European Review for Medical and Pharmacological Sciences 2014; 18: 2042-2047

Oncostatin-M as a novel biomarker in colon cancer patients and its association with clinicopathologic variables

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Abstract. – OBJECTIVE: The aim of this study was to evaluate preoperative serum oncostatin M (OSM) concentration as a diagnostic marker in colon cancer patients and its association with clinicopathologic variables.

PATIENTS AND METHODS: Preoperative serum OSM concentrations were measured in 100 colon cancer patients and 70 healthy volunteers by enzyme-linked immunosorbent assay (ELISA).

RESULTS: Serum OSM concentrations were significantly higher in colon cancer patients than in controls ($p < 0.001$). Serum OSM concentrations increased significantly with higher T stage ($p < 0.001$) and were significantly higher in patients with increased tumor burden, lymphovascular involvement, and lymph node and distant metastasis ($p < 0.001$ for each).

CONCLUSIONS: To our knowledge, this is the first report showing that elevated OSM concentration was associated with colon cancer and its clinicopathologic variables, including invasion and metastasis. These findings indicate that serum OSM may serve as a novel biomarker in the diagnosis of colon cancer.

Key words:
Oncostatin M, Colon cancer, Clinicopathologic variables.

Introduction

Colon carcinoma was the third most common type of malignancy and the third leading cause of cancer-related deaths for both genders worldwide in 2011[1]. The pathogenesis of colon cancer has not been completely determined. Early detection and treatment provide the most promising approach to increasing long-term survival of colon cancer patients[2,3].

Screening for colon cancer can include fecal occult blood tests, sigmoidoscopy and colonoscopy[4]. In many countries, the fecal occult blood test is most widely used, despite its lack of sensitivity and specificity[5]. Sigmoidoscopy and colonoscopy are invasive and inconvenient procedures, reducing their use in colon cancer screening[6]. Thus, additional non-invasive methods with greater sensitivity and specificity are needed for colon cancer screening. Biomarkers specific to colon cancer may, therefore, provide non-invasive and economically advantageous methods for the early detection of colon cancer.

Serum contains many biomarkers and proteins that could potentially be of use in the sensitive and specific detection of colon cancer[7]. New biomarkers, however, are needed in the diagnosis and prognosis of this disease[8]. Oncostatin M (OSM) is a pleiotropic cytokine encoded by the *OSM* gene[9,10]. OSM acts by binding to two cell surface receptors, both of which include the protein gp130. The type I receptor is composed of gp130 and LIFR, whereas the type II receptor is composed of gp130 and OSMR[11]. Upon binding, OSM activates JAK1, JAK2, and Tyk2[11,12]. OSM plays a pro-inflammatory role in endothelial cells, stimulating their production of IL-6 and P-selectin[13] and modulating the expression of acute phase proteins[14] and of cell growth[10,15]. To assess the role of OSM in colon cancer, we assayed OSM expression, angiogenesis, and metastasis in patients with colon cancer, showing that increased expression of OSM correlates with clinicopathologic variables and increased lymph invasion in these patients.

Patients and Methods

Patients

Preoperative blood samples were collected from colon cancer patients and controls between January 2010 and May 2013. Patients with a his-

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tory of familial adenomatous polyposis, hereditary non-polyposis colon cancer, hypertension, any other tumors, or evident inflammatory disease, were not included. None of the patients received chemotherapy, radiotherapy, or immunotherapy before surgery. Clinicopathologic characteristics of each patient were collected, including age, gender, tumor size, tumor grade and tumor-node-metastasis stage (Table I). The control group consisted of 70 healthy age-matched volunteers. Written informed consent was obtained from each subject following a detailed explanation of the objectives and protocol of the study which was conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" and approved by the Institutional Ethics Committee.

All blood samples were immediately centrifuged at 1000 x g for 15 minutes at 2-8° C. Serum samples were assayed immediately or stored at –80° C before assay.

ELISA of OSM

OSM concentrations were measured by sandwich ELISA (sELISA) using commercially available kits according to the manufacturer's instructions (Abcam, Cambridge, MA, USA, Catalog no: a100619). Each serum sample was assayed in duplicate, with the mean used for analysis. This assay has high sensitivity (1 pg/mL) and specificity for human OSM, with no significant cross-reactivity or interference between human OSM and analogues, and a detection range of 1.37-1000 pg/mL.

Statistical Analysis

Data are reported as mean and standard deviation; median, minimum, and maximum; or frequency and percentage. The normal distribution of results was assessed by Kolmogorov-Smirnov statistics, followed by Shapiro Wilk tests. Normally distributed variables were compared by *t*-tests for independent samples, whereas non-normally distributed variables were compared using Mann Whitney U-tests, Kruskal-Wallis one-way analysis of variance and Bonferroni-corrected Mann-Whitney U tests. Gender distribution of the two groups was compared by chi-square tests. All comparisons were two-sided, with significance set at $p < 0.05$. All statistical analyses were performed using SPSS 21.0 software (SPSS Inc., Chicago, IL, USA).

Results

Table I shows the demographic and clinicopathologic characteristics of the patient and control groups. The patient group included 100 pa-

Table I. Clinicopathologic features of patients and controls.

	Controls (n=70)	Patients (n=100)	p value
Age (y)	56.48 ± 4.47	57.26 ± 4.98	<0.05
Gender (m/f)	37/33	56/44	
	52.9%/47.1%	56%/44%	
Tumor size			
≤ 4 cm		24	
> 4 cm		76	
Tumor-Node-Metastasis stage			
T1		24	
T2		15	
T3		21	
T4		37	
Invasion			
T1		25	
T2		16	
T3		21	
T4		38	
Lymph node metastasis			
N0		31	
N1		27	
N2		42	
Metastasis			
Present		69	
Not present		31	

Table II. Preoperative serum OSM concentrations in patients and controls (Mean ± SD) (Min-Max).

	Controls (n=70)	Patients (n=100)	*p* value
OSM (pg/ml)	3.08 ± 0.72 (1.5-4.6)	101.92 ± 70.59 (9.5-253.7)	< 0.001

tients with colon cancer (56 men and 44 women), of mean age 57.26 ± 4.98 years (range, 40-72 years). Twenty four patients (24%) had tumors < 3 cm, and 69 (69%) had distant metastases. Their postoperative T stages were T1, T2, T3, and T4 in 25, 16, 21, and 38 patients, respectively. The control group consisted of 70 healthy subjects (37 men and 33 women), of mean age of 56.48 ± 4.47 years (range, 41-71 years).

Table II shows the serum OSM concentrations in the patient and control groups. Mean ± SD serum OSM concentration was significantly higher in the patient than in the control group (101.92 ± 70.59 pg/mL vs. 3.08 ± 0.72 pg/mL, $p < 0.001$), whereas age ($p = 0.289$) and gender distribution ($p = 0.685$) were similar (Figure 1).

Table III shows the relationship between serum OSM concentrations in patients with colon cancer and their clinicopathologic variables. Serum OSM concentrations increased significantly with higher T stage, from T1 to T4 ($p < 0.001$) (Figure 2), with each stage differing significantly from all other stages ($p < 0.001$). In addition, serum OSM concentrations increased significantly with increasing tumor size, lymph vascular involvement, distant metastasis, and lymph node metastasis ($p < 0.001$).

We also found that serum OSM concentrations were significantly higher in patients with larger (>3 cm) than smaller (≤ 3 cm) tumors, and in patients with than without distant metastasis ($p = 0.004$), lymph node metastasis ($p = 0.005$) and lymph vascular involvement ($p = 0.003$).

Discussion

OSM is a 227-amino acid polypeptide, of molecular weight 28 kDa, that is structurally and functionally similar to the IL-6 subclass of cytokines. OSM is secreted by monocytes and T cells and acts as a proinflammatory cytokine. The signal transduction pathways stimulated by OSM appear are similar to those stimulated by other growth factors that activate tyrosine kinases[16]. OSM stimulates tyrosine phosphorylation, increases diacylglycerol and inositol phosphatase activities, and increases the message levels of immediate-early genes[16].

In addition to acting as a mitogen, OSM has been reported to act as an autocrine growth factor in cells derived from autoimmune immunodeficiency syndrome-associated Kaposi sarcomas[17]. OSM is also expressed in prostate cancer cells[18,19].

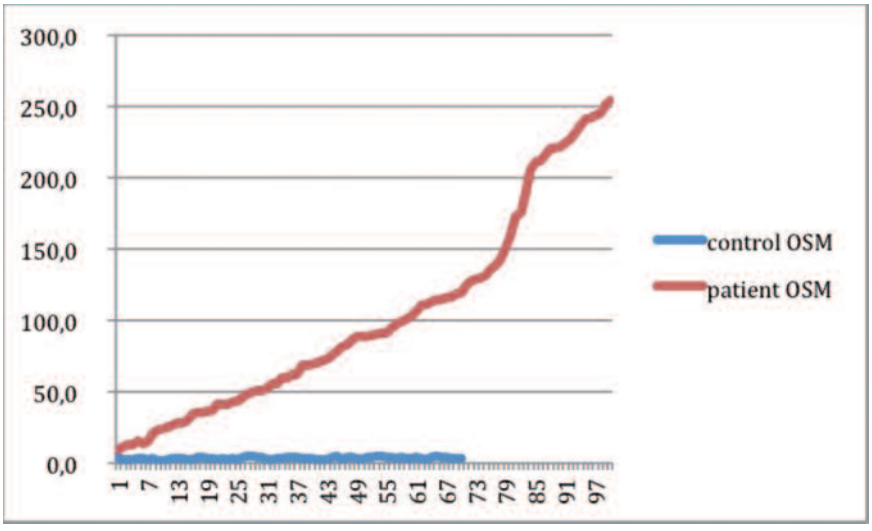

Figure 1. OSM concentrations in colorectal cancer patients and controls.

Table III. Relationship between preoperative serum OSM concentrations and clinicopathologic variables in colorectal cancer patients (Mean ± SD) (Minimum-Maximum).

	OSM (pg/mL)
Tumor size	
≤ 4 cm	26.3 ± 10.88 (9.5-42.5)
> 4 cm	125.8 ± 64.29 (43.0-253.7)
Tumor-Node-Metastasis stage	
T1	25.7 ± 10.92 (9.5-42.5)
T2	54.32 ± 10.73 (43.0-69.8)
T3	85.61 ± 10.74 (69.8-105.7)
T4	173.91 ± 52.01 (109.8-253.7)
Invasion	
T1	26.97 ± 11.16 (9.5-43.0)
T2	57.48 ± 8.09 (45.5-69.8)
T3	89.42 ± 10.55 (71.3-109.8)
T4	176.86 ± 51.58 (110.5-253.7)
Lymph node metastasis	
N0	31.26 ± 13.41 (9.5-51.5)
N1	77.19 ± 13.54 (54.8-99.0)
N2	169.98 ± 53.5 (100.5-253.7)
Metastasis	
Present	355.04 ± 183.19 (113.5-724.3)
Not present	63.07 ± 31.27 (21.3-110.5)

OSM has been reported to increase the levels of phosphorylated MAPK extracellular signal-regulated kinases-1 and -2 in cancer cells[20-22], as well as to induce RANKL expression by osteoblastic and stromal cells[23-25]. OSM can also modulate the first stage of differentiation and enhance apoptosis[25,26].

Despite its biologic activities as a cytokine and growth factor[16,9,27], less is known about the role of OSM on vascular and endothelial cells and on extracellular matrix. OSM has been found to increase DNA synthesis, whereas peptides with sequence homology, such as leukemia inhibition factor and granulocyte colony-stimulating factor, did not[16].

OSM is expressed by T-cells, monocytes, neutrophils, and osteoblasts, as well as in the testes, brain and kidneys[28,29]. OSM derived from activated T lymphocytes and macrophages, was shown to inhibit the proliferation of various human cancer cells and to be involved in inflammatory events[10,30]. For example, OSM was found to induce the expression of adhesion molecules in endothelial cells, thereby modulating leukocyte adhesion[30].

Conclusions

Because it is a product of macrophages and endothelial cells, we hypothesized that OSM could contribute to tumor cell invasion and metastasis, as well as to angiogenesis, in cancer patients. Indeed, ELISA analyses in patients with colon cancer suggested that OSM may be an independent biomarker associated with TNM stage and metastases to lymph nodes and distant organs. These findings suggest that additional studies exploring the role of OSM in the colon cancer development and invasion are warranted. Moreover, these findings may suggest cause and effect relationships that offer clues to the biological functions of OSM and its roles in host responses to tumor growth signaling and angiogenesis.

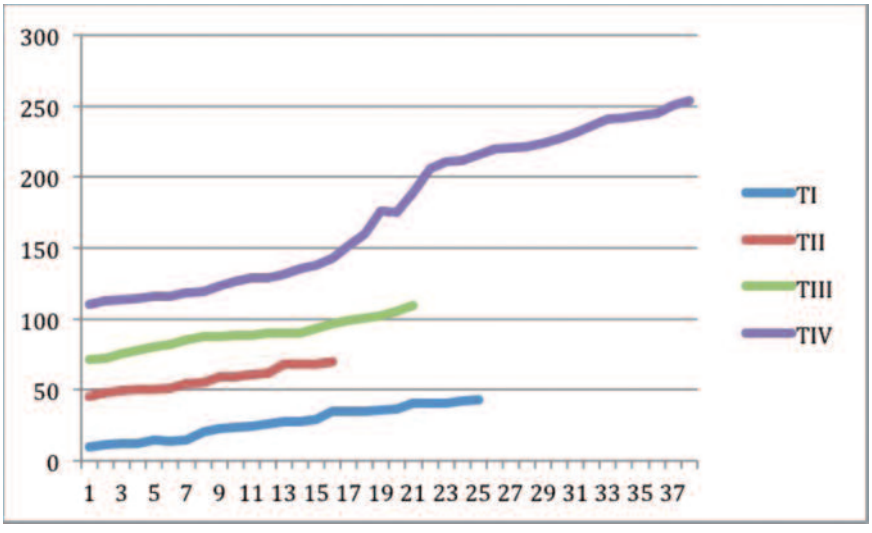

Figure 2. OSM concentrations relative to Tumor-Node-Metastasis stages in colorectal cancer patients.

Conflict of interest

The authors declare that there are no conflicts of interest.

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