Circulating vitamin D binding protein, total, free and bioavailable 25-hydroxyvitamin D and risk of colorectal cancer

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Epidemiological investigation have suggested that there is a significantly inverse association between circulating 25-hydroxyvitamin D (25(OH)D) and the risk for developing colorectal cancer (CRC) in humans. However, little is known about the role of vitamin D binding protein (VDBP) in colorectal carcinogenesis. Blood samples were collected from 212 CRC patients and 212 controls matched with age, gender and blood collection time. We used logistic regression to calculate the odds ratios and 95% confidence intervals for further estimation of the association of the quartiles of VDBP, total, free and bioavailable 25(OH)D with CRC risk. The results revealed that there was no significant association between circulating VDBP concentrations and CRC in the present study, and that a negative association existed between total 25(OH)D and the risk of CRC, which was unchanged after adjustment for VDBP. Higher levels of free and bioavailable 25(OH)D were significantly associated with decreased risk of CRC. After stratifying by VDBP, high levels of total, free and bioavailable 25(OH)D were associated significantly with decreased CRC risk among participants with circulating VDBP below the median. These findings indicate that VDBP is not directly associated with the risk of CRC, but it modulates circulating free and bioavailable 25(OH)D concentration.

Colorectal cancer (CRC) is the third most prevalent cancer and the fourth most common cause of cancer mortality worldwide1. Accumulating epidemiological studies suggest that high vitamin D intake may influence the risk of CRC2,3, which is an interesting cancer prevention strategy that has raised public concerns globally. Although vitamin D can be obtained from diet and dietary supplements, most vitamin D is synthesized by human skin exposed to the sunlight. Due to reduced outdoor activity, the absence of adequate sun exposure and limited resource of vitamin D supplements from diet, vitamin D deficiency is a common phenomenon both in healthy individuals and in CRC cases4.

25-hydroxyvitamin D (25(OH)D), the precursor of the active form of vitamin D, is recognized as the optimal indicator of vitamin D metabolic status with a relatively long half-life and high concentration in plasma5. 25(OH)D is commonly bound to vitamin D binding protein (VDBP) and albumin(Alb), which can be converted into hormonally active 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) in the kidney, colon and several other tissues6–9. The majority of 25(OH)D and 1,25(OH)2D3 are primarily bound to VDBP, approximately 10–15% to Alb, free 25(OH)D and 1,25(OH)2D3 only account for less than 1%10. Since the affinity of Alb to 25(OH)D or 1,25(OH)2D3 is weaker than that of VDBP, the loosely binding fraction and the free fraction consist of bioavailable 25(OH)D11. Vitamin D receptor (VDR), a key nuclear receptor, can only be activated by the free form of 1,25(OH)2D3, and regulates the transcription and expression of numerous vitamin D targeted genes that are related to cell proliferation, differentiation, invasion and angiogenesis.

VDBP, which is synthesized and secreted primarily by the liver, includes three functional domains. Although 25(OH)D and 1,25(OH)2D3 are mainly bound to VDBP domain I, circulating VDBP and Alb levels considerably exceed the concentrations of 25(OH)D and 1,25(OH)2D312. VDBP also plays an important role in the elimination of dead or injured cell, macrophage activation and neutrophil chemotaxis in inflammatory condition12. As inflammation is deemed to be an important contributor to carcinogenesis, while inflammatory-cells such as...
macrophage and neutrophil are the main components to defense against cancer cell\(^1\), we thus speculate that VDBP may be involved in carcinogenesis and cancer progression.

Recently, several studies have reported the relationship among VDBP, 25(OH)D and the risk of cancer. Two studies showed that circulating VDBP was inversely associated with the risk of pancreatic cancer and renal cell carcinoma\(^4,6\). Another study reported no association between VDBP and bladder cancer\(^6\). In addition, study by Stephanie et al\(^7\) indicated that VDBP might modulate the impact of vitamin D status on prostate cancer. However, there is no study reporting the influence of VDBP on the association between circulating total, free, and bioavailable 25(OH)D and the risk of CRC. Therefore, in this study, we measured the circulating VDBP, total, free and bioavailable 25(OH)D in 212 CRC patients and 212 well-matched healthy controls and further explored the relationship between total, free, bioavailable 25(OH)D and VDBP, and the risk of CRC.

**Methods**

All subjects in our study were from a health assessment cohort population in Nanjing First Hospital (Nanjing, Jiangsu, China). The health assessment cohort population comprised 25616 routine physical examination of individuals who were performed annually at Nanjing First Hospital from 2010 to 2012. All participants were healthy, cancer-free individuals, didn’t show clinical CRC symptoms, rectal digital examination and fecal occult blood test were normal and tumor protein biomarkers (plasma CEA, CA199, CA50 and CA242) were lower than the reference values when they were enrolled in the cohort, with an average follow-up time of 3.4 years until diagnosis. The case group consisted of 212 CRC patients, including 115 colon and 97 rectal cancer patients, and ranging in age from 37 to 83 years. All CRC patients were all initially diagnosed by colonoscopy and CT detections, and confirmed with histological evidence from biopsies performed from 2013 to 2014 in Nanjing First Hospital. Patients with acute or chronic renal and liver failures, nutritional problem (malnutrition, anorexia and inappetence), active infection, or without histological confirmation were excluded from the present study. The controls were composed of 212 blood collections from healthy individuals with matched gender, age (±5 year) and blood collection time. Due to unwillingness of the controls and its invasiveness, colonoscopy was not used to exclude the CRC in the controls. However, all the controls were clinical symptom-free of CRC, rectal digital examination, fecal occult blood test and tumor protein biomarkers (plasma CEA, CA199, CA50 and CA242) were normal. All participants enrolled in our study did not use vitamin D or calcium supplement and drugs that might influence circulating calcium, phosphorus and 25(OH)D concentrations in recent six months. Relative clinical data were obtained from the hospital medical record. Blood samples of enrolled subjects in our study were collected during the first time health check-up in Nanjing First Hospital between November and February from 2010 to 2012. Samples were drawn between 8:00 and 10:00 each morning, and plasma samples were stored at \(-80\) °C until measurement. This study was approved by the Institution Ethics Commission of Southeast University, written informed consents were obtained from all participants and the methods were carried out in accordance with the approved guidelines.

**Results**

A total of 424 individuals including 212 newly diagnosed CRC patients and 212 healthy participants matched with gender and age (±5 year) as well as blood collection time were enrolled in this study. Baseline characteristics of cases and controls were described in Table 1. No significant difference was found in BMI value, frequencies of smoking, drinking, diabetes and hypertension as well as concentration of circulating VDBP when compared with two groups. However, the plasma total, free and bioavailable 25(OH)D in CRC cases were significantly lower in comparison with the controls (p-value \(< 0.001, = 0.018\) and 0.001).

The results of associations among plasma VDBP, total, free and bioavailable 25(OH)D and the risk of CRC were listed in Table 2. Plasma VDBP concentration was not observed to be directly associated with the risk of CRC, further adjustment for 25(OH)D did not alter the quartile risk estimates (p-trend = 0.944, OR = 0.93, 95%CI = 0.51–1.69 for Q4:Q1). Adjustment for VDBP did not alter the inverse association between plasma total 25(OH)D and the risk of CRC (p-trend = 0.027, OR = 0.53, 95%CI = 0.29–0.98 for Q4:Q1). Moreover, negative associations were also observed between free 25(OH)D (p-trend < 0.001, OR = 0.42, 95%CI = 0.22–0.82 for Q4: Q1), bioavailable 25(OH)D (p-trend = 0.006, OR = 0.29, 95%CI = 0.15–0.56 for Q4: Q1) and the risk of CRC. No significant risk association was found between VDBP and CRC stratifying by 25(OH)D (p-interaction = 0.280, p-trend = 0.490, OR = 0.72, 95%CI = 0.33–1.57). On the contrary, plasma VDBP modified the association between total, free and bioavailable 25(OH)D and CRC, with lower risk for elevated total, free and bioavailable 25(OH)D appeared to be restricted to individuals with lower concentrations of VDBP (total 25(OH)D Q4:Q1 p-interaction = 0.001, p-trend < 0.001, OR = 0.14, 95%CI = 0.05–0.41; free 25(OH)D Q4:Q1 p-interaction = 0.001, p-trend < 0.001, OR = 0.10, 95%CI = 0.04–0.22; bioavailable 25(OH)D Q4:Q1 p-interaction = 0.001, p-trend < 0.001, OR = 0.03, 95%CI = 0.01–0.08) (Table 3).

**Discussion**

In this study, we found strong inverse associations between total, free and bioavailable 25(OH)D and the risk of CRC. However, we did not find a direct association between circulating VDBP and the risk of CRC. Interestingly, higher circulating total, free and bioavailable 25(OH)D concentrations were inversely associated with CRC only when circulating VDBP below the median. Our results suggest that higher VDBP level may sequester more 25(OH)D leading to less free and bioavailable 25(OH)D, and free and bioavailable 25(OH)D may be more biologically relevant to the risk of CRC in comparison with total 25(OH)D.

VDBP, a glycosylated-globulin, plays an important role not only in transporting vitamin D and circulating metabolites, but also in other biological functions to affect carcinogenesis. It is a member of the circulating actin scavenger system that prevents formation of F-actin and biological functions to affect carcinogenesis. It is a member of the
Weinstein et al. indicated that circulating 25(OH)D was inversely associated with CRC and DBP did not modify the association between 25(OH)D and CRC. Consistent with these studies, our results did not conclude that VDBP was associated with CRC, but low levels of total, free and bioavailable 25(OH)D were associated with elevated CRC risk, whereas the negative associations between total, free bioavailable 25(OH)D and CRC were found only when individual with lower level of VDBP (<median). These findings indicate that VDBP is not involved in colorectal carcinogenesis through such mechanisms, but support the “free hormone hypothesis” that lower concentration of VDBP may bind less 25(OH)D contributing to higher level of circulating free and bioavailable 25(OH)D, while free and bioavailable 25(OH)D have more biological effects on colorectal tissue. It has been reported that 1,25(OH)2D3 and its analogues could inhibit growth and promote apoptosis, proliferation and differentiation of colon cancer cell in a dose-dependent manner. 25(OH)D can be transformed into 1,25(OH)2D3 in colorectal cell and forms a 1,25(OH)2D3-VDR complex to regulate a series of genes that are involved in colorectal carcinogenesis. Moreover, VDBP may only have its canonical role in transporting 25(OH)D, not directly relating to colorectal carcinogenesis. Furthermore, previous studies have indicated that VDBP was markedly deglycosylated in CRC patients. A higher level of deglycosylated VDBP results in a lower level of GcMAF. Consequently, eliminated GcMAF cannot perform its biological activities to macrophages and phagocytic activation, anti-angiogenesis effect and anti-tumor activity. In addition, more than 120 single nucleotide polymorphisms were identified in VDBP gene, rs7041 and rs4588 has been demonstrated to influence the affinity of VDBP for VD and were associated with the concentration of 25(OH)D, VDBP who carried allele C of rs4588 has been illustrated to be less able to convert 25(OH)D to 1,25(OH)2D3. VDBP gene may affect its secondary construction and function, eliminating its functions of bound to VDBP by interaction of VDBP with megalin and cubulin. The metabolite of 1,25(OH)2D3 binds to VDR is expressed in colorectal cell and forms a 1,25(OH)2D3-VDR complex to regulate a series of genes that are involved in colorectal carcinogenesis. Therefore, genetic variation of VDBP gene may affect its secondary construction and function, eliminating its functions of antitumorigenesis.

| Table 1 | Baseline characteristics of colorectal cancer patients and controls

| Characteristics | Cases (n = 212) | Controls (n = 212) | p-value
|----------------|----------------|--------------------|------------------|
| Age (years)    | Median (range) | 63 (56–73)         | 63 (57–73)       | 0.958 |
| BMI (kg/m²)    | Median (range) | 22.6 (20.8–23.8)   | 23.2 (22.0–24.4) | 0.122 |
| Smoking (%)    | Yes            | 50 (23.6%)         | 64 (30.2%)       | 0.125 |
| Drinking (%)   | Yes            | 25 (11.8%)         | 33 (15.6%)       | 0.258 |
| Diabetes (%)   | Yes            | 187 (88.2%)        | 179 (84.4%)      | 0.312 |
| Hypertension (%)| Yes            | 181 (85.4%)        | 188 (88.7%)      | 0.334 |
| VDBP (ug/ml)   | Quartile 1     | 212.27 (168.59–285.60) | 215.74 (172.36–288.15) | 0.271 |
| Plasma 25(OH)D ng/ml | Quartile 2 | 9.71 (5.70–17.73) | 14.6 (7.28–28.84) | <0.001 |
| Free 25(OH)D (pg/ml) | Quartile 3 | 0.022 (0.013–0.042) | 0.033 (0.014–0.062) | 0.018 |
| Bioavailable 25(OH)D (pg/ml) | Quartile 4 | 1.25 (0.72–2.57) | 2.02 (0.88–3.69) | 0.001 |

| Table 2 | Odds ratios and 95% confidence interval for the association of plasma VDBP, total, free and bioavailable 25(OH)D with the risk of colorectal cancer

| Quartile | VDBP, range (ug/ml) | OR (95%CI) | OR (95%CI) | OR (95%CI) | p-trend |
|----------|---------------------|------------|------------|------------|---------|
| Quartile 1 | <168.59 | 1 | 0.79(0.46–1.38) | 0.82(0.71–0.95) | 1.48(0.77–2.85) | <0.001 |
| Quartile 2 | 168.59–212.27 | 48/53 | 0.75(0.44–1.30) | 0.77(0.44–1.34) | 0.95(0.53–1.69) | 0.45–1.35 |
| Quartile 3 | 212.27–285.60 | 49/53 | 0.78(0.45–1.35) | 0.83(0.47–1.46) | 0.93(0.51–1.69) | 0.615 |
| Quartile 4 | 285.60–<285.60 | 51/53 | 0.69(0.42–1.16) | 0.72(0.42–1.20) | 0.53(0.29–0.98) | 0.494 |

| Total 25(OH)D, range (ng/ml) | OR (95%CI) | OR (95%CI) | OR (95%CI) | p-trend |
|-------------------------------|------------|------------|------------|---------|
| Quartile 1 | 168.59–212.27 | 80/53 | 0.65(0.38–1.11) | 0.67(0.39–1.16) | 0.62(0.35–1.12) | <0.001 |
| Quartile 2 | 212.27–285.60 | 49/53 | 0.62(0.36–1.07) | 0.63(0.36–1.09) | 0.67(0.38–1.20) | 0.027 |
| Quartile 3 | 285.60–<285.60 | 46/53 | 0.67(0.36–1.09) | 0.63(0.36–1.09) | 0.53(0.29–0.98) | 0.027 |
| Quartile 4 | 285.60–<285.60 | 37/53 | 0.60(0.33–1.07) | 0.60(0.33–1.07) | 0.53(0.29–0.98) | 0.027 |

| Free 25(OH)D, range (pg/ml) | OR (95%CI) | OR (95%CI) | OR (95%CI) | p-trend |
|-----------------------------|------------|------------|------------|---------|
| Quartile 1 | <0.0136 | 58/53 | 0.136–0.0329 | 0.0329–0.062 | 0.062 |
| Quartile 2 | 0.0136–<0.0329 | 82/53 | 0.91(0.52–1.58) | 0.91(0.52–1.58) | 0.032 |
| Quartile 3 | 0.0329–<0.062 | 49/53 | 0.42(0.22–0.79) | 0.42(0.22–0.79) | 0.001 |
| Quartile 4 | 0.062–<0.062 | 23/53 | 0.42(0.02–0.82) | 0.42(0.02–0.82) | 0.001 |

| Bioavailable 25(OH)D, range (pg/ml) | OR (95%CI) | OR (95%CI) | OR (95%CI) | p-trend |
|-------------------------------------|------------|------------|------------|---------|
| Quartile 1 | <0.88 | 73/53 | 0.088–0.20 | 0.088–0.20 | 0.369 |
| Quartile 2 | 0.88–<2.02 | 59/53 | 0.84(0.49–1.43) | 0.84(0.49–1.43) | 0.30(0.16–0.56) | 0.007 |
| Quartile 3 | 2.02–<3.69 | 60/53 | 0.84(0.49–1.43) | 0.84(0.49–1.43) | 0.29(0.15–0.56) | 0.006 |

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; OR, odds ratio; VDBP, vitamin D binding protein.

**Condition on age and gender.**

**Condition on age and gender, and adjusted for BMI, smoking, drinking, history of diabetes and hypertension.**

**Condition on age and gender, and adjusted for BMI, smoking, drinking, history of diabetes and hypertension as well as either total 25(OH)D or VDBP.**
Table 3 | Odds ratios and 95% confidence interval for the association between plasma VDBP stratifying by total 25(OH)D or total, free and bioavailable 25(OH)D stratifying by VDBP and the risk of colorectal cancer

| VDBP, range (μg/ml) | Quartile1 | Quartile2 | Quartile3 | Quartile4 | p_trend | p_interaction |
|---------------------|-----------|-----------|-----------|-----------|---------|--------------|
| 25(OH)D above median |           |           |           |           |         |              |
| No. of cases/no. of controls | 10/19 | 28/37 | 20/25 | 25/25 |         |              |
| ORa (95%CI) | 1 | 1.63(0.62-4.30) | 1.37(0.49-3.81) | 1.84(0.64-5.26) | 0.360 |         |              |
| ORb (95%CI) | 1 | 1.64(0.61-4.41) | 1.49(0.51-4.30) | 2.28(0.68-7.56) | 0.413 |         |              |
| ORc (95%CI) | 1 | 1.78(0.60-5.32) | 1.76(0.58-5.38) | 2.27(0.66-7.88) | 0.211 | 0.280 |              |
| 25(OH)D below median |           |           |           |           |         |              |
| No. of cases/no. of controls | 54/34 | 20/16 | 29/28 | 26/28 |         |              |
| ORa (95%CI) | 1 | 0.82(0.36-1.86) | 0.65(0.32-1.30) | 0.60(0.29-1.21) | 0.163 |         |              |
| ORb (95%CI) | 1 | 0.81(0.35-1.91) | 0.66(0.33-1.35) | 0.65(0.31-1.35) | 0.197 |         |              |
| ORc (95%CI) | 1 | 1.17(0.46-2.98) | 0.79(0.37-1.66) | 0.72(0.33-1.57) | 0.490 |         |              |
| Total 25(OH)D, range (ng/ml) | <7.29 | 7.29 - <14.61 | 14.61 - <28.84 | ≥28.84 |         |              |
| VDBP above median |           |           |           |           |         |              |
| No. of cases/no. of controls | 25/31 | 30/25 | 18/23 | 27/27 |         |              |
| ORa (95%CI) | 1 | 1.41(0.65-3.08) | 1.17(0.47-2.93) | 1.24(0.55-2.78) | 0.983 |         |              |
| ORb (95%CI) | 1 | 1.42(0.65-3.12) | 1.04(0.41-2.66) | 1.27(0.59-2.94) | 0.946 |         |              |
| ORc (95%CI) | 1 | 1.96(0.80-4.79) | 1.38(0.48-3.95) | 1.67(0.68-4.13) | 0.624 | 0.001 |              |
| VDBP below median |           |           |           |           |         |              |
| No. of cases/no. of controls | 55/22 | 19/28 | 28/30 | 10/26 |         |              |
| ORa (95%CI) | 1 | 0.29(0.13-0.64) | 0.36(0.17-0.75) | 0.15(0.06-0.39) | <0.001 |         |              |
| ORb (95%CI) | 1 | 0.30(0.13-0.69) | 0.35(0.16-0.77) | 0.13(0.05-0.35) | <0.001 |         |              |
| ORc (95%CI) | 1 | 0.31(0.14-0.71) | 0.37(0.16-0.86) | 0.14(0.05-0.41) | <0.001 |         |              |
| Free 25(OH)D, range (ng/ml) | <0.0136 | 0.0136 - <0.0329 | 0.0329 - <0.062 | ≥0.062 |         |              |
| VDBP above median |           |           |           |           |         |              |
| No. of cases/no. of controls | 22/33 | 45/37 | 21/22 | 12/14 |         |              |
| ORa (95%CI) | 1 | 1.56(0.82-2.97) | 1.46(0.76-2.83) | 1.76(0.86-3.61) | 0.477 |         |              |
| ORb (95%CI) | 1 | 1.47(0.76-2.85) | 1.63(0.77-3.46) | 2.13(0.87-5.24) | 0.492 | 0.001 |              |
| VDBP below median |           |           |           |           |         |              |
| No. of cases/no. of controls | 36/20 | 37/16 | 28/31 | 11/39 |         |              |
| ORa (95%CI) | 1 | 1.32(0.66-2.65) | 0.44(0.23-0.84) | 0.12(0.06-0.25) | <0.001 |         |              |
| ORb (95%CI) | 1 | 1.59(0.76-3.33) | 0.49(0.25-0.97) | 0.10(0.04-0.22) | <0.001 |         |              |
| Bioavailable 25(OH)D, range (ng/ml) | <0.88 | 0.88 - <2.02 | 2.02 - <3.69 | ≥3.69 |         |              |
| VDBP above median |           |           |           |           |         |              |
| No. of cases/no. of controls | 31/40 | 29/30 | 31/24 | 9/12 |         |              |
| ORa (95%CI) | 1 | 1.10(0.59-2.05) | 1.62(0.91-2.88) | 1.14(0.58-2.22) | 0.541 |         |              |
| ORb (95%CI) | 1 | 1.05(0.53-2.00) | 1.60(0.84-3.03) | 1.13(0.52-2.47) | 0.647 | 0.001 |              |
| VDBP below median |           |           |           |           |         |              |
| No. of cases/no. of controls | 42/13 | 30/23 | 29/29 | 11/41 |         |              |
| ORa (95%CI) | 1 | 0.41(0.20-0.85) | 0.27(0.13-0.56) | 0.04(0.02-0.10) | <0.001 |         |              |
| ORb (95%CI) | 1 | 0.50(0.23-1.09) | 0.35(0.16-0.77) | 0.03(0.01-0.08) | <0.001 |         |              |

*aConditioned on age and gender.
*bConditioned on age and gender, and adjusted for BMI, smoking, drinking, history of diabetes and hypertension.
*cConditioned on age and gender, and adjusted for BMI, smoking, drinking, history of diabetes and hypertension as well as either total 25(OH)D or VDBP.

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; OR, odds ratio; VDBP, vitamin D binding protein.

To our knowledge, it is the first investigation studying the association of plasma VDBP, free and bioavailable 25(OH)D with the risk of CRC. Our prospective measurement of circulating VDBP, Alb, 25(OH)D to assess the association of VDBP, total, free and bioavailable 25(OH)D with CRC risk is a strength of our study. Moreover, all plasma samples were collected between 8:00 and 10:00 each morning in winter season in Nanjing first hospital, which could maximize reduce the influence from sunlight, intensity of ultraviolet ray, and diurnal and postprandial fluctuations. However, several limitations of this study should be addressed. Due to limited available plasma samples, small sample size of our study could not represented the whole population and would reduce statistical power in our study. Although VDBP was supposed to be stable in adulthood, each plasma sample of the individual was only collected and measured at one point, which could not reflect the comprehensive status of total circulating vitamin D and DBP in all participants. Furthermore, we did not obtain detailed information concerning multiple potential confounding factors of each participant, such as physical activity, dietary vitamin intake and exposure to sunshine.

In summary, although we did not observe a direct association between VDBP and the risk of CRC, high levels of circulating total, free, bioavailable 25(OH)D were associated with a decreased risk of CRC, particularly when VDBP concentration was below the median. These findings suggest that VDBP is just a VD transporter which may not affect colorectal carcinogenesis, but can modulate the impact of vitamin D status on CRC. Thus the simultaneous detection and investigation of plasma total, free, bioavailable 25(OH)D and VDBP may be important for studying the relationship between vitamin D and the risk of CRC.
7. Cross, H. S., Peterlik, M., Reddy, G. S. & Schuster, I. Vitamin D metabolism in human colon adenocarcinoma-derived Caco-2 cells: expression of 25-hydroxyvitamin D3-1alpha-hydroxylase activity and regulation of side-chain metabolism. *J Steroid Biochem Mol Biol* 62, 21–28 (1997).

8. Bareis, P., Bises, G., Bischof, M. G., Cross, H. S. & Peterlik, M. 25-hydroxy-vitamin D metabolism in human colon cancer cells during tumor progression. *Biochem Biophys Res Commun* 285, 1012–1017 (2001).

9. Tangričević, V. et al. 25-hydroxyvitamin D-1alpha-hydroxylase in normal and malignant colon tissue. *Lancet* 357, 1673–1674 (2001).

10. Bilde, D. D. et al. Assessment of the free fraction of 25-hydroxyvitamin D in serum and its regulation by albumin and the vitamin D-binding protein. *J Clin Endocrinol Metab* 63, 954–959 (1986).

11. Brown, A. J. & Coyne, D. W. Bioavailable vitamin D in chronic kidney disease. *Kidney Int* 82, 5–7 (2012).

12. Chishimba, L., Thickett, D. R., Stockley, R. A. & Wood, A. M. The vitamin D axis and its regulation by albumin and the vitamin D-binding protein. *J BIol Chem* 279, 53328–53327 (2004).

13. Anic, G. M., Steinweis, S. J., Moya, K. A., Mannisto, S. & Albanes, D. Vitamin D-binding protein, circulating vitamin D and risk of renal cell carcinoma. *Int J Cancer* 134, 2699–2706 (2014).

14. Mondul, A. M., Steinweis, S. J., Virtamo, J. & Albanes, D. Influence of vitamin D-binding protein on the association between circulating vitamin D and risk of bladder cancer. *Br J Cancer* 107, 1589–1594 (2012).

15. Wei, S. J. et al. Circulating 25-hydroxyvitamin D, vitamin D-binding protein and risk of prostate cancer. *Int J Cancer* 132, 2940–2947 (2013).

16. Bhan, I. et al. Bioavailable vitamin D is more tightly linked to mineral metabolism than total vitamin D in incident hemodialysis patients. *Kidney Int* 82, 84–92 (2012).

17. Zhang, J. & Kreis, R. R. Identification of a region in the vitamin D-binding protein that mediates its C5a chemotactic cofactor function. *J Biol Chem* 279, 18070–18078 (2004).

18. Anic, G. M., Steinweis, S. J., Mondul, A. M., Mannisto, S. & Albanes, D. Serum vitamin D, vitamin D-binding protein, and risk of colorectal cancer. *Plos One* 9, e102966 (2014).

19. Steinweis, S. J. et al. Serum 25-hydroxyvitamin D, vitamin D-binding protein and risk of colorectal cancer in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. *Int J Cancer*, doi:10.1002/ijc.29157 (2014).

20. Diaz, D. G., Paraskeva, C., Thomas, M. G., Binderup, L. & Hague, A. Apoptosis is induced by the active metabolite of vitamin D3 and its analogue EB1089 in colorectal adenocarcinoma-derived Caco-2 cells: possible implications for prevention and therapy. *Cancer Res* 60, 2304–2312 (2000).

21. Zhao, X. & Feldman, D. Regulation of vitamin D receptor abundance and responsiveness during differentiation of HT-29 human colon cancer cells. *Endocrinology* 132, 1808–1814 (1993).

22. Nykjaer, A. et al. An endocytic pathway essential for renal uptake and activation of the steroid 25-(OH) vitamin D3. *Cell* 96, 507–515 (1999).

23. Nykjaer, A. et al. Cubulin dysfunction causes abnormal metabolism of the sterol hormone 25(OH) vitamin D(3). *Proc Natl Acad Sci USA* 98, 13895–13900 (2001).

24. Cross, H. S. et al. 25-Hydroxyvitamin D(3)-1alpha-hydroxylase and vitamin D receptor gene expression in human colonic mucosa is elevated during early cancerogenesis. *Stoerids* 66, 287–292 (2001).

25. Peng, X. et al. Regulation of CYP24 splicing by 1,25-dihydroxyvitamin D(3) in human colon cancer cells. *Endocrinology* 212, 207–215 (2012).

26. Rehder, D. S., Nelson, R. W. & Borges, C. R. Glycosylation status of vitamin D binding protein in cancer patients. *Protein Sci* 18, 2036–2042 (2009).

27. Yamamoto, N., Suyama, H., Nakazato, H. & Koga, Y. Immunotherapy of metastatic colorectal cancer with vitamin D-binding protein-derived macrophage-activating factor, GcMAF. *Cancer Immunol Immunother* 57, 1007–1016 (2008).

28. Nagasawa, H., Sasaki, H., Uto, Y., Kubo, S. & Hori, H. Association of the macrophage activating factor (MAF) precursor activity with polymorphism in vitamin D-binding protein. *Anticancer Res* 24, 3361–3366 (2004).

29. Kanda, S., Mochiraki, Y., Miyata, Y., Kanetake, H. & Yamamoto, N. Effects of vitamin D(3)-binding protein-derived macrophage activating factor (GcMAF) on angiogenesis. *J Natl Cancer Inst* 94, 1311–1319 (2002).

30. Koga, Y., Naraparaju, V. R. & Yamamoto, N. Antitumor effect of vitamin D-binding protein-derived macrophage activating factor on Ehrlich ascites tumor-bearing mice. *Proc Soc Exp Biol Med* 220, 20–26 (1999).

31. Nonaka, K. et al. Vitamin D binding protein-macrophage activating factor inhibits HCC in SCID mice. *J Surg Res* 172, 116–122 (2012).

32. Speeckaert, M., Huang, G., Delanghe, J. R. & Taes, Y. E. Biological and clinical aspects of the vitamin D binding protein (Gg-globulin) and its polymorphism. *Clin Chim Acta* 372, 33–42 (2006).

33. Yamamoto, N. & Homma, S. Vitamin D3 binding protein (group-specific component) is a precursor for the macrophage-activating signal factor from lysophosphatidylcholine-treated lymphocytes. *Proc Natl Acad Sci U S A* 88, 8539–8543 (1991).

34. Sonderman, J. S., Munro, H. M., Blot, W. J. & Signorello, L. B. Reproducibility of serum 25-hydroxyvitamin d and vitamin D-binding protein levels over time in a prospective cohort study of black and white adults. *Am J Epidemiol* 176, 615–621 (2012).

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### Author contributions

H.Q.Y. performed the laboratory detection, wrote the manuscript. H.L.S. performed the laboratory detection. B.S.H. and Y.Q.P. collected the blood sample and clinical data. F.W. and H.Q.Y. performed the laboratory detection, wrote the manuscript. H.L.S. performed the study and reviewed and approved the manuscript.

### Additional information

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