RESEARCH COMMUNICATION

Clinical Application of Serum Tumor Associated Material (TAM) from Non-small Cell Lung Cancer Patients

Cheng-Guang Li[1], Xin-En Huang[1]*, Lin Xu[2*], Ying Li[1], Yan-Yan Lu[1]

Abstract

Objective: To explore the associations of serum tumor associated material (TAM) with other common tumor markers like carcinoembryonic antigen (CEA), carbohydrate antigen 125 (CA125), carbohydrate antigen19-9 (CA19-9) and its clinical application in non-small cell lung cancer (NSCLC) patients. Methods: A total of 87 patients were enrolled into this study, all with histologically or cytologically confirmed NSCLC. With the method of chemical colorimetry, the level of TAM was determined and compared, while chemiluminescence was used to measure the levels of common tumor markers. Results: The level of TAM decreased after chemotherapy compared with before chemotherapy when CT or MRI scans showed disease control. Furthermore, it increased when disease progessed and there was no statistically significant difference in monitoring of TAM and common tumor markers (P>0.05). Conclusions: Detecting TAM in NSCLC patients has a higher sensitivity and specificity, so it can be used as an indicator for clinical monitoring of lung cancer chemotherapy.

Keywords: Tumor associated material - tumor marker - lung cancer - NSCLC

Asian Pacific J Cancer Prev, **13**, 301-304

Introduction

Altered glycosylation patterns are a hallmark of tumor phenotype. This phenomenon was first described by Meezan et al in 1969 with the demonstration that healthy fibroblasts have smaller membrane glycoproteins than their transformed counterparts (Meezan et al., 1969). This finding was later corroborated with histological evidence that lectins show differential binding to healthy compared with malignant tissue (Turner, 1992; Saussez et al., 1998). More recently, cancer-associated cell-surface glycans have been directly characterized using specific monoclonal antibodies and mass spectrometry (Shriver et al., 2004), further illuminating the molecular changes that occur upon malignant transformation (Matsushita et al., 1990; Pancino et al., 1991).

We now know that changes in glycosylation include both the under- and overexpression of naturally-occurring glycans, as well as neoexpression of glycans normally restricted to embryonic tissues. These structures most often arise from changes in the expression levels of glycosyltransferases in the Golgi compartment of cancerous cells. Changes in glycosyltransferase levels can lead to modifications in the core structure of N-linked and O-linked glycans. One of the most common changes is an increase in the size and branching of N-linked glycans. This increased branching is often attributed to increased activity of N-acetylglucosaminyltransferase V (GlcNAc-TV, also known as or MGAT5; the enzyme that leads to β1, 6GlcNAc branching) (Dennis et al., 1987).

The increased branching creates additional sites for terminal sialic acid residues, which, in conjunction with a corresponding upregulation of sialyltransferases, ultimately leads to an increase in global sialylation (Kim et al., 1997).

In addition to changes in the corestructures of glycans, altered terminal structures are also associated with malignancy. Glycosyltransferases (for example, sialyltransferases and fucosyltransferases) involved in linking terminating residues on glycans tend to be overexpressed in tumour tissue. The increase in activity of these glycosyltransferases in turn leads to the overexpression of certain terminal glycans. Examples of terminal glycan epitopes commonly found on transformed cells include sialyl Lewis x (sLex), sialyl Tn (sTn), Globo H, Lewis y (Ley) and polysialic acid (prostate-specific antigen, PSA) (Gabius, 1988; Sell, 1990; Taylor-Papadimitriou et al., 1994; Hakomori et al., 1997). Many of these epitopes are observed in malignant tissues throughout the body, including the brain, breast, colon and prostate (Orntoft et al., 1999).

Another common feature of tumors is the overproduction of certain glycoproteins and glycolipids. For example, epithelial tumors often overproduce mucin glycoproteins, which are characterized by dense clusters of O-linked glycans. Mucins are used as diagnostic markers of cancer and can also func-tion as scaffolds for most of the above-listed cancer-associated epitopes

*1Department of Chemotherapy, 2Department of Thoracic Surgery,The Affiliated JiangSu Cancer Hospital of Nanjing Medical University, Nanjing, China *For correspondence: huangxinen06@yahoo.com.cn, xulin83@vip.sina.com*

(Hollingsworth et al., 2004). Additionally, cancer tissues can display an increase in ganglioside expression. For example, complex gangliosides (for example, GD2, GD3 and fucosyl GM1) are found at elevated levels in small-cell lung carcinomas, neuroblastomas and melanomas (Hakomori et al., 1997; Hakomori, 2000). Although gross changes in glycosylation of tumor tissues are apparent, no single change seems to distinctly differentiate normal and malignant cells. Instead, each type of malignant tissue is characterized by a distinct set of changes in glycan expression (Zhang et al., 1997).

Glycan changes that indicate malignancy can be used for diagnosis. Indeed, the commonly used 'CEA' test for colon cancer monitors serum levels of an antibody specific for a cancer-associated glycan (sLea) (MacDonald, 1999). Glycan analyses of other serum markers, such as CA125 (Kui Wong et al., 2003)and prostate-specific antigen (Basu et al., 2003; Peracaula et al., 2003), reveal distinct changes in glycosylation in ovarian and prostate tumour tissue, respectively. These studies suggest that specific changes in glycosylation could be useful as diagnostic tools. For many cancers, however, there are no serum markers available.

Serum tumor associated material (TAM) testing kit was a new test kit,developed by QINGDAO BO-XING Biotechnology Co., Ltd,can be quickly and easily used for cancer early detection, screening, and monitoring the efficacy of cancer treatment.

Detection of serum TAM by a special polymer carrier to identify a specific oligosaccharide sequence to identify specific glycan terminal epitope (eg, Lewis oligosaccharides X (sLex), etc.), the end of the sialic acid, and with the reactions. At present most used tumor markers are glycoproteins glycolipids, oligosaccharides contain specific sequences, TAM polymer carrier identified by the sequence of these oligosaccharides with a variety of markers to achieve the reaction. TAM removed most of the macromolecular protein complex (at 450nm can colour in the non-tumor-related material) that could affect the test results by sedimentation centrifuge, to make accurate of the test results (Serum tumor associated material (TAM) testing kit, QINGDAO BO-XING Biotechnology Co., Ltd).

The aim of this study was to explore the application of serum tumor associated material (TAM) before and after chemotherapy in non-small cell lung cancer (NSCLC) patients.

Table 1. Characteristics of the Patients

		NSCLC
Number		87
Sex	Male	61
	Female	26
Age(yr)Median (Range)		59(33-79)
Adjuvant therapy		12
Palliative therapy		75

NSCLC, non- small cell lung cancer

Materials and Methods

To be included in the study, patients who were hospital inpatients had to have histologically or cytologically confirmed NSCLC,and blood collected before and after treatment, as described elsewhere (Zhou et al., 2009; Yan et al., 2010).

Measured by fasting blood 2ml, separation of serum, -20 °C to save, backup. Strict accordance with the clinical serum tumor associated materials (TAM) detection kit operating requirements. In the colorimetric under 450nm, calculated measured value.According QINGDAO BO-XING Biotechnology Co., Ltd provides criteria: TAM detection value≥95U/ml demonstrates positive, <95U/ml negative.(Serum tumor associated material (TAM) testing kit, QINGDAO BO-XING Biotechnology Co., Ltd)

Several blood tumor markers e.g.carcinoembryonic antigen (CEA), carbohydrate antigen 125 (CA125), carbohydrate antigen 19-9 (CA19-9), were measured by chemiluminescence method. If CEA <3.5ng/mL, it is defined as negative, CA125<35U/mL is negative, CA19-9 < 39U/mL 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).

Table 2. The Serum Tumor Associated Material(TAM) Level and Several Tumor Markers after Chemotherapy in Non-small Cell Lung Cancer Patients

	Disease control (CR+PR+SD)		Disease progression (PD)		OR (95%CI)	OR* (95%CI)
	Increase N(%)	Decrease N(%)	Increase N(%)	Decrease N(%)		
TAM*	16(23.2)	53(76.8)	10(55.6)	8(44.4)	4.14 (1.40-12.3)	4.01 (1.35-12.0)
CEA**	31(44.9)	38(55.1)	9(50)	9(50)	1.23 (0.43-3.46)	1.23 (0.43-3.51)
CA125***	25(36.2)	44(63.8)	12(66.7)	6(33.3)	3.52 (1.18-10.5)	3.66 (1.21-11.1)
CA19-9****	30(43.5)	39(56.5)	11(61.1)	7(38.9)	2.04 (0.71-5.90)	1.98 (0.67-5.85)

CEA, carcinoembryonic antigen; CA, carbohydrate antigen; TAM, serum tumor associated material; CR, complete response; PR, partial response; SD, stable disease (SD); PD, progressive disease (PD); *P=0.018, TAM after chemotherapy compare with before; **P=0.061, TAM compare with CEA; ***P=0.351, TAM compare with CA125; ****P=0.089, TAM compare with CA199; OR, Odds Ratio; *OR, adjusted for age and sex; 95%CI, 95% Confidence Interval

Statistical analysis

Continuous variables were summarized by descriptive statistics, categorical variables by frequency. Count data by Chi-square test; measurement data as mean ± standard deviation. $P<0.05$ was considered statistically signifcant. The study data was analyzed through the STATA 8.0 software (Stata Corporation, 4905 Lakeway Drive College Station, Texas 77845 USA).

Results

From April 1st to July 31st 2011, 87 NSCLC patients were enrolled into this study,men 61,women 26, with age ranging from 33-79. All patients had histologically or cytologically confirmed NSCLC (Table 1). The level of TAM decreased after chemptherapy compared with before chemotherapy when CT or MRI scan showed disease controlled,in contrary, it increased when disease progessed ($P<0.05$), and there was no statistically significant difference in monitoring of TAM and common tumor markers ($P>0.05$) (Table 2).

Discussion

Serum tumor associated material (TAM) is a glycoprotein detection kit, substances such as glucosamine and L-hydroxyproline and other amino acids as the main detected objects. Sialic acid and other glycosaminoglycans substances are the main materials which constitute glycolipids and glycoproteins, closely relating with intercellular adhesion, contact, inhibition of cancerous cells,tumor metastasis and proliferation (Serum tumor associated material (TAM) testing kit, QINGDAO BO-XING Biotechnology Co., Ltd).

About 85% of cancer patients with elevated serum sialic acid, serum saliva acid changes in the level of disease was positively correlated with cancer, continued to rise indicated a poor prognosis (Wu et al., 1983). In tumor recurrence or metastasis, the serum sialic acid content increased may be several weeks before the clinical diagnosis, which was significant for early detection and promptly treatment. The elevation level of hydroxyproline, bone and collagen catabolin, indicated of bone metastases, especially bone metastases of breast cancer. Liu et al., showed that the serum free hydroxyproline in patients with malignant bone tumors were significantly high; peptide with hydroxyproline can identify malignant and benign bone tumors (Liu et al., 1986). As the reagent for sialic acid and hydroxyproline and other tumor-related substances were detected at the same time, color overlay, and thus improve the detection sensitivity.

Furthermore,TAM removed most of the macromolecular protein complex (at 450nm can colour in the non-tumor-related material) that could affect the test results by sedimentation centrifuge, to make accurate of the test results. So that, it can detect more tumor marker in serum sample. Earlier authors showed that TAM was higher in malignant tumors than benign tumors and healthy tissues (Zou et al., 2004; Deng et al., 2010; Jiang et al., 2010). It had been reported that CEA, CA125, CA19-9 could monitor the treatment efficacy in NSCLC (Diez et al., 1996; Vinolas et al., 1998; Foa et al., 1999). So we compared TAM and these tumor makers in monitoring treatment efficacy in NSCLC. In our study the level of TAM decreased after chemotherapy compared with before chemotherapy when CT or MRI scan showed disease control. In contrast, it increased when disease progessed, and there was no statistically significant difference in monitoring of TAM and common tumor markers such as CEA, CA125, CA19-9 ($P>0.05$).Since the limitation of poor specificity of common tumor makers in efficacy monitoring, TAM had a good effect of monitoring non-small cell lung cancer treatment with common tumor makers, because of its accuraty.

However, the detection of glycosamine substances also composed of the body's immune response protein, Zou et al. found that patients with autoimmune disease's positive rate of up to 47.62%, significantly higher (Zou et al., 2004), so the identification of clinical applications should be noted.

In conclusion, serum tumor associated material (TAM) is a new serum tumor marker detecting technology,which is sensitive in monitoring NSCLC treatment, deserving of further clinical study.

Acknowledgements

Dr Xin-En Huang is supported in part by a grant from Jiangsu Provincial Administrtion of Chinese Medicine (LZ11091), and in part from a special research fund of Organization Department of Jiangsu Provincial Party Committee, Talent Work Leading Group of Jiangsu Province (333 High-level Talents Training Project).

References

Basu PS, Majhi R, Batabyal SK (2003). Lectin and serum-PSA interaction as a screeninng test for prostate cancer. *Clin Biochem*, **36**, 373-6.

Dennis JW, Laferté S, Waghorne C, et al (1987). β1-6 branching of asn-linked oligosaccharides is directly associated with metastasis. *Science*, **236**, 582–5.

Deng H, Zhong BY, Peng GH, et al (2010). Application of Bo Xin SerumTumor Associated Material (BXTM) in Malignant Tumor. *Prac Prev Med*, **17**, 2493-5.

Diez M, Torres A, Maestro ML, et al (1996). Prediction of survival and recurrence by serum and cytosolic level sof CEA, CA125 and SCC antigens in resectable non-small-cell lung cancer. *Br J Cancer*, **73**, 1248-54.

Foa P, Fornier M, Miceli R, et al (1999). Tumour markers CEA, NSE, SCC, TPA and CYFRA21.1 in resectable non-small cell lungcancer. *Anticancer Res*, **19**, 3613-8.

Gabius HJ (1988). Tumor lectinology- at the intersection of carbohydrate chemistry, biochemistry, cell biology, and oncology. *Angew Chem*, **27**, 1267–76.

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.

Hakomori S (2000). Traveling for the glycosphingolipid path. *Glycoconj J*, **17**, 627–47.

Hakomori S, Zhang Y (1997). Glycosphingolipid antigens and cancer therapy. *Chem Biol*, **4**, 97-104.

Hollingsworth MA, Swanson BJ (2004). Mucins in cancer: Protection and control of the cell surface. *Nat Rev Cancer*,

4, 45-60.
Jiang M, Pu R (2010). Study on serum tumor related material (BXTM) with early diagnosis in malignant tumor. *National Medical Frontiers of China,* **5**, 80-1.
Kim YJ, Varki A (1997). Perspectives on the significance of altered glycosylation of glycoproteins in cancer. *Glycoconj J*, **14**, 569–76.
Kui Wong N, Easton RL, Panico M, et al (2003). Characterization of the oligosaccharides associated with the human ovarian tumor marker CA125. *J Biol Chem*, **278**, 28619–34.
Liu ZM, et al (1986). Serum free proline and free hydroxyproline and its peptide-binding determination of hydroxyproline in the evaluation of bone tumors. *Chinese J Laboratory Med*, **9**, 129-33 (Chinese).
MacDonald JS (1999). Carcinoembryonic antigen screening: Pros and cons. *Sem Oncol*, **26**, 556–60.
Matsushita Y, Cleary KR, Ota DM, et al(1990).Sialyl-dimeric Lewis-X antigen expressed on mucin-like glycoproteins in colorectal-cancer metastases. Lab Invest,63, 780–91.
Meezan E, Wu HC, Black PH, et al (1969). Comparative studies on carbohydrate-containing membrane components of normal and virus-transformed mouse fibroblasts. Separation of glycoproteins and glycopeptides by sephadex chromatography. *Biochemistry*, **8**, 2518–24.
Orntoft TF, Vestergaard EM (1999). Clinical aspects of altered glycosylation of glycoproteins in cancer. *Electrophoresis*, **20**, 362–71.
Pancino G, Osinaga E, Charpin C, et al (1991). Purification and characterization of a breast-cancer-associated glycoprotein not expressed in normal breast and identified by monoclonal antibody 83D4. *Brit J Cancer*, **63**, 390–8.
Peracaula R, Tabarés G, Royle L, et al (2003). Altered glycosylation pattern allows the distinction between prostate-specifc antigen (PSA) from normal and tumor origins. *Glycobiology*, **13**, 457–70.
Saussez S, Marchant H, Nagy N, et al (1998). Quantitative glycohistochemistry defines new prognostic markers for cancers of the oral cavity. *Cancer*, **82**, 252–60.
Sell S (1990). Cancer-associated carbohydrates identified by monoclonal antibodies. *Human Path*, **21**, 1003–19.
Serum tumor associated material (TAM) testing kit, QINGDAO BO-XING Biotechnology Co., Ltd. http://www.cqtrueway.com.
Shriver Z, Raguram S, Sasisekharan R (2004). Glycomics: a pathway to a class of new and improved therapeutics. *Nat Rev Drug Disc*, **3**, 863–73.
Taylor-Papadimitriou J, Epenetos AA (1994). Exploiting altered glycosylation patterns in cancer- progress and challenges in diagnosis and therapy. *Trends Biotech*, **12**, 227–33.
Turner GA (1992). N-Glycosylation of serum-proteins in disease and its investigation using lectins. *Clin Chim Acta*, **208**, 149–71.
Vinolas N, GalanMC, Casas F, et al (1998). Tumor markers inresponse monitoring and prognosis of non small cell lungcancer. *Preliminaryreport Anticancer Res*, **18**, 631-4.
Wu HX, et al (1983). Encyclopedia of Chinese Medical Oncology. *Shanghai Science and Technology Press*, 63 (Chinese).
Yan PW, Huang XE, Jiang Y, et al (2010). A clinical comparison on safety and efficacy of Paclitaxel/Epirubicin (NE) with Fluorouracil/Epirubicin/Cyclophosphamide (FEC) as postoperative adjuvant chemotherapy in breast cancer. *Asian Pac J Cancer Prev*, **11**, 1115-8.
Zhang S, Cordon-Cardo C, Zhang HS, et al (1997). Selection of tumor antigens as targets for immune attack using immunohistochemistry: I.Focus on gangliosides. *Int J Cancer*, **73**, 42-9.
Zhang S, Zhang HS, Cordon-Cardo C, et al (1997). Selection of tumor antigens as targets for immune attack using immunohistochemistry: II. Blood group–related antigens. *Int J Cancer*, **73**, 50-6.
Zhou JN, Huang XE, Ye Z, et al (2009). Weekly paclitaxel/Docetaxel combined with a paltinum in the treatment of advanced non-samll cell lung cancer: a study on efficacy, safety and pre-medication. *Asian Pac J Cancer Prev*, **10**, 1147-50.
Zou X, Zhang XS, Sui L, et al (2004). Role of tulnor associated material (BXTM) in early diagnosing malignant tumor. *Qilu J Med Laboratory Sci*, **15**, 4-6.