Research Paper

Impact and Prognosis of Complement 1q (A, B, C) on Colorectal Cancer

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In order to investigate the relationship between complement component 1q (A, B, C) and prognosis of colorectal cancer, we analyzed C1q (A, B, C) expression, clinical stage and methylation level in colorectal cancer from the Cancer Genome Atlas database and Gene Expression Omnibus database, including the prognosis of colorectal cancer. Complement 1q (A, B, C) were overexpressed (p=6.16e-07, p=4.01e-06 and p=3.28e-08, respectively) and showed low methylation levels (p=2.51e-11, p<1e-12 and p=1.64e-12, respectively) in colorectal cancer as per the cancer genome atlas database. The expression of C1q (A, B, C) were high in tumor tissues than normal tissues as per gene expression omnibus database (p=0.0044, p=8.9e-07 and p=5.2e-07, respectively). Additionally, C1q (A, B, C) and microsatellite instability have statistical significance (p=2.96e-08, p=1.08e-06 and p=4.18e-07, respectively). Finally, high expression of C1q (A, C) denotes poor survival in 5 y (p=0.026 and p=0.041, respectively) while high and low expression of C1q B have no significant difference survival in 5 y (p=0.082). However, the survival time is more than 3000 d and the prognosis of high expression of C1qB is poor. C1q (A, B, C) were overexpressed and showed low methylation levels in colorectal cancer. In addition, close correlation of C1q (A, B, C) with microsatellite instability has been explored and high expression of C1q (A, C) depicted poor survival in 5 y.

Key words: Complement component 1q, mutations, methylation, colorectal cancer

Colorectal Cancer (CRC) is one of the common malignant tumors in the world[1]. About 10 % of the patients are diagnosed with CRC and cancer-related deaths worldwide every year[2]. The diagnosis rate in women is higher than that of men. However, in women, incidence and mortality are approximately 25 % lower than in men[1]. With the development of medical technology, it is estimated that the incidence rate of CRC will be about 250 million worldwide by 2035. Sex and age are high risk factors of CRC[3]. However, the hereditary and environmental risk factors also play a part in the development of CRC. Approximately 10 %-20 % of patients have a family history[4]. According to studies, CRC has 12 %-35 % rate of heritability from twins and family[5].

Microsatellite Instability (MSI) is characterized by a high quantity of mutations in microsatellite locations[6]. The presence of MSI implies the disabling of Mismatch Repair (MMR) mechanisms[7]. MSI is recognized as one of the major carcinogenetic pathways and it has been confirmed that MSI mutation affects the prognosis of CRC[8]. At present, there are more and more target drugs for MSI mutations[9].

Colonoscopy has been well established as the gold standard for CRC screening with high sensitivity and specificity[1]. However, colonoscopy has some limitations like intestinal cleaning is required, manpower requirement, requiring experienced endoscopists and patient adherence[10]. Currently, with the development of molecular technology, proteins, Deoxyribonucleic Acid (DNA) (for the detection of mutations and methylation markers), Ribonucleic Acid (RNA) (mainly microRNAs) technology is becoming more and more mature. Discovery of new molecular targets is helpful to differentiate the precancerous lesions of colon cancer and improve prognosis.

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Complement component 1q (C1q) contains three types of chains namely A, B and C which play a key role in immune complexes[11]. In addition to autoimmunity, C1q can also defense against infection. In prostate cancer, C1qA and C1qB shows high expression and prolongs the survival time[12]. C1q can induce apoptosis and has anti-angiogenic effect in breast cancer research[13]. Therefore, we assume that the expression of C1q is related to the prognosis and may become a target molecule of CRC.

We analyzed the expression of C1q (A, B and C) and prognosis in CRC from The Cancer Genome Atlas (TCGA) database and Gene Expression Omnibus (GEO) database. Further, we have also clarified the correlation between C1q and MSI, to provide an evidence for finding new molecular targets for CRC.

MATERIALS AND METHODS

Data collection and workflow illustration:

We obtained the expression levels, methylation levels and prognosis of C1q (A, B, C) for CRC from TCGA database (https://portal.gdc.cancer.gov). We have verified the expression of C1q (A, B, C) from GEO database (https://www.ncbi.nlm.nih.gov/geo/). MSI mutation was obtained from TCGA database (fig. 1).

University of Alabama at Birmingham Cancer data Analysis (UALCAN) online network:

We utilized UALCAN online network (http://ualcan.path.uab.edu/) and studied the expression, methylation levels and prognosis of C1q (A, B, C) for CRC from TCGA database.

Gene expression profile and MSI mutation:

We obtained the expression profiling of C1q (A, B, C) by clusterProfiler package in R version 4.0.2 (http://www.r-project.org/) for series GSE37182 from GEO database. Further, we have obtained MSI mutation through cBioPortalData package in R version 4.0.2 from TCGA database. We used Spearman's correlation analysis (ρ) to describe the correlation between quantitative variables without a normal distribution.

Statistical analysis:

The Student's t-test (R function t-test) was used to determine whether there were significant differences between the two groups and $p<0.05$ was considered to be statistically significant. Statistical Package for Social Sciences (SPSS) 21.0 was used to draw the Receiver Operating Characteristic (ROC) curve and survival curve.

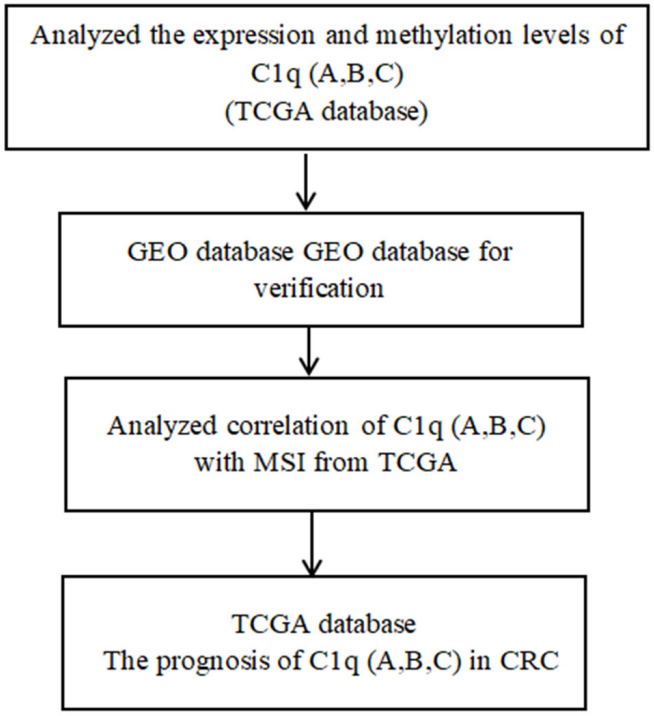

Fig. 1: Flow chart showing the analysis of the expression and methylation levels of C1q (A, B, C)

RESULTS AND DISCUSSION

We analyzed C1q (A, B, C) expression in CRC from TCGA database through UALCAN online network. The C1q (A, B, C) were overexpressed in tumor tissues (p=6.16e-07, p=4.01e-06 and p=3.28e-08, respectively) (fig. 2A-fig. 2C). Subsequently, we verified the expression of C1q (A, B, C) in series GSE37182 from GEO database. We found that the expression of C1q (A, B, C) were high in tumor tissues than normal tissues (p=0.0044, p=8.9e-07 and p=5.2e-07, respectively) (fig. 3A-fig. 3C). At the same time, we analyzed the methylation of C1q (A, B, C) in CRC sample from TCGA database. We found that the expression of methylation levels of C1q (A, B, C) were low in tumor tissues than normal tissues in CRC (p<2.51e-11, p<1e-12 and p<1.64e-12, respectively) (fig. 4A-fig. 4C).

The expression of C1q (A, B, C) significantly decreased in normal tissues (p<0.05). In addition, the expression of C1q (A, B, C) were significantly different in Stage II, Stage III and Stage IV. For the correlation of C1qA, Stage II *vs.* Stage IV were compared, (p=2.2e-03) and Stage III *vs.* Stage IV were compared, where p=2.25e-02. Similarly for the correlation of C1qB, Stage II *vs.* Stage IV were compared (p=1.28e-03) and then Stage III *vs.* Stage IV was also compared (p=2.53e-02). While for the correlation of C1qC, Stage II *vs.* Stage IV was compared, (p=1.04e-03) and Stage III *vs.* Stage IV was also compared (p=1.19e-02). We analyzed the correlation between C1q (A, B, C) and MSI in CRC (fig. 5A-fig. 5C). In 455 samples, C1q (A, B, C) and MSI has showed statistical significance (p=2.96e-08, p=1.08e-06 and p=4.18e-07, respectively) and the correlation coefficient were noted respectively as $\rho_{Spearman}$=0.26, Confidence Interval (CI) 95 % (0.17, 0.35), $\rho_{Spearman}$=0.23, CI 95 % (0.14, 0.32), $\rho_{Spearman}$=0.24, CI 95 % (0.15, 0.33).

We analyzed C1q (A, B, C) expression and impact of C1q (A, B, C) on the prognosis of CRC from TCGA database. Among 279 samples, 71 samples showed low expression while 208 samples showed high expression of C1q (A, B, C). High expression of C1q (A and C) depicted poor survival in 5 y of time (p=0.026 and p=0.041, respectively) while high and low expression of C1q B have no significant difference of survival in 5 y of time (p=0.082) (fig. 6A-fig. 6C). However, the survival time is more than 3000 d and the prognosis of high expression of C1qB is poor.

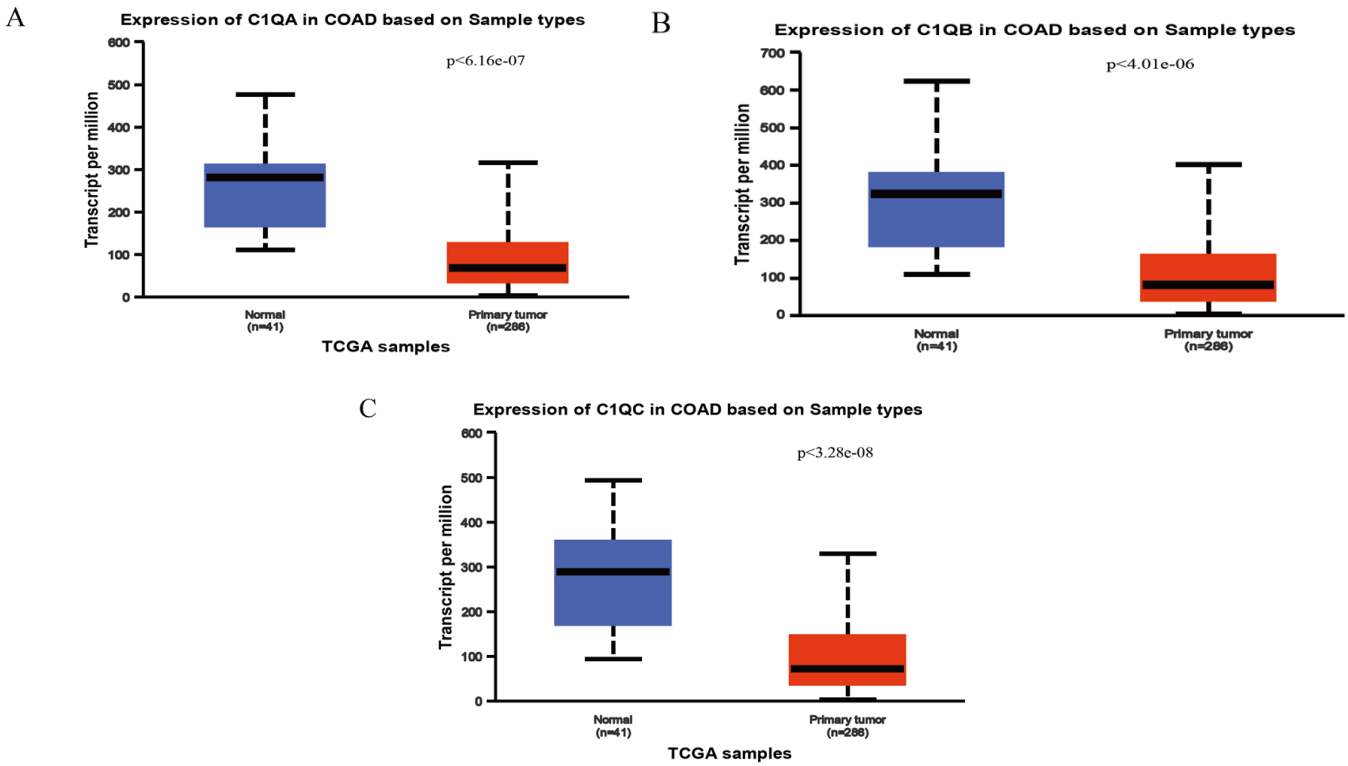

Fig. 2: Analysis of C1q (A, B, C) expression in CRC from TCGA database through UALCAN online network
Note: Overexpression of C1q (A, B, C) in tumor tissues where p=6.16e-07, p=4.01e-06 and p=3.28e-08, respectively

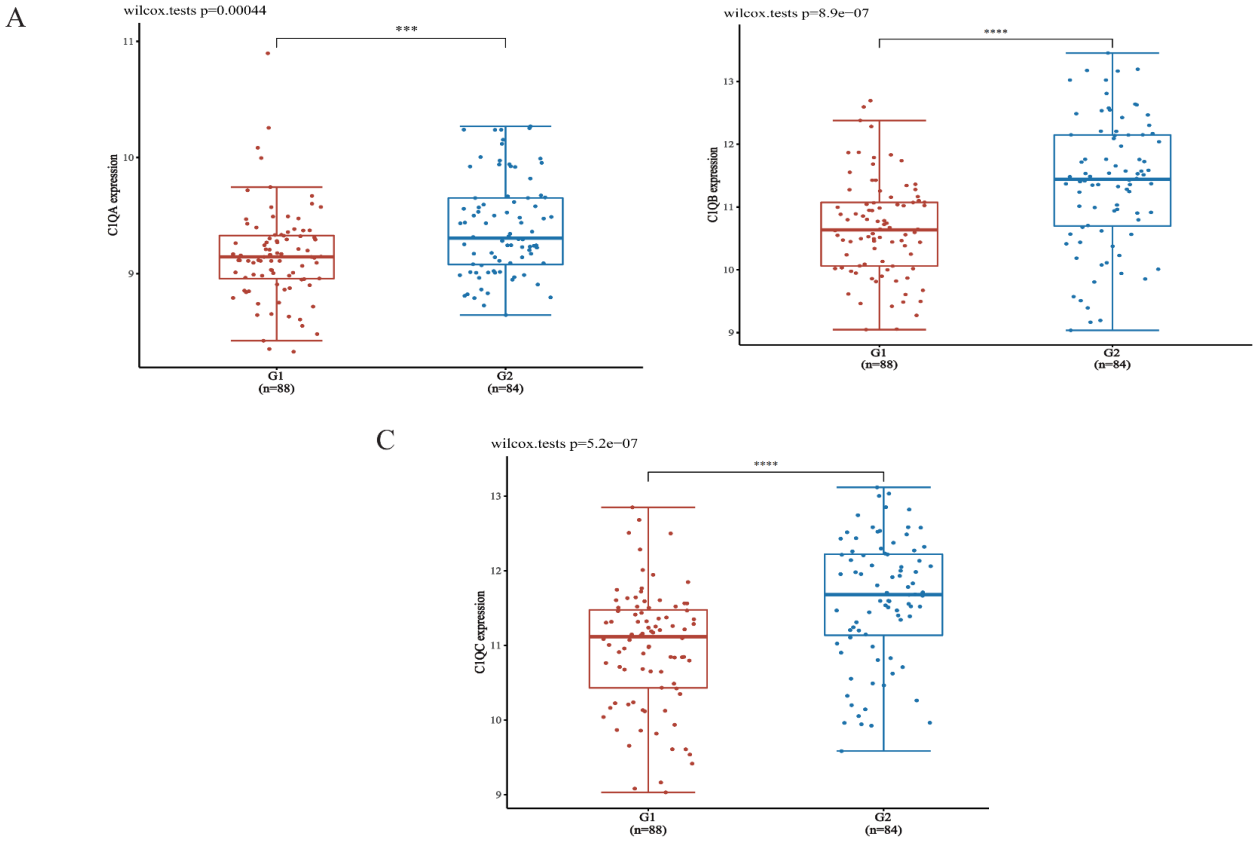

Fig. 3: Analysis of high expression of C1q (A, B, C) in tumor tissues than normal tissues in series GSE37182 from GEO database (p=0.0044, p=8.9e-07 and p=5.2e-07, respectively)
Note: ( ): G1 (n=88); ( ): G2 (n=84)

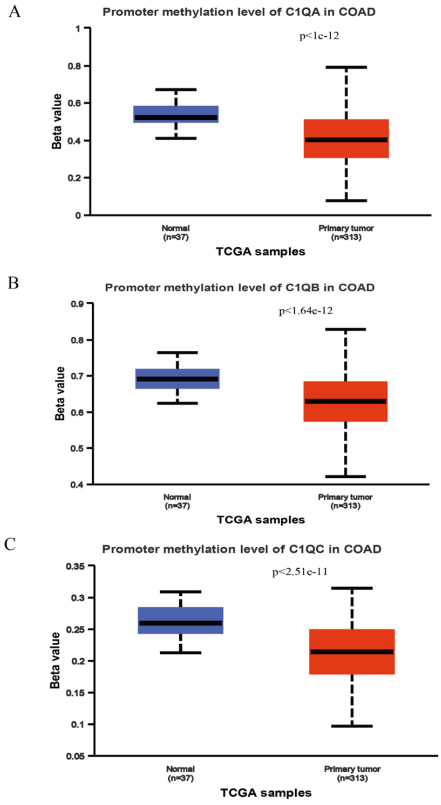

Fig. 4: Analysis the methylation of C1q (A, B, C) in CRC sample from TCGA database
Note: Methylation levels of C1q (A, B, C) were low expressed in tumor tissues where p<1e-12, p<1.64e-12 and p<2.51e-11, respectively

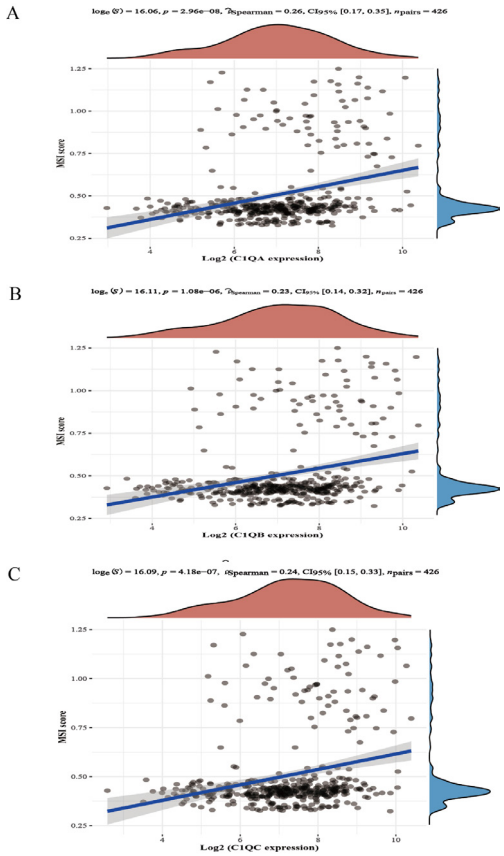

Fig. 5: Statistical significance of C1q (A, B, C) and MSI in 455 samples (p=2.96e-08, p=1.08e06 and p=4.18e-07, respectively) and correlation coefficient were noted respectively as $\rho_{Spearman}$=0.26, CI 95 % (0.17, 0.35), $\rho_{Spearman}$=0.23, CI 95 % (0.14, 0.32) and $\rho_{Spearman}$=0.24, CI 95 % (0.15, 0.33)
Note: (■): Log2 (C1QC expression) and (■): High MSI Score

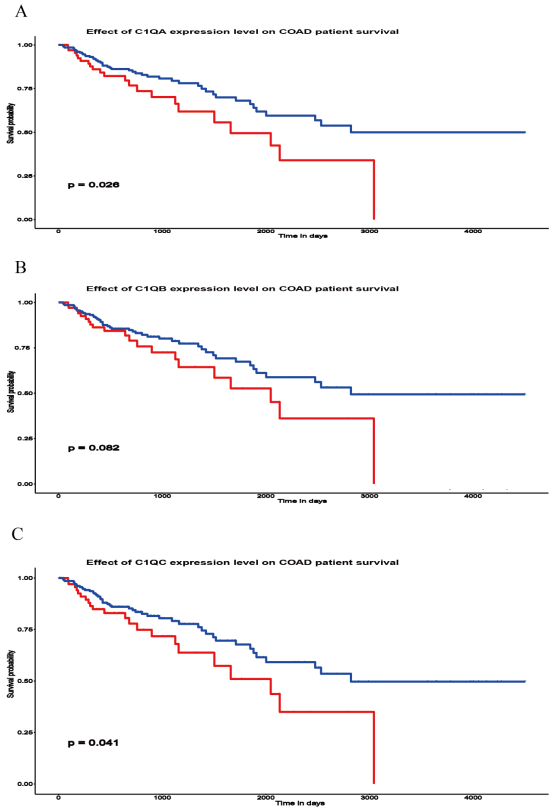

Fig. 6: Low and high expression levels of C1q (A, B, C) in 279 samples
Note: (———) High expression (n=71); (———) Low/Medium expression (n=208)

In our study, while analyzing C1q (A, B, C) expression, methylation and prognosis of CRC cancer through TCGA database and GEO database, we found the overexpression of C1q (A, B, C) in tumor tissues. At this time, the methylation levels of C1q (A, B, C) were low in tumor tissues. The prognosis of C1q with overexpression and low methylation significantly had poor prognosis than that of C1q (A, B, C) with low expression and high methylation. Subsequently, we analyzed the correlation between C1q (A, B, C) and MSI, where we found that C1q (A, B, C) and MSI are closely related to each other. Therefore, we consider C1q (A, B, C) as a novel target molecule in CRC.

C1q can synthesize matrix proteins that can promote tumor growth and metastasis in tumor microenvironment[14]. It is well known that C1q is associated with infection and autoimmunity[13]. At present, some studies indicate that C1q as a cancer-promoting factor. It can show impact on tumor biology including vascularization and metastatic spread[15].

Further, in various cancers including prostate cancer, ovarian cancer and melanoma, the expression of C1q has been proven to relate with the correlation and cancer prognosis[16]. C1q (A, B, C) overexpression plays a positive role that can prolong the Overall Survival (OS) in osteosarcoma[17]. At this time, Bandini *et al.*[18] confirmed that the overexpression of C1q (A, B, C) shows good prognosis in breast cancer which was contrary to our results. In our study, the overexpression of C1q (A, B, C) plays poor role in prognosis of CRC. However, the C1q levels are increased when compared with normal tissues and these levels are negatively associated with survival of glioblastoma multiforme[19]. In prostate cancer also, C1q plays poor role for prognosis[12].

It is well known that MSI mutation affects the prognosis of CRC. MSI detection has been widely used in clinical practice in patients with CRC, while the exploration in other tumors is still continuing[20]. MSI may also play a role in endometrial, ovarian, skin, brain and upper gastrointestinal tumors[21]. In clinical study, the results showed that the OS and Disease-Free Survival (DFS) of MSI-positive tumor patients were better and were independent of prognostic factors comparatively at different stages[22,23]. In addition, when stage I/II CRC patients were compared with MSI-High (MSI-H) patients showed worse pathological types and survival outcomes[22,23]. In patients with CRC, MSI is also a predictor of ineffective 5-fluorouracil (5-FU) based adjuvant chemotherapy[24]. In addition, other studies suggest that the level of MMR indicates the effectiveness of receiving Programmed Cell Death Protein 1 (PD-1)/PD Ligand 1 (PD-L1) immune checkpoint inhibitors[25].

In our study, we found that C1q (A, B, C) and MSI are closely related and C1q (A, B, C) can impact the prognosis of CRC. Therefore, we speculate that C1q may become a new target molecule for predicting CRC. In future, CRC tissues and cells might be useful to detect the expression of C1q and methylated tissues for validation. It is suggested that C1q can be applied in clinical practice to predict the prognosis of CRC.

Conflict of interests:

The authors declared no conflict of interest.

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