The application of C12 biochip in the diagnosis and monitoring of colorectal cancer: Systematic evaluation and suggestion for improvement

Chen C, Chen LQ[1], Yang GL, Li Y

Department of Oncology, Zhongnan Hospital of Wuhan University, Hubei Key Laboratory of Tumour Biological Behaviors and Hubei Cancer Clinical Study Center, Wuhan, 430 071, [1]Department of Oncology, Central Hospital of Xian'ning City, Xian'ning, Hubei, 437 100, P. R. China

Correspondence:
Li Yan
E-mail: liyansd2@163.com

Received : 28-02-07
Review completed : 01-04-08
Accepted : 06-05-08
pubMed ID : 18626164
J Postgrad Med 2008;54:186-90

ABSTRACT

Background: The 12 tumor markers' (TMs) biochip diagnostic (C12) system has been proven useful in some previous studies but its value for colorectal cancer (CRC) only was not systematically investigated. Aims: To evaluate the value of C12 system for CRC. Settings and Design: The associations between TMs and clinicopathological characteristics were evaluated. The most relevant TMs, the most useful combinations, and the correlations between TM levels were assessed. Materials and Methods: The TMs detected by the C12 system in the sera of 170 pathologically confirmed CRC patients were analyzed. One or more TMs higher than or equal to reference value were defined as positive. Statistical Analysis: Chi-square test, Spearman rank correlation test and Receiver-operating characteristic (ROC) curves were used for the analysis. Results: The overall positive rate was 41.76%, and was low in stage 0- Ⅰ (12.90%). Carcinoembryonic cantigen (CEA) had the highest positive rate of 36.47%. The positive rates were significantly correlated to clinical stages and lymph node status, but not to age, sex, tumor location and pathological types. Any combinations of the five highest positive TMs did not have significantly improvements. The levels of three most related TMs (CEA, CA19-9, CA242) of CRC had positive correlation with each other. CA242 and β-HCG levels were associated with lymph node metastasis. Conclusions: C12 system has some value in advanced CRC, but not in early CRC.

KEY WORDS: Clinical staging, colorectal cancer, early diagnosis, protein biochip tumor markers

Colorectal cancer (CRC) is the third most common malignant tumor in the world.[1] In China, CRC is the fifth most common malignancy, and with changes in disease spectrum and diet structures, the incidence of CRC has been steadily increasing, especially in urban areas, making it one of the key diseases in China's anti-cancer campaign.[2] Tumor markers (TMs) play an important role in the diagnosis, prognosis, treatment selection and monitoring of cancer.[3] However, the sensitivity of TMs for early diagnosis needs to be improved,[4] especially in CRC.[5] A combination of several TMs appears to be a promising strategy for improving the diagnosis.[4]

In an effort to improve the screening and early diagnosis, a 12-TM protein chip system (C12) was developed in China,[6] which covers a wide range of common tumors, including CRC. The C12 system detects 12 common TMs in the serum, including cancer antigen 125 (CA125), cancer antigen 15-3 (CA15-3), cancer antigen 19-9 (CA19-9), cancer antigen 242 (CA242), carcinoembryonic cantigen (CEA), alpha fetoprotein (AFP), prostate specific antigen (PSA), free prostate specific cantigen (Fr-PSA), human growth hormone (HGH), β-human chorionic gonadotropin (β-HCG), neuron-specific enolase (NSE) and ferritin. This system has been tested in some relatively large-scale studies on a variety of cancers,[7-9] but up to now, no systematic experience in using such a biochip system exclusively for the diagnosis of CRC has ever been reported. This study was to conduct a systemic evaluation of this system on 170 consecutive CRC patients.

Materials and Methods

Patient population

Among all the CRC patients admitted for surgery at the Department of Oncology, Zhongnan Hospital of Wuhan University from January 2003 to June 2007, 170 patients were enrolled. The main clinicopathological characteristics of these patients are summarized in Table 1; the mean age was 57 years (range 22 to 87 years). All the patients had biopsy confirmation before surgery whenever possible, and the post-surgical stage was classified based on the pathological criteria edition of the American Joint Committee on Cancer (AJCC). The TMs of all the patients were detected in their sera using the C12 system before operation. One or more TMs higher than or equal to the reference value were defined as positive.

Table 1: Main clinicopathological characteristics of the 170 colorectal cancer patients and the correlation with positive tumor markers'

Items	Detection of C12			
	Number of patients (%)	Negative number (%)	Positive number (%)	*P* value
Sex				
Male	108 (63.53)	63 (58.33)	45 (41.27)	0.973
Female	62 (36.47)	36 (58.06)	26 (41.94)	
Age				
<60 years	93 (54.71)	50 (53.76)	43 (46.24)	0.194
≥60 years	77(45.29)	49 (63.64)	28 (36.36)	
Location				
Colon	69 (40.59)	41 (59.42)	28 (40.58)	0.796
Rectum	101 (59.41)	58 (57.43)	43 (42.57)	
Lymph node				
Negative	86 (50.59)	61 (70.93)	25 (29.07)	0.042
Positive	52 (30.59)	28 (53.85)	24 (46.15)	
Unknown[#]	32 (18.82)	10 (31.25)	22 (68.75)	
Clinical stages				
Stage 0-I	31 (18.23)	27 (87.10)	4 (12.90)	0.000
Stage II	52 (30.59)	32 (61.54)	20 (38.46)	
Stage III	44 (25.88)	26 (59.09)	18 (40.91)	
Stage IV	36 (21.18)	11 (30.56)	25 (69.44)	
Unknown[‡]	7 (4.12)	3 (42.86)	4 (57.14)	
Pathological types				
Adenocarcinoma, highly differentiated	29 (17.06)	21 (73.31)	8(27.59)	0.470
Adenocarcinoma, intermediately differentiated	110 (64.71)	6 5(59.09)	45(40.91)	
Adenocarcinoma, poorly differentiated	6 (3.53)	3 (50.00)	3 (50.00)	
mucinous adenocarcinoma	11 (6.47)	5 (45.45)	6 (54.55)	
Others*	4 (2.35)	2 (50.00)	2 (50.00)	
Unknown[†]	10 (5.88)	3 (30.00)	7 (70.00)	
Total	170	99 (58.24)	71 (41.76)	-

[#]Referral patients or patients with widespread metastases without specific treatment or patients discharged from hospital without operation; [‡]Patients discharged from hospital without operation; *Others included signet-ring cell carcinoma, two cases; carcinoid, one case; undifferentiated carcinoma, one case; [†]Referral patients or patients with widespread metastases without specific treatment

Blood sample collection and detection of serum tumor markers

Three milliliters of fasting blood was taken from each patient in the next morning following admission, and the serum was separated for the detection of TMs using the C12 biochip system according to the manufacturer's instruction (Shanghai HealthDigit Co., Ltd. Shanghai, China).

Statistical analysis

The positive rates of TMs were calculated, and the associations between positive rates of TMs and clinicopathological characteristics were assessed with the Chi-square test. Preliminary descriptive statistics revealed that the distribution of 12 TMs' levels did not follow a normal distribution. Consequently, the correlation between serum levels of various markers was assessed with the Spearman rank correlation test. Receiver-operating characteristic (ROC) curves were used to evaluate the serum TMs levels between patients with lymph node metastasis and without metastasis, and the area under the curve (AUC) values were determined. Statistical analyses were performed using SPSS 13.0 software (SPSS Inc. Chicago, IL) and *P* values of less than 0.05 were considered statistically significant.

Results

Main clinicopathological characteristics of 170 CRC patients

As shown in Table 1, the ratio of male to female was nearly 2 to 1 and more than half of the patients were less than 60 years old. Most tumors were located in the rectum and nearly one-third of CRC patients had lymph node metastases. Only 18.23% (31/170) of these patients were in stage 0-I. The main pathological type was adenocarcinoma, accounting for more than 80% of 170 CRC patients.

Positive rates of TMs

The associations between positive rates of TMs with clinicopathological characteristics are also summarized in Table 1. The data of characteristics which were defined as unknown were excluded from the analysis. There were no correlations between positive rates of TMs and sex (male, female), age (≥60, <60 years old), tumor location (colon, rectum) and pathological types (highly, intermediately and poorly differentiated adenocarcinoma, mucinous adenocarcinoma, others) ($P > 0.05$).

The positive rate of TMs was statistically higher in those with positive lymph nodes (positive vs. negative, $P < 0.05$) [Table 1]. There were significant differences of the positive rates of TMs among different clinical stages (contingency table chi-square test, $P = 0.000$) [Table 1]. On further analysis, other than the statistically significant differences in the positive rates of stage I vs stage IV ($P = 0.000$) and stage II vs stage IV ($P = 0.004$), the differences in positive rates among all the other stages were statistically not significant ($P > 0.05/6$).

The positive rate of each TM in the C12 system is listed in Table 2. Only a few TMs were positive in CRC. The five most

Table 2: Positive rates of each TM in 170 CRC patients

Items	Normal range	Positive numbers (%)	Test results
CEA	<5.0 ng/ml	62 (36.47)	5.05-219.78 ng/ml
CA242	<20.0 KU/L	34 (20.00)	20.44-174.05 KU/L
CA19-9	<35.0 KU/L	32 (18.82)	35.4-266.21 KU/L
CA125	<35.0 KU/L	15 (8.82)	36.81-267.51 KU/L
Fr-PSA	<1.0 ng/ml	7 (4.12)	1.1-8.38 ng/ml
PSA	<5.0 ng/ml	5 (2.94)	5.92-15.53 ng/ml
AFP	<20.0 ng/ml	4 (2.35)	22.49-129.41 ng/ml
CA15-3	<35.0 KU/L	3 (1.73)	67.57-160.94 KU/L
HGH	<7.5 ng/ml	3 (1.73)	10.26-18.94 ng/ml
β-HCG	<3.0 ng/ml	2 (1.18)	4.18-5.99 ng/ml
Ferritin	<322 ng/ml	1 (0.59)	365.86 ng/ml
NSE	<13.0 ng/L	0 (0)	-

common TMs for CRC detection were CEA (36.47%), CA242 (20.00%), CA19-9 (18.82%), CA125 (8.82%) and Fr-PSA (4.12%). The combination of these five TMs is shown in Table 3. Compared with CEA, the most sensitive TM in this system, any combination of TMs could not significantly increase the positive rates ($P > 0.05$ for all comparisons with CEA). Combination of four markers (CEA + CA242 + CA125 + Fr-PSA or CEA + CA19-9 + CA125 + Fr-PSA) was as good as 12 markers in terms of diagnosis.

These results were confirmed by the Spearman correlation analysis, which showed that CEA, CA19-9 and CA242, the three most frequent TMs of this study, were positively correlated with correlation coefficients (R) of 0.666 ($P = 0.000$) for CEA vs. CA242, 0.553 ($P = 0.000$) for CEA vs. CA19-9, and 0.844 ($P = 0.000$) for C242 vs. CA19-9.

The associations of highest single and combined TMs' positive rates with different clinical stages are illustrated in Figure 1. The positive rates in each group were significantly associated with the clinical stage of CRC ($P = 0.000$). On further analysis, the differences between stage 0-I and stage IV in each group were statistically significant ($P = 0.000$). In addition, the differences between stage 0-I and stage III, stage 0-I and stage III were statistically significant in CEA, CEA + CA242, and CEA + CA19-9 group. But in CEA + Fr-PSA group, only stage 0-I and stage II achieved statistically significant ($P = 0.005$). The differences in four or five TMs' combination group were the same with 12 TMs combination.

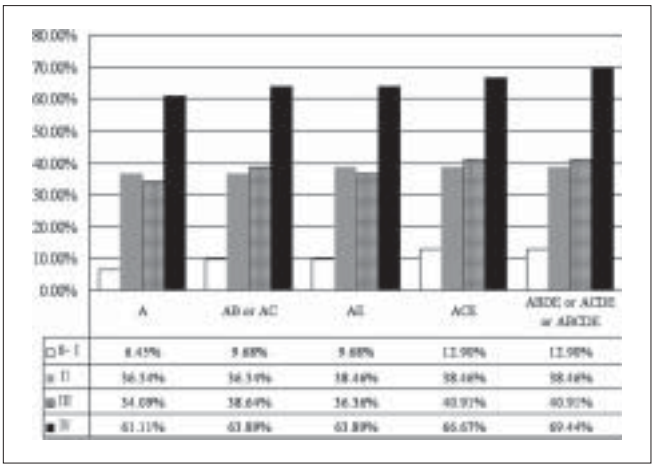

Figure 1: The highest single and combined positive rates of TMs group in different clinical stages. A, CEA; B, CA242; C, CA19-9; D, CA125; E, Fr-PSA.

Association of TMs levels with positive lymph nodes

The ROC curves analysis of TMs levels by lymph node status is shown in Figure 2. Other than Fr-PSA, ferritin and CA153, the serum levels of other TMs showed a tendency to increase in patients with lymph node metastasis (the area under ROC curves [AUC] > 0.5), but only CA242 (AUC = 0.601, 95% confidence interval [95%CI] = 0.501-0.704, $P = 0.047$) and β-HCG (AUC = 0.628, 95%CI = 0.530-0.727, $P = 0.012$) achieved statistical significance. Higher levels of these two markers meant greater probabilities of lymph node metastasis. Therefore, in patients with confirmed CRC, CA242 and β-HCG levels could predict lymph node metastasis better than any other markers in this C12 system.

Discussion

The biochip diagnostic system C12 developed in China[6] is a relatively successful system in screening and detecting cancers.[7-11] Recently, there have been reports on using C12 system for the diagnosis of CRC cancers, as summarized in Table 4. The overall positive rates ranged from 48.1-79.9%, higher than 41.76% in our series of 170 patients with well-defined CRC. But our study achieved a positive rate of 69.44% in stage IV CRC, similar to other reports in overall positive

Table 3: The contribution of five most frequent TMs in C12 system to improve diagnosis*

1 marker		2 markers		3 markers		4 or 5 markers	
Items	Positive numbers (%)	Items	Positive numbers (%)	Items	Positive numbers (%)	Items	Positive numbers (%)
A	62 (36.47)	AB	66 (38.82)	ACE	70 (41.18)	ACDE	71 (41.76)
B	34 (20.00)	AC	66 (38.82)	ABE	69 (40.59)	ABDE	71 (41.76)
C	32 (18.82)	AE	66 (38.82)	ACD	67 (39.41)	ABCE	70 (41.18)
D	15 (8.82)	AD	63 (37.06)	ABD	67 (39.41)	ABCD	67 (39.41)
E	7 (4.12)	BE	40 (23.53)	ADE	67 (39.41)	BCDE	41 (24.12)
		CE	38 (22.35)	ABC	66 (38.82)	ABCDE	71 (41.76)
		BD	36 (21.18)	BDE	41 (24.12)		
		CD	35 (20.59)	BCE	40 (23.53)		
		BC	34 (20.00)	CDE	40 (23.53)		
		DE	21 (12.35)	BCD	36 (21.18)		

*A, CEA; B, CA242; C, CA19-9; D, CA125; E, Fr-PSA

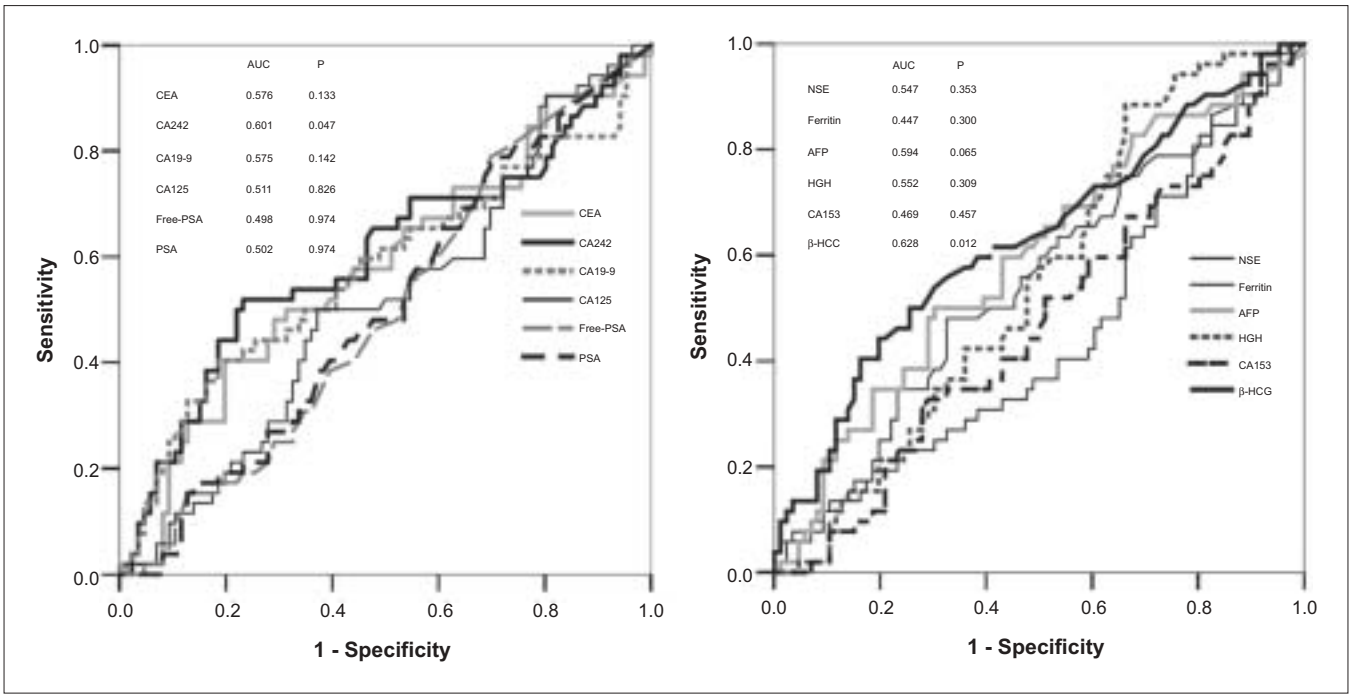

Figure 2: ROC curves of 12 TMs assays for CRC patients with lymph node metastasis in relation to those without metastasis. AUC, area under ROC curve.

Table 4: Summary of studies evaluating multiple tumor markers' combination for the diagnosis of colorectal cancer

Items	Author (year)	Number of CRC patients	Combined positive rate	Positive rate of single TM (%)		
C12#	Sun, *et al.*[6] (2004)	158	48.1	27.6	17.1	23.8
	Yu[7] (2005)	229	61.1	38.8	27.0	28.0
	Liang, *et al.*[8] (2005)	124	50.0	33.0	25.8	27.4
	Jiang, *et al.*[9] (2005)	98	61.2	32.7	23.5	27.6
	Mou, *et al.*[10] (2005)	139	79.9	48.2	28.1	32.4
	Zhong, *et al.*[11] (2007)	239	72.0	53.5	42.5	43.5
CA72-4 + CEA	Carpelan-Holmstrom, *et al.*[17] (2004)	204	NP*	CA72-4, 27.0; CEA, 43.6		
G-CSF + CEA	Mroczko, *et al.*[18] (2006)	76	55.0	G-CSF, 26.0; CEA,37.0		
VEGF + CEA	Tsai, *et al.*[19] (2006)	279	73.5	VEGF,63.0; CEA, 42.5		
P53 + CEA	Abdel-Aziz, *et al.*[20] (2007)	48	60.4	P53, 39.6; CEA, 31.3		
EGFR + CEA	-	48	81.2	EGFR, 70.8; CEA, 31.3		

#The positive single TM represented CEA, CA242 and CA19-9, respectively; G-CSF, Granulocyte-colony stimulating factor; VEGF, Vascular endothelial growth factor; EGFR, Epidermal growth factor receptor *NP = Not provided

rates. However, except for this study, none of the other studies correlated C12 results with clinicopathological stages of the patients, making it impossible to assess the value of C12 in diagnosing tumors at various stages. Moreover, the positive rate in early-stage cancer was not high, as indicated in the original development report, this C12 system did not work well in screening and diagnosing early-stage CRC.[6]

In order to evaluate whether the combination of key TMs could enhance the positive rate, we tested the combination of two, three, four, or five TMs against a single marker, CEA, which proved to be the most sensitive marker in this diagnosis system. Any of the combinations did not significantly enhance the positive rate compared with CEA alone.

The limited value of C12 system for early diagnosis of CRC might due to two reasons. First, the sensitivity and specificity of TMs remain limited, and there was almost no single marker which is sensitive and specific enough to perform an accurate diagnosis,[4] especially in CRC.[5] In this study, the three highest positive TMs were CEA, C242 and CA19-9, but their positive rates, especially in the early stage, were not high. Even for CEA, which was the only marker widely accepted to be of positive value in CRC,[4-5] the positive rate was only 36.47%, and less than 7% in early stage. Therefore, a better use of CEA could be in prognosis and monitoring.[4-5,12] Discovering new CRC TMs, especially those related with early CRC detection was one of the strategies to solve this problem. Fortunately, with the development of technology,[13] especially the proteomics techniques,[14] some progress has already been made recently.[15-16]

Second, the TMs selected in this C12 system were not the

optimal combination for CRC. Only several related TMs of CRC in this system, and moreover, the Spearman correlation analysis indicated that the three highest positive TMs (CEA, CA242, CA19-9) were positively correlated with each other, and their combinations could not improve the positive rates, as shown by Carpelan-Holmstrom *et al*.[17] In other words, these markers are redundant but not complementary. Therefore, optimizing the combination of TMs, especially with new markers which could provide additional information, was another possible strategy for improving the positive rate for early CRC, and several promising combinations are summarized in Table 4.[17-20]

The ROC analysis is now widely recognized as the best approach for measuring the quality of diagnostic information and diagnostic decisions.[21] In this study, this method was used to evaluate the serum TMs' levels between patients by lymph node status. We found that CA242 and β-HCG were associated with lymph node metastasis, whereas the other 10 TMs showed no such relationships, even for CEA and CA19-9, which are in agreement with Mroczko's report.[22] Therefore, CA242 could be used in the future chip development to indicate the lymph node status.

In conclusion, this is the first systematic evaluation on the value of C12 biochip diagnostic system for CRC, and the findings suggest that this system has some value in the diagnosis of advanced CRC, but not in early CRC. Discovering new TMs and optimizing the combination of TMs might help improve the diagnosis.

Acknowledgments

Supported by New-Century Excellent Talents Supporting Program of the Ministry of Education of China (NCET-04-0669), Foundation for the Author of National Excellent Doctoral Dissertation of PR China (FANEDD-200464), Natural Science Funding of China (20675058), and Wuhan Innovation Study Project (20066002054).

References

1. Kamangar F, Dores GM, Anderson WF. Patterns of cancer incidence, mortality, and prevalence across five continents: Defining priorities to reduce cancer disparities in different geographic regions of the world. J Clin Oncol 2006;24:2137-50.
2. Dong ZW, Qiao YL, Li LD, Chen YD, Wang RT, Lei TH, *et al*. Report of Chinese cancer control strategy. China Cancer 2002;11:250-60.
3. Ludwig JA, Weinstein JN. Biomarkers in cancer staging, prognosis and treatment selection. Nat Rev Cancer 2005;5:845-56.
4. Voorzanger-Rousselot N, Garnero P. Biochemical markers in oncology, Part I: molecular basis, Part II: clinical uses. Cancer Treat Rev 2007;33:230-83.
5. Duffy MJ, van Dalen A, Haglund C, Hansson L, Holinski-Feder E, Klapdor R, *et al*. Tumour markers in colorectal cancer: European Group on Tumour Markers (EGTM) guidelines for clinical use. Eur J Cancer 2007;43:1348-60.
6. Sun ZH, Fu XL, Zhang L, Yang X, Liu F, Hu G. A protein chip system for parallel analysis of multi-tumor markers and its application in cancer detection. Anticancer Res 2004;24:1159-66.
7. Yu DY. Application of multi-tumor markers protein chip assistance diagnostic system for detection of various tumors. China Trop Med 2005;5:414-16.
8. Liang Z, Wang HF, Wu AZ, Fu SM. Clinical value of 12 tumor markers protein biochip detection of digestive system neoplasm. China Trop Med 2005;5:407-09.
9. Jiang ZJ, Zhou W X, Lin W, Huang QX. Application of multi-tumor markers protein chip diagnose system in the diagnosing of intestinal neoplasms. J Mod Lab Med 2005;20:17-19.
10. Mou JH, Li ZP, Wang D, Ma Y, Xiao HL, Zhang QH. Combined detection of multiple tumor marker and its diagnostic value in colorectal cancer. J Digest Surg 2005;4:268-70.
11. Zhong ZY, Wang D, Li ZP, Li MX, Dai N, Cao XJ, *et al*. Clinical significance of C-12 multiple tumor marker protein chip detective system in diagnosing colorectal carcinoma. Chongqing Med 2007;36:2406-08.
12. Locker GY, Hamilton S, Harris J, Jessup JM, Kemeny N, Macdonald JS, *et al*. ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. J Clin Oncol 2006;24:5313-27.
13. Hundt S, Haug U, Brenner H. Blood markers for early detection of colorectal cancer: a systematic review. Cancer Epidemiol Biomarkers Prev 2007;16:1935-53.
14. Bitarte N, Bandres E, Zarate R, Ramirez N, Garcia-Foncillas J. Moving forward in colorectal cancer research, what proteomics has to tell. World J Gastroenterol 2007;13:5813-21.
15. Madoz-Gurpide J, Lopez-Serra P, Martinez-Torrecuadrada JL, Sanchez L, Lombardia L, Casal JI. Proteomics-based validation of genomic data: applications in colorectal cancer diagnosis. Mol Cell Proteomics 2006;5:1471-83.
16. Cowan ML, Vera J. Proteomics: advances in biomarker discovery. Expert Rev Proteomics 2008;5:21-23.
17. Carpelan-Holmstrom M, Louhimo J, Stenman UH, Alfthan H, Jarvinen H, Haglund C. Estimating the probability of cancer with several tumor markers in patients with colorectal disease. Oncology 2004;66:296-302.
18. Mroczko B, Groblewska M, Wereszczynska-Siemiatkowska U, Kedra B, Konopko M, Szmitkowski M. The diagnostic value of G-CSF measurement in the sera of colorectal cancer and adenoma patients. Clin Chim Acta 2006;371:143-47.
19. Tsai WS, Changchien CR, Yeh CY, Chen JS, Tang R, Chiang JM, *et al*. Preoperative plasma vascular endothelial growth factor but not nitrite is a useful complementary tumor marker in patients with colorectal cancer. Dis Colon Rectum 2006;49:883-94.
20. Abdel-Aziz MM, Lotfy M, El-Kady IM, Abozaid M. Mutant p53 protein in the serum of patients with colorectal cancer: Correlation with the level of carcinoembryonic antigen and serum epidermal growth factor receptor. Cancer Detect Prev 2006;Epub ahead of print.
21. Swets JA. Measuring the accuracy of diagnostic systems. Science 1988;240:1285-93.
22. Mroczko B, Groblewska M, Wereszczynska-Siemiatkowska U, Okulczyk B, Kedra B, Laszewicz W, *et al*. Serum macrophage-colony stimulating factor levels in colorectal cancer patients correlate with lymph node metastasis and poor prognosis. Clin Chim Acta 2007;380:208-12.

Source of Support: New-Century Excellent Talents Supporting Program of the Ministry of Education of China (NCET-04-0669), Foundation for the Author of National Excellent Doctoral Dissertation of PR China (FANEDD-200464), Natural Science Funding of China (20675058), and Wuhan Innovation Study Project (20066002054)., **Conflict of Interest:** Not declared.