Saudi Journal of Biological Sciences (2017) **24**, 466–476

King Saud University

**Saudi Journal of Biological Sciences**

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ORIGINAL ARTICLE

# Establishment of apoptotic regulatory network for genetic markers of colorectal cancer

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Received 3 November 2016; revised 25 December 2016; accepted 6 January 2017
Available online 26 January 2017

**KEYWORDS**

Colorectal cancer;
Genetic marker;
Meta analysis;
GO analysis;
KEGG analysis

**Abstract** Our purpose is to screen out genetic markers applicable to early diagnosis for colorectal cancer and to establish apoptotic regulatory network model for colorectal cancer, thereby providing theoretical evidence and targeted therapy for early diagnosis of colorectal cancer. Taking databases including CNKI, VIP, Wanfang data, Pub Med, and MEDLINE as main sources of literature retrieval, literatures associated with genetic markers applied to early diagnosis of colorectal cancer were searched to perform comprehensive and quantitative analysis by Meta analysis, hence screening genetic markers used in early diagnosis of colorectal cancer. Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were employed to establish apoptotic regulatory network model based on screened genetic markers, and then verification experiment was conducted. Through Meta analysis, seven genetic markers were screened out, including WWOX, K-ras, COX-2, p53, APC, DCC and PTEN, among which DCC shows highest diagnostic efficiency. GO analysis of genetic markers found that six genetic markers played role in biological process, molecular function and cellular component. It was indicated in apoptotic regulatory network built by KEGG analysis and verification experiment that WWOX could promote tumor cell apoptotic in colorectal cancer and elevate expression level of p53. The apoptotic regulatory model of colorectal cancer established in this study provides clinically theoretical evidence and targeted therapy for early diagnosis of colorectal cancer.
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## 1. Introduction

Colorectal cancer, as a common malignant tumor in digestive system, ranks third among male common malignant tumors and ranks second among female common malignant tumors in terms of morbidity in worldwide. In 2008, there were 1.2 million new cases of colorectal cancer globally, among which 609 thousand died of the disease (Jemal et al., 2011). In China, colorectal cancer mainly attacks people aged 40–60 years old and due to its occult onset and low awareness,

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Peer review under responsibility of King Saud University.

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http://dx.doi.org/10.1016/j.sjbs.2017.01.014
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most patients have been in advanced stage when diagnosed, and metastasis has occurred in about 25% patients when first diagnosed. Therefore, elevation of early diagnostic rate for early treatment and improvement of prognosis for colorectal cancer are focuses in current and further colorectal cancer prevention and control.

Meta analysis refers to a quantitative literature review which takes multiple independent research results for the same topic as objects, and based on strict design, it employs proper statistical methods to perform systematic, objective, quantitative and comprehensive analysis, aiming to promote statistical test efficacy, evaluate inconsistency or contradiction of research results and discover disadvantages in individual research. In addition, it can process a large quantity of literatures without number limitation. Therefore, Meta analysis plays a significant role in clinical diagnosis, treatment, risk assessment, prevention and intervention, health service as well as decision-making (Zhou et al., 2010). Meta analysis not only promotes efficacy of statistical inference thus lessening inconsistency of single research and draw more comprehensive and reliable conclusions (Zhang et al., 2013; Chaiyakunapruk et al., 2014), but also puts forward some novel research subjects, guiding direction for further study.

Gene Ontology (GO) database (Hill et al., 2016) is a structured standard biological model established recently by GO organization, aiming to build a standard system of genes and their biological productions to analyze genes and their cellular component, molecular function and biological processes they are involved in.

Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa and Goto, 2000) is a database that integrates genome, chemistry, and information of system function, which links gene catalogs obtained from genome that has been completely sequenced to system function of higher level of cell, species and ecosystem. It is characterized by powerful image function, enabling people to have an intuitive and comprehensive understanding of the metabolic pathways they study.

Taking databases including CNKI, VIP, Wanfang data, Pub Med, and MEDLINE as main sources of literature retrieval, literatures associated with genetic markers that are applied to early diagnosis of colorectal cancer were searched to perform comprehensive and quantitative analysis by Meta analysis, hence screening genetic markers which can be used in early diagnosis of colorectal cancer. Regarding the screened seven genetic markers, including WWOX, K-ras, COX-2, P53, APC, DCC and PTEN, their apoptotic regulatory network model in colorectal cancer was established by GO analysis and KEGG analysis, and then verification experiment was conducted. The model defines programed death regulatory mechanism for colorectal cancer cell, hence directing the individual diagnosis and targeted therapy of colorectal cancer.

## 2. Material and methods

### 2.1. Subjects

With CNKI, VIP and Wanfang databases were regarded as primary sources for Chinese literatures retrieval, literatures published between 1st January 1990 and 31 December 2013 were searched under key words of colorectal cancer, genetic markers, and early diagnosis. Regarding English literatures, Pub Med and MEDLINE were considered as main sources, and literatures published between 1st January 1990 and 31 December 2013 were searched with key words "colorectal cancer", "genetic markers" and "diagnosis". All literatures meeting inclusion criteria were carefully read, including the whole text and references, and related literatures were searched as well. The full text of included literatures were either in Chinese or in English and concerning researches made by the same institution or on the same subject but published on different journals, the latest and the most complete report was adopted.

Inclusion criteria for literatures: (1) the literature should be in English or in Chinese, with content of application of genetic markers in early diagnosis of colorectal cancer; (2) the research type is retrospective study; (3) the gold standard in literature is histopathology or operative diagnosis, and the literature takes patients with colorectal cancer as experimental group and healthy people or patients with benign tumor as control group, and objects with no restriction of nation, age as well as sex; (4) literature should provide diagnose results of colorectal cancer separately diagnosed by genetic markers; (5) true positive (TP), false positive (FP), false negative (FN) and true negative (TN) of patients with colorectal cancer that is separately diagnosed by genetic markers can be obtained directly according to the literature or by calculation; (6) the literature employs correct methods and the study has normative process, and regarding researches multiply made by the same institution or on the same subject but published on different journals, the latest and the most complete report was adopted. All included literatures in this study were published full text in Chinese or in English and all data were obtained from the original text.

Exclusion criteria for literatures: (1) the literature involves either an unoriginal or repetitive research, or serious design defect, or incomplete data; (2) the type of literature is review or abstract; (3) cases are not diagnosed by gold standard; (4) subject is colon cancer or rectal cancer; (5) no control group is set in the study; (6) the literature studies application of genetic markers in postoperative recurrence diagnosis of colorectal cancer; (7) the literature shows no results of separate diagnosis but only combined diagnosis results of genetic markers for colorectal cancer.

### 2.2. Data extraction and quality assessment

Data extraction of included in literatures: (1) general data, including authors, published time, published journal, title, the numbers of cases in experimental group and in control group; (2) methodological characteristics: cutoff value; (3) characteristics of research results: diagnostic results of genetic markers for colorectal cancer, including TP, FP, FN and TN.

Quality assessment of included literatures: included literatures were separately and independently assessed and performed cross-check by two professional reviewers using quality assessment of diagnostic accuracy studies (QUADAS) developed by Whiting et al. (2003). QUADAS consists of 14 assessment indicators. Regarding each indicator, "Yes" indicates meeting the standard; "No" indicates not meeting the standard, "Not clear" indicates insufficient information can be got from the literature to determine whether the standard is met.

### 2.3. Meta analysis

All data were performed two-sided test of Meta analysis, in which $P < 0.05$ indicates statistical difference and $P < 0.01$ indicates extremely significant difference. Meta analysis of related results was performed by Meta-Disc software and the results were shown as forest graph and SROC figure.

### 2.4. GO analysis

To conduct GO analysis, the home page of AmiGO (http://geneontology.org/) was visited. With "Homo sapiens" as filter criteria, preliminary analysis of GO annotation was performed on seven genetic markers obtained from Meta analysis and the seven genetic markers are WWOX, K-ras, COX-2, p53, APC, DCC and PTEN.

### 2.5. KEGG analysis

To perform KEGG analysis on genetic markers, the homepage of KEGG signaling pathway database (http://www.kegg.jp/kegg/pathway.html) was visited. Then, taking "hsa" as screening criteria, and seven genetic markers including WWOX, K-ras, COX-2, p53, APC, DCC, and PTEN as keywords, signaling pathways of genetic markers in patients with colorectal cancer or linked to apoptosis were searched.

### 2.6. Verification experiment for apoptotic regulatory network of colorectal cancer

#### 2.6.1. Experiment materials

pcDNA4.0/Myc-WWOX recombinant plasmid was constructed and preserved at laboratory in the First Affiliated Hospital of Xi'an Jiaotong University, and it was purified by Qiagen plasmid purification kits before being determined purification and concentration by ultraviolet spectrophotometer. Human colorectal cancer cell line Colo205 was purchased in China Center for Type Culture Collection, and verification experiment was conducted after cells' arrival. The cells were grown in RPMI 1640 supplemented with 2.5 g/L glucose, 1.5 g/L sodium bicarbonate, 0.11 g/L sodium pyruvate and 10% fetal bovine serum; and they were routinely screened for Mycoplasma contamination. The primers were synthesized by Shanghai Shenggong Bioengineering Co., Ltd. The sequences of primers were as follows: WWOX, upstream primer was 5′-GATAATCCGACCAAGCCAAC-3′, downstream was 5′-ACTGCTTCACTCGCCCTTG-3′, length of amplification product was 209 bp; p53, upstream primer was 5′-GGCCCACTTCACCGTACTAA-3′, downstream primer was 5′-TAAAACGCAGCTCAGTAACAGTCCG-3′, length of amplification product was 186 bp; housekeeping gene β-actin, upstream primer was 5′-TGGAATCCTGTGGCATCCATGAAAC-3′, downstream primer was 5′-TAAAACGCAGCTCAGTAACAGTCCG-3′. Transfection reagents were from Qiagen.

#### 2.6.2. Cell transfection

Cell transfection was conducted referring to the introduction of liposomes transfection reagent when culture fluids for Colo205 cell line reached to 60–80%. The ratio of liposome and plasmid was 10:1; meanwhile, the empty vector pcDNA4.0/Myc-His and the non transfected Colo205 cell lines were set as control groups.

#### 2.6.3. Apoptosis detection by flow cytometry

With pcDNA4.0/Myc-WWOX as transfection group, pcDNA4.0/Myc-His empty vector-transfected group and Colo205 cell line control group, cell apoptosis rate was analyzed with the FACSalibur software of flow cytometry.

#### 2.6.4. RNA extraction

Total RNA was extracted according to instructions of total RNA extraction kit by Trizol method. Ultraviolet spectrophotometer was employed to detect ultraviolet absorption of total RNA at 260 nm, 280 nm, and 230 nm to determine purification and concentration of total RNA. Integrity of total RNA was tested on 1% agarose gel.

#### 2.6.5. Synthesis of cDNA by reverse transcription

One μg RNA with good purification and integrity was taken respectively from each group as template for reverse transcription which was carried out in accordance with steps on Takara RT-PCR kit.

#### 2.6.6. Polymerase chain reaction (PCR) reaction

Twenty-five μL PCR reaction system was added according to kit instructions:

| | |
|---|---|
| cDNA template | 2 μL |
| 10 mM primer I upstream primer (20 p mol/μL) | 1 μL |
| 10 mM primer II downstream primer (20 p mol/μL) | 1 μL |
| 2 × Master mix | 12.5 μL |
| dd$H_2O$ | 9.5 μL |

The reaction was performed under 94 °C for 45 s, 55 °C for 45 s and 72 °C for 60 s for 33 cycles before being extended at 72 °C for 7 min.

## 3. Results

### 3.1. Meta analysis on genetic markers for early diagnosis of colorectal cancer

#### 3.1.1. Included literatures

A total of 394 Chinese literatures and 1030 English literatures were retrieved by computer, among which 44 literatures (see Appendix) were eventually selected and included in Meta analysis. The flow chart of literature search and screening process is shown in Fig. 1.

#### 3.1.2. Meta analysis results of genetic marker p53

*3.1.2.1. Data extraction from included literatures linked to genetic marker p53.* Taking Meta analysis results of genetic marker p53 for example, a total of 13 literatures, including 11 Chinese literatures and 2 English literatures, were included. Totally, there were 773 patients with colorectal cancer and 524 controls in included literatures and specific data are shown in Table 1.

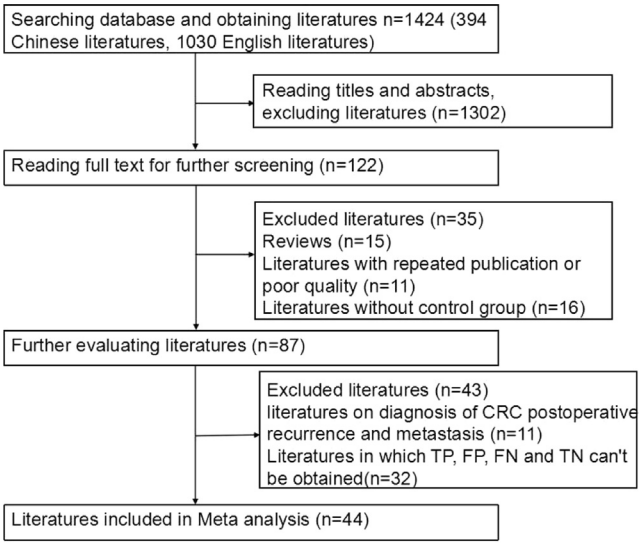


**Figure 1** Flow chart of literature search and screening process of meta analysis on genetic markers for early diagnosis of colorectal cancer.

*3.1.2.2. Meta analysis on p53 for early diagnosis of colorectal cancer*. Taking Meta analysis results of genetic marker p53 for instance, Figs. 2–4 are sensitivity forest plot, specificity forest plot and diagnostic odds ratio (DOR) graph of p53 on colorectal cancer respectively. According to the figures, in 13 literatures, the sensitivity of p53 for colorectal cancer diagnosis was 24–85% and pooled sensitivity 0.57 (0.53, 0.60); the specificity was 80–100%, pooled specificity 0.93 (0.91, 0.95); the diagnostic ratio was 17.42 (9.30, 32.62). Fig. 5 is the summary receiver operating characteristic curve (SROC) of p53 for colorectal cancer, which indicates that area under SROC (AUC) is 0.8305 and standard error 0.0563.

*3.1.2.3. Bias analysis*. Liner regression method was used for bias detection, and DEEK graph was drawn as shown in Fig. 6. Results indicated that $P = 0.74 > 0.05$, which means there was no bias.

### *3.1.3. Meta analysis results of seven genetic markers*

Meta analysis results of seven genetic markers are listed in Table 2. As shown, the DOR of WWOX, K-ras, COX-2, P53, APC, DCC and PTEN in Meta analysis were 7.56 (4.97, 11.50), 12.56 (6.33, 24.90), 10.29 (4.00, 26.45), 17.42 (9.30, 32.62), 25.40 (7.37, 87.50), 4.41 (11.28, 262.54) and 22.39 (10.69, 46.88), respectively, indicating that all seven factors had high diagnostic efficiency for colorectal cancer, among which DCC had the best diagnostic efficiency.

### *3.2. GO analysis results of genetic markers*

In reference to GO analysis, the roles that gene and protein play in cell are classified into three parts, biological process, molecular function and cellular component. Tables 3 and 4 show GO analysis results of genetic markers DCC and PTEN. As shown, DCC plays a key role in biological process and cellular component and participates in apoptotic signaling pathway with positive regulation. And PTEN covered biological process, molecular function as well as cellular component, significantly acting in T cell receptor signaling pathway and biological process such as inositol phosphate metabolic process and phospholipid metabolic process.

Tables 5 and 6 demonstrate GO analysis results of genetic markers COX-2 and p53. As indicated, COX-2 played a crucial part in biological process and molecular function and participates in cyclooxygenase pathway, inflammatory response, regulation of blood pressure and other biological processes. And p53 was included in biological process and cellular component, getting involved in apoptotic process and playing positive regulation in apoptotic signaling pathway.

Tables 7 and 8 exhibit GO analysis results of gene markers APC and WWOX. As presented, both APC and WWOX covered biological process, molecular function and cellular component functions. APC was involved in apoptotic process with positive function; WWOX participated in intrinsic apoptotic signaling pathway by p53 class mediator, Wnt signaling pathway and so on; whereas functional annotation of human gene K-ras was not found in GO database.

### *3.3. Establishment of apoptotic regulatory network of genetic markers for colorectal cancer*

Through KEGG analysis of seven genetic markers based on KEGG signaling pathway database, it was found that p53, APC, DCC and K-ras were involved in regulatory network of colorectal cancer, as shown in Fig. 7. Combining results

**Table 1** General data of included literatures related to p53.

| Number | Author | Colorectal cancer group (case) | Control group (case) | TP | FP | FN | TN |
|---|---|---|---|---|---|---|---|
| 1 | Chen Haiwei | 40 | 40 | 29 | 11 | 3 | 37 |
| 2 | Wang Wenxing | 95 | 57 | 65 | 30 | 9 | 48 |
| 3 | Chaar Ines | 59 | 108 | 20 | 39 | 9 | 99 |
| 4 | Zhan Qiang | 40 | 20 | 23 | 17 | 0 | 20 |
| 5 | Li Weiwei | 31 | 10 | 10 | 21 | 0 | 10 |
| 6 | Chung-Chuan Chan | 94 | 54 | 23 | 71 | 1 | 53 |
| 7 | Wang Yuhuan | 68 | 40 | 34 | 34 | 1 | 39 |
| 8 | Zhao Jianling | 35 | 15 | 14 | 21 | 1 | 14 |
| 9 | Zhang Yanxia | 80 | 40 | 53 | 27 | 8 | 32 |
| 10 | Hou Hui | 80 | 80 | 68 | 12 | 0 | 80 |
| 11 | Xiao Chaowen | 40 | 20 | 32 | 8 | 0 | 20 |
| 12 | Zhang Jiping | 45 | 25 | 31 | 14 | 4 | 21 |
| 13 | Chen Ling | 66 | 15 | 38 | 28 | 0 | 15 |

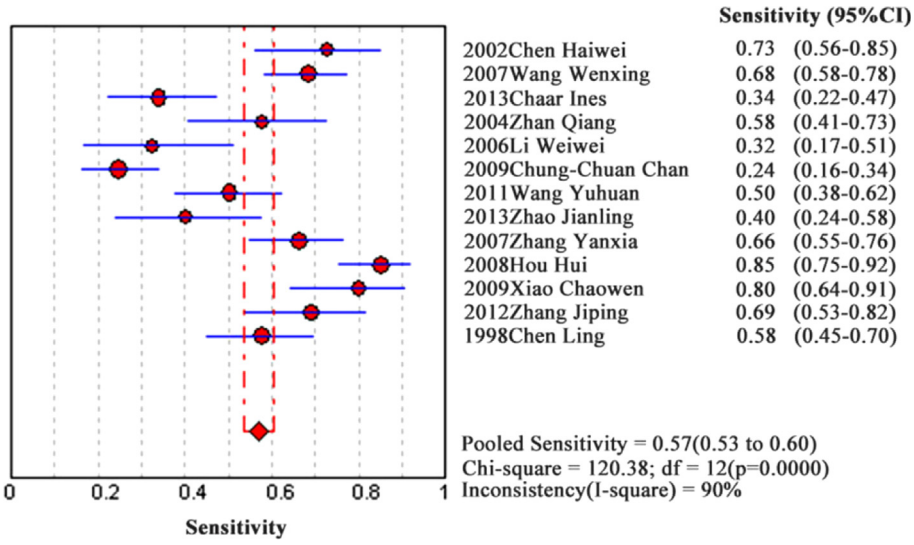


**Figure 2** Sensitivity forest plot of p53 for diagnosis of colorectal cancer.

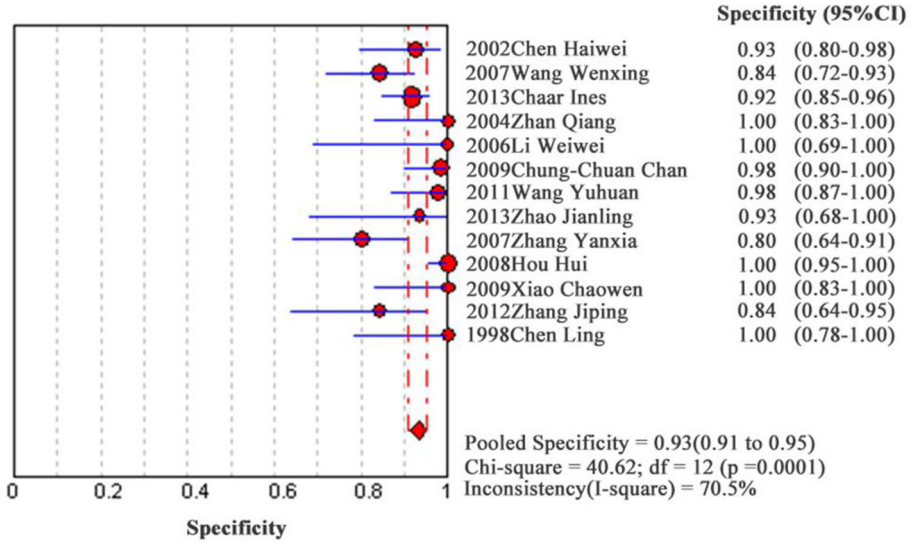


**Figure 3** Specificity forest plot of p53 for diagnosis of colorectal cancer.

of GO analysis and KEGG analysis, genetic markers PTEN and COX-2 were added to establish primary apoptotic regulatory network of genetic markers for colorectal cancer, as shown in Fig. 8. WWOX is a newly discovered tumor suppressor factor, and the apoptotic regulatory signaling pathway of colorectal cancer that WWOX was involved in is still unclear. It was found in GO analysis that WWOX was involved in apoptotic signaling pathway by p53 class mediator, so related verification experiment was supplied.

### *3.4. Verification experiment for apoptotic regulatory network of colorectal cancer*

#### *3.4.1. Effect of WWOX transfection on cell apoptosis of colorectal cancer*

As indicated in Fig. 9, after transfection of gene WWOX into Colo205 cell line, apoptosis rate of pcDNA4.0/Myc-WWOX transfection, pcDNA4.0/Myc-His empty vector-transfected group and Colo205 cell line control group were (12.63 ± 0.43)%, (2.31 ± 0.58)%, (2.20 ± 0.36)%, respectively. pcDNA4.0/Myc-WWOX group was different from the two control groups in apoptosis rate with statistical significance ($P < 0.01$), indicating that the transfection of pcDNA4.0/Myc-WWOX elevated apoptosis rate of Colo205 cell line and that gene WWOX promoted apoptosis of colorectal cancer tumor cells.

#### *3.4.2. RT-PCR analysis on effect of WWOX on p53 mRNA expression*

Fig. 10 demonstrates RT-PCR analysis on effect of WWOX on p53 mRNA expression. As indicated, after transfection of pcDNA4.0/Myc-WWOX into the tumor cell, Colo205 cell, mRNA expression level of gene WWOX in transfected cell Colo205 was higher than that in pcDNA4.0/Myc-His empty

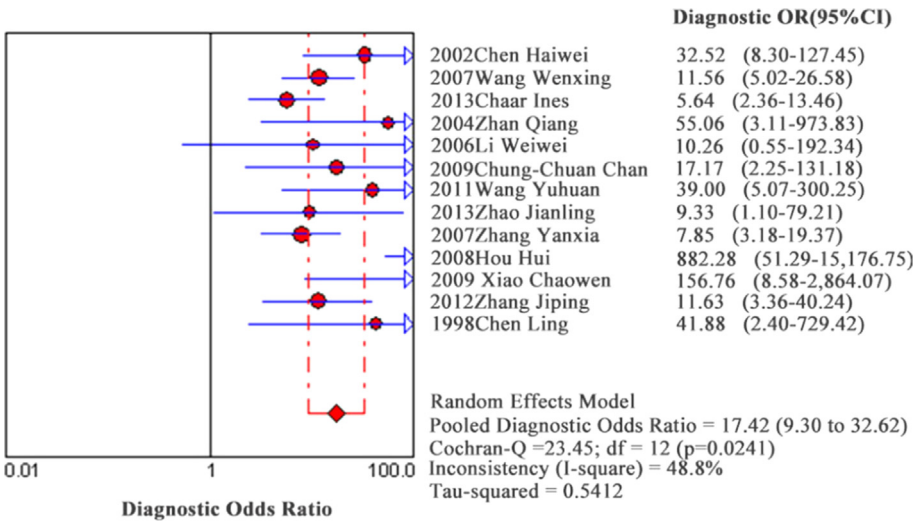


**Figure 4** Diagnostic odds ratio graph of p53 for diagnosis of colorectal cancer.

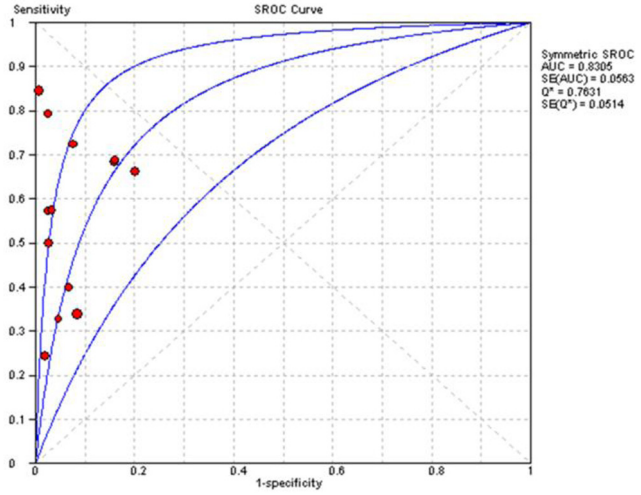


**Figure 5** SROC curve of p53 for diagnosis of colorectal cancer.

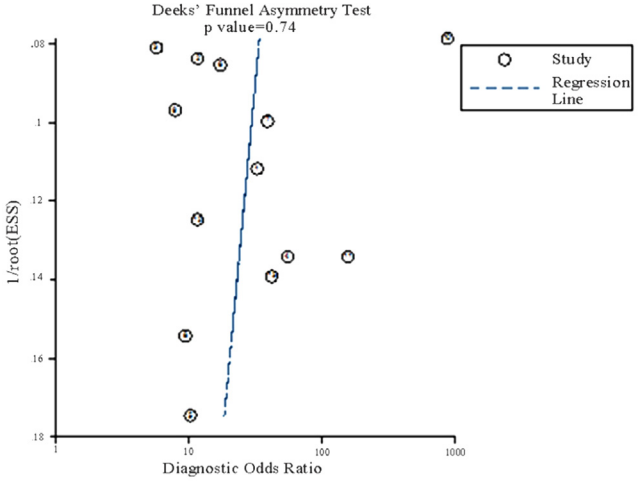


**Figure 6** Bias assessment of published literatures on colorectal cancer diagnosis.

vector-transfected group and Colo205 cell line control group. Normally, wild-type p53 expresses low in Colo205 cell line; after transfection of pcDNA4.0/Myc-WWOX, however, it expressed higher in transfected Colo205 cell line than that in pcDNA4.0/Myc-His empty vector-transfected group and that in Colo205 cell line control group, which indicated that WWOX can elevate expression level of p53.

## 4. Discussion

Colorectal cancer, a genetic disease, is caused by the multi-phase and long-term process in which proto-oncogene is activated and suppressor gene is inactivated under the environmental effect. The onset of colorectal cancer is latent, leading to a low degree of symptom awareness, so most patients have already been in advanced phase when diagnosed. Up to 50% of newly diagnosed patients eventually developed into metastatic colorectal cancer, with five-year survival rate less than 5%. Besides, patients with intermediate and advanced colorectal cancer always have poor therapeutic results and the bad prognosis impairs their life quality, at the same time, imposes them large economic burden (Al-Shuneigat et al., 2011).

Colorectal cancer growth is correlated to pathways like gene mutation, gene repair, signal transduction and metastasis and invasion. At present, commonly used serum markers for early diagnosis of colorectal cancer in clinic have low diagnostic value because many patients have already been in advanced phase when diagnosed, which severely affected their treatment and prognosis. Thus it's necessary to find diagnostic methods with high sensitivity and specificity. The molecular model of colorectal cancer morbidity was posed by Fearon and Jones (1992) in 1992, and with the development of molecular biology techniques, molecular mechanism in the model was expanded and it's found that mutations in growth and development of colorectal cancer are sequential, which makes it feasible to diagnose colorectal from respects of oncogenes and tumor suppressor genes. Sugai and Habano (2016) discussed the genetic mechanisms of colorectal cancer and the relationship of these alterations with emerging biomarkers for pathological diagnosis, patient prognosis, and the prediction of treatment

**Table 2** Meta analysis results of seven genetic markers.

| Genetic marker | Number of literatures (case) | Number of patients (case) | Control group (cases) | Pooled sensitivity | Pooled specificity | DOR |
|---|---|---|---|---|---|---|
| K-ras | 5 | 270 | 76 | 0.70 (0.64, 0.75) | 0.82 (0.71, 0.90) | 12.56 (6.33, 24.90) |
| COX-2 | 8 | 449 | 230 | 0.79 (0.75, 0.83) | 0.66 (0.56, 0.72) | 10.29 (4.00, 26.45) |
| p53 | 13 | 773 | 524 | 0.57 (0.53, 0.60) | 0.93 (0.91,0.95) | 17.42 (9.30, 32.62) |
| APC | 5 | 381 | 297 | 0.61 (0.56,0.66) | 0.94 (0.91,0.96) | 25.40 (7.37,87.50) |
| DCC | 6 | 361 | 225 | 0.57 (0.51,0.62) | 0.98 (0.95,0.99) | 54.41 (11.28,262.54) |
| PTEN | 5 | 256 | 198 | 0.58 (0.52,0.64) | 0.96 (0.92,0.98) | 22.39 (10.69,46.88) |
| WWOX | 7 | 391 | 225 | 0.65 (0.60, 0.69) | 0.79 (0.73, 0.84) | 7.56 (4.97, 11.50) |

**Table 3** GO analysis results of genetic marker DCC.

| Gene ontology | GO number | Name |
|---|---|---|
| Biological process | GO:0043065 | Positive regulation of apoptotic process |
| | GO:0007411 | Axon guidance |
| | GO:0097190 | Apoptotic signaling pathway |
| Cellular component | GO:0005829 | Cytosol |
| | GO:0005886 | Plasma membrane |

responses, which provided significant evidence for early diagnosis and treatment of tumor.

Related literatures about genetic markers used in early diagnosis of colorectal cancer were searched through CNKI database, VIP database, Wanfang database, Pub Med database and MEDLINE database. And then through Meta-analysis of diagnostic test, it was found that the DORs of WWOX, K-ras, COX-2, P53, APC, DCC and PTEN respectively were 7.56 (4.97, 11.50), 12.56 (6.33, 24.90), 10.29 (4.00, 26.45), 17.42 (9.30, 32.62), 25.40 (7.37,87.50), 54.41 (11.28,262.54) and 22.39 (10.69,46.88), which suggested that these seven genetic markers had high diagnostic efficacy with DCC highest and WWOX lowest.

GO and KEGG databases were used to conduct GO functional analysis and KEGG signaling pathway analysis on the seven genetic markers so as to establish a primary apoptotic regulatory network, which showed that DCC was involved in the apoptotic signaling pathway with positive regulation. And over these years DCC was reported as one of the key tumor suppressor genes (Kazemzadeh et al., 2015) and was closely correlated to growth and development of colorectal cancer. And it was found that DCC gene can inhibit cell proliferation and cause degraded carcinoembryonic antigen (CEA) expression in rectal cancer cell line SW1116 (Jiang et al., 2015). Therefore, detection of DCC protein in caner tissues is important for colorectal cancer patients' prognosis assessment and usage of assistant treatment. PTEN is a key tumor suppressor gene having phosphatase activity, which involves in biological process, molecular function and cell components and it plays an important role in biological processes including T cell receptor signaling pathway, inositol phosphate metabolic process and phospholipid metabolic process, etc. According to the apoptotic regulatory network in this study, PTEN was involved in apoptotic pathway with negative regulation to serine/threonine protein kinase B(PKB/Akt), which is in line with the report by Zeng et al. (2016). Besides, COX-2, called "quick responsive gene", is an inducible enzyme. It expresses low in normal tissues while the expression increases rapidly under internal and external stimulus and COX-2 is, in a large part, involved in the development of tumor, reported by Tabriz et al. (2016) In addition, p53 playing an important role in biological process and cell components is involved in apoptotic process with positive regulation. Al-Saran et al. (2016) found that Zinc can up-regulate

**Table 4** Go analysis results of genetic marker PTEN.

| Gene ontology | GO number | Name |
|---|---|---|
| Biological process | GO:0050852 | T cell receptor signaling pathway |
| | GO:0048011 | Neurotrophin TRK receptor signaling pathway |
| | GO:0043647 | Inositol phosphate metabolic process |
| | GO:0007173 | Epidermal growth factor receptor signaling pathway |
| | GO:0008543 | Fibroblast growth factor receptor signaling pathway |
| | GO:0006661 | Phosphatidylinositol biosynthetic process |
| | GO:0048015 | Phosphatidylinositol-mediated signaling |
| | GO:0044281 | Small molecule metabolic process |
| | GO:0006644 | Phospholipid metabolic process |
| | GO:0038095 | Fc-epsilon receptor signaling pathway |
| | GO:0045087 | Innate immune response |
| Cellular component | GO:0005829 | Cytosol |
| Molecular function | GO:0016314 | Phosphatidylinositol-3,4,5-trisphosphate3-phosphatase activity |
| | GO:0051717 | Inositol-1,3,4,5-tetrakisphosphate 3-phosphatase activity |
| | GO:0051800 | Phosphatidylinositol-3,4-bisphosphate 3-phosphatase activity |

**Table 5** GO analysis results of genetic marker COX-2.

| Gene ontology | GO Number | Name |
|---|---|---|
| Biological process | GO:0019371 | Cyclooxygenase pathway |
| | GO:0006954 | Inflammatory response |
| | GO:0008217 | Regulation of blood pressure |
| | GO:0006979 | Response to oxidative stress |
| | GO:0055114 | Oxidation-reduction process |
| Molecular function | GO:0004601 | Peroxidase activity |
| | GO:0004666 | Prostaglandin-endoperoxide synthase activity |
| | GO:0020037 | Heme binding |

**Table 6** GO analysis results of genetic marker p53.

| Gene ontology | GO number | Name |
|---|---|---|
| Biological process | GO:1900740 | Positive regulation of protein insertion into mitochondrial membrane involved in apoptotic signaling pathway |
| | GO:0007219 | Notch signaling pathway |
| | GO:0007596 | Blood coagulation |
| | GO:0006915 | Apoptotic process |
| | GO:0000075 | Cell cycle checkpoint |
| | GO:0033554 | Cellular response to stress |
| | GO:0097193 | Intrinsic apoptotic signaling pathway |
| | GO:0006977 | DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest |
| Cellular component | GO:0005654 | Nucleoplasm |

**Table 7** GO analysis results of genetic marker APC.

| Gene ontology | GO number | Name |
|---|---|---|
| Biological process | GO:0043065 | Positive regulation of apoptotic process |
| | GO:0008285 | Negative regulation of cell proliferation |
| | GO:0016477 | Cell migration |
| | GO:0000281 | Mitotic cytokinesis |
| | GO:0007026 | Negative regulation of microtubule depolymerization |
| | GO:0030178 | Negative regulation of Wnt signaling pathway |
| | GO:0007050 | Cell cycle arrest |
| | GO:0006974 | Cellular response to DNA damage stimulus |
| Cellular component | GO:0005737 | Cytoplasm |
| | GO:0005634 | Nucleus |
| Molecular function | GO:0008013 | Beta-catenin binding |

**Table 8** GO analysis results of genetic marker WWOX.

| Gene ontology | GO number | Name |
|---|---|---|
| Biological process | GO:0072332 | Intrinsic apoptotic signaling pathway by p53 class mediator |
| | GO:0016055 | Wnt signaling pathway |
| | GO:0001649 | Osteoblast differentiation |
| | GO:2001241 | Positive regulation of extrinsic apoptotic signaling pathway in absence of ligand |
| | GO:0097191 | Extrinsic apoptotic signaling pathway |
| | GO:0048705 | Skeletal system morphogenesis |
| Cellular component | GO:0005829 | Cytosol |
| | GO:0005794 | Golgi apparatus |
| Molecular function | GO:0005515 | Protein binding |

expression of p53 and p21, resulting in apoptosis of human breast cancer MCF-7 cell. Analysis of genomic data suggested that p53 is linked to incidence of cancer (Stracquadanio et al., 2016). He et al. (2011) found that curcumin can speed up tumor cell apoptosis and improve patients' health through increasing p53 expression. What's more, APC and WWOX both play a role in biological process, molecular function and cell components, and APC participates in apoptosis progress with function of promoting cell apoptosis and WWOX is involved in apoptotic signaling pathway and Wnt signaling pathway induced by p53. There was report indicating that as a negatively regulatory protein, APC can abnormally activate Wnt signaling transduction pathway when it's not expressed, suggesting that APC as one of cancer suppressive factors of colorectal cancer is involved in the development of colorectal cancer (Blundon et al., 2016; Xu et al., 2016). A new cancer suppressor gene named as WWOX was found by Bednarek et al. (2000) in 2000 through Shotgun method. WWOX gene is correlated to tumor infiltration degree, lymphnode metastasis and pathological stage and it always expresses low in many tumor cells but its over-expression may induce tumor cell apoptosis (Xiong et al., 2010; Baykara et al., 2010). Moreover, recent research reported that K-ras mutation is the negative factor for growth, development and prognosis of colorectal cancer and it's closely correlated to targeted treatment. K-ras is a tumor gene, whose mutation is the early event of colorectal cancer, thus K-ras gene have drawn people's attention more and more in tumor treatment. And detection of K-ras gene mutation is helpful for individual treatment of cancer, which is crucial in treatment of colorectal cancer (Liu and Fu, 2012).

Experimental verification of WWOX showed that WWOX participated in apoptotic signaling pathway of colorectal cancer by activating p53 signaling pathway. According to study by Chang et al. (2001), stable transfection of WWOX gene into L929 cells resulted in an elevated expression of WWOX in L929 tumor cells, a reduced expression of more than 85% of anti-apoptosis factor Bc-1 2 and Bc-1 xL and an increased expression of 200% of pro-apoptotic factor p53 as well as an increased TNF's cell toxicity which is in good agreement with partial results of this study.

## 5. Conclusions

A primary apoptotic regulatory network of colorectal cancer composed of p53, APC, DCC, K-ras, PTEN, WWOX and

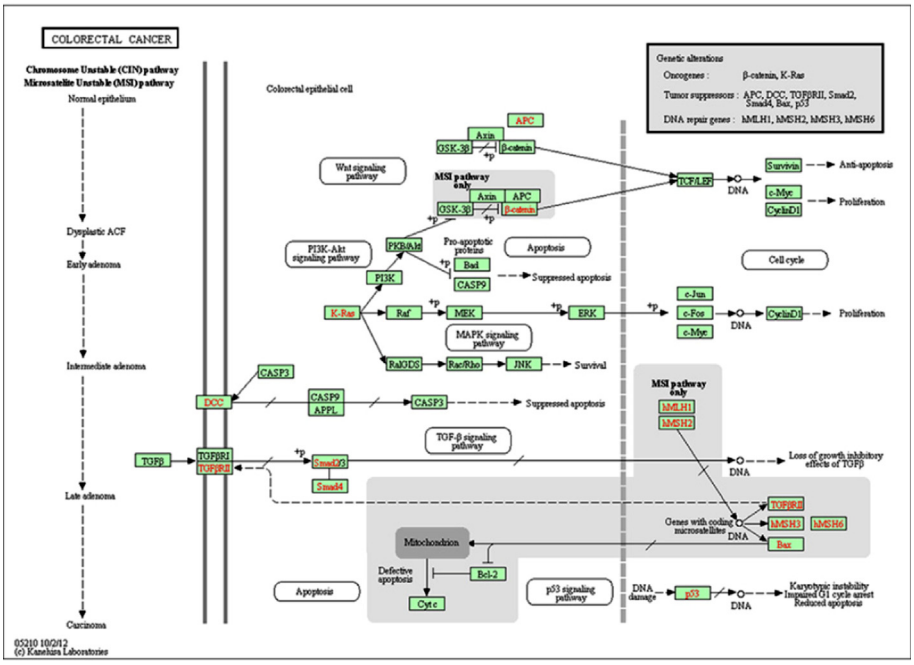


**Figure 7** Primary apoptotic regulatory network of genetic markers for colorectal cancer.

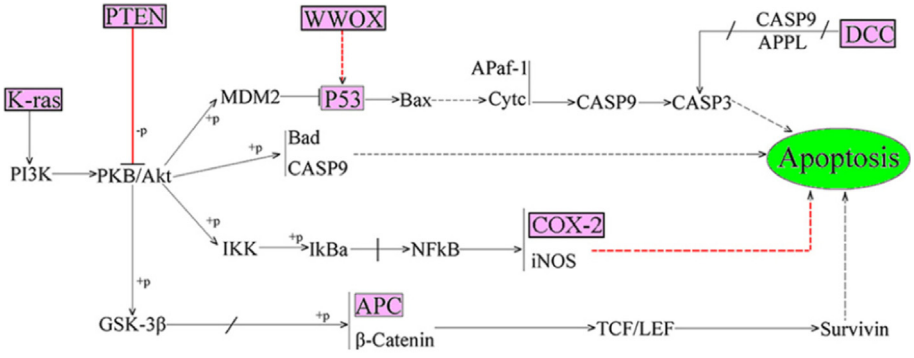


**Figure 8** Apoptotic regulatory network of genetic markers for colorectal cancer.

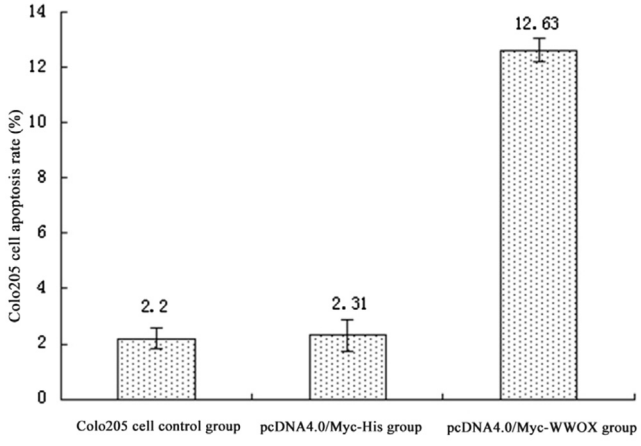


**Figure 9** Effect of transfecting WWOX into Colo205 cell line on cell apoptosis.

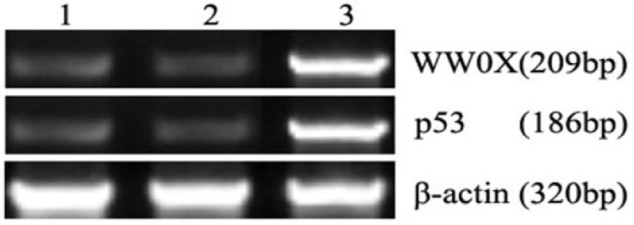


**Figure 10** RT-PCR analysis on effect of WWOX on p53 mRNA expression; Note: 1: Colo205 cell line control group; 2: pcDNA4.0/Myc-His empty vector-transfected group; 3: pcDNA4.0/Myc-WWOX transfection group.

COX-2 and other related genes were established in this study by Meta analysis combined with Go functional analysis and KEGG signal pathway analysis. And it's proved by experiment that WWOX is involved in apoptotic signaling pathway of colorectal cancer through activation of p53 signaling pathway

by elevating p53 expression. Apoptotic regulatory network of colorectal cancer can provide a theoretical basis for early diagnosis and targeted treatment of colorectal cancer in clinic.

**Acknowledgment**

The authors acknowledge the financial support from the National Key Technology R&D Program (Grant: 2016YFC1303200).

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**Appendix: Literatures included in Meta analysis**

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