Genes & Diseases (2024) 11, 101093

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RAPID COMMUNICATION

Complement C1q induces the M2-polarization of tumor-associated macrophages in lung adenocarcinoma

Complement C1q was proved to be able to regulate the polarization of macrophages to the anti-inflammatory phenotype (M2 polarized), acting as an anti-inflammation molecule independent of the classical complement pathway. A high level of C1q expression has been detected in the tumor microenvironment (TME) of diverse tumors, including non-small cell lung cancer, in which C1q could be considered a predictor of poor prognosis.[1] Prominently, researchers have found that C1q was positively correlated with the M2 polarization of tumor-associated macrophages (TAMs) in mouse renal clear cell carcinoma and human osteosarcoma. M2-TAMs are significantly associated with immunosuppressive TME and poor prognosis in non-small cell lung cancer. Hence, we investigated the mutual effects between C1q and TAMs in lung adenocarcinoma. The findings of this study could be extracted as below: (i) The expression of C1qA was positively correlated with M2-TAMs in lung adenocarcinoma. (ii) M2-TAMs could express abundant C1q, and C1q in lung adenocarcinoma was mainly from TAMs, especially M2-TAMs. (iii) C1qA could promote the proportion of M2-TAMs. (iv) C1qA led to the reduced phosphorylation of JAK2, STAT1, and STAT5, but augmented the levels of p52, phospho-p65, and phospho-IκBα belonging to NF-κB pathway. (v) C1q promoted the growth of LLC xenograft.

C1q consists of 18 polypeptide chains, including 6 C1qA, 6 C1qB, and 6 C1qC. Research has revealed that defects in the C1qA, C1qB, or C1qC genes were associated with disfunction of C1q, generating very similar responses. In this study, the bioinformatic analysis through Pearson and Spearman correlation test showed C1qA expression was positively correlated with M2-TAMs gene signatures in the lung adenocarcinoma database (Fig. 1A). Given the results of bioinformatic analysis, C1qA active protein was applied for the following *in vitro* experiments. The ELISA experiment indicated the C1q concentration of IL-4 induced M2-TAMs supernatant was the highest among groups (Table S2), signifying M2-TAMs expressed a major amount of C1q (Fig. 1B). We confirmed C1qA induced the M2 polarization of TAMs by detecting molecule markers using flow cytometry, quantitative real-time PCR (qRT-PCR), and Western blot (Fig. 1C–E). To explore the possible pathway of M2 polarization induced by C1qA, the phosphorylation levels of JAK2, STAT1, and STAT5 and the activation of NF-κB pathway in each group were detected (Fig. 1F). The results demonstrated C1qA inhibited the phosphorylation of JAK2, STAT1, and STAT5, and activated the NF-κB pathway in TAMs (Fig. 1G). The growth curves generated in *in vivo* investigation verified C1q promoted the growth of LLC xenograft, and antagonizing C1q treatment could lessen the proliferation of tumors (Fig. 1H). Furthermore, immunohistochemistry targeted C1qA and immunofluorescence targeted C1qA plus CD206 conducted in the paraffin section of LLC xenograft proved C1qA was distinctly expressed in the xenograft and the expression of C1qA and CD206 was essentially simultaneous (Fig. 1I, J), meaning TAMs, especially M2-TAMs were the main source of C1q.

Initially, C1q functions as an anti-inflammatory molecule, restraining the release of proinflammatory cytokines in macrophages and meditating the inhibition of effector T lymphocyte activation. The complement system has been found activated to varying degrees in most tumors, and C1q has been detected in a variety of tumors. Complement activation in tumors eventually forms chronic inflammation, promoting the suppressing immune microenvironment and leading to tumor progression.[2] Therefore, TAMs expressing C1q in some tumors may also induce chronic inflammation in TME, resulting in immunosuppressive TME and tumor progression.

This study tentatively explored the connection between C1q and M2-TAMs by using the public mRNA-Seq database of

Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2023.101093

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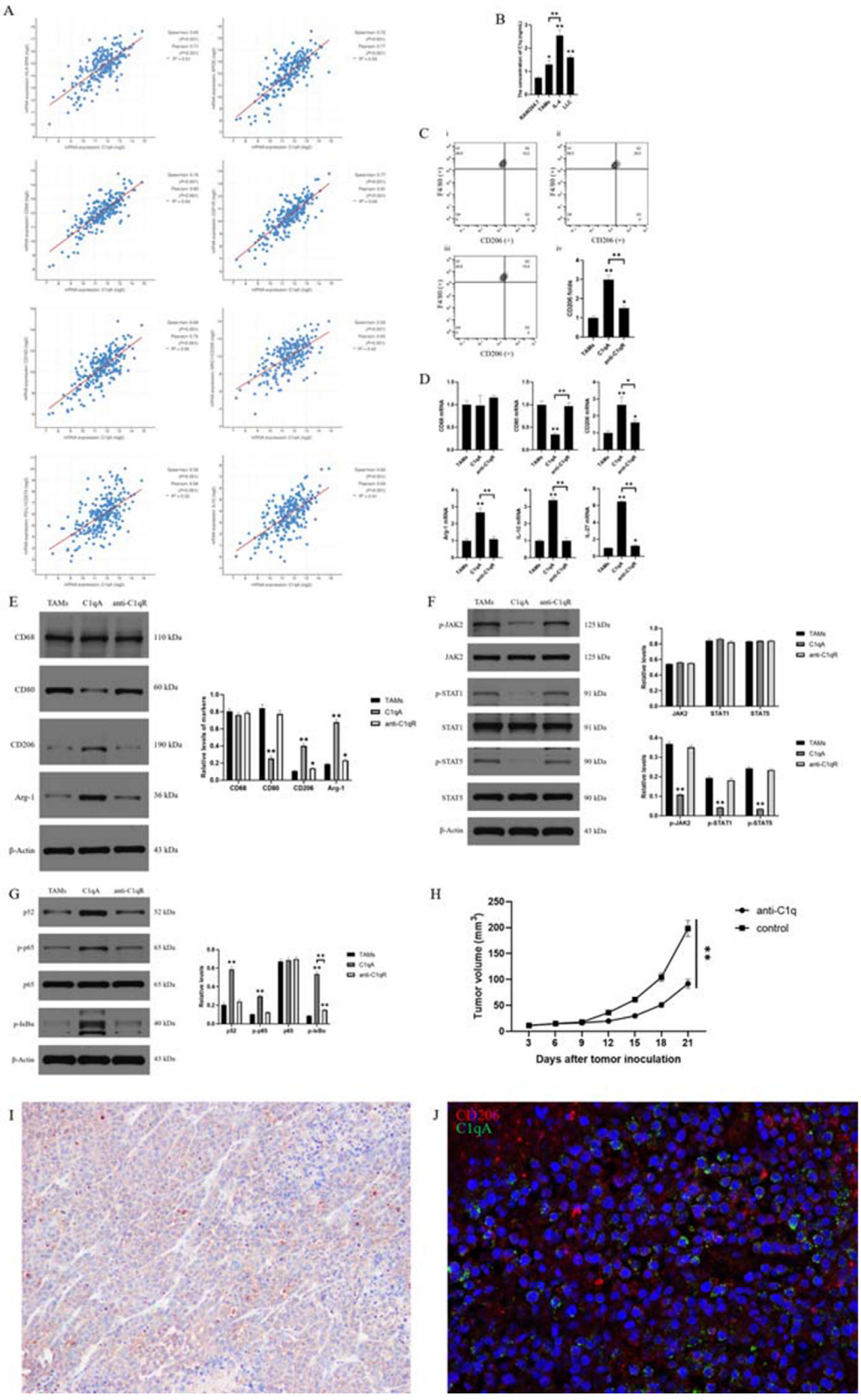

Figure 1 The mutual relation between C1q and M2-TAMs in lung adenocarcinoma. **(A)** Correlation between C1qA mRNA expression and M2-TAMs gene signatures in lung adenocarcinoma (all $P < 0.001$). The lung adenocarcinoma dataset (TCGA, Firehose Legacy) consisted of 230 cases of lung adenocarcinoma, all of which were used for Spearman and Pearson correlation analysis. The results indicated that the expression of C1qA mRNA was significantly correlated with M2-TAM marker genes, involving HLA-DRA

lung adenocarcinoma. Consistent with existing omics studies, the transcriptome analysis of this study suggested that the expression of C1q was strongly correlated with the expression of TAM markers such as HLA-DR, APOE, CD68, and CSF1R. Therefore, it can be inferred that C1q in lung adenocarcinoma is mainly from TAMs. Besides, the ELISA experiment exhibited M2-TAMs stimulated by IL-4 secreted a large amount of C1q. The results of ELISA and transcriptome analysis manifested that M2-TAMs of lung adenocarcinoma were the major source of C1q.

Research has revealed that complement activation in TME is continuous and forms chronic inflammation, accelerating T cell depletion, thus immunosuppressive TME is created. C1q can trigger the complement cascade, so TAMs expressing C1q ($C1q^+$ TAMs) may have a pro-tumor effect by activating the complement. Macrophage-derived C1q has been studied to be a poor prognostic predictor in several tumors such as renal clear cell carcinoma, hepatocellular carcinoma, and osteosarcoma. Recent single-cell sequencing studies further clarified that $C1q^+$ TAMs were significantly enriched in melanoma and basal cell skin cancer patients who did not respond to immune checkpoint inhibitor treatment. Moreover, $C1q^+$ TAMs in diverse tumors simultaneously showed up-regulation of MRC1 (encoding CD206).[3]

In this study, we investigated the specific effect of C1qA, a functional subunit of C1q, on the polarization of TAMs. The flow cytometry, qPCR, and Western blot results displayed that C1qA could significantly induce M2 polarization of TAMs and elevated the transcription of inflammatory

(Spearman: 0.65, Pearson: 0.71), APOE (Spearman: 0.72, Pearson: 0.77), CD68 (Spearman: 0.76, Pearson: 0.80), CSF1R (Spearman: 0.77, Pearson: 0.81), CD163 (Spearman: 0.68, Pearson: 0.81) 0.75), CD206 (Spearman: 0.59, Pearson: 0.65), PD-L1 (Spearman: 0.58, Pearson: 0.58), and IL-10 (Spearman: 0.60, Pearson: 0.64). **(B)** Effects of different interventions on complement C1q concentration in culture supernatants. $^{**}P < 0.01$ *vs*. RAW264.7, $^{*}P < 0.05$ *vs*. RAW264.7. The concentration of C1q in the culture supernatant of RAW264.7 cells, TAMs group, IL-4 group, and LLC cells were 0.725 ± 0.048 ng/mL, 1.298 ± 0.166 ng/mL, 2.551 ± 0.239 ng/mL, and 1.602 ± 0.091 ng/mL, respectively. Compared with RAW264.7 cells, the concentration of C1q in LLC cells culture supernatant was higher. The concentration of C1q in the culture supernatant of the TAMs group was higher than that of RAW264.7 cells. Compared with RAW264.7 cells, the concentration of C1q in the culture supernatant of the IL-4 group (TAMs stimulated by IL-4) was significantly increased, and significantly higher than the TAMs group. **(C)** Effects of C1qA on the expression of CD206, the M2 polarization marker, in TAMs. **(i)** TAMs group; **(ii)** C1qA group; **(iii)** anti-C1qR group; **(iv)** relative folds of $CD206^+$ TAMs. $^{**}P < 0.01$ *vs*. TAMs, $^{*}P < 0.05$ *vs*. TAMs. F4/80-FITC and CD206-PE antibodies were used to co-stain TAMs in each group. F4/80 and CD206 double positive cells detected by Flow cytometry were calculated, which represented the proportion of M2-TAMs. Compared with the TAMs group, the proportion of M2-TAMs in the C1qA group significantly increased. The percentage of M2-TAMs was significantly lower in the C1qA group than in the anti-C1qR group, but still higher than the TAMs group. **(D)** Effects of C1qA on mRNA levels of M1/M2 polarization markers in TAMs. $^{**}P < 0.01$ *vs*. TAMs, $^{*}P < 0.05$ *vs*. TAMs. qRT-PCR was used to detect the mRNA levels of macrophage markers in each group. The mRNA level of CD68, a non-specific marker, was not significantly different among the three groups. Compared with the TAMs group, the mRNA level of M1 polarization marker CD80 was significantly inhibited in the C1qA group. The mRNA level of CD80 in the anti-C1qR group was significantly higher than that in the C1qA group. As for the expression of M2 polarization marker CD206 and Arg-1, compared with the TAMs group, C1qA could significantly increase the mRNA expression of CD206 and Arg-1 in TAMs; besides, the mRNA expression of CD206 in the anti-C1qR group was significantly lower than that in C1qA group. Though, the expression of CD206 in the anti-C1qR group was still higher than that in the TAMs group. The expression of other M2 polarization markers such as IL-10 and IL-27 was further detected. Compared with the TAMs group, the mRNA levels of IL-10 and IL-27 in the C1qA group were significantly increased. The mRNA levels of IL-10 and IL-27 in the C1qA group were significantly lower than in the anti-C1qR group. **(E)** Effects of C1qA on proteins of M1/M2 polarization markers in TAMs. $^{**}P < 0.01$ *vs*. TAMs, $^{*}P < 0.05$ *vs*. TAMs. Western blot was conducted to detect the protein expression of CD68, CD80, CD206, and Arg-1 in TAMs of each group. There was no significant difference in the expression of CD68 among the three groups. Compared with the TAMs group, the expression of CD80 in the C1qA group was significantly inhibited. The expression of CD80 in the anti-C1qR group was significantly higher than that in the C1qA group, and there existed no significant difference between the anti-C1qR group and the TAMs group. The expression of CD206 and Arg-1 in the C1qA group was significantly higher than those of the TAMs group. Meanwhile, the expression of CD206 and Arg-1 in the anti-C1qR group was higher than those of the TAMs group. **(F)** Effects of C1qA on the phosphorylation of JAK2, STAT1, and STAT5 in TAMs. $^{**}P < 0.01$ *vs*. TAMs. There was no significant difference in the expression of JAK2, STAT1, and STAT5 proteins among groups. Compared with the TAMs group, the levels of phospho-JAK2, phospho-STAT1, and phospho-STAT5 proteins in the C1qA group were significantly decreased. Nevertheless, C1qA had no significant effect on the phosphorylation of JAK2, STAT1, and STAT5 in the anti-C1qR group. **(G)** Effects of C1qA on NF-κB pathway in TAMs. $^{**}P < 0.01$ *vs*. TAMs. Compared with the TAMs group, the expression levels of p52, phospho-p65, and phospho-IκBα in the C1qA group were significantly increased, indicating that NF-κB pathway was activated. When it came to the anti-C1qR group, the expression of p52 and phospho-p65 in TAMs were not significantly different from those in the TAMs group; though the expression of phospho-IκBα was increased, it was still significantly beneath that of C1qA group, suggesting NF-κB pathway was not apparently activated. **(H)** Effects of antagonizing C1q on the growth of LLC xenograft. $^{**}P < 0.01$ *vs*. control. After the treatment of antagonizing C1q, the growth speed and volume of LLC xenograft were significantly lessened. **(J, K)** Immunohistochemistry and immunofluorescence results of paraffin sections of LLC xenograft. The LLC xenograft was harvested and made into paraffin sections, which were subjected to immunohistochemistry targeting C1qA and immunofluorescence targeting C1qA plus CD206, showing C1qA was distinctly expressed and C1qA and CD206 expression were essentially simultaneous in LLC xenograft.

inhibitory cytokines IL10 and IL27. This research confirmed that C1q could play an immunomodulatory role in TME, and such tight association and interaction between C1q and M2-TAMs may be the reason for the inefficient response to immunotherapy and the low overall survival rate.

In tumor immunity, the continuous up-regulation of STAT1 could promote anti-tumor immunity, on the other way, some tumor cells could prevent M1 polarization of TAMs by stimulating the expression of negative regulators against JAK2/STAT5, thereby avoiding tumor cell killing. Also, many studies have shown that both the pro- and anti-inflammatory effects of macrophages are closely related to the phosphorylation of STAT5.[4]

We found that the expression of phospho-JAK2, phospho-STAT1, and phospho-STAT5 was significantly inhibited in TAMs cultured with C1qA. Blocking C1qA binding to the receptor not only impaired the M2 polarization of TAMs, but the phosphorylation of JAK2, STAT1, and STAT5 also was not influenced. These results revealed that C1q mediated the M2-polarization of TAMs by inhibiting the phosphorylation of JAK2, STAT1, and STAT5, which are vital molecules for inflammatory effect.

The effect of NF-κB activation in inflammation and innate immunity is usually to promote inflammatory response. However, there also existed evidence that M2-TAMs were also dependent on the activation of NF-κB pathway, which inhibited the expression of inflammatory factors and released IL-10, transforming growth factor-β, and vascular endothelial growth factor, leading to tumor proliferation and angiogenesis, thereby making tumor cells develop drug resistance. NF-κB activation in TAMs is an important factor in maintaining M2-TAMs and immunosuppressive TME.[5]

This study discovered that NF-κB pathways were activated. Blocking the binding of C1qA to TAMs not only prevented the M2-polarization of TAMs but also the activation of NF-κB pathway, prompting that the activation of NF-κB pathway may be a crucial intermediate link of the C1q-mediated M2-TAMs in lung adenocarcinoma.

Taking the distinct growth curves and immunological experiments of LLC xenograft into account, conclusions could be drawn: (i) M2-TAMs are the main source of C1q. (ii) C1q can induce M2-TAMs, involved in inhibiting the phosphorylation of JAK2, STAT1, and STAT5. (iii) Activation of NF-κB is an important process in C1q inducing M2-TAMs. (iv) C1q and M2-TAMs can participate in the tumor proliferation and immunosuppressive tumor microenvironment by over-expressing and relying on C1q. (v) C1q may be a potential therapeutic target of lung adenocarcinoma.

Conflict of interests

The authors declare no conflict of interests.

Funding

This research was funded by the National Natural Science Foundation of China (No. 82272928), CSCO (Chinese Society of Clinical Oncology)-BMS (Bristol-Myers Squibb) Cancer Immunotherapy Research Foundation (No. Y-BMS2019-003), and the Knowledge Innovation Program of Wuhan-Basic Research (China) (No. 2022020801010475).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.101093.

References

1. Kou W, Li B, Shi Y, et al. High complement protein C1q levels in pulmonary fibrosis and non-small cell lung cancer associated with poor prognosis. *BMC Cancer*. 2022;22(1):110.
2. Magrini E, Minute L, Dambra M, Garlanda C. Complement activation in cancer: effects on tumor-associated myeloid cells and immunosuppression. *Semin Immunol*. 2022;60:101642.
3. Yang J, Lin P, Yang M, et al. Integrated genomic and transcriptomic analysis reveals unique characteristics of hepatic metastases and pro-metastatic role of complement C1q in pancreatic ductal adenocarcinoma. *Genome Biol*. 2021;22(1):4.
4. Song Y, Wu Q, Jiang H, et al. The effect of shionone on *Sepsis*-induced acute lung injury by the ECM1/STAT5/NF-κB pathway. *Front Pharmacol*. 2022;12:764247.
5. Wanderley CW, Colón DF, Luiz JPM, et al. Paclitaxel reduces tumor growth by reprogramming tumor-associated macrophages to an M1 profile in a TLR4-dependent manner. *Cancer Res*. 2018;78(20):5891–5900.

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28 December 2022