Saudi Journal of Biological Sciences (2017) **24**, 634–643

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 and optimal selection of traditional Chinese medicine target

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Received 27 October 2016; revised 28 December 2016; accepted 7 January 2017
Available online 26 January 2017

KEYWORDS

Colorectal cancer;
Meta analysis;
KEGG analysis;
Traditional Chinese medicine

Abstract The paper aimed to screen out genetic markers applicable to early diagnosis for colorectal cancer and establish apoptotic regulatory network model for colorectal cancer, and to analyze the current situation of traditional Chinese medicine (TCM) target, thereby providing theoretical evidence for early diagnosis and targeted therapy 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 that are applied to early diagnosis of colorectal cancer were searched and performed comprehensive and quantitative analysis by Meta analysis, hence screening genetic markers used in early diagnosis of colorectal cancer. KEGG analysis was employed to establish apoptotic regulatory network model based on screened genetic markers, and optimization was conducted on TCM targets. Through Meta analysis, seven genetic markers were screened out, including WWOX, K-ras, COX-2, P53, APC, DCC and PTEN, among which DCC has the highest diagnostic efficiency. Apoptotic regulatory network was built by KEGG analysis. Currently, it was

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2017.01.036

reported that TCM has regulatory function on gene locus in apoptotic regulatory network. The apoptotic regulatory model of colorectal cancer established in this study provides theoretical evidence for early diagnosis and TCM targeted therapy of colorectal cancer in clinic.

1. Introduction

Worldwide, 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 2008, there were 1.2 million new cases of colorectal cancer in the world, 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 people's low awareness of the disease, most patients have been in advanced stage when diagnosed, and metastasis has occurred in about 25% patients when first diagnosed. Therefore, the prevention and control of colorectal cancer for now and the future should be focused on increasing early diagnostic rate, early treatment and improving prognosis of colorectal cancer.

Meta analysis refers to a quantitative literature review which takes multiple independent research results on the same topic as objects, and based on strict design, it employs proper statistical methods to perform systematic, objective, quantitative and comprehensive analysis, playing a crucial role in clinical diagnosis, treatment, risk evaluation, prevention and intervention, health service and decision (Zhou et al., 2010). And this method not only promotes efficacy of statistical inference thus lessening inconsistency of single research and drawing more comprehensive and reliable conclusions (Zhang et al., 2013; Chaiyakunapruk et al., 2014), but also puts forward some novel research subjects and guides direction for further study.

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 levels 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.

With the development of traditional Chinese medicine (TCM), active components of Chinese herbs play an important part in the whole treatment of colorectal cancer; specifically, they work positively in inducing cell apoptosis, inhibiting cell proliferation, impacting invasiveness of tumor cells and drug-resistance reversion, which indicates unique advantages of TCM over colorectal cancer treatment.

Taking databases including CNKI, VIP, Wanfang, Pub Med, and MEDLINE as main sources of literature retrieval, literatures related to genetic markers that are applied to early diagnosis of colorectal cancer were searched and performed comprehensive and quantitative analysis by Meta analysis, hence screening genetic markers which can be used in early diagnosis of colorectal cancer. Regarding 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 KEGG analysis. The model defines regulatory mechanism of colorectal cancer cell's programmed death, and the current situation of TCM target in the established network was analyzed, hence directing individual diagnosis and targeted therapy of colorectal cancer.

2. Material and methods

2.1. Subjects

With CNKI, VIP and Wanfang databases as major sources for Chinese literatures retrieval, literatures published from 1st January, 1990 to 31th December, 2013 were searched under keywords of colorectal cancer, genetic marker, and (early diagnosis. Regarding English literatures, Pub Med and MEDLINE were considered as main sources, and literatures published from 1st January, 1990 to 31th December, 2013 were searched with keywords "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 or 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 there is no restriction of nation, age and sex for all subjects; (4) literature should provide diagnostic 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 or the most complete report was adopted. All included literatures in this study were published in 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) subjects are 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 literatures: (1) general data, including authors, published time, published journal, title, the number 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 obtained from the literature to determine whether the standard is met.

2.3. Meta analysis

All data underwent 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 conducted by Meta-Disc software, and the results were shown as forest graph and SROC figure.

2.4. KEGG analysis

To perform KEGG analysis for genetic markers, the homepage of KEGG PATHWAY Database (http://www.kegg.jp/kegg/pathway.html) was visited. Then, taking "hsa" as filter 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.

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 were included, including 11 Chinese literatures and 2 English literatures. Totally, there were 773 patients with colorectal cancer and 524 controls in included literatures, and specific results are shown in Table 1.

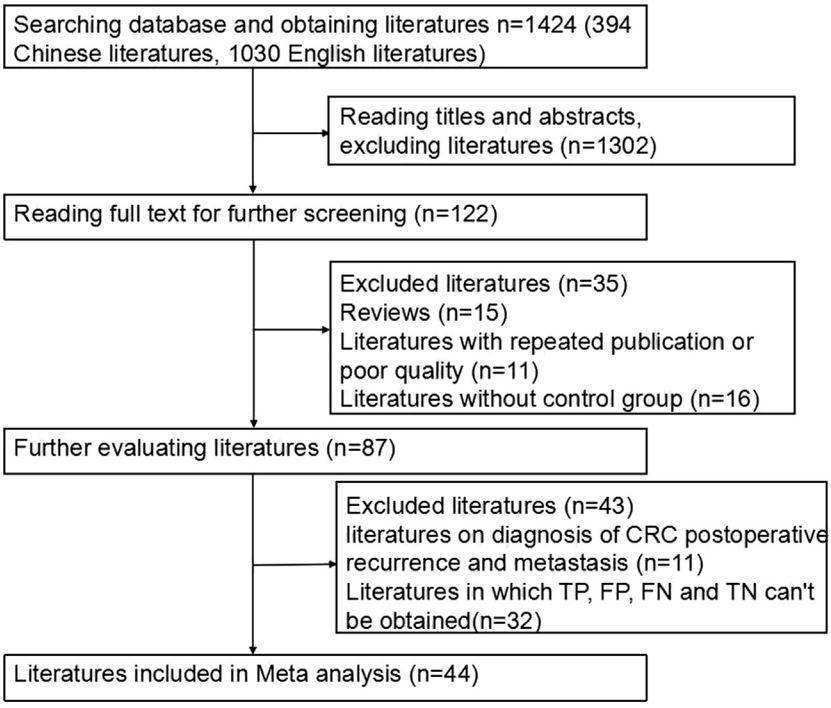

Figure 1 Flow chart of literature search and screening process of Meta analysis on genetic markers for early diagnosis of colorectal cancer.

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

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. According to figures, in the 13 literatures, the sensitivity of p53 for colorectal cancer diagnosis was 24–85%, 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, standard error 0.0563.

3.1.2.3. Bias analysis. Linear 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 diagnostic efficiency of DCC was the highest.

3.2. Establishment of apoptotic regulatory network of genetic markers for colorectal cancer

Through KEGG analysis of seven genetic markers based on KEGG 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. According to results of KEGG analysis of WWOX, PTEN and COX-2 gene, these three genes were added to establish primary apoptotic regulatory network of genetic markers for colorectal cancer, as shown in Fig. 8.

3.3. Optimal selection of TCM targets in the regulatory network

There are seven genetic markers taking part in the apoptosis in the regulatory network established in this study. At present, it

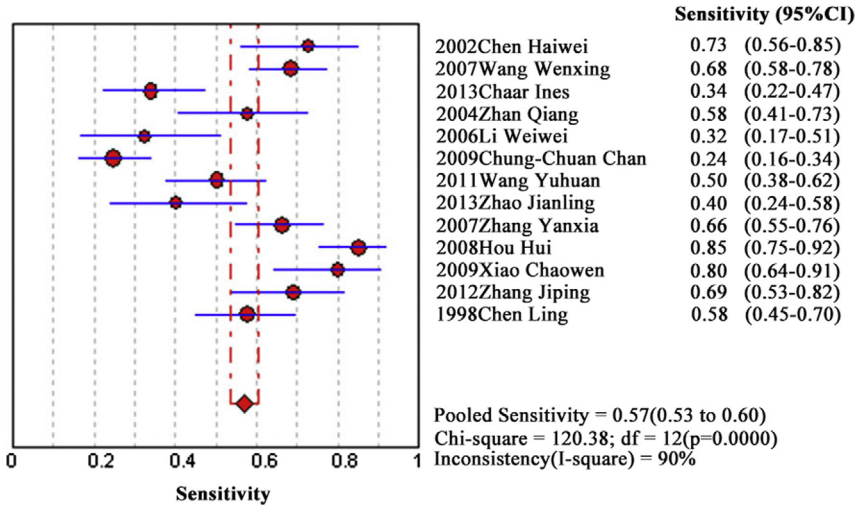

Figure 2 Sensitivity forest plot of p53 for diagnosis of colorectal cancer.

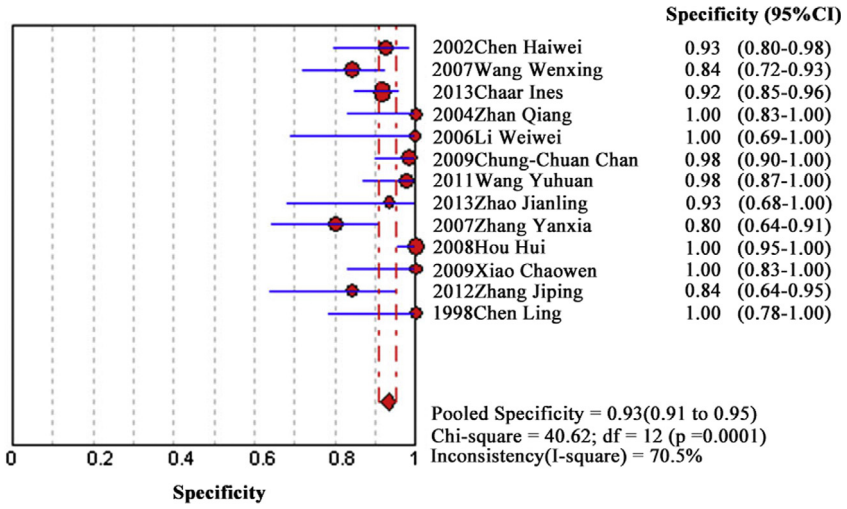

Figure 3 Specificity forest plot of p53 for diagnosis of colorectal cancer.

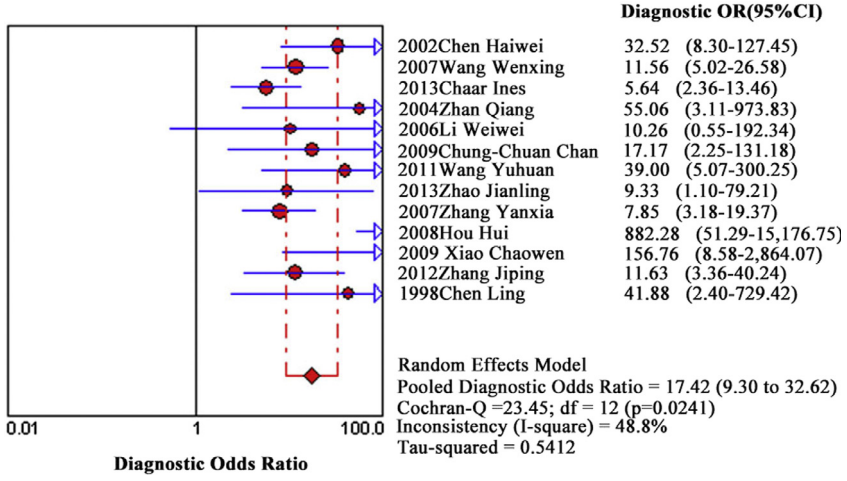

Figure 4 Diagnostic odds ratio graph of p53 for diagnosis of colorectal cancer.

has been reported that the active components in Chinese herb help induce apoptosis of colorectal cancer cells through regulating gene locus. Study by Li et al. (2009) shows that after 24 h cultivation in kaempferol of 15–120 μmol/L, levels of p53 protein and phosphorylation in cell increased with dosage. And study by Mo (2015) suggests that matrine could induce apoptosis of colorectal cancer cells and it is, to some extent, related to K-ras gene mutation. And low-concentration matrine group (0.125 mg/mL, 0.25 mg/mL) showed the function of drifting early apoptotic cell to advanced stage, while high-concentration matrine group (0.5 mg/mL, 1 mg/mL) indicates the function of promoting tumor cell into early apoptotic stage. Li et al. (2014) reported that after cultivation of paeonol, apoptotic colorectal cancer cells increased from 15.4% to 38.6%, and the difference was statistically significant as compared to control group. In order to explore relationship between cox-2 and paeonol, LoVo cells were cultured for 48 h respectively in paeonol with concentration of 0, 30, 60 and 120 mg/L, and it was found that cox-2's expression level was lower than that in control group when cultured in paeonol with concentration of 30 mg/L and it decreased further in paeonol of 120 mg/L. What's more, study by Chen et al. (2013) found that in HCT116 PTEN+/+ cells, curcumin caused G2/M arrest, while it induced G0/G1 arrest in HCT116PTEN−/− cells.

4. Discussion

Colorectal cancer, a genetic disease, is a multi-phase and long-term process in which proto-oncogene is activated and suppressor gene is inactivated under the environmental effect. Since the onset of colorectal cancer is latent and public awareness of its symptoms is low, most patients have been in advanced phase when diagnosed. Up to 50% of newly diagnosed patients eventually develop into metastatic colorectal cancer with five-year survival rate less than 5%. And patients with intermediate and advanced colorectal cancer always have poor therapeutic results, and the bad prognosis impairs their

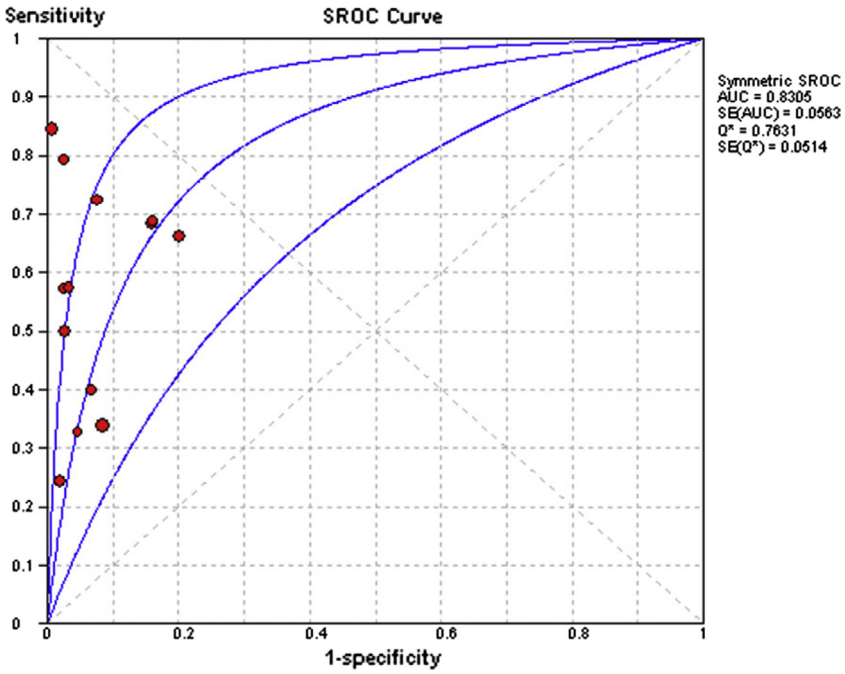

Figure 5 SROC curve of p53 for diagnosis of colorectal cancer.

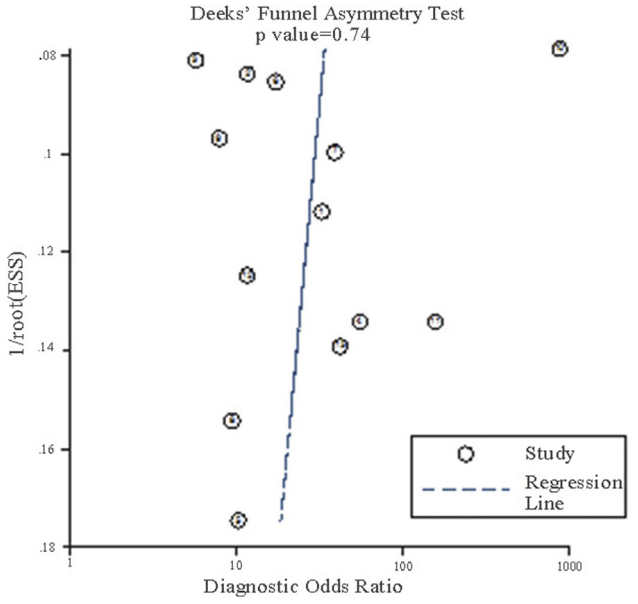

Figure 6 Bias assessment of published literatures on colorectal cancer diagnosis.

life quality, at the same time, it also imposes them large economic burden (Ikeguchi et al., 1999).

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 been in advanced phase when diagnosed and their treatment and prognosis are severely affected. Thus it's necessary to find a diagnostic method 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 has been expanded; in addition, 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 mechanism of colorectal cancer and the relationship of these alterations with emerging biomarkers for pathological diagnosis, patient prognosis and the prediction of treatment responses, which provides significant evidence for early diagnosis and treatment of tumor.

Literatures associated with genetic markers that are 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 suggests that these seven genetic markers have high diagnostic efficacy with DCC highest and WWOX lowest.

KEGG database was used to conduct KEGG signaling pathway analysis on the seven genetic markers (WWOX, K-ras, COX-2, p53, APC, DCC and PTEN) and then to establish a primary apoptotic regulatory network.

As a form of cell death, apoptosis is important for body. Disturbance of apoptosis is a key factor leading to tumor. And during the formation of tumor, cell apoptotic ability reduces gradually; therefore, inducing tumor cell to apoptosis is one of effective methods for tumor treatment.

Kaempferol is a kind of flavanoid extracted from Zingiberaceae Rhizoma Kaempferiae and Santalaceae Herba Thesii and it widely exists in various vegetables and fruits (Zhou

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)

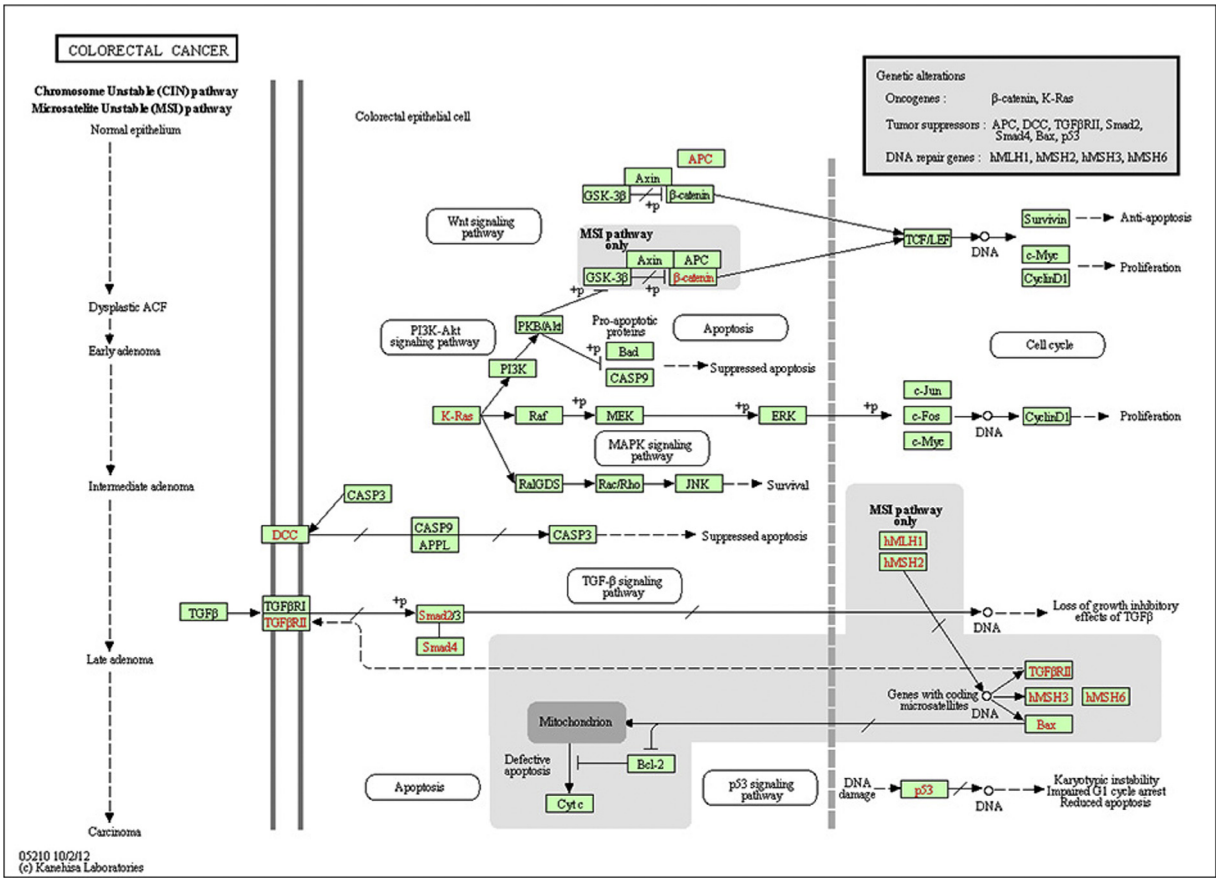

Figure 7 Primary apoptotic regulatory network of genetic markers for colorectal cancer.

et al., 2015). P53 plays an important role in biological progress and cell components and takes part in apoptosis with positive regulation in apoptotic signaling pathway (Stracquadanio et al., 2016). When kaempferol acts on human colorectal cancer SW48 cells, levels of p53 protein and phosphorylation increase with dosage, Bcl-2 level reduces and expression of Bax protein increases significantly, consequently inducing tumor cell's apoptosis (Li et al., 2009).

Matrine is an active pharmaceutical ingredient extracted from Chinese herb legumina Sophora flavescens (Zhang and Chen, 2009). Over these years, there are more and more anti-tumor researches on matrine, and it was found by many studies that matrine can inhibit multiple malignant tumors and its anti-tumor mechanism is to inhibit tumor cell proliferation and metastasis, to induce tumor cell differentiation and apoptosis, to inhibit the activity of telomerase and to inhibit tumor angiogenesis, etc. And as a newly found antitumor drug with low toxicity, matrine has broad applicable prospect. K-ras is one of proto-oncogenes of intracellular signaling protein. And K-ras gene mutation is not only the adverse factor of the growth and development of colorectal cancer but also the early event for colorectal cancer; at the same time, it is closely correlated to targeted therapy, playing a crucial role in the treatment of colorectal cancer (Liu and Fu, 2012). Study by Mo (2015) indicated that after 24-h cultivation of K-ras gene mutant SW480 cell in matrine, SW480 cell showed typical apoptotic changes under optical microscope. Through Annexin V-FITC/PI staining, it was found that induction of

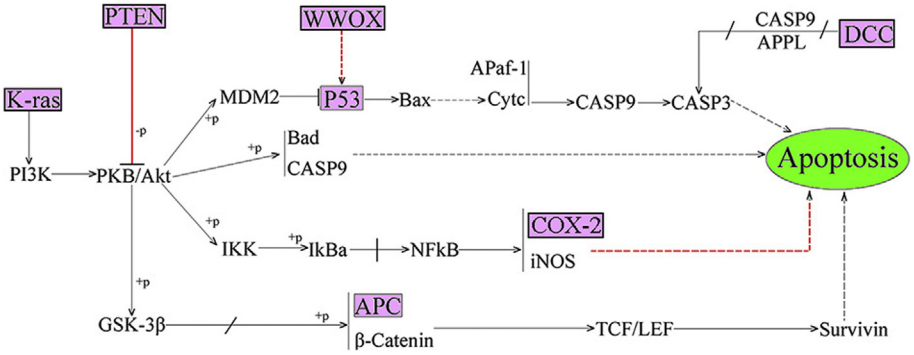

Figure 8 Apoptotic regulatory network of genetic markers for colorectal cancer.

matrine on SW480 cell apoptosis is concentration-dependence. And matrine can inhibit cell proliferation of colorectal cancer by K-ras gene mutation, which was dose-and time-dependent.

Paeonol is the main component of Paeonia suffruticosa (Hu and Xu, 2014). COX-2 called as "quick reaction gene" is an inducible enzyme which takes part in epoxidase pathway, inflammatory response and blood pressure regulation. And COX-2 expresses low in health tissues but it is elevated rapidly when simulated by various internal and external factors. Tabriz et al. (2016) reported that COX-2 is involved in the development of tumor. Besides, study by Li et al. (2014) suggested that paeonol is able to inhibit cell proliferation of colorectal cancer and its inducible efficacy of colorectal cancer cells is correlated to the reduction of COX-2 expression level. Immunostaining analysis indicated that paeonol inhibits activation of upstream regulator NF-κB pathway protein of COX-2, and can inhibit gene transcription correlated to COX-2 and PGE2 caused by entrance of NF-κB protein into karyon. With the elevation of paeonol dosage, pro-apoptotic factor Bax expresses increasingly and antiapoptosis factor Bcl-1 expresses decreasingly.

Curcuminoid is extracted from rhizomes of some Zingiberaceae and Araceae plants and it has diketone pigment which rarely exists in plant kingdom (Zhang et al., 2011). Curcuminoid has various pathological functions, like blood fat lowering, anti-tumor, anti-inflammation, choleretic effect, anti-oxidation, anti-hepatotoxin, anti-rheumatism and antibacterial function, etc. PTEN is an important oncogene with alkaline phosphatase activity and it covers three domains, biological process, molecular function and cellular components, crucially functioning in T cell receptor signaling pathway, inositol phosphate metabolism and phospholipid metabolism. According to the apoptotic regulatory network model established in this study, PTEN can negatively regulate serine-threonine kinases B (PKB/Akt) and takes part in the apoptotic pathway, which is in line with the report of Zeng et al. (2016). In the study of Chen et al. (2013), the inhibition difference of curcuminoid on colorectal cancer under different status was analyzed, and the study indicated that the damage of PTEN genes doesn't affect the sensitivity of colorectal cancer cells to 5-FU, irinotecan, oxaliplatin but it increases the sensitivity of colorectal cancer cell to curcuminoid and increases the toxicity of curcuminoid to colorectal cancer cell. At the same time, PETEN gene defect changes the model of curcuminoid, thereby inducing cell cycle arrest. Besides, in HCT116 PTEN+/+ cells and HCT116PTEN−/− cells, curcuminoid respectively causes G2/M and G0/G1 arrests, indicating that curcuminoid has potentially therapeutic function on colorectal cancer with PTEN gene mutation.

Traditional Chinese Medicine Integrated Database (TCMID) (web site: http://www.megabionet.org/tcmid/) collected about 47,000 prescriptions and 8159 traditional Chinese medicines and their 25,210 ingredients; according to their main components, they were connected with 3791 diseases, 6828 medicines and 17,521 disease-associated proteins (Xue, 2013). And it's found that APC and p53 are, to some extent, correlated to colorectal cancer; there are 11 TCM components related to APC, which are apigenin, Emodin and Pedatisectin B, etc., and 197 TCM components linked to p53 which are astaxanthin, oroxylin, Chrysin, aescin, campherol, quercitrin and aloe emodin, etc. What's more, in the regulatory network of colorectal cancer established in this study, APC and p53 are key critical nodes.

A primary apoptotic regulatory network of colorectal cancer based on p53, APC, DCC, K-ras, PTEN, WWOX and COX-2 were established in this study by Meta analysis combined with KEGG signal pathway analysis, and the current situation of TCM targets in the network was analyzed as well, providing a theoretical foundation for early diagnosis and targeted treatment of colorectal cancer in clinic. To date, idea of "multiple-target medicine" based on molecular network has been a new trend, which changes the traditional model of "one medicine, one gene and one disease". TCM Network Pharmacology shows that researching components of TCM and its targets' function under background of cell network helps understand the general, dialectical and coordinated TCM medication principle. We hold the belief that expounding the mechanism of TCM on the basis of molecular network regulation may conversely develop new multi-component medicines from efficient clinical TCM, which will also positively drive the development of modernization of TCM (Zhao et al., 2014; Hopkins, 2008).

Acknowledgments

This study was supported by the National Natural Science Foundation of China (Grant: 81373879), the Open cooperation project of Henan Province, China (Grant No. 132106000064), the Program of research in base and cutting-edge technologies of Henan Province, China (152300410151), and the National 12th "Five-Year" Technology Support Programs (2013BAI02B00). I thank all authors who have con-

tributed to this paper for advice and comments and thank Cancer Hospital Affiliated to Zhengzhou University for providing technical support.

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Appendix (Literatures included in Meta analysis)

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