# Diabetes associated with abnormal p53 immunohistochemical patterns in colorectal cancer

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## Abstract

# Background

Previous study has suggested a link between diabetes and colorectal cancer (CRC), but the specific molecule for the link has not been well-understood. Abnormal p53 immunohistochemical (IHC) pattern is an accurate predictor for TP53 gene mutation. The present study aimed to investigate the relationship between type 2 diabetes mellitus (T2DM) and p53 IHC patterns in CRC.

# Methods

We analyzed p53 protein expression of 742 cases of CRC with radical colectomy by immunohistochemistry. The patients were grouped into subsets of non-diabetes (n = 570) and diabetes (n = 172), and further divided into subgroups of 1 normal p53 IHC pattern (p53 wild type or WT) and 3 abnormal p53 IHC patterns which included heterogeneous pattern (HT), overexpression (OE) and complete absence (CA).

# Results

The ratios of p53 abnormal pattern in groups of T2DM and non-T2DM were 70.9% and 50.9% ($P < 0.001$). Univariately, groups of both T2DM and prediabetes (FPG: 6.1 ~ 6.9 mmol/L) were significantly associated with abnormal p53 pattern, compared with normal FPG control ($P < 0.05$ and $P < 0.001$). Moreover, T2DM was significantly associated with abnormal p53 patterns in cases with microsatellite instability (MSI) stable (MSS)/MSI-low phenotype ($P < 0.001$) and distal colon/rectum location, but not in cases with MSI-high phenotype and proximal colon location ($P > 0.05$). Multivariate analysis retained the above significance. Furthermore, abnormal p53 IHC patterns were positively associated with risk of lymph node metastasis and high tumor-node-metastasis (TNM) stage of CRC, which suggested a link between the abnormal p53 IHC patterns and aggressive clinical outcome.

# Conclusion

Diabetes is associated with risk of abnormal p53 IHC patterns in CRC. It was suggested that diabetes might influence carcinogenesis, progression and prognosis via inducing TP53 mutation and abnormal p53 expression in CRC.

## Introduction

Epidemiology has suggested a link between diabetes and colorectal cancer (CRC) [1, 2], but the special molecule for the link has not been well-understood. P53 protein is a tumor suppressor encoded by TP53 gene. As one of the most characteristic alterations in the conventional colorectal adenoma-carcinoma

pathway [3], TP53 mutation was found in about 43% cases of sporadic CRC (IARC TP53 database; https://p53.iarc.fr). Wild-type p53 protein maintains genome stability by arresting cell cycle and inducing apoptosis of damaged cells, while mutant p53 protein promotes carcinogenesis and progression of cancer via dominant-negative mechanism or gain-of-function [4]. Wild type p53 protein is maintained at low level within cells by its interaction with E3 ubiquitin ligase MDM2 which mediates degradation of p53 [5].

Abnormal p53 immunohistochemical (IHC) pattern is surrogate for TP53 mutation [6–8]. Generally, wild-type p53 can be detected in non-neoplastic tissue such as fibroblasts and lymphocytes with a scattered nuclear positive pattern, which acts as a perfect internal control. Abnormal accumulation of p53 protein or complete loss of immunoreactivity of p53 due to TP53 mutation leads to abnormal p53 IHC pattern [9]. Mutant p53 is stabilized in cancer cells, while cancer cells carrying mutant p53 acquire selective advantages for clone expansion and finally become the dominant cell population [10], which lead to overexpression (OE) or complete absence (CA) of p53 IHC patterns. The main p53 IHC patterns contain 1 normal pattern or wild type (WT) pattern, and 3 abnormal patterns including OE, CA and heterogeneous pattern (HT) [9, 11].

Diabetes is a metabolic disorder and a component of metabolic syndrome (MetS). Recent research indicated sera levels of tumor-associated anti-p53 antibody were significantly increased in patients with diabetes or diabetic patients with cancer in comparison with non-diabetes control [12]. In the present study, we aimed to investigate the relationship between type 2 diabetes mellitus (T2DM) and p53 IHC patterns in order to reveal the role of specific molecule in the link between diabetes and CRC.

## Patients and Methods

### 1. Ethics

The study was reviewed and approved by the Ethics Committee of Zhejiang Provincial People's Hospital (KY2019012). Anonymous clinical data was used in the study.

### 2. Patients

The study included cases of 742 primary CRC (all were adenocarcinoma) with radical colectomy from 2015 to 2020 in Zhejiang Provincial People's Hospital. Cases with preoperative chemotherapy and/or radiotherapy were excluded in the study. The basic clinical data including gender, age, weight, height, blood pressure (or hypertension history), fasting plasma glucose (FPG) or diabetes history, fasting plasma triglycerides (TG), and fasting plasma high-density lipoprotein cholesterol (HDL), etc, were retrospectively collected from the electronic medical records. Body mass index (BMI) values of 18 cases were unavailable. Pathological parameters including invasion depth (T stage), lymph node (LN) status and Tumor-node-metastasis (TNM) stage of the patients were acquired from the pathological reports and/or medical records.

### 3. Immunohistochemistry

Monoclonal antibody for p53 (D0-7, Cat: ZM0408), MLH1, PMS2, MSH2, and MSH6 were purchased from Beijing Zhongshan Golden Bridge Biotechnology (Beijing, China). Archived paraffin-embed specimens of CRC were sectioned and stained by EnVison method. Appropriate positive and negative controls were run concurrently for all the markers tested.

P53 IHC patterns were classified 4 subgroups[9]: (a) WT: scattered strong and/or moderate nuclear staining of neoplastic cells within background of weak and/or negative staining tumor cells (positive tumor cells < 20% in most cases and rarely > 50%); (b) OE: majority of the tumor cells (> 60%, virtually almost 100% of the tumor cells in most cases) are diffuse and strong nuclear positive; (c) CA: the tumor cells are complete absent for nuclear staining, but the internal controls such as fibroblasts and lymphocytes are scattered positive; (d) HT: admixture of WT pattern and abnormal pattern (commonly OE and occasionally CA). Cases with p53 cytoplasmic staining were excluded from the study due to very small sample size (n = 9). To reduce the interobserver variability, the p53 IHC patterns were independently evaluated by 2 pathologists who did not previously know the clinical condition such as diabetic status. If there is disagreement for a specific case, the third pathologist would perform the evaluation. The final classifications of p53 patterns of the cases were determined according the agreement of at least 2 pathologists.

Microsatellite instability (MSI) status of the cases were classified into 2 subgroups [13]: (a) MSI unstable (MSI-H): anyone of the 4 mismatch repair (MMR) proteins (MLH1, PMS2, MSH2 and MSH6) was totally nuclear negative for cancer cells, while the internal control such as normal mucosal epithelial cells, fibroblasts and lymphocytes was positive; (b) MSI stable (MSS) or MSI low (MSI-L): all the 4 MMR proteins were positive.

4. Criteria for T2DM and prediabetes

T2DM was determined according the following criteria: (a) Patients who have been previously diagnosed as T2DM and documented in medical record before the diagnosis of CRC; (b) The patients do not have a diabetic history but met the criteria for diabetes of American diabetes Association (ADA) [14]: FPG ≥ 7.0 mmol/L or 2-h plasma glucose (PG) ≥ 11.1 mmol/L during oral glucose tolerance test or Hemoglobin A1C≥ 6.5%. Medical conditions such as dextrose and corticosteroids treatment which may lead to elevated blood glucose levels were excluded. Prediabetes was defined as FPG in the range of 6.1 ~ 6.9 mmol/L [15].

5. Criteria for MetS and its individual component

MetS was defined as the presence of any 3 or more of the following 4 factors according the criteria of China diabetes Society (CDS) [16]: (a) Central obesity: BMI ≥ 25.0 kg/$m^2$; (b) Hyperglycemia: FPG ≥ 6.1 mmol/L or diagnosed as T2DM previously; (c) Dyslipidemia: hypertriglyceridemia (fasting plasma TG ≥ 1.7 mmol/L) and/or low HDL (fasting plasma HDL < 0.9 mmol/L for men or < 1.0 mmol/L for women); (d) Hypertension: systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg and/or previously diagnosed as hypertension and under antihypertensive drug administration.

6. Tumor invasion

Tumor invasion (or pT-stage) was recorded according to American Joint Cancer Committee (AJCC) (8th edition) and further divided into subgroups of deep invasion (pT-stage 3 ~ 4) and non-deep invasion (pT-stage 1 ~ 2) [17].

7. Tumor stage

Tumor stages were recorded according to TNM staging described in AJCC and further classified into subgroups of low TNM stage (AJCC stage I ~ II) and high TNM stage (AJCC stage III ~ IV) in the present study [17].

8. Statistical analysis

Age was expressed as mean ± standard deviation (SD) and analyzed by T-test. Categorized data were calculated as frequency or percentage and analyzed by Chi-square test. Multinominal or binary logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) of p53 IHC patterns in association with diabetes and other individual component of MetS, as well as pathological features. Two-sided value of $P < 0.05$ was considered statistically significant. All statistical analysis was performed with SPSS software version 19.0 (IBM Corp., Armonk, NY).

# Results

1. Baseline characteristics

As showed in Table 1, the mean age of the patients was 64.8 years-old (23–95 years). There are 570 cases of non-diabetes and 172 cases of T2DM (43 ~ 91 years). Among the 172 cases of diabetes, 130 cases were previously diagnosed as T2DM, while 42 cases met the criteria of T2DM (FPG ≥ 7.0 mmol/L, n = 31, hemoglobin A1C ≥ 6.5%, n = 11).

Table 1
Basic characteristics according p53 IHC patterns of colorectal cancer

| Characteristic | P53 IHC pattern | | Total | *P* |
|---|---|---|---|---|
| | WT (%) | Abnormal (%) | | |
| Age (mean ± s) | 65.5 ± 13.8 | 64.3 ± 11.4 | 64.8 ± 12.5 | 0.221 |
| Gender | | | | |
| Femal | 133 (40.3) | 177 (43.0) | 310 | 0.465 |
| Male | 197 (59.7) | 235 (57.0) | 432 | |
| MetS | | | | |
| No | 280 (84.8) | 305 (74.0) | 585 | < 0.001 |
| Yes | 50 (15.2) | 107 (26.0) | 157 | |
| BMI ≥ 25 kg/m$^2$ | | | | |
| No | 246 (76.4) | 286 (71.1) | 532 | 0.110 |
| Yes | 76 (23.6) | 116 (28.9) | 192 | |
| Hypertension | | | | |
| No | 168 (50.9) | 187 (45.4) | 355 | 0.135 |
| Yes | 162 (49.1) | 225 (54.6) | 387 | |
| TG > 1.7 mmol/L | | | | |
| No | 265 (80.3) | 311 (75.5) | 576 | 0.116 |
| Yes | 65 (19.7) | 101 (24.5) | 166 | |
| Low-HDL | | | | |
| No | 211 (63.9) | 288 (69.9) | 499 | 0.086 |
| Yes | 119 (36.1) | 124 (30.1) | 243 | |
| FPG | | | | |
| < 6.1 mmol/L | 261 (79.1) | 254 (61.7) | 515 | < 0.001 |
| 6.1 ~ 6.9 mmol/L | 19 (5.8) | 36 (8.7) | 55 | |
| ≥7.0 mmol/L or T2DM | 50 (15.1) | 122 (29.6) | 172 | |
| T2DM | | | | |

Statistics: Chi-square test; MSS/MSI-L: microsatellite instability (MSI) stable/MSI low; MSI-H: MSI high. FPG: fasting plasma glucose; T2DM: type 2 diabetes.

| Characteristic | P53 IHC pattern | | Total | *P* |
|---|---|---|---|---|
| | WT (%) | Abnormal (%) | | |
| No | 280 (84.8) | 290 (70.4) | 570 | < 0.001 |
| Yes | 50 (15.2) | 122 (29.6) | 172 | |
| Tumor Location | | | | |
| Proximal colon | 199 (60.3) | 314 (76.2) | 513 | < 0.001 |
| Distal colon/rectum | 131 (39.7) | 98 (23.8) | 229 | |
| MSI status | | | | |
| MSS/MSI-L | 259 (78.5) | 373 (90.5) | 632 | < 0.001 |
| MSI-H | 71 (21.5) | 39 (9.5) | 110 | |
| Statistics: Chi-square test; MSS/MSI-L: microsatellite instability (MSI) stable/MSI low; MSI-H: MSI high. FPG: fasting plasma glucose; T2DM: type 2 diabetes. | | | | |

The distribution of normal and abnormal p53 patterns were remarkably different according to MetS status, PFG, T2DM, tumor location, and MSI status ($P < 0.001$), but not significantly different according to age, gender, BMI value, hypertension, hypertriglyceridemia and low HDL in CRC ($P > 0.05$) (Table 1).

Four p53 IHC patterns were observed in CRC, which included WT (Fig. 1a), HT (Fig. 1b), OE (Fig. 1c) and CA (Fig. 1d). Among the 3 abnormal p53 patterns, OE was the most frequent form which accounted for 58.1% (240/413), while CA and HT accounted for 24.0% (99/413) and 17.9% (74/413).

2. Association between p53 IHC patterns and diabetes or prediabetes in CRC

To determine the relationship between diabetic status and abnormal p53 IHC patterns, we assayed the association between T2DM (or prediabetes) and p53 IHC patterns. As indicated in Fig. 2, the ratio of abnormal p53 IHC pattern in T2DM group was remarkably higher than that of non-T2DM group (70.9% vs. 50.9%, $P < 0.001$). The ratio of abnormal p53 IHC pattern in either prediabetes group or T2DM group was also remarkably higher than that of normal FPG group (65.5% or 70.9% vs. 49.3%, $P < 0.001$).

Univariately, both groups of T2DM (OR = 2.51, 95% CI: 1.73–3.64, P < 0.001) and prediabetes (OR = 2.06, 95% CI: 1.14–3.73, $P = 0.016$) were significantly associated with abnormal p53 patterns in comparison with normoglycemic group (Table 2). Multivariate analysis retained the above significance. The results suggested that hyperglycemia in diabetic or prediabetic status was an independent risk factor for abnormal p53 patterns in CRC.

Table 2
Association among abnormal p53 IHC patterns and FPG levels or individual component of metabolic syndrome in colorectal cancer

| Factors | Variables | Univariable | | Multivariable | |
|---|---|---|---|---|---|
| | | OR (95% CI) | *P* | OR (95% CI) | *P* |
| AP-p53 | FPG < 6.1 | Reference | | 1 | |
| | FPG: 6.1 ~ 6.9 | 2.06 (1.14–3.73) | 0.016 | 2.08 (1.12–3.84) | 0.020 |
| | Hypertension | | | 1.18 (0.81–1.72) | 0.380 |
| | BMI > 25 | | | 1.08 (0.71–1.62) | 0.728 |
| | TG ≥ 1.7 | | | 1.04 (0.68–1.58) | 0.861 |
| | Low HDL | | | 0.64 (0.44–0.94) | 0.021 |
| | Male | | | 0.90 (0.64–1.27) | 0.548 |
| | Age | | | 0.99 (0.98–1.01) | 0.281 |
| AP-p53 | FPG < 6.1 | Reference | | 1 | |
| | FPG ≥ 7.0 or T2DM | 2.51 (1.73–3.64) | < 0.001 | 2.62 (1.77–3.89) | < 0.001 |
| | Hypertension | | | 1.10 (0.78–1.56) | 0.584 |
| | BMI ≥ 25 | | | 0.99 (0.69–1.44) | 0.974 |
| | TG ≥ 1.7 | | | 1.22 (0.83–1.79) | 0.323 |
| | Low HDL | | | 0.69 (0.49–0.97) | 0.031 |
| | Male | | | 0.95 (0.69–1.31) | 0.776 |
| | Age | | | 0.99 (0.98-1.00) | 0.142 |

Statistics: Binominal logistic regression; AP-p53: abnormal p53 IHC pattern including heterogeneous pattern, overexpression and complete absence.

3. Association between diabetes and p53 IHC patterns in CRC with different MSI status and tumor location

To determine whether the association between p53 IHC patterns and diabetes was influenced by MSI status and tumor location, we assessed the associations between p53 patterns and diabetes in different subgroups with different MSI phenotypes or tumor locations. Ratio of abnormal p53 patterns in MSI-H group was remarkably higher than that of MSS/MSI-L group (64.5% vs. 41.0%, $P < 0.01$) (Table 1), while ratio of WT p53 pattern in distal colon/rectum location was remarkably lower than that of proximal colon group (38.8% vs. 57.2%, $P < 0.01$),

As showed in Table 3, T2DM were significantly associated with abnormal p53 IHC patterns in cases with MSS/MSI-L phenotype (OR = 2.42, 95% CI: 1.61–3.63, $P < 0.001$), distal colon/rectum location (OR = 2.58, 95% CI: 1.63–4.09, $P < 0.001$), and in overall patients (OR = 2.36, 95% CI: 1.63–3.40, $P < 0.001$), compared with non-T2DM. The above significant association was also detected in multivariate analysis. However, the above associations between diabetes and p53 IHC patterns were not detected in groups with MSI-H phenotype ($P > 0.05$) and proximal colon location ($P > 0.05$). The results suggested that the association between diabetes and abnormal p53 IHC patterns was stronger in cases with MSS/MSI-L phenotype or distal colon/rectum location than corresponding cases with MSI-H phenotype or proximal colon location.

Table 3
Association among abnormal p53 IHC patterns and diabetes or individual components of metabolic syndrome in colorectal cancer with different MSI status and location

| Factors (Subgroups) | Variables | Univariable | | Multivariable | |
|---|---|---|---|---|---|
| | | OR (95% CI) | *P* | OR (95% CI) | *P* |
| AP-p53 (MSI-H) | T2DM | 1.88 (0.72–4.93) | 0.199 | 1.88 (0.62–5.72) | 0.263 |
| | Hypertension | 1.85 (0.84–4.09) | 0.126 | 2.38 (0.84–6.73) | 0.102 |
| | BMI ≥ 25 | 0.72 (0.29–1.79) | 0.485 | 0.61 (0.22–1.66) | 0.336 |
| | TG ≥ 1.7 | 1.35 (0.55–3.30) | 0.509 | 1.19 (0.44–3.21) | 0.725 |
| | Low-HDL | 0.49 (0.22–1.10) | 0.082 | 0.39 (0.16–0.96) | 0.041 |
| AP-p53 (MMS, MSI-L) | T2DM | 2.42 (1.61–3.63) | < 0.001 | 2.43 (1.58–3.71) | < 0.001 |
| | Hypertension | 1.15 (0.84–1.59) | 0.376 | 1.10 (0.77–1.57) | 0.613 |
| | BMI ≥ 25 | 1.49 (1.03–2.17) | 0.036 | 1.31 (0.89–1.94) | 0.171 |
| | TG ≥ 1.7 | 1.36 (0.92–2.02) | 0.121 | 1.25 (0.83–1.88) | 0.277 |
| | Low-HDL | 0.90 (0.64–1.27) | 0.540 | 0.80 (0.56–1.15) | 0.228 |
| AP-p53 (Distal colon) | T2DM | 2.58 (1.63–4.09) | < 0.001 | 2.75 (1.70–4.47) | < 0.001 |
| | Hypertension | 1.24 (0.87–1.78) | 0.229 | 1.19 (0.79–1.80) | 0.395 |
| | BMI ≥ 25 | 1.15 (0.76–1.74) | 0.502 | 1.03 (0.67–1.58) | 0.890 |
| | TG ≥ 1.7 | 1.15 (0.75–1.77) | 0.516 | 1.12 (0.72–1.74) | 0.627 |
| | Low-HDL | 0.65 (0.45–0.96) | 0.030 | 0.56 (0.37–0.83) | 0.004 |
| AP-p53 (Proximal colon/rectum) | T2DM | 1.89 (0.99–3.61) | 0.054 | 1.75 (0.86–3.57) | 0.123 |

Statistics: Multinomial logistic regression; AP-p53: abnormal p53 IHC pattern.

| Factors (Subgroups) | Variables | Univariable | | Multivariable | |
|---|---|---|---|---|---|
| | | OR (95% CI) | *P* | OR (95% CI) | *P* |
| | Hypertension | 1.09 (0.65–1.84) | 0.746 | 0.88 (0.86–3.57) | 0.682 |
| | BMI ≥ 25 | 1.84 (1.01–3.34) | 0.047 | 1.56 (0.80–3.01) | 0.189 |
| | TG ≥ 1.7 | 1.79 (0.95–3.36) | 0.072 | 1.61 (0.82–3.18) | 0.169 |
| | Low-HDL | 1.18 (0.69–2.03) | 0.545 | 1.05 (0.58–1.91) | 0.875 |
| AP-p53 (Overall) | T2DM | 2.36 (1.63–3.40) | < 0.001 | 2.42 (1.64–3.56) | < 0.001 |
| | Hypertension | 1.25 (0.93–1.67) | 0.135 | 1.20 (0.86–1.67) | 0.278 |
| | BMI ≥ 25 | 1.31 (0.94–1.84) | 0.112 | 1.15 (0.81–1.63) | 0.432 |
| | TG ≥ 1.7 | 1.32 (0.93–1.88) | 0.118 | 1.23 (0.85–1.77) | 0.274 |
| | Low-HDL | 0.76 (0.56–1.04) | 0.086 | 0.67 (0.48–0.93) | 0.016 |
| Statistics: Multinomial logistic regression; AP-p53: abnormal p53 IHC pattern. | | | | | |

4. Association between diabetes and special p53 IHC pattern in CRC

To determine whether T2DM was associated with special abnormal p53 IHC pattern, we further analyzed the relationship between diabetes and p53 IHC patterns by multinominal logistic regression analysis. As showed in Table 4, univariately, T2DM was significantly associated with p53 patterns of HT (OR = 3.68, 95% CI: 2.11–6.43, $P < 0.001$), OE (OR = 1.99, 95% CI: 1.31–3.03, $P < 0.001$), and CA (OR = 2.73, 95% CI: 1.62–4.59, $P < 0.001$) in comparison with WT p53 pattern. Multivariate analysis including hypertension, high BMI, hypertriglyceridemia, low HDL, gender and age as covariate, retained the above significance. Interestingly, low HDL was found to be negatively associated with HT p53 pattern (OR = 0.50, 95% CI: 0.27–0.90, $P = 0.021$) and OE p53 pattern (OR = 0.67, 95% CI: 0.46–0.97, $P = 0.034$) in multivariate analysis. Other factors including hypertension, high BMI and hypertriglyceridemia were not significantly associated with abnormal p53 IHC patterns ($P > 0.05$). The data suggested that T2DM was an independent risk factor for all the 3 abnormal p53 patterns in CRC.

Table 4
Association among special p53 IHC pattern and type 2 diabetes or individual components of metabolic syndrome in colorectal cancer

| p53 patterns | Variables | Univariate (n = 742) | | Multivariate (n = 742) | |
|---|---|---|---|---|---|
| | | OR (95% CI) | *P* | OR (95% CI) | *P* |
| WT | Reference | 1 | | 1 | |
| HT | T2DM | 3.68 (2.11–6.43) | < 0.001 | 3.97 (2.19–7.21) | < 0.001 |
| | Hypertension | | | 0.81 (0.45–1.44) | 0.471 |
| | BMI ≥ 25 | | | 1.24 (0.69–2.24) | 0.474 |
| | TG ≥ 1.7 | | | 1.59 (0.88–2.88) | 0.126 |
| | Low-HDL | | | 0.50 (0.27–0.90) | 0.021 |
| | Male | | | 0.50 (0.29–0.85) | 0.010 |
| | Age | | | 1.00 (0.97–1.02 | 0.776 |
| OE | T2DM | 1.99 (1.31–3.03) | 0.001 | 2.01 (1.29–3.13) | 0.002 |
| | Hypertension | | | 1.30 (0.89–1.91) | 0.174 |
| | BMI ≥ 25 | | | 1.26 (0.85–1.86) | 0.249 |
| | TG ≥ 1.7 | | | 1.30 (0.86–1.95) | 0.214 |
| | Low-HDL | | | 0.67 (0.46–0.97) | 0.034 |
| | Male | | | 1.16 (0.81–1.66) | 0.410 |
| | Age | | | 0.98 (0.97-1.00) | 0.022 |
| CA | T2DM | 2.73 (1.62–4.59) | < 0.001 | 2.81 (1.62–4.85) | < 0.001 |
| | Hypertension | | | 1.35 (0.81–2.25) | 0.253 |
| | BMI ≥ 25 | | | 0.81 (0.46–1.41) | 0.457 |
| | TG ≥ 1.7 | | | 0.81 (0.45–1.46) | 0.481 |
| | Low-HDL | | | 0.78 (0.47–1.27) | 0.313 |
| | Male | | | 0.81 (0.51–1.29) | 0.372 |
| | Age | | | 0.99 (0.97–1.01) | 0.306 |

Statistics: Multinomial logistic regression and WT p53 pattern as conference; HT: heterogeneous pattern; OE: overexpression; CA: complete absence.

5. Association between p53 IHC patterns and pathological features of CRC.

To determine whether the abnormal p53 IHC patterns were associated with adverse pathological features of CRC, we examined the relationship between p53 IHC patterns and pathological features including tumor invasion, LN status and TNM stage. As showed in Table 5, the distribution of p53 IHC patterns was remarkably different among LN status and TNM stage ($P < 0.001$), but not tumor invasion ($P > 0.05$). As indicated in Fig. 3, the rate of abnormal p53 pattern in group with LN positive was higher than that of LN negative group (64.6% vs. 35.4%, $P < 0.001$), while the rate of abnormal p53 pattern in group with high TNM stage was higher than that of low TNM stage (64.4% vs. 35.6%, $P < 0.001$). The data demonstrated that the abnormal p53 patterns of CRC were link to unfavorable clinical outcome in the present study.

Table 5
Association between p53 IHC patterns and pathological features in colorectal cancer

| Factors | Total (%) | p53 pattern (n = 742) | | | | *P* |
|---|---|---|---|---|---|---|
| | | WT (%) | HT (%) | OE (%) | CA (%) | |
| Tumor invasion | | | | | | |
| T1 ~ T2 | 174 (23.5) | 83 (25.2) | 17 (23.0) | 54 (22.5) | 20 (20.2) | 0.728 |
| T3 ~ T4 | 568 (76.5) | 246 (74.8) | 57 (77.0) | 186 (77.5) | 79 (79.8) | |
| LN metastasis | | | | | | |
| No | 426 (57.4) | 217 (66.0) | 40 (54.1) | 122 (50.8) | 47 (47.5) | < 0.001 |
| Yes | 316 (42.6) | 112 (34.0) | 34 (45.9) | 118 (49.2) | 52 (52.5) | |
| TNM stage | | | | | | |
| Low | 416 (56.1) | 213 (64.7) | 38 (51.4) | 120 (50.0) | 45 (45.5) | < 0.001 |
| High | 326 (43.9) | 116 (35.3) | 36 (48.6) | 120 (50.0) | 54 (54.5) | |
| Statistics: Chi-square test. | | | | | | |

# Discussion

Extensive researches have been performed to clarify the link between diabetes and cancers. Majority of TP53 mutations are missense mutations resulted from single residue replacements of DNA-binding domain[18]. TP53 missense mutation with “gain-of-function” leads to p53 abnormal accumulation and p53 OE pattern is highly suggestive for TP53 missense mutation. Similarly, p53 CA pattern is a predictor for functional loss of wild type p53 protein due to nonsense or truncation mutation of TP53.

Hyperglycemia is characteristic of T2M. Accumulation of mutant p53 protein was observed in diabetic mice in comparison with normal control during oral oncogenesis [19]. High level glucose was showed to promote mutagenesis in human lymphoblastoid cells [20]. Mutant p53 protein level was downregulated via dietary glucose restriction [21]. Our present results demonstrated that either diabetes or prediabetes

was positively associated with risk of abnormal p53 patterns in CRC. The above reports and our present results support the notion that diabetes/hyperglycemia might be a causal factor for TP53 mutation.

Site-specific difference of CRC risk among people with T2DM has been reported previously [22]. TP53 mutation was observed in distal colon and rectal tumors at higher frequencies, while it was observed in proximal tumors at lower frequencies[23]. Our present results indicated that the abnormal p53 patterns were also positively associated with distal colon/rectum location in CRC. Moreover, T2DM patients with colon/rectum tumor location were associated with a higher risk of abnormal p53 IHC patterns in comparison with non-T2DM patients. However, the above associations were not obvious in patients with proximal colon tumor location.

P53 expressions were also associated with MSI status of CRC [24]. CRC with MSI-H phenotype were linked to WT TP53 gene[23]. Our present results also showed that the ratio of p53 WT pattern in MSI-H group is remarkably higher than that of MSS/MSI-L group. The positive association between T2DM and abnormal p53 patterns was also detected in overall patients and cases with MSS/MSI-L phenotype. However, only a weak positive association between T2DM and abnormal p53 patterns can be detected in patients with MSI-H phenotype. It is well known that CRCs with MSI-H phenotype are tend occur in proximal colon and represent a distinctive carcinogenic pathway different from conventional adenoma-carcinoma pathway. The site- and MSI-specific differences in T2DM-associated risk of abnormal p53 patterns observed in our study were likely to be related to the different molecular pathways in carcinogenesis of CRC.

The mechanism for the link between T2DM and p53 abnormal expression or TP53 mutation is not clear. Recently, high glucose was reported to increase protein O-GlcNAcylation in cells of diabetic mice, and trigger nucleotide imbalance through O-GlcNAcylation of key enzymes (the ribonucleotide reductase) activity, which led to deficiency in dNTP pools and gene mutation in pancreatic cells [25]. Whether this mechanism was involved the diabetes-associated p53 abnormal expression or TP53 mutation needs further investigation.

Although not all abnormal p53 IHC patterns is caused by TP53 mutation, the fact that all the 3 abnormal p53 IHC patterns were significantly associated with T2DM in the present study was highly suggestive for a link between T2DM and TP53 mutation. In a recent study [26], the rate of TP53 nonsynonymous SNVs (mutation) were found in 80.2% of p53-strong expression group (> 50% of tumor cells were positive), while rates of stop-gain mutation and indels were found in 38.2% and 14.7% of p53-no group (complete absence) of CRC. In another study using 20% as cut-off value for p53 positive [27], TP53 mutation was found in 79.6% (39/49) of p53 positive CRC and 97.4% (38/39) of the mutation was missense mutation.

Abnormal p53 patterns were reported to be associated with unfavorable prognosis of some cancers including squamous cell carcinoma and high-grade serous carcinomas [28, 29]. P53 HT pattern is an intermediate form of abnormal and WT patterns [9, 11]. Although p53 HT pattern has been described in cancers such as endometrioid cancer, which has not been reported in CRC so far. Our present data demonstrated that p53 HT pattern were common and easily recognized in CRC. Formation of subclone

with new p53 mutation was regarded to be the cause of HT p53 pattern [11]. Morphologically, HT p53 pattern is a admixture of WT and abnormal pattern, which was likely to be a transitional status of WT p53 pattern to OE (or CA) pattern. Our results showed that T2DM was associated with increased risk for p53 HT patterns in CRC. Giving the crucial role of p53 in conventional colorectal adenoma-carcinoma pathway, T2DM might play an important role in carcinogenesis of CRC via promoting p53 abnormal expression or TP53 mutation.

Previous study has demonstrated that p53 overexpression (or positive) was associated with poor prognosis of cancers [30, 31]. Our present data also demonstrated that the abnormal p53 IHC patterns were linked to more aggressive behavior such as LN metastasis and high TNM stage of CRC. It was suggested that T2DM-associated increase of abnormal p53 expression might result in unfavorable clinical outcome.

In addition, our data also showed that low HDL was inversely correlated with HT and OE p53 pattern in CRC. HDL has antioxidant and anti-inflammation properties and showed to be associated with the development of CRC [32, 33]. However, in diabetic condition, glycation and oxidation of HDL could promote metastasis of cancer [34]. Because p53 can be activated by diabetes-associated oxidant stress and HDL can alleviate the oxidant press and damage [35], it was reasonable that low HDL may increase the oxidant press and promoting TP53 mutation and/or p53 abnormal patterns in CRC.

In summary, this study characterized the significant association between T2DM and abnormal pattern in CRC. T2DM is significantly associated with p53 abnormal IHC patterns, especially in CRC with distal colon/rectum location and MSS/MSI-L phenotype. The abnormal p53 IHC pattern is associated with aggressive behaviors such as LN metastasis and high TNM stage of CRC. It was suggested that diabetes might influence carcinogenesis and progression via promoting TP53 mutation and abnormal p53 expression in CRC.

# Declarations


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### Authors' contributions

Yang Z designed the study and wrote the paper; Ma J collected and analyzed the data; Zhang X and Qi G performed the research.

### Conflict of interest statement

None declared.

### Consent for publication

The present manuscript does not contain any individual person's data including individual name, images and other detail.

### Data availability

The data used in the study are available from the corresponding author on reasonable request.

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# Figures

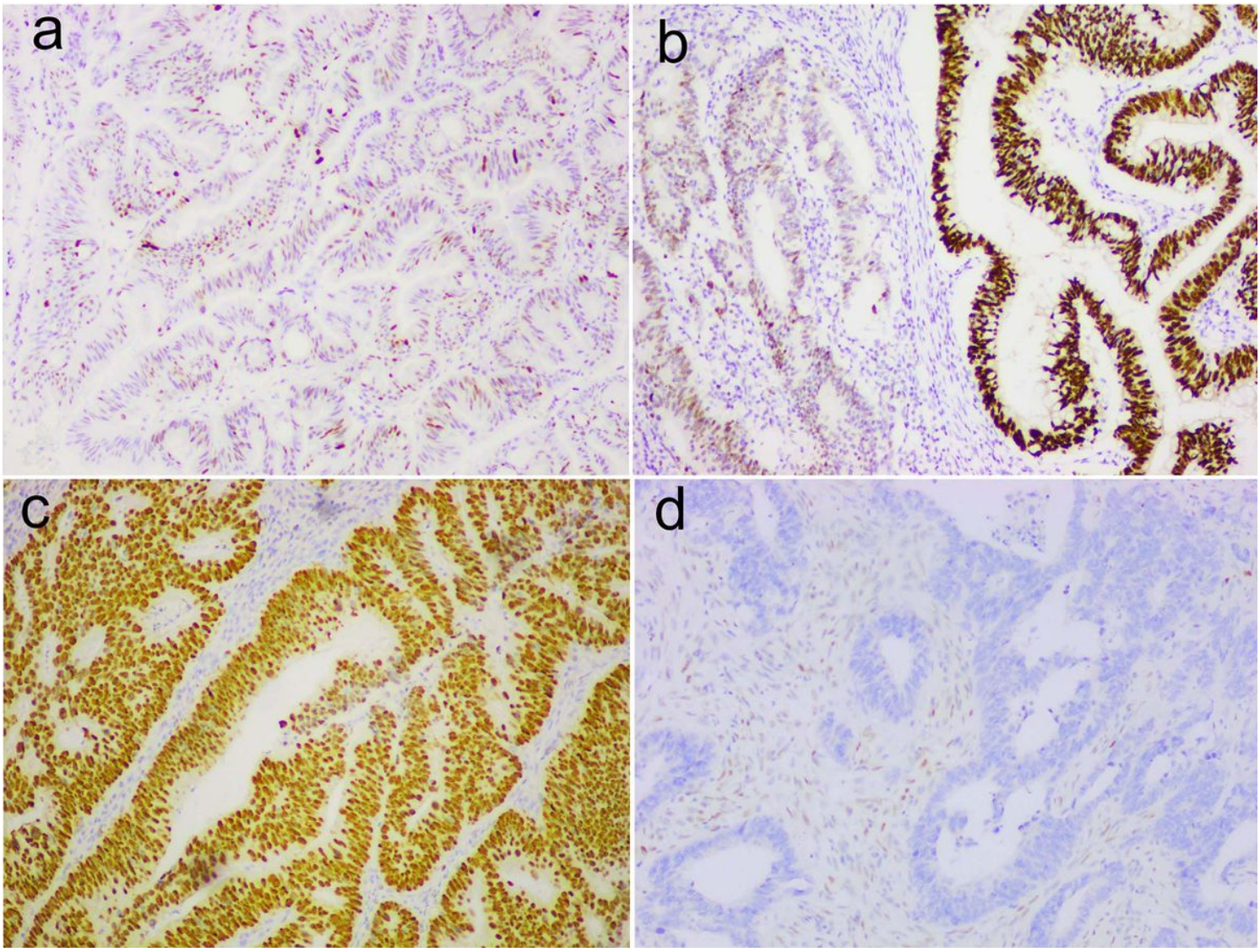


**Figure 1**

Immunohistochemistry of colorectal cancer. A. Wild type p53 pattern. The neoplastic cells are scattered moderate-strong positive on the background of weak positive and negative tumor cells, ×100; B. Heterogeneous pattern of p53. Left field presents WT pattern and right field presents overexpression pattern), ×100; C. Overexpression pattern of p53, ×100; almost 100% of neoplastic cells is strong nuclear positive; D. Complete absence pattern of p53. The neoplastic cells are complete negative, while internal control such as fibroblasts are scattered positive, ×100.

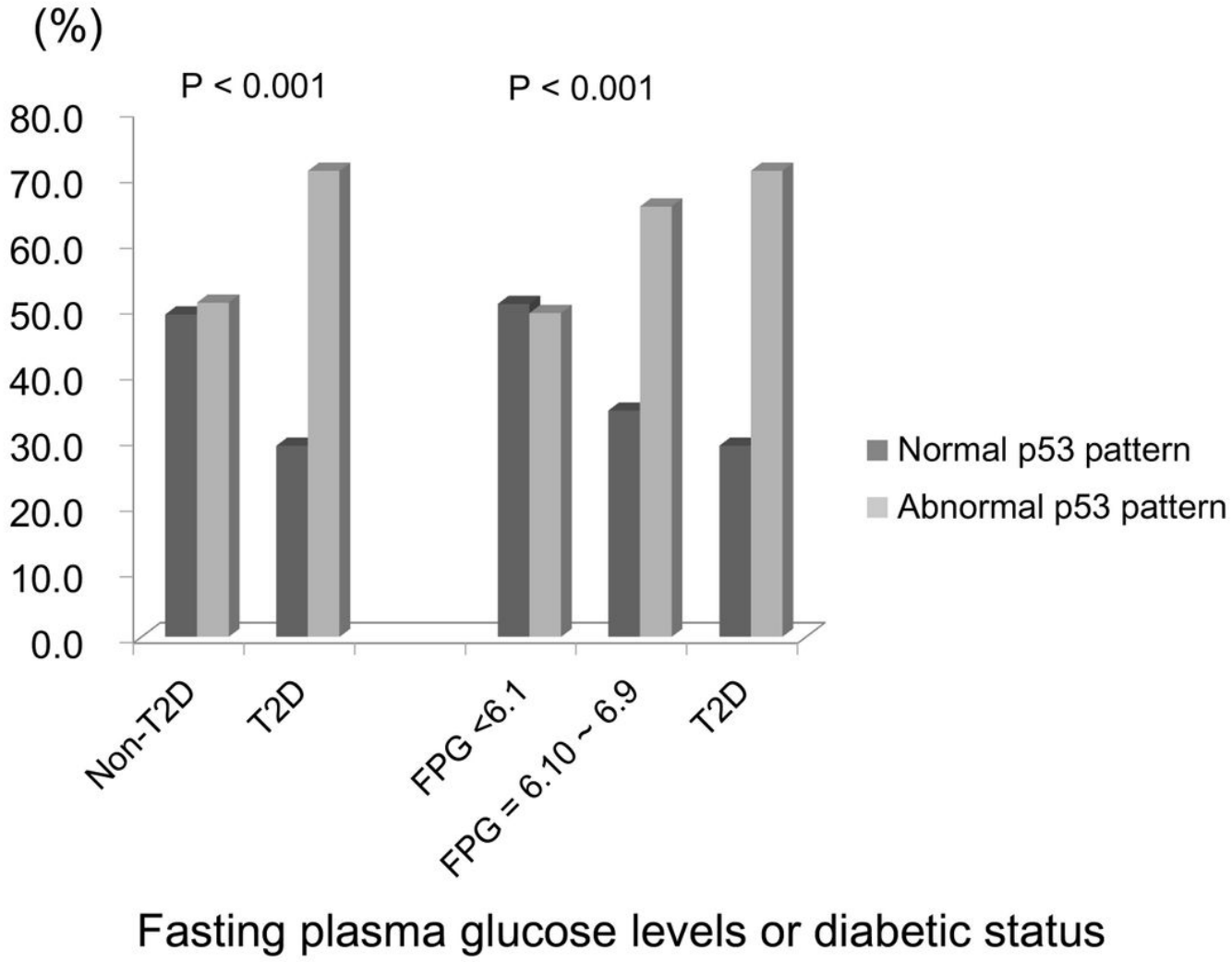


**Figure 2**

Association between diabetes and p53 IHC pattern in colorectal cancer.

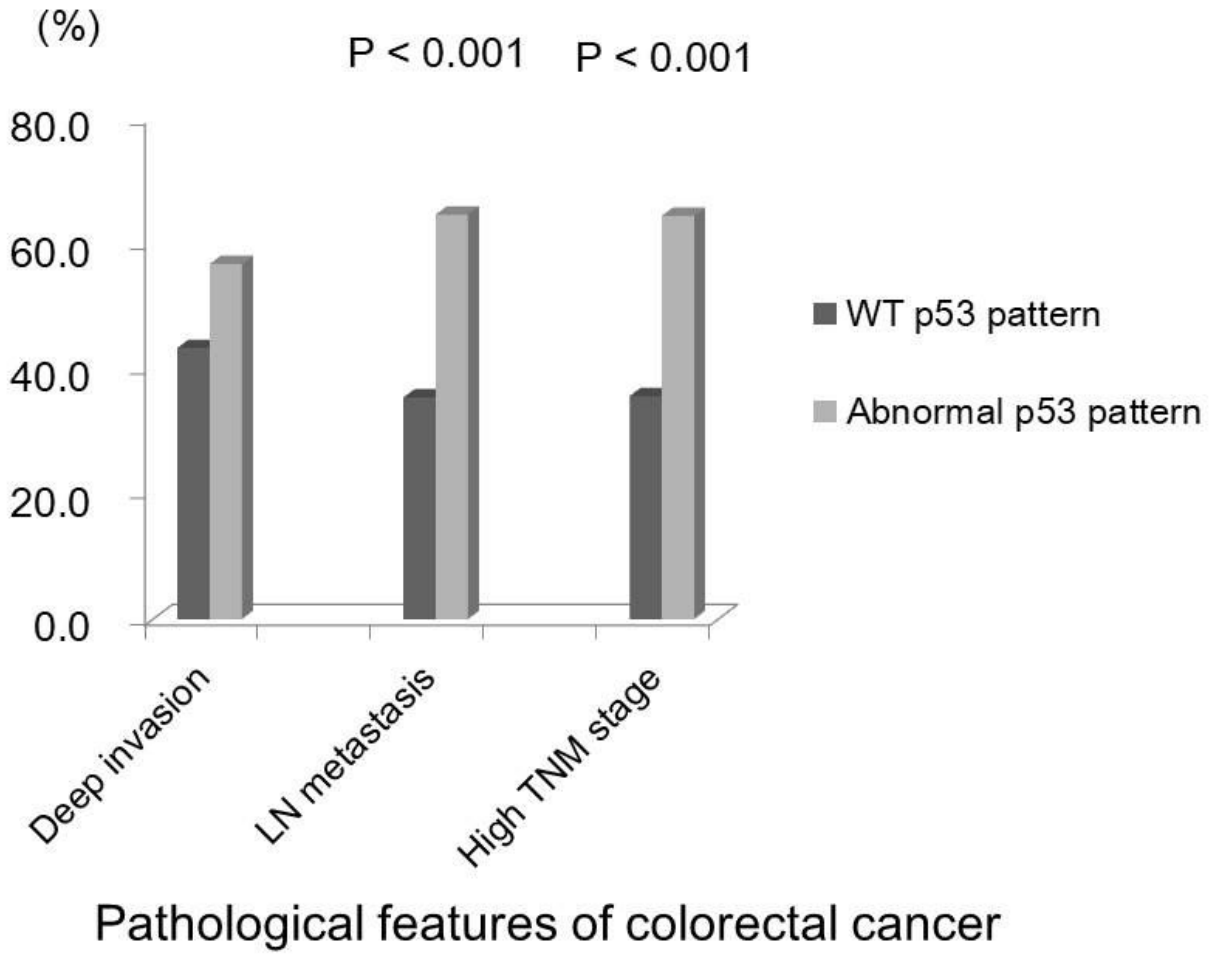


**Figure 3**

Association between p53 IHC pattern and pathological features in colorectal cancer.