Immunohistochemical Investigation of TMSG-1 Protein in Colorectal Cancer

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Abstract

Aim: We sought to study the expression of tumor metastasis suppressor gene-1(TMSG-1) in colorectal cancer and analyze the data using statistical methods. Our purpose was to report our results and combine them with available data at the international literature, in an attempt to understand the role of this gene in tumor biology.

Materials and Methods: Immunohistochemical analysis in paraffin sections obtained from 200 cases of colorectal cancer (100 cases with Duke's stage B cancer and 100 cases with metastatic stage C cancer) was performed. Tissue samples of stage C cancer were received from the lymph node metastases as well. The stained samples collected after the use of specific monoclonal antibodies were evaluated under the microscope in order to define the level of TMSG-1 expression in cancer cells.

Results: The analysis of our findings revealed significant association between the stage of the tumor and TMSG-1 expression ($p<0.0001$). Negative TMSG-1 expression correlates with stage C cancer. Other parameters, such as age, sex, tumor size, grade and BCL-2 expression did not correlate with TMSG-1 expression ($P>0.05$).

Conclusions: TMSG-1 could be implicated in inhibition of cell growth, infiltrating ability and metastatic potential in colorectal cancer. Further investigation with cellular and molecular methods is warranted in order to explore its mechanism of action in this type of cancer and its potential role in clinical practice.

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Introduction

Colorectal cancer (CRC) is the third most common cancer in men and the second most common cancer in women worldwide. [1] Fifty five percent of all cases have been reported in developed regions. [1] Colorectal cancer can be associated with several genetic and environmental factors, such as increased fat intake, low fiber diet, alcohol consumption, tobacco, chronic inflammatory bowel disease, family history of cancer and history of adenomas. The histologic type, the grade and stage of cancer remain the most helpful parameters for tumor classification and prognosis. However, research during the last years is oriented to the identification of molecular diagnostic and prognostic markers, that could be easily used in daily clinical routine and give us information regarding tumor's behavior.

TMSG-1 is located on chromosome 1q11. [2] It was initially cloned in a laboratory in Peking University several years ago when they used the mRNA differential display method in order to compare gene expression levels in cancer cell lines with different metastatic potential. [3] In 2003, the TMSG-1 gene complementary DNA (cDNA) was cloned into plasmids and they were transfected in prostate cancer cell lines with high metastatic potential. In vitro analysis did not show significant difference in cell growth capacity. However, the infiltrating ability and colony formation potential of the transfected cell lines were decreased compared to control cells. [3,4] In other studies that have been conducted over the last decade, the presence of TMSG-1 has been associated with a significant reduction in prostate cancer cell proliferation and infiltrating ability in vitro and with deceleration of prostate cancer metastasis in vivo. [4-6] Aside prostate cancer cells the TMSG-1 was also detected in breast, colon, ovarian and pancreatic cancers. [7] A study that included immunohistochemical analysis in 41 tissue samples in patients with colon cancer showed that metastatic tumors had weakly positive or negative stain associated with low TMSG-1 expression, while non-metastatic tumors were positively stained indicating high TMSG-1 expression. [7]

The same study sought to understand better the molecular mechanism of metastasis, using monoclonal antibodies against tumor metastasis suppressor gene-1. An antigenic epitope synthesized by the gene was injected into mice and cell lines with anti-TMSG-1 specific antibodies were generated. The application of Western blot in these lines revealed the role of a protein (45KB protein coded by TMSG-1) which was inversely correlated to cell growth in vitro and to tumor metastatic potential in vivo. [7] A study conducted few years later in prostate cancer cell line PC-3M-1E8 (high metastatic potential) reported the same results regarding the negative correlation of the protein with cell proliferation and metastatic potential. [5]

The present study was to assess the expression of TMSG-1 gene with the method of immunohistochemistry (monoclonal antibodies against human tumor metastasis suppressor gene-1) in 200 tissue samples with colorectal cancer and study the correlation of the gene's expression with other parameters such stage, grade, age and gender. The purpose of this study is to report our results and briefly summarize the available data from the literature in order to explore a potential role of the gene’s protein as a biomarker in CRC.

Materials and Methods

This is a prospective study conducted in a period of five years (2000-2005) in the Department of Pathology, at “Tzaneio General Hospital”, (480

beds). A number of 200 cases with colorectal cancer were collected: 100 with Duke's stage B (N0, tumor did not infiltrate lymph nodes) and 100 with stage C (N1-2, tumor invaded 1-2 lymph nodes). From every case paraffin sections with 4μm thickness were obtained. In cases with stage C cancer, sections were obtained from the infiltrated lymph nodes as well. Hematoxylin-Eosin stains were re-evaluated in all cases to confirm the diagnosis.

For the immunohistochemistry method the monoclonal Mouse-anti MTA1 Antibody (clone 1F3F12, isotype IgG2b-kappa, supplied by ThermoFisher SCIENTIFIC Company, Greece) was used. It was produced from tissue culture and diluted in phosphate buffered saline (PBS), PH 7,4 and 1% bovine serum albumin (BSA) with 0.1% sodium azide as preservative. As positive control sample, tissue from breast cancer was used. The stain pattern was nuclear. Regarding the level of expression, we set the following cut-off points: tissue samples with expression in less than 10% of tumor cells were considered to have negative TMSG-1 expression, with 10-50% positive tumor cells had low TMSG-1 expression and with more than 50% positive tumor cells were considered to have high expression. The results from immunohistochemistry in the primary tumors and in the lymph node metastases were statistically analyzed.

The analysis was conducted with statistical PASW 18 program (SPSS Inc, Chicago, Illinois, USA). Results were considered statistically significant when p value <0.05. The x2 Pearson test (with correction for 2x2 tables) was used to compare the expression of TMSG-1 in the primary and metastatic sites with other parameters such as Duke's stage, tumor size, grade, sex and BCL-2 expression. ANOVA and student's t-test were conducted to compare the mean age of patients, according to the expression of TMSG-1 in the primary and metastatic tumor sites respectively.

Results

The study included 200 cases of colorectal cancer. From these cases tissue samples were obtained (100 in Duke's stage C and 100 in Duke's stage B) and studied immunohistochemically (monoclonal antibodies against TMSG-1 gene) in order to evaluate the expression of TMSG-1 gene. Tissue samples in stage C cancer were received from both the primary site and the metastatic lymph node site. Twenty one samples (10.5%) were defined as low grade adenocarcinomas, 171(85.5%) as intermediate grade and the 8 (4%) were high grade adenocarcinomas. Regarding the tumor size, 42 samples (21%) were up to 3cm, 120 (60%) were between 3cm and 7cm and 38 (19%) were at least 7cm.

Regarding theTMSG-1 expression in the primary site: 61 samples were negative, 57 showed low expression and 82 showed high expression (Fig. 1, High TMSG-1 expression in primary site of colon cancer). In lymph node metastases, the gene's expression was observed in 32 samples (the 32% of stage C tumors) (Fig. 2, Positive TMSG-1 expression in lymph node). The tissue samples characteristics are shown in Table 1.

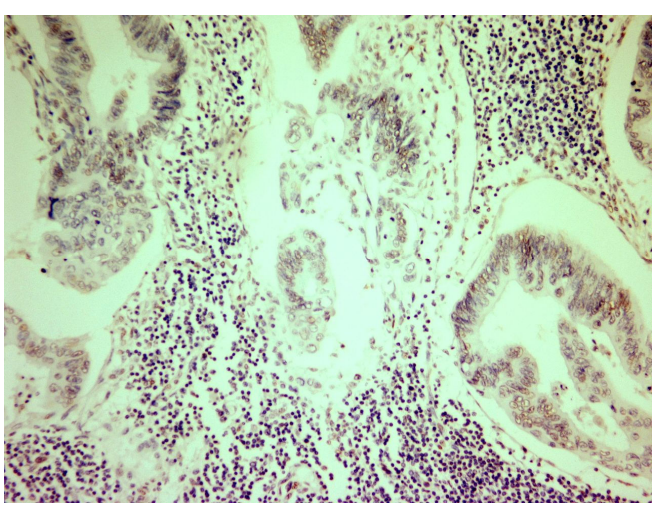

Figure 1. High TMSG-1 expression in primary site of colon cancer

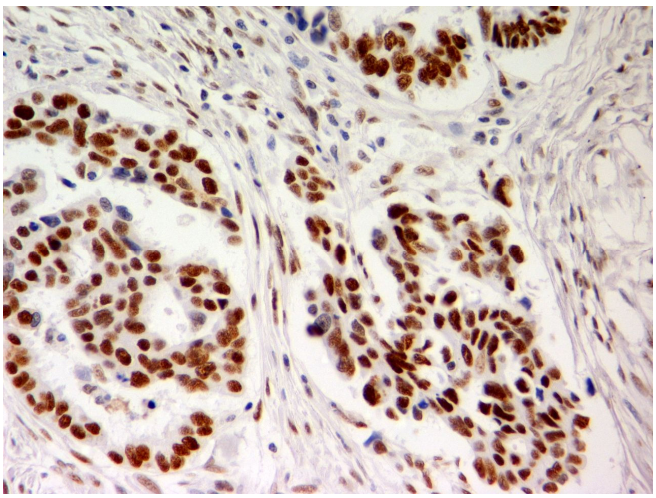

Figure 2. Positive TMSG-1 expression in lymph node

The results demonstrated that negative expression of TMSG-1 correlates with Duke's stage C while high expression was correlates with Duke's B stage tissue samples obtained from the primary sites ($p < 0.0001$). Other parameters, such as sex, tumor size, grade and BCL-2 expression did not correlate with TMSG-1 expression ($p > 0.05$). TMSG-1 lymph node expression did not correlate with any factor ($P > 0.05$). Mean age of the patients did not differ significantly according to TMSG-1 expression in primary sites (ANOVA, $p=0.113$) and in metastatic sites (t-test, $p=0.673$). The results of the univariate statistical analysis according to the parameters evaluated are presented below (Table 2).

Table 1. TMSG-1 expression and tissue samples characteristics

Immunohistochemistry No. of cases(percentage)		
anti-TMSG-1 Antibodies	Stage B (100 cases)	Stage C(100 cases) (primary site results)
≤10% of the tumor cells(negative)	7 (7%)	54 (54%)
10-50% of the tumor cells(low expression)	34 (34%)	23 (23%)
≥50% of the tumor cells(high expression)	59 (59%)	23 (23%)
Grade(200 cases)		
Low	21 (10.5%)	
Intermediate	171 (85.5%)	
High	8 (4%)	
Tumor Size (diameter)/200 cases		
< 3cm	42 (21%)	
3 -7 cm	120 (60%)	
> 7cm	38 (19%)	

Table 2. Univariate analysis of factors associated with TMSG-1 expression

FACTORS	p value (TMSG-1 EXPRESSION IN PRIMARY SITES)	p value(TMSG-1 EXPRESSION IN LYMPH NODES)
Sex	0.396	0.868
Tumor size	0.116	0.431
Grade	0.416	0.165
Duke's stage	<0.0001	0.1
BCL-2 expression	0.660	0.134

Discussion

TMSG-1 was discovered 16 years ago, while researchers in Peking University were studying prostate cancer cell lines with high metastatic potential. The same time period, LASS2 gene (homo sapiens longevity assurance homologue 2 of yeast LAG1), a human homologue of the LAG1 gene that was identified in human liver cDNA, was found to have high homology with TMSG-1. [2] Since then, several methods have been tried to identify the mechanism of action of TMSG-1/LASS2. Pei F et al (7), used an antigen solution containing a TMSG-1 epitope in order to immunize mice. The cell lines generated were screened with ELISA method for specific anti-TMSG-1 antibodies. Subsequently, the cells characteristics were studied with Western blotting. The tests confirmed that the reading frame of TMSG-1 had 380 amino acids and produced a protein with molecular weight 45kD. The protein was localized in the cytoplasm and the cytoplasmic membrane and it was inversely correlated with tumor metastatic potential. [7]

In the same study, paraffin section immunohistochemistry in 52 breast carcinomas (25 non-metastatic, 27 metastatic) and 41 colon carcinomas (21 non-metastatic, 20 metastatic) revealed that TMSG-1 expression was high in non-metastatic tumors and low in metastatic tumors. All the results were analyzed with t-test and were found statistically significant. [7] In our study, we sought to compare the TMSG-1 expression in 200 cases of colorectal cancer (100 non-metastatic, 100 with lymph node metastases) using the method of immunohistochemistry as well. Our results showed that TMSG-1 was highly expressed in non-metastatic (stage B) tumors, while low expression of the gene was observed in stage C

tumors in tissue samples received from the primary tumor sites ($p < 0.0001$).

The pattern of TMSG-1 expression in the initial studies revealed that the number of the gene's transcripts was very high in prostate cancer cells. [8] Ma C et al. transfected TMSG-1 to prostate cancer cell line PC-3M-1E8 which has high metastatic potential and studied the effects on the cells. The results demonstrated that TMSG-1 up regulation in prostate cancer cells decreased Vacuolar ATPase (V-ATPase) activity and inhibited cell growth. TMSG-1 also inhibited the expression of BCL-2 gene, which was an important finding considering the fact that BCL-2 inhibits cell apoptosis. [8] Su J et al. conducted a similar study in the same prostate cell line and showed that TMSG-1 protein significantly suppressed cell proliferation and cell invasion in vitro. [5]

Other studies have gone further in exploration of mechanisms that TMSG-1 uses to inhibit tumor invasion and metastasis. The direct interaction of TMSG-1 protein with the C subunit of V-ATPase pump proved to be a very important finding. [6, 9] TMSG-1 protein leads to inactivation of the pump. In cancer cells, V-ATPase causes hydrogens to leave the intracellular space and as a result the extracellular PH is decreased. This is considered to induce secretion of proteases that degrade extracellular matrix, like metalloproteinases MMP-2 and MMP-9 and promote tumor invasion. [2, 6, 9] The role of the V- ATPase in mediating cell sensitivity to signal molecules that are involved in cell proliferation and adhesion is also under investigation. [2, 10] In addition to the above, TMSG-1(LASS2) has been shown to increase ceramide C22:0 and C24:0 synthesis. Ceramides have been implicated to inactivation of cell cycle in several cancers. [2, 11]

The biological effects of LASS2/TMSG-1 were studied in breast cancer cells as well. [8] They transfected plasmids with LASS2/TMSG-1 cDNA to breast cancer cell line MCF-7 and compared the results with the non-transfected cells. The TMSG-1 overexpression led to inhibition of cell growth in vitro by decreasing V-ATPase and arresting cell cycle. Also the metalloproteinase-2(MMP-2) that degrades extracellular matrix was down-regulated in breast cells with high gene expression compared to the control cells. [12]

Some other theories that have been formulated the past few years implicate two pathways; the caspase and cytochrome c pathways and make efforts to confirm their role in cell apoptosis mediated by TMSG-1. [2] In 2015, Su et al. have used human embryonic kidney cell lines HEK293 and 293T, they transfected them with the plasmids containing the gene and finally analyzed the results from the TMSG-1 expression with several methods. [13] The Western blot test showed that the activation of procaspase-3 and procaspase-9 in the cells overexpressing TMSG-1 as well as the release of cytochrome c from mitochondria to cytoplasm ended up in increased apoptosis compared to control cell lines. Flow cytometry has also demonstrated that TMSG-1 overexpression mediates prolongation of G0/G1 phase of the cell cycle and decreases the proportion of S and G2/M phases. [13]

Conclusions

Several studies have been conducted the last decade regarding TMSG-1 role in the mechanisms of tumor invasion and metastasis. The molecular mechanisms of TMSG-1 expression and the actions of TMSG-1 protein in colorectal cancer cells remain in the shadow. Further research is warranted in order to shed light in the molecular pattern of TMSG-1 expression and also in the biological behavior of TMSG-1 protein. The protein may hold promise for its use as a prognostic tumor biomarker in colorectal cancer.

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