Mutations of C-Reactive Protein (CRP) -286 SNP, APC and p53 in Colorectal Cancer: Implication for a CRP-Wnt Crosstalk

Hai-Xiang Su1,2*, Hai-Hong Zhou1,2*, Ming-Yu Wang3*, Jin Cheng3, Shi-Chao Zhang3, Feng Hui3, Xue-Zhong Chen1,2, Shan-Hui Liu3, Qin-Jiang Liu1,2, Zi-Jiang Zhu1,2, Qing-Rong Hu1,2, Yi Wu3,4+, Shang-Rong Ji3*

1 Gansu Provincial Academic Institute for Medical Research, Lanzhou, P.R. China, 2 The Gansu Provincial Tumor Hospital, Lanzhou, P.R. China, 3 MOE Key Laboratory of Cell Activities and Stress Adaptations, School of Life Sciences, Lanzhou University, Lanzhou, P.R. China, 4 Key Laboratory of Preclinical Study for New Drugs of Gansu Province, Lanzhou University, Lanzhou, P.R. China

Abstract

C-reactive protein (CRP) is an established marker of inflammation with pattern-recognition receptor-like activities. Despite the close association of the serum level of CRP with the risk and prognosis of several types of cancer, it remains elusive whether CRP contributes directly to tumorigenesis or just represents a bystander marker. We have recently identified recurrent mutations at the SNP position -286 (rs3091244) in the promoter of CRP gene in several tumor types, instead suggesting that locally produced CRP is a potential driver of tumorigenesis. However, it is unknown whether the -286 site is the sole SNP position of CRP targeted for mutation and whether there is any association between CRP SNP mutations and other frequently mutated genes in tumors. Herein, we have examined the genotypes of three common CRP non-coding SNPs (rs7553007, rs1205, rs3093077) in tumor/normal sample pairs of 5 cancer types (n = 141). No recurrent somatic mutations are found at these SNP positions, indicating that the -286 SNP mutations are preferentially selected during the development of cancer. Further analysis reveals that the -286 SNP mutations of CRP tend to co-occur with mutated APC particularly in rectal cancer (p = 0.04; n = 67). By contrast, mutations of CRP and p53 or K-ras appear to be unrelated. These results underscore the functional importance of the -286 mutation of CRP in tumorigenesis and imply an interaction between CRP and Wnt signaling pathway.

On the other hand, single nucleotide polymorphisms (SNPs) that associate with genetically elevated concentrations of CRP do not confer an increased cancer risk to the general population [15]. This suggests that circulating CRP is not causally involved in tumorigenesis. Intriguingly, in contrast to the aforementioned pro-cancer activities, early studies have also documented anti-cancer actions of CRP through activation of macrophage/monocyte [16–18]. Consequently, it has been difficult to define whether CRP is solely a passive marker or an active player in cancer, or to dissect the exact contribution of CRP in tumorigenesis. Serum CRP is produced by hepatocytes of the liver; however, accumulating evidence also reveals a local production of CRP by extra-hepatic cells [3,19]. Interestingly, we have recently found that the promoter of CRP is specifically mutated at the SNP position (rs3091244) 286 bp upstream the transcription start site in 109 out of 453 tumor samples but not in the matched normal controls [19]. These mutations are associated with enhanced local
CRP induction in tumors likely via disruption of the conserved CpG methylation motif. Moreover, most of the cancer types examined harbor the -286 mutation and the fraction of the mutated allele is high (0.487, 95% CI: 0.477–0.517). These findings thus support the role of CRP produced in situ as a potential cancer driver that is probably involved in general mechanisms favoring tumorigenesis [19].

Besides the -286 SNP, there are several additional common non-coding SNPs that significantly affect the baseline levels of serum CRP. The representatives include rs7553007, rs1205, and rs3093077 [15,20,21]. It is therefore of interest whether these SNP sites are also targeted for mutation in tumors. We show here by genotyping of 141 tumor/normal sample pairs that no recurrent mutations occur at the 3 CRP SNP sites, thus highlighting that the -286 mutations are highly specific to tumorigenesis. We further examined whether there is any correlation between the -286 mutations of CRP and other frequently mutated genes in tumors. The identified association between the -286 and APC mutations implies an interaction of CRP with Wnt signaling.

### Materials and Methods

Frozen tumor/normal tissue sample pairs were obtained from the tissue bank of Gansu Provincial Tumor Hospital. Genomic DNA was isolated from tissues or blood samples using DNAiso Reagent or Blood Genome DNA Extraction Kit (Takara) according to the manufacturer’s instructions. For the identification of gene mutations, genomic DNA was amplified with specific primers (human CRP: forward: 5’-AGGGGGAGGGATAGCATTAGAA-3’; reverse: 5’-CGTCTCTGCTGGACGTATACAAG-3’; human p53: forward: 5’-CTGTCCTTCCCA- GAAACCT-3’; reverse: 5’-CTGTCCTTCCCA- GAAACCT-3’; human APC: forward: 5’-TTAACTCGCTGAAATAGCAGAATTA-3’; human K-ras: forward: 5’-ATGACTGAATA-TAAACTTGTGGTA-3’; reverse: 5’- CAACACCCTGCTGGTCTTGTT-3’; followed by sequencing.

### Table 1. Clinicopathologic features of 141 cancer patients whose tumor/normal sample pairs were genotyped.

| Allele frequencies, % | Number of patients (%) rs3091244 rs7553007 rs1205 rs3093077 |
|-----------------------|-------------------------------------------------------------|
| **N**                 | 141                                                         |
| Age                   |                                                             |
| <58 y                 | 66 (47)                                                     |
| ≥58 y                 | 75 (53)                                                     |
| Gender                |                                                             |
| Female                | 38 (27)                                                     |
| Male                  | 103 (73)                                                    |
| Tumor Stage           |                                                             |
| 0–2                   | 57 (40)                                                     |
| 3–4                   | 84 (60)                                                     |
| Chemotherapy status   |                                                             |
| Naïve                 | 113 (80)                                                    |
| Prior treatment       | 28 (20)                                                     |

DOI:10.1371/journal.pone.0102418.t001

Figure 1. Percentage of patients with somatic mutations at the indicated SNP sites in tumors. 3 CRP SNPs (rs7553007, rs1205, and rs3093077) and 21 additional SNPs of 141 tumor/normal sample pairs were genotyped by Sequenom. These samples were collected from 37 gastric, 12 lung, 27 esophagus, 24 colon and 41 rectal cancer patients. (A) The mutation frequencies at each SNP sites. None of these sites is recurrently mutated in tumors. The frequency of the CRP-286 SNP (rs3091244) mutation in these samples is shown for comparison. (B) The pooled mutation frequencies of SNPs with or without associated genes. Gene-associated SNP sites tend to exhibit lower mutation frequencies albeit without reaching statistical significance (two sample t test, two-tailed, p = 0.47).

DOI:10.1371/journal.pone.0102418.g001
rs1568645, rs929689, rs2254896, rs1951096, rs1843026, rs1543193, rs718015, rs16091) was performed by the mass spectrometry-based Sequenom service (Genergy Biotechnology, Shanghai, China). Written informed consent was obtained from patients. All patients are Chinese. The study was approved by the Ethic Committee of the Gansu Provincial Tumor Hospital.

**Results**

No recurrent somatic mutations occur at 3 common CRP SNP sites in tumors

To see whether other non-coding SNP sites of CRP are mutated in tumors, we determined the genotypes of 3 CRP common SNPs (rs7553007, rs1205, and rs3093077) together with 21 additional SNPs in 141 tumor/normal sample pairs of 5 cancer types, i.e.

Table 2. Clinicopathologic features of 35 colon cancer patients whose tumor/normal sample pairs were examined for p53 mutations.

|                         | Number of Patients (%) | Number of patients with CRP-286 mutation (%) | p* | Number of patients with p53 mutation (%) | p* |
|-------------------------|------------------------|---------------------------------------------|----|------------------------------------------|----|
| **N**                   | 35                     |                                             |    |                                          |    |
| Age                     |                        |                                             |    |                                          |    |
| <57 y                   | 17 (49)                | 9 (56)                                      | 0.51|9 (53)                                    | 0.74|
| ≥57 y                   | 18 (51)                | 7 (44)                                      | 0.64|8 (47)                                    | 1   |
| Gender                  |                        |                                             |    |                                          |    |
| Female                  | 13 (37)                | 6 (37.5)                                    | 0.64|6 (35)                                    | 0.64|
| Male                    | 22 (63)                | 10 (62.5)                                   | 0.64|11 (65)                                   | 1   |
| Tumor Stage             |                        |                                             |    |                                          |    |
| 0–2                     | 19 (54)                | 9 (56)                                      | 0.64|8 (47)                                    | 0.51|
| 3–4                     | 16 (46)                | 7 (44)                                      | 0.64|9 (53)                                    | 1   |
| Chemotherapy status     |                        |                                             |    |                                          |    |
| Naïve                   | 30 (86)                | 13 (81)                                     | 0.64|15 (88)                                   | 1   |
| Prior treatment         | 5 (14)                 | 3 (19)                                      | 0.64|2 (12)                                    | 1   |

*Fisher’s exact test, two-tailed.

doi:10.1371/journal.pone.0102418.t002
gastric, lung, esophagus, colon and rectal cancers. The frequencies of alleles associated with lower CRP levels are 48.9% for A allele of rs7553007, 47.9% for T allele of rs1205, and 79.1% for T allele of rs3093077 in normal samples (Table 1), thus providing sufficient sample sizes for detection of recurrent mutations. Therefore, we identified only 1 case of G>A mutation at rs7553007, 0 case of mutation at rs1205, and 2 cases of G>T mutations at rs3093077 in the matched tumor samples. Such a low incidence of somatic mutation was also found for 21 other examined non-coding SNP sites distributed across 9 different chromosomes (Figure 1). These indicate that, in contrast to the highly recurrent CRP-286 SNP (rs3091244) mutations [19], the 3 CRP SNP sites assayed herein are only randomly mutated in tumors at the background mutation frequency.

According to the genotyping results, the mutation frequencies of SNP sites with and without associated genes are 0.97% (95% CI: 0.35–1.59%) and 1.30% (95% CI: 0.78–1.82%), respectively. Although not statistically significant, this suggests that gene-associated SNP sites tend to be less prone to random mutation than those with unknown association, possibly due to constraints that limit damages to genomic loci with functional importance. Of the gene-associated SNPs, rs1143627 and rs4073 are two promoter SNPs that locate at 31 and 199 bp upstream of the transcription start sites of IL-1β and IL-8, respectively. Their low mutation frequencies (0.7–1.4%) argue that the promoter localization per se is not likely the cause of somatic hypermutation at the CRP-286 SNP -site in tumors; rather, the high incidence of the -286 mutation would be the result of functional consequences related to the enhanced induction of CRP, which may confer host cell clones sufficient advantage to survive and expand in the development of cancer.

CRP-286 SNP mutation is associated with mutated APC in rectal cancer

The CRP-286 SNP mutation is most prevalent in colon cancers [19], in which p53, K-ras and APC are among the most frequently mutated genes that promote tumorigenesis via distinct mechanisms [22–24]. We thus sought to examine whether there is any association between these mutation events. Mutated p53, K-ras and APC were identified by sequencing of their respective hotspot mutation regions, i.e. 301–1044 of p53, 24–442 of K-ras, and 3922–4453 of APC in cDNA sequence ranges, according to the statistics

### Table 3. Clinicopathologic features of 35 colon cancer patients whose tumor/normal sample pairs were examined for K-ras mutations.

|          | Number of Patients (%) | Number of patients with CRP-286 mutation (%) | p*   | Number of patients with K-ras mutation (%) | p*   |
|----------|------------------------|---------------------------------------------|------|--------------------------------------------|------|
| N        | 35                     |                                             |      |                                            |      |
| Age      | <56 y                  | 17 (49)                                     | 0.32 | 9 (43)                                     | 0.50 |
|          | ≥56 y                  | 18 (51)                                     | 7 (41)| 12 (57)                                    |      |
| Gender   | Female                 | 12 (34)                                     | 6 (35)| 1 (43)                                     | 0.28 |
|          | Male                   | 23 (66)                                     | 11 (65)| 12 (57)                                   |      |
| Tumor Stage | 0–2                  | 20 (57)                                     | 10 (59)| 13 (62)                                    | 0.51 |
|          | 3–4                    | 15 (43)                                     | 7 (41)| 8 (38)                                     |      |
| Chemotherapy status | Naïve              | 29 (83)                                     | 13 (76)| 20 (95)                                    | 0.03 |
|          | Prior treatment        | 6 (17)                                      | 4 (24)| 1 (5)                                      |      |

*p Fisher’s exact test, two-tailed.

### Table 4. Clinicopathologic features of 36 esophagus cancer patients whose tumor/normal sample pairs were examined for p53 mutations.

|          | Number of Patients (%) | Number of patients with CRP-286 mutation (%) | p*   | Number of patients with p53 mutation (%) | p*   |
|----------|------------------------|---------------------------------------------|------|------------------------------------------|------|
| N        | 36                     |                                             |      |                                          |      |
| Age      | <61 y                  | 16 (44)                                     | 8 (57)| 10 (59)                                   | 0.18 |
|          | ≥61 y                  | 20 (56)                                     | 6 (43)| 7 (41)                                    |      |
| Gender   | Female                 | 4 (11)                                      | 2 (14)| 3 (18)                                    | 0.33 |
|          | Male                   | 32 (89)                                     | 12 (86)| 14 (82)                                   |      |
| Tumor Stage | 0–2                  | 21 (58)                                     | 5 (36)| 10 (59)                                   | 1    |
|          | 3–4                    | 15 (42)                                     | 9 (64)| 7 (41)                                    |      |
| Chemotherapy status | Naïve              | 32 (89)                                     | 10 (71)| 16 (94)                                   | 0.61 |
|          | Prior treatment        | 4 (11)                                      | 4 (29)| 1 (6)                                     |      |

*p Fisher’s exact test, two-tailed.

* doi:10.1371/journal.pone.0102418.t003

* doi:10.1371/journal.pone.0102418.t004
of the COSMIC database. Despite their high incidences (about 50%), the CRP-286 SNP mutation shows no apparent association with mutated $p53$ ($n = 35$; Table 2 and Fig. 2A) or K-ras ($n = 35$; Table 3 and Fig. 2B). The lack of association between $p53$ and the CRP-286 SNP mutations was also confirmed in esophagus cancer ($n = 36$; Table 4 and Fig. 2C), wherein $p53$ represents the most frequently mutated gene.

By contrast, a two-fold enrichment of mutant $APC$ were observed in colon tumors with the concurrent CRP-286 SNP mutations ($n = 38$; Table 5 and Figure 3A). However, such a correlation did not reach the statistical significance probably owing to the limited sample size that we could obtain. We thus further examined 67 tumor/normal sample pairs of rectal cancer (Table 6 and Figure 3B), which is very similar to colon cancer in both the cell type origin and genomic alterations [25] showing high incidence of both $APC$ [25] and the CRP-286 SNP mutations [19]. Indeed, the co-occurrence of these two mutations in this sample set became more evident (odds ratio: 5.56, 95% CI: 1.17–26.36) and significant ($p = 0.04$). These results thus suggest that CRP and APC may cooperate in overlapping pathways during the development of colorectal cancer.

**Discussion**

The in vitro activities of CRP [3,4,6,11,13,14], including the recognition of endogenous or exogenous danger signals, regulation of complement activation, induction of proinflammatory cell responses, lead to the idea that CRP may function as a soluble pattern recognition receptor in the innate immunity and host defense [6,7]. However, the lack of consistent support by research on animal models [26–34], human subjects [35,36] and genetic epidemiology [15,20,21,37] makes it uncertain whether CRP plays any significant role in chronic inflammation in vivo or simply represents a nonspecific marker as hinted by its acute phase expression pattern. In this regard, the identification of the highly recurrent CRP-286 SNP mutations in multiple types of human cancer [19] provides a compelling evidence that this protein is a

### Table 5. Clinicopathologic features of 38 colon cancer patients whose tumor/normal sample pairs were examined for APC mutations.

|               | Number of Patients (%) | Number of patients with CRP-286 mutation (%) | $p^*$ | Number of patients with PC mutation (%) | $p^*$ |
|---------------|------------------------|---------------------------------------------|-------|----------------------------------------|-------|
| **N**         | 38                     |                                             |       |                                        |       |
| **Age**       |                         |                                             |       |                                        |       |
| $<56$ y       | 19 (50)                | 11 (61)                                     | 0.33  | 2 (20)                                 | 0.06  |
| $\geq 56$ y   | 19 (50)                | 7 (39)                                      |       |                                        |       |
| **Gender**    |                         |                                             |       |                                        |       |
| Female        | 13 (34)                | 7 (39)                                      | 0.73  | 3 (30)                                 | 1     |
| Male          | 25 (66)                | 11 (61)                                     | 7 (70) |                                        |       |
| **Tumor Stage**|                        |                                             |       |                                        |       |
| 0–2           | 23 (61)                | 11 (61)                                     | 1     | 8 (80)                                 | 0.26  |
| 3–4           | 15 (39)                | 7 (39)                                      |       |                                        |       |
| **Chemotherapy status** |          |                                             |       |                                        |       |
| Naïve         | 31 (82)                | 14 (78)                                     | 0.69  | 7 (70)                                 | 0.35  |
| Prior treatment | 7 (18)              | 4 (22)                                      |       |                                        |       |

$^*$Fisher’s exact test, two-tailed.

doi:10.1371/journal.pone.0102418.t005
potential driver of tumorigenesis and a core component of the regulatory network of inflammation.

Promoter mutations in TERT [38,39] and CRP [19] constitute the first examples that non-coding regulatory regions can also be targeted to promote tumorigenesis by modulating the expression instead of the activities of key genes. However, it is somewhat unique in case of CRP that the mutation occurs at a common SNP site. This raises the concern whether SNP sites are generically more vulnerable to genetic alterations, leading to the high incidence of passenger mutations. To address this concern, we genotyped 24 SNPs of 141 tumor/normal sample pairs. These SNPs are located on 9 distinct chromosomes, and consist of 3 SNPs of CRP, 2 promoter SNPs of inflammatory cytokines, 1 SNP of a non-coding gene, 18 SNPs with unknown association. Despite that, all of the SNP sites were found to be mutated in tumors with only low background frequency. Therefore, the highly recurrent mutation at the CRP-286 SNP site is most likely the result of the selection by cancer development, but not simply due to general properties associated with SNP site or genomic location. It is, however, still possible that the -286 mutation is just a consequence of tumorigenesis and further functional assays are required to clarify this point.

Nonetheless, it is intriguing that although the 4 examined CRP SNPs all affect the serum level of CRP, only the -286 SNP is targeted by tumorigenesis. This would suggest that the effects of the other 3 SNPs are secondary to the -286 SNP, which may in part be explained by the dependence of CRP expression on promoter CpG methylation, an essential epigenetic mechanism in gene silencing [40]. Indeed, we have recently shown that high CRP expression is correlated with low promoter methylation, and vice versa [19]. Of the 5 CpG motifs in CRP promoter, the evolutionarily conserved -286 CpG appears to be the key, particularly for extrahepatic cell types, in determining the basal level of CRP expression [19]. As the majority of the -286 mutations are C>A/T transitions that disrupt the methylation motif, it is conceivable that such genetic alterations will in turn contribute to switching on the promoter activity of CRP likely via lowering the inhibitory methylation signal and facilitating the binding of transcription factors to the underlying E-box sequence [41]. These may eventually allow the subsequent participation of distal regulatory elements containing the other CRP SNPs.

The high recurrence and pervasiveness of the CRP-286 SNP mutations in tumors suggest that locally produced CRP, instead of circulating CRP, drives the development of cancer. This paradox may be explained by the tight dependence of the actions of CRP on inflammatory microenvironments [3,13,14,36]. Circulating CRP is produced by the liver as a pentamer primarily showing anti-inflammatory activities [7,42,43]. Besides hepatocytes, extra-hepatic cells are also able to secrete CRP locally in response to inflammatory stimuli. Moreover, triggers enriched in inflammatory loci will induce prompt conformation changes in the pentameric CRP post its in situ production [44–49], to release the full potential in ligand binding [47,48,50], complement regulation [46,48,51–54] and stimulation of proinflammatory and angiogenic cell responses [48,49,55–61]. As such, the local abundance of CRP and its interactions with the stressful microenvironment should be more relevant to disease progression; while circulating CRP levels mainly mirror the underlying inflammatory status.

The dysregulation of Wnt signaling pathway is the most frequent event observed in colorectal cancer, which is usually manifested by inactivating mutations of APC or activating mutations of β-catenin [25]. One direct consequence of APC inactivation is the stabilization of β-catenin and the aberrant activation of the downstream target genes [62]. It is therefore of interest that CRP has been shown to be a target of β-catenin [63]. Moreover, our results reveal that the CRP-286 SNP mutations tend to co-occur with mutant APC in colon and rectal tumors. These would imply that the two secretory molecules, i.e. CRP and Wnt, may act in feed-back and cooperative manners to promote tumorigenesis, which deserves further investigations. Given the aberrantly activated Wnt signaling and highly induced CRP expression in tumors, topical targeting both molecules may be a potential option for colorectal cancer therapy.

**Acknowledgments**

We thank Mr. Jing Zhao for his excellent technical assistance.

**Author Contributions**

Conceived and designed the experiments: SRJ YW HXS. Performed the experiments: HXS HHZ MYW JC SCZ FH SHL. Analyzed the data: SRJ...

---

**Table 6.** Clinicopathologic features of 67 rectal cancer patients whose tumor/normal sample pairs were examined for APC mutations.

|                       | Number of Patients (%) | Number of patients with CRP-286 mutation (%) | p* | Number of patients with APC mutation (%) | p* |
|-----------------------|------------------------|---------------------------------------------|----|----------------------------------------|----|
| N                     | 67                     |                                             |    |                                        |    |
| Age                   |                        |                                             |    |                                        |    |
| <58 y                 | 31 (46)                | 3 (37.5)                                    | 0.72 | 7 (54)                                 | 0.56 |
| ≥58 y                 | 36 (54)                | 5 (62.5)                                    | 0.43 | 7 (54)                                 | 0.12 |
| Gender                |                        |                                             |    |                                        |    |
| Female                | 23 (34)                | 4 (50)                                      | 1   | 4 (31)                                 | 0.22 |
| Male                  | 44 (66)                | 4 (50)                                      |    | 6 (46)                                 |    |
| Tumor Stage           |                        |                                             |    |                                        |    |
| 0–2                   | 32 (48)                | 4 (50)                                      |    | 1                                      | 0.19 |
| 3–4                   | 35 (52)                | 4 (50)                                      |    | 9 (69)                                 | 1   |
| Chemotherapy status   |                        |                                             |    |                                        |    |
| Naïve                 | 50 (75)                | 4 (50)                                      |    | 10 (77)                                | 1   |
| Prior treatment       | 17 (25)                | 4 (50)                                      |    | 3 (23)                                 |    |

*Fisher’s exact test, two-tailed.

doi:10.1371/journal.pone.0102418.t006
References

1. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144: 646–674.
2. Grivennikov SI, Greten FR, Karin M (2010) Inflammation, immunity, and cancer. Cell 140: 883–899.
3. Ma X, Ji SR, Wu Y (2013) Regulated conformation changes in C-reactive protein exert angiogenic effects in atherosclerosis. Chinese Sci Bull 58: 1642–1656.
4. Pepys MB, Hirschfeld GM (2000) C-reactive protein: a critical update. J Clin Invest 111: 1805–1812.
5. Allin KH, Nordestgaard BG (2011) Elevated C-reactive protein in the diagnosis, prognosis, and cause of cancer. Crit Rev Clin Lab Sci 48: 155–170.
6. Bottazzi R, Doni A, Garlenda C, Mantovani A (2010) An integrated view of humoral innate immunity: pentraxins as a paradigm. Am Rev Respir Dis 180: 157–183.
7. Du Clos TW (2013) Pentraxins: Structure, Function, and Role in Inflammation. Springer; ISBN 1-4612-6151-3.
8. Yang J, Weizenman M, Zhang X, Lin P, Wang M, et al. (2007) Human C-reactive protein binds activating Fcgamma receptors and protects myeloma tumor cells from apoptosis. Cancer Cell 12: 252–265.
9. Kim ES, Cho Y, Ham M, Jung J, Kim SG, et al. (2013) Inflammatory lipid sphingosine-1-phosphate upregulates C-reactive protein via C/EBPbeta and potentiates breast cancer progression. Oncogene.
10. Turu MM, Stevin M, Matos S, West D, Hopewell JC, et al. (2009) Genetic and environmental interactions determine CRP levels: a Mendelian randomization study. Nat Genet 41: 1391–1397.
11. Wang MY, Ji SR, Bai CJ, El Kebir D, Li HY, et al. (2011) A redox switch in C-reactive protein modulates classic complement activation on necrotic cells. FASEB J 25: 232–242.
12. Gershov D, Kim S, Elkon KB (2000) C-Reactive protein binds to cell membranes and cell membranes associated signalling pathways and gene expression. BMC Cell Biol 9: 47.
13. Mold C, Gewurz H, Du Clos TW (1999) Regulation of complement activation by C-reactive protein. Immunopharmacology 42: 23–30.
14. Greizen E, Kim D, Kottke E, and Knot K (2005) C-reactive protein binds to apoptotic cells, protects the cells from assembly of the terminal complement components, and sustains an antiinflammatory innate immune response: implications for systemic autoimmunity. J Exp Med 192: 1353–1364.
15. Singh SK, Suresh MV, Valeri R, Agrawal A (2008) The connection between C-reactive protein and atherosclerosis. Ann Med 40: 110–120.
16. Eisenhardt SU, Hahsberger J, Peter K (2009) Monomeric C-reactive protein generation on activated platelets: the missing link between inflammation and atherosclerosis. Trends Cardiovasc Med 19: 232–237.
17. Allin KH, Nordestgaard BG, Zacho J, Tybjerg-Hansen A, Bojesen SE (2010) C-reactive protein and the risk of cancer: a mendelian randomization study. J Natl Cancer Inst 102: 202–209.
18. Barna BP, Deodhar KD (1987) Activation of human monocyte monocordial activity by C-reactive protein. Cancer Res 47: 3959–3963.
19. Barna BP, Deodhar SD, Gaultam S, Yen-Lieberman B, Roberts D (1984) Macrophage activation and generation of tumouricidal activity by liposome-associated human C-reactive protein. Cancer Res 44: 953–961.
20. Deodhar SD, James K, Deodhar KD (1982) Inhibition of human lymphocyte mitogenic activity by C-reactive protein. Cancer Res 42: 5084–5089.
21. Wang MY, Zhou HH, Zhang SC, Hu F, Zhu W, et al. (2014) Recurrent mutations at C-reactive protein gene promoter SNP position -286 in human cancers. Cell Res 24: 505–508.
22. Zacho J, Tybjerg-Hansen A, Jensen JS, Grande P, Sillehus E, et al. (2000) Genetically elevated C-reactive protein and ischamic vascular disease. N Engl J Med 348: 1907–1917.
23. Elliott P, Chambers JC, Zhang W, Clarke R, Hopewell JC, et al. (2009) Genetic loci associated with C-reactive protein levels and risk of coronary heart disease. JAMA 302: 37–48.
24. Armaghany T, Wilson JD, Chu Q, Mills G (2012) Genetic alterations in colorectal cancer. Gastrointest Cancer Res 5: 19–27.
25. Feuer EJ (2011) Molecular genetics of colorectal cancer. Annu Rev Pathol 6: 479–567.
26. Goel A, Boland CR (2010) Recent insights into the pathogenesis of colorectal cancer. Curr Opin Gastroenterol 26: 47–52.
27. Cancer Genome Atlas (2012) Characterization of human colon and rectal cancer. Nature 479: 350–357.
28. Paul A, Ko KW, Li L, Yecheov Y, McCroy MA, et al. (2004) C-reactive protein accelerates the progression of atherosclerosis in apolipoprotein E-deficient mice. Circulation 109: 647–653.
29. Hirschfeld GM, Gallimore JR, Kahn MC, Hutchison WL, Sabim CA, et al. (2005) Transgenic human C-reactive protein is not proatherogenic in apolipoprotein E-deficient mice. Proc Natl Acad Sci U S A 102: 8309–8314.
30. Kovacs A, Tornvall P, Nilsson R, Tegner J, Hamsten A, et al. (2007) Human C-reactive protein does not accelerate atherosclerosis development in a mouse model with human-like hypercholesterolemia. Proc Natl Acad Sci U S A 104: 13768–13773.
31. Teupser D, Weber O, Rao TN, Saa K, Thiery J, et al. (2011) No reduction of atherosclerosis in C-reactive protein (CRP)-deficient mice. J Biol Chem 286: 6372–6379.
32. Carlucci F, Cook HT, Garg A, Pepys MB, Bottoni M (2010) Lack of effect of a single injection of human C-reactive protein on murine lupus or nephrotic nephritis. Arthritis Rheum 62: 245–249.
56. Khreiss T, Jozsef L, Potempa LA, Filep JG (2005) Loss of pentameric symmetry in C-reactive protein induces interleukin-8 secretion through peroxynitrite signaling in human neutrophils. Circ Res 97: 690–697.

57. Molins B, Pena E, Vilahur G, Mendieta C, Slevin M, et al. (2008) C-Reactive Protein Isoforms Differ in Their Effects on Thrombus Growth. Arterioscler Thromb Vasc Biol 28: 2239–2246.

58. Ji SR, Ma L, Bai CJ, Shi JM, Li HY, et al. (2009) Monomeric C-reactive protein activates endothelial cells via interaction with lipid raft microdomains. FASEB J 23: 1806–1816.

59. Slevin M, Matou-Nasri S, Turu M, Luque A, Rovira N, et al. (2010) Modified C-reactive protein is expressed by stroke neovessels and is a potent activator of angiogenesis in vitro. Brain Pathol 20: 151–163.

60. Boncler M, Rysanik J, Szymanski J, Potempa LA, Rychlik B, et al. (2011) Modified C-reactive protein interacts with platelet glycoprotein Ibalpha. Pharmacol Rep 63: 464–475.

61. Li HY, Wang J, Wu YX, Zhang L, Liu ZP, et al. (2014) Topological localization of monomeric C-reactive protein determines pro-inflammatory endothelial cell responses. J Biol Chem 289: 14283–14290.

62. Clevers H, Nusse R (2012) Wnt/beta-catenin signaling and disease. Cell 149: 1192–1205.

63. Choi YS, Hur J, Jeong S (2007) Beta-catenin binds to the downstream region and regulates the expression C-reactive protein gene. Nucleic Acids Res 35: 5511–5519.