac-1'), or inactivated-C3b (iC3b) receptor 3 ('CR3').	POMC	preproprotein that undergoes extensive, tissue-specific, post-translational processing via cleavage by subtilisin-like enzymes known as prohormone convertases
UBE2C	required for the destruction of mitotic cyclins and for cell cycle progression, and may be involved in cancer progression	CCNB2	binds to transforming growth factor beta RII and thus cyclin B2/cdc2 may play a key role in transforming growth factor beta-mediated cell cycle control
CCNB1	a regulatory protein involved in mitosis	ADCY4	membrane-associated enzymes that catalyze the formation of the secondary messenger cyclic adenosine monophosphate
THBS1	an adhesive glycoprotein that mediates cell-to-cell and cell-to-matrix interactions		

Table 3. Cox proportional hazard model of immune cell infiltration in gastric cancer.

	coef	HR	se(coef)	95% CI	95% CI_u	z	p value
T cell CD8+	-0.159	0.853	0.494	0.324	2.247	-0.322	0.748
T cell CD4+	0.209	1.233	0.433	0.528	2.880	0.484	0.629
Macrophage	2.34	10.377	0.664	2.825	38.117	3.525	0.000
B cell	-0.945	0.389	0.799	0.081	1.861	-1.183	0.237
Neutrophil	-0.717	0.488	1.266	0.041	5.841	-0.566	0.571
Myeloid dendritic cell	-0685	0.504	0.411	0.225	1.128	-1.666	0.096

Figures

Figure 1

A. Genetic status of C3AR1 gene in gastric cancer, containing normal, amplification, missense mutation, truncating mutation and deep deletion (TCGA sample n=440). **B.** Genetic status of C3AR1 dependence on various types of stomach adenocarcinoma. **C.** Observed mutation sites of C3AR1 protein in TCGA patients. **D.** mRNA expression levels of C3AR1 gene in different types of cancer and normal tissue. **E.** Protein expression levels of C3AR1 in normal stomach tissue and tumor tissue obtained from the Human Protein Atlas database. *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001.

Figure 2

Kaplan-Meier survival of overall survival (**A**, OS), first progression (**B**, FP), post progression survival (**C**, PPS) curves and overall dependence on tumor stage (I-IV), respectively. The median follow-up duration was list in the table.

Figure 3

mRNA expression levels of C3AR1 gene in gastric cancer dependence on promoter methylation level (A), TP53 mutation status (B), H.pylori infection status (C), individual cancer stages (D), tumor grade (E) and nodal metastasis status (F).

Figure 4

Heatmap of top ranked genes dependence on down-regulation and up-regulation process.

Figure 5

A. Protein-protein interaction network of differentially expressed genes in gastric cancer and hub genes.
B. Interaction network of enriched pathways in gastric cancer.

Figure 6

The protein-protein interaction and functional annotations dependence on top 5 hub genes.

Figure 7

Correlation regression of hub genes and C3AR1 expression level in gastric cancer: CDK1, UBE2C, CCNB2, CCNB1, ITGAM, THBS1, ADCY4, CXCL12, POMC. These images were obtained from cBioportal website using TCGA database. Regression curve was employed using spearman method.

Figure 8

Gene ontology analysis of down-regulated (A-C) and up-regulated (D-E) genes by cellular components (A), biological process (B and D) and molecular function (C and E). Threshold value considered as significance: FDR q-value <0.05.

Figure 9

Gene set enrichment analysis score profile of up-regulated and down-regulated pathways using GSEA software with Hallmark gene set. Threshold value considered as significance: NOM p-value <0.05 & FDR q-value <0.05 & |NES|>1.0.

Figure 10

Correlation between *C3AR1* expression level and immune cell infiltration in gastric cancer dependence on various types of immune cells: **A)**, T cell CD8+; **B)**, T cell CD4+; **C)**, macrophage; **D)**, B cell; **E)**, neutrophil; **F)**, myeloid dendritic cell. Overall survival curve of immune cell infiltration levels and C3AR1 expression.

Figure 11

Correlation between *C3AR1* expression levels and cellular biomarkers of macrophage with different subtypes (tumor-associated macrophage (TAM), M1 and M2) in gastric cancer.