

RESEARCH ARTICLE

Clinical Application of Serum Tumor Abnormal Protein (TAP) in Colorectal Cancer Patients

Xue-Yan Wu, Xin-En Huang*

Abstract

Objective: To explore the association of serum tumor abnormal protein (TAP) with other serological biomarkers e.g. carcinoembryonic antigen (CEA), carbohydrate antigen 125 (CA125), carbohydrate antigen 19-9 (CA19-9) and its clinical application in colorectal cancer (CRC) patients. **Methods:** Patients (N=98) were enrolled into this study with histologically or cytologically confirmed CRC. Using a test kit, the level of TAP was determined, while chemiluminescence was used to measure the levels of some other common serological biomarkers e.g. CEA, CA125 and CA19-9. **Results:** The area of TAP condensed particulate matter decreased after chemotherapy compared with before chemotherapy when CT or MRI scans showed disease control. In contrast, it increased with disease progression ($P<0.05$). Furthermore, a statistically significant difference was confirmed in monitoring of TAP and common serological biomarkers e.g. CEA and CA19-9 ($p<0.05$). **Conclusions:** Detecting TAP in CRC patients has high sensitivity and specificity and can be used as a new independent indicator for clinically monitoring CRC patients in the course of chemotherapy.

Keywords: Tumor abnormal protein (TAP) - serological biomarkers - colorectal cancer (CRC)

Asian Pac J Cancer Prev, 16 (8), 3425-3428

Introduction

Colorectal cancer (CRC) is one of the commonest cancers in both men and women with an estimated annual incidence of 1 million new cases (Ferlay et al., 2010), and the third most commonly diagnosed cancer and the third leading cause of cancer death world-wide (Jemal et al., 2011). The prognosis in CRC patients is dependent on the stage at which the disease is diagnosed. But due to a frequent lack of early diseasespecific symptoms and a reluctance to seek medical investigation, many CRC cases present late when the disease is at a relatively advanced stage. In the US over the past 10 years there has been progress in reducing the incidence of colorectal cancer through prevention and early detection of the disease. However, in Asia there has been an increase in the morbidity and mortality due to CRC due to changes in lifestyle and diet (Jemal et al., 2011).

A number of methods could be used to screen for CRC. A meta-analysis of trials based on screening with fecal occult blood (FOB) tests indicated a 25% reduction in CRC-related mortality (Hewitson et al., 2008). Similarly, recent results of flexible sigmoidoscopy-based screening programs have shown dramatic reductions in CRC incidence and mortality (Elmunzer et al., 2012). Early detection of CRC in its localized or preinvasive form is therefore likely to represent the most realistic approach to reducing the number of cancer-related deaths (Brenner

et al., 2014). Current screening strategies that are based around FOB testing or colonoscopy suffer from poor uptake based on a lack of patient acceptability. An alternative strategy is to develop reliable and specific biomarkers detectable in a more readily accessible medium, such as the peripheral blood, that can accurately and reliably detect CRC in its earliest stages when treatment options can be maximized (Brenner et al., 2013).

Clinically applicable biomarkers of CRC are not only required for the early detection of the disease, they are also needed for accurate diagnosis, prognostic stratification, and surveillance of CRC following treatment (Luo et al., 2013; Martinez-Aguilar et al., 2013). CEA is a serum glycoprotein and currently is the most widely used marker for colon cancer, which is commonly secreted by tumors located in hollow organs and has a specificity and sensitivity of 36 and 87 %, respectively, in screening for colon cancer (McKeown et al., 2014). CA19-9 is an antigen that is elevated in many types of gastrointestinal cancer including colorectal cancer, esophageal cancer, and hepatocellular carcinoma (Perkins et al., 2003). CA19-9 has a sensitivity and specificity of 23 and 96 % for colorectal cancer (Goldberg et al., 1989). CA125 is a glycoprotein antigen that was first found associated with ovarian cancer (Bast et al., 1983).

Serum tumor abnormal protein (TAP) testing kit is a new test kit, developed by ZHEJIANG RUI SHENG MEDICAL TECHNOLOGY, can be quickly and easily

¹Department of Chemotherapy, ²Department of Research, the Affiliated Jiangsu Cancer Hospital of Nanjing Medical University and Jiangsu Institute of Cancer Research, Nanjing, China *For correspondence: apjcpuangxinen@163.com

used for cancer early detection, screening, and monitoring the efficacy of cancer treatment.

Aberrant glycosylation occurs in essentially all types of experimental and human cancers, as has been observed for over 35 years. Many recent studies indicate that some, if not all, aberrant glycosylation is a result of initial oncogenic transformation, as well as a key event in induction of invasion and metastasis. When the aberrant glycosylation occurred, various glycoproteins with abnormal glycan structure generated on the surface of cell. Detection of serum TAP by a group of special agglutinin to assist and promote various glycoproteins inter coagulation and form specific crystalloid condensates (serum tumor abnormal protein (TAP) testing kit, ZHEJIANG RUIHENG MEDICAL TECHNOLOGY). The specific crystalloid condensates can be counted under TAP image analysis system or biological microscope and show significant different with normal sundries generated in blood. Numbers of TAP crystalloid condensate in test blood can used for early detection, accurate diagnosis, prognostic stratification, and monitor the efficacy of CRC treatment.

We designed this study to explore the application of serum TAP before and after two period chemotherapy in CRC patients.

Materials and Methods

Patients and Materials

All patients enrolled were hospital inpatients who had histologically or cytologically confirmed CRC, and blood collected before and after chemotherapy, as described elsewhere (Lu et al., 2014; Wu et al., 2014).

Fasting blood (2ml) was collected from the cubital vein in the morning. All blood samples were examined with TAP testing kit, and then searched and measured the condensed particulate matter. According ZHEJIANG RUIHENG MEDICAL TECHNOLOGY provides criteria: TAP detection value condensed area 0-121 μ m² normal, \geq 121 μ m² abnormal; in abnormal, TAP detection value condensed area 121-225 μ m² as smaller condensation and \geq 225 μ m² as bigger. (serum tumor abnormal protein (TAP) testing kit, ZHEJIANG RUIHENG MEDICAL TECHNOLOGY).

Several serological biomarkers e.g. carcinoembryonic antigen (CEA), carbohydrate antigen 125 (CA125), carbohydrate antigen 19-9 (CA19-9) were measured by chemiluminescence method. According to the manufacturer's instructions, CEA<3.5ng/ml is defined as negative, CA125<35U/ml is negative, CA19-9<39U/ml is negative.

Tumor response was assessed every two cycles after chemotherapy, according to World Health Organization criteria and was assessed by CT/MRI or by the same tests used initially to stage the tumor (Gehan et al., 2000). A complete response (CR) was defined as the disappearance of all clinical and radiologic evidence of tumor for at least four weeks; a partial response (PR) was defined as a decrease of 50 percent or more in the sum of the products of the longest perpendicular diameters of all measurable lesions for at least four weeks; and progressive disease

(PD) was defined as an increase of more than 25 percent in the sum of the products of the perpendicular diameters of all measurable lesions or the appearance of new lesions. All other circumstances were considered to indicate stable disease (SD).

Statistical analysis

Continuous variables were summarized by descriptive statistics, categorical variables by frequency. Count data by Chi-square test; measurement data as mean \pm standard deviation. $P < 0.05$ was considered statistically significant. The study data was analyzed through the STATA 8.0 software (Stata Corporation, 4905 Lakeway Drive College Station, Texas 77845 USA). We have enough experience in conducting medical researches, and have published some result elsewhere (Huang et al., 2014; Ji et al., 2014; Liu et al., 2014; Tian et al., 2014; Wang et al., 2015; Wu et al., 2015; Yang et al., 2015).

Results

From September 1st 2014 to February 28th 2015, a total of 98 CRC patients (male/female 59:39, mean age: 57) were enrolled in the study. Most CRC patients (68.4%) accepted palliative therapy and the rest 31 patients (31.6%) accepted adjuvant therapy. 32.7% (32/98) of CRC tumors were poorly differentiated; 60.2% (59/98) and 7.1% (7/98) were moderately and well differentiated, respectively. 13.3% (13/98) of all CRC patients confirmed early stage Dukes B, 18.4 (18/98) confirmed Dukes C and 68.3 (67/98) confirmed Dukes D. The demographics and clinical characteristics of all CRC patients are summarized in Table 1.

The area of TAP condensed particulate matter decreased after chemotherapy compared with before chemotherapy when CT or MRI scan showed disease controlled, in contrary, it increased when disease progressed ($P < 0.05$). The level of CEA and CA19-9 in serum showed positive relationship with disease control. It decreased after chemotherapy when disease controlled and increased when disease progressed ($p < 0.05$). In addition,

Table 1. Patient Demographics and Clinical Characteristics

Variables	N=98
Age, median (Range) years	57 (22-80)
Gender	
Males	59 (60.2)
Females	39 (39.8)
Degree of Differentiation	
Poorly Differentiation	32 (32.7)
Intermediate Differentiation	59 (60.2)
High Differentiation	7 (7.1)
Chemotherapy	
Adjuvant Therapy	31 (31.6)
Palliative Therapy	67 (68.4)
Dukes Stage	
A	0 (0.0)
B	13 (13.3)
C	18 (18.4)
D	67 (68.3)

Table 2. The Serum Tumor Abnormal Protein (TAP) Level and Several Serological Biomarkers after Chemotherapy in CRC Patients

	Disease Control (CR+PR+SD)		Disease Progression (PD)		OR (95%CI)	P Value
	Increase N (%)	Decrease N (%)	Increase N (%)	Decrease N (%)		
TAP	16 (21.3)	59 (78.7)	21 (91.3)	2 (8.7)	38.72 (8.2-182.8)	0.000* [#]
CEA**\$\$\$	17 (22.7)	58 (77.3)	18 (78.3)	5 (21.7)	12.28 (3.97-37.97)	0.000* [#]
CA125***\$\$	7 (9.3)	68 (90.7)	3 (13)	20 (87)	1.46 (0.34-6.16)	0.609 [#]
CA19-9****\$	8 (10.7)	67 (89.3)	11 (47.8)	12 (52.2)	.68 (2.56-23.03)	0.0002* [#]

CRC, colorectal cancer; CEA, carcinoembryonic antigen; CA, carbohydrate antigen; TAP, serum tumor abnormal protein; CR, complete response; PR, partial response; SD, stable disease (SD); PD, progressive disease (PD); *, $p < 0.05$; [#]TAP increasing compare with disease progression; **, $p = 0.000$; \$\$\$, TAP compare with CEA; ***, $p = 0.877$; \$\$, TAP compare with CA125; ****, $P = 0.001$; \$, TAP compare with CA19-9; OR, Odds Ratio; 95%CI, 95% Confidence Interval

statistically significant differences were confirmed in monitoring of TAP and common serological biomarkers e.g. CEA and CA19-9 ($p < 0.05$).

Discussion

CRC is one of the most commonly diagnosed cancers in the world, and despite its several screening methods, the morbidity and mortality of CRC are still high (Siegel et al., 2012). Many of CRC patients are diagnosed at advanced stages of the disease by which existent therapies are ineffectual. For a good outcome of colorectal cancer therapy, it is essential to detect the disease on-time in early stages may be through candidate biomarkers, which play important functions in cancer progression (Kim et al., 2008; Newton et al., 2012). CEA is the most commonly used tumor marker in patients with colorectal cancer (Filella et al., 1992). CEA has a specificity for colorectal cancer of 90%, but a sensitivity of only 40% to 75%. There is also a transient increase of approximately 20% of CEA level in 12% to 15% of the patients with colorectal cancer during chemotherapy (Sorbye et al., 2004; Li et al., 2009). An additional marker to monitor colorectal cancer is carcinoma antigen (CA) 19-9. CA 19-9 was described by Koprowski et al in 1979 as a monoclonal antibody, raised against a human colorectal cancer cell line. CA19-9 has a sensitivity and specificity of 23 and 96 % for colorectal cancer (Goldberg et al., 1989). Several studies have reported that the serum CEA measurement is an insensitive test for the early detection and screening, because it is often low in early stages of CRC progression (Winawer et al., 2003; Eleftheriadis et al., 2009). Many evidences have also indicated that accumulation of genetic and epigenetic alterations plays major roles in initiation and progression of CRC (Migliore et al., 2011). Different genes with altered levels of promoter methylation are defined as useful biomarkers for CRC tumorigenesis, such as APC genes (APC), O6-methylguanine-DNA methyltransferase (MGMT) (Lee et al., 2009), Septin 9 gene (SEPT9) (Grutzmann et al., 2008), the genes ALX homeobox 4 (ALX4) and two follistatin-like domains 2 (TMEFF2) (Huang et al., 2010). Furthermore, a number of peripheral blood biomarkers have been identified for CRC detection, including CEA, Cytokeratin 19 (CK19) and Cytokeratin 20 (CK20) (Xu et al., 2006), although these biomarkers have insufficient sensitivity for detecting primary CRC (Zieglschmid et al., 2005). In addition, in

line with several reports that demonstrated microRNA signature as potential biomarkers for various diseases (including cancer), (Chen et al., 2008) found that plasma miR-29a and miR-92a have significant diagnostic value for early detection of CRC (Huang et al., 2010).

We designed this study to find out a new serum indicator to help monitor the efficacy of CRC chemotherapy. Serum tumor abnormal protein (TAP) is a detection to coagulate various abnormal glycoproteins formed when aberrant glycosylation occurred. Lectin is a kind of carbohydrate-binding protein, can exclusively identify and combine with a specifically glycosylated sequences in a monosaccharide or oligosaccharide with specific configuration. In TAP testing kit, the major ingredients are a group of special agglutinin which can assist and promote various glycoproteins inter coagulation and form specific crystalloid condensates (serum tumor abnormal protein (TAP) testing kit, ZHEJIANG RUI SHENG MEDICAL TECHNOLOGY). Then through finding and counting these crystalloid condensates, we can more accurately to make prognostic stratification, and monitor the efficacy of CRC treatment.

The present study evaluated the sensitivity and specificity of TAP detection in patients with CRC. We found that The area of TAP condensed particulate matter decreased after chemotherapy compared with before chemotherapy when CT or MRI scan showed disease controlled, in contrary, it increased when disease progressed ($P < 0.05$). We also compared TAP and some other serological biomarkers e.g. CEA, CA125, CA19-9 respectively. Statistically significant difference were confirmed in monitoring of TAP and common serological biomarkers e.g. CEA and CA19-9 ($p < 0.05$). Since the limitation of poor specificity of common tumor makers in efficacy monitoring, TAP had a good effect of monitoring CRC chemotherapy with common tumor makers, because of its accuracy. TAP test also had significant difference with CEA and CA19-9, which could not be replaced.

However, the data of TAP was only collected before and after chemotherapy in our study. Add up with data pre and post-surgery and at disease recurrence can make more sufficient analysis.

In conclusion, serum TAP is a new serum tumor marker detecting technology, which is sensitive in monitoring CRC chemotherapy, deserving of further clinical research. This study can open a novel avenue for validation of TAP detection to improve CRC screening and monitoring.

Acknowledgements

Dr. Xin-En Huang is supported by the project of science and technology of Nanjing science committee (Grant 201303046), Jiangsu Provincial Special Program of Medical Science (BL2014092) and Jiangsu Province fourth stage “333 high-level Personnel Training Project” third levels of talent cultivating object.

References

- Bast RC Jr, Klug TL, St John E, et al (1983). A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. *N Engl J Med*, **309**, 883-7.
- Brenner H, Kloor M, Pox CP (2014). Colorectal cancer. *Lancet*, **383**, 1490-502.
- Chen X, Ba Y, Ma L, et al (2008). Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res*, **18**, 997-1006.
- Coghlin C, Murray GI (2013). Progress in the identification of plasma biomarkers of colorectal cancer. *Proteomics*, **13**, 2227-8.
- Eleftheriadis N, Papaloukas C, Pisteveu-Gompaki K (2009). Diagnostic value of serum tumor markers in asymptomatic individuals. *J Buon*, **14**, 707-10.
- Elmunzer BJ, Hayward RA, Schoenfeld PS, et al (2012). Effect of flexible sigmoidoscopy-based screening on incidence and mortality of colorectal cancer: a systematic review and meta-analysis of randomized controlled trials. *PLoS Med*, **9**, e1001352.
- Ferlay J, Shin HR, Bray F, et al (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*, **127**, 2893-917.
- Filella X, Molina R, Grau JJ, et al (1992). Prognostic value of CA 19-9 levels in colorectal cancer. *Ann Surg*, **216**, 55-9.
- Gehan EA, Tefft MC (2000). Will there be resistance to the RECIST (Response Evaluation Criteria in Solid Tumors)? *J Natl Cancer Inst*, **92**, 179-81.
- Goldberg EM, Simunovic LM, Drake SL, et al (1989). Comparison of serum ca 19-9 and cea levels in a population at high risk for colorectal cancer. *Hybridoma*, **8**, 569-75.
- Grutzmann R, Molnar B, Pilarsky C, et al (2008). Sensitive detection of colorectal cancer in peripheral blood by septin 9 DNA methylation assay. *PLoS One*, **3**, e3759.
- Hewitson P, Glasziou P, Watson E, Towler B, Irwig L (2008). Cochrane systematic review of colorectal cancer screening using the fecal occult blood test (hemoccult): an update. *Am J Gastroenterol*, **103**, 1541-9.
- Huang XE, Cao J, Qian ZY, et al (2014). Leucogen tablets at 60 mg three times per day are safe and effective to control febrile neutropenia. *Asian Pac J Cancer Prev*, **15**, 8495-7.
- Huang Z, Huang D, Ni S, Peng Z, Sheng W, Du X (2010). Plasma microRNAs are promising novel biomarkers for early detection of colorectal cancer. *Int J Cancer*, **127**, 118-26.
- Jemal A, Bray F, CenterMM et al (2011). Global cancer statistics. *CA Cancer J Clin*, **61**, 69-90.
- Ji ZQ, Huang XE, Wu XY, et al (2014). Safety of Brucea javanica and cantharidin combined with chemotherapy for treatment of NSCLC patients. *Asian Pac J Cancer Prev*, **15**, 8603-5.
- Kim HJ, Yu MH, Kim H, Byun J, Lee C (2008). Noninvasive molecular biomarkers for the detection of colorectal cancer. *BMB Rep*, **41**, 685-92.
- Lee BB, Lee EJ, Jung EH, et al (2009). Aberrant methylation of APC, MGMT, RASSF2A, and Wif1 genes in plasma as a biomarker for early detection of colorectal cancer. *Clin Cancer Res*, **15**, 6185-91.
- Li YH, An X, Xiang XJ (2009). Clinical significance of a transient increase in carcinoembryonic antigen and carbohydrate antigen 19-9 in patients with metastatic colorectal cancer receiving chemotherapy [in Chinese]. *Ai Zheng*, **28**, 939-44.
- Liu J, Huang XE (2014). Efficacy of Bifidobacterium tetragenous viable bacteria tablets for cancer patients with functional constipation. *Asian Pac J Cancer Prev*, **15**, 10241-4.
- Lu YY, Huang XE, et al (2014). Clinical observations on associations between the UGT1A1 genotype and severe toxicity of irinotecan. *Asian Pac J Cancer Prev*, **15**, 3335-41.
- Luo Y, Wang L, Wang J (2013). Developing proteomics-based biomarkers for colorectal neoplasms for clinical practice: opportunities and challenges. *Proteomics Clin Appl*, **7**, 30-41.
- Martínez-Aguilar J, Chik J, Nicholson J, et al (2013). Quantitative mass spectrometry for colorectal cancer proteomics. *Proteomics Clin Appl*, **7**, 42-54.
- McKeown E, Nelson DW, Johnson EK, et al (2014). Current approaches and challenges for monitoring treatment response in colon and rectal cancer. *J Cancer*, **5**, 31-43.
- Migliore L, Migheli F, Spisni R, Coppede F (2011). Genetics, cytogenetics, and epigenetics of colorectal cancer. *J Biomed Biotechnol*, **2011**, 792362.
- Newton KF, Newman W, Hill J (2012). Review of biomarkers in colorectal cancer. *Colorectal Dis*, **14**, 3-17.
- Perkins GL, Slater ED, Sanders GK, et al (2003). Serum tumor markers. *Am Fam Physician*, **68**, 1075-82.
- Siegel R, Naishadham D, Jemal A (2012). Cancer statistics, 2012. *CA Cancer J Clin*, **62**, 10-29.
- Sorbye H, Dahl O (2004). Transient CEA increase at start of oxaliplatin combination therapy for metastatic colorectal cancer. *Acta Oncol*, **43**, 495-8.
- Tian GY, Miu M, Huang XE (2014). Systematic analysis of pemetrexed-based chemoradiotherapy for patients with locally advanced or metastatic esophageal cancer. *Asian Pac J Cancer Prev*, **15**, 8475-8.
- Wang L, Huang XE (2015). Clinical study on safety and efficacy of JiSaiXin (recombinant human granulocyte colony stimulating factor injection manufactured in China) for Chinese undergoing chemotherapy. *Asian Pac J Cancer Prev*, **16**, 299-301.
- Winawer S, Fletcher R, Rex D, et al (2003). Colorectal cancer screening and surveillance: clinical guidelines and rationale-Update based on new evidence. *Gastroenterology*, **124**, 544-60.
- Wu XY, Huang XE (2015). Screening for patients with non-small cell lung cancer who could survive long term chemotherapy. *Asian Pac J Cancer Prev*, **16**, 647-52.
- Wu XY, Huang XE, et al (2014). A predictive model for evaluating responsiveness to pemetrexed treatment in patients with advanced colorectal cancer. *Asian Pac J Cancer Prev*, **15**, 5941-4.
- Xu D, Li XF, Zheng S, Jiang WZ (2006). Quantitative real-time RTPCR detection for CEA, CK20 and CK19 mRNA in peripheral blood of colorectal cancer patients. *J Zhejiang Univ Sci*, **B7**, 445-51.
- Yang L, Sun Y, Huang XE, Yu DS, et al (2015). Carcinoma Microsatellite Instability Status as a Predictor of Benefit from Fluorouracil-Based Adjuvant Chemotherapy for Stage II Rectal Cancer. *Asian Pac J Cancer Prev*, **16**, 1545-51.
- Zieglschmid V, Hollmann C, Bocher O (2005). Detection of disseminated tumor cells in peripheral blood. *Crit Rev Clin Lab Sci*, **42**, 155-96.