Clinical significance of the loss of *MATS1* mRNA expression in colorectal cancer

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Abstract. Human Mob as tumor suppressor 1 (MATS1) has been shown to play a crucial role in tumor growth by regulating cell proliferation and apoptosis. We evaluated the expression of *MATS1* in colorectal cancer patients and assessed its clinicopathological significance. *MATS1* mRNA expression in paired tumor/normal samples from 72 cases of colorectal cancer was evaluated, using real-time RT-PCR analysis. MATS1 protein expression was determined by immunohistochemical staining. *MATS1* mRNA expression was significantly lower in tumor tissues than in normal colorectal tissues ($p<0.05$). Clinicopathologically, factors such as incidence of large tumor size, deep invasion, lymphatic permeation and liver metastasis were significantly more frequent in the low *MATS1* expression group ($p<0.05$). Prognosis of the low expression group tended to be poorer than that of the high expression group. MATS1 protein expression was diminished in tumor cells compared with corresponding non-cancer colon epithelial cells. *MATS1* mRNA expression is suppressed in tumor tissue and its low expression is associated with tumor growth, invasion and metastasis of colorectal cancer. Specifically, low MATS1 expression may be a good marker of liver metastasis, a life-threatening indicator for colorectal cancer patients.

Introduction

Human *Mob as tumor suppressor 1* (*MATS1*) is a homolog of *Drosophila MATS* (a member of the Mob protein superfamily) and was reported to be a novel tumor suppressor gene (1). MATS1 interacts with one of the nuclear Dbf2-related (NDR) family members, *large as tumor suppressor 1* (*LATS1*), and activates protein kinase activity of LATS1 (2,3). The mechanism of cell proliferation and apoptosis by LATS and MATS was elucidated in *Drosophila* (Fig. 1A). The Hippo (Hpo) - Salvador (Sav) - LATS - MATS pathway functions to coordinate cell proliferation and cell death by regulating levels of key molecules such as Cyclin E (cell cycle accelerator) and DIAP1 (apoptosis inhibitor). These subsequently control organ size (4-6). A similar mechanism was proposed for humans (Fig. 1B). Mammalian Ste20-like (MST2) and SAV1 are human homologs of *Drosophila* Hpo and Sav, respectively. The MST2 - SAV1 - LATS1 - MATS1 pathway also controls cell proliferation and apoptosis through key molecules such as CDC2 and BAX - HTRA2 (7-12). Imbalances in this pathway may therefore contribute to the development of tumors. Indeed, abnormal control of the *LATS1* gene causes cancer (13-17) and loss of *MATS1* expression causes human skin melanoma (1). *MATS1* expression has not been studied in human colorectal cancer.

We studied *MATS1* gene expression in human colorectal cancer and showed that its expression was down-regulated in colorectal cancer tissue. Expression status was associated with several clinical and pathological factors, including liver metastasis.

Materials and methods

Clinical samples and laser micro-dissection samples. A total of 72 patients with colorectal cancer (28 male, 44 female), who had surgery at our institutes between 1993 and 1999, were involved in the present study. Written informed consent was obtained from all patients. All 72 patients were clearly diagnosed with colorectal cancer based on clinicopathological criteria described by the Japanese Society for Cancer of the Colon and Rectum (18). Resected tumor and paired non-tumor tissue specimens were immediately frozen in liquid nitrogen and kept at -80˚C until analysis. The tumor center in the resected specimen was extracted for examination, with care taken to avoid necrotic tissue. The tissues from eight of 72 patients with colorectal cancer were selected for laser microdissection (LMD). For LMD, 5-micron frozen sections were fixed in 70% ethanol for 30 sec, stained with hematoxylin and eosin and dehydrated as follows: 5 sec each in 70%, 95% and 100% ethanol and a final 5 min in xylene. Sections were air-dried, then micro-dissected with the LMD system (Leica Microsystems, Wetzlar, Germany). Target cells were excised, at least 100 cells per section, and bound to the transfer film.

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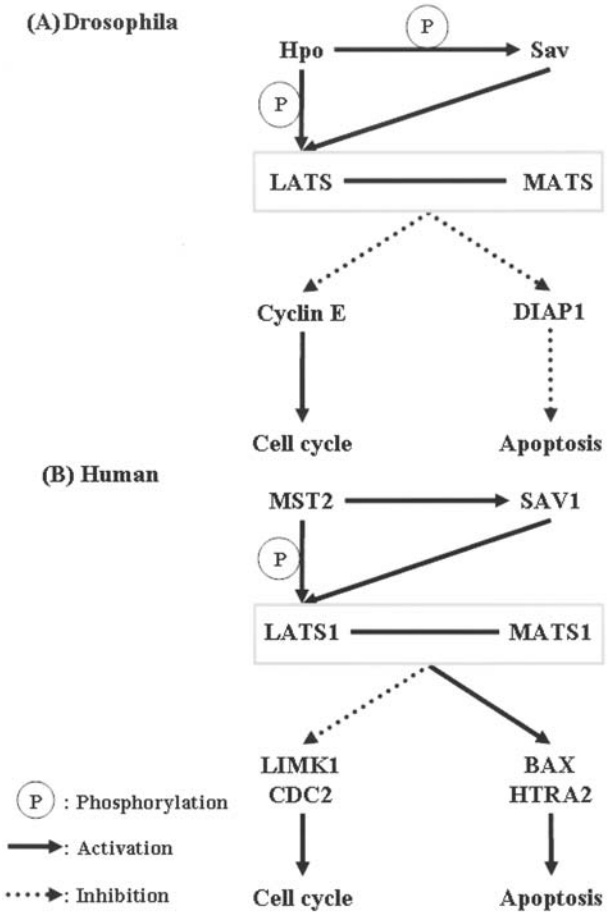

Figure 1. The pathway of *Drosophila* MATS (A) and human MATS1 (B) controls cell cycle and apoptosis. The Hippo (Hpo) - Salvador (Sav) - LATS - MATS pathway functions to coordinate cell proliferation and cell death by regulating levels of key molecules such as Cyclin E (cell cycle accelerator) and DIAP1 (apoptosis inhibitor). This subsequently controls organ size. Mammalian Ste20-like (MST2) and SAV1 are human homologs of *Drosophila* Hpo and Sav, respectively. The MST2 - SAV1 - LATS1 - MATS1 pathway also controls cell proliferation and apoptosis through levels of key molecules such as CDC2 and BAX - HTRA2.

This was repeated on 20 sections, for each of the 8 samples, and total RNA was extracted (19).

Total RNA extraction. Frozen tissue specimens were homogenized in guanidinium thiocyanate and total RNAs were obtained by ultracentrifugation through a cesium chloride cushion as described previously (20,21). Cultured cells from each cell line were dissolved in 350 μl of buffer RLT containing 1% ß-mercaptoethanol and total RNA was extracted and purified using the RNeasy mini kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's protocols.

Cell lines. Human colon cancer cell lines, LOVO, DLD-1, CCK81, Colo205, Colo320DM, WiDr, HT-29 and LS174T were obtained from the Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan and the Japanese Cancer Research Bank, Tokyo, Japan. Cell lines were maintained in RPMI-1640 (DLD1, Colo205, Colo320DM, WiDr), Ham's F-12 (Lovo), McCoy's 5a (HT29) or DMEM (CCK81, LS174T) medium (Invitrogen Corp., Carlsbad, CA) supplemented with 10% fetal bovine serum (Equitec-Bio, Ingram, TX), 100 units/ml penicillin G and 100 μg/ml streptomycin (Invitrogen Corp.). The cells were incubated in 5% CO_2 at 37˚C and passaged every three days.

Real-time quantitative RT-PCR. The sequences of *MATS1* primers were as follows: sense primer, 5'-CCTCAACACC TCCTTTAAGCAC-3'; and antisense primer, 5'-AGACAC AGGCAATGGGTATCTT-3'. *Glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) was used as an internal control and the sequences of *GAPDH* primers were as follows: sense primer, 5'-TTGGTATCGTGGAAGGACTCTA-3'; and antisense primer, 5'-TGTCATATTTGGCAGGTT-3'. cDNA was synthesized from 8.0 μg of total RNA as described previously (22). Real-time monitoring of PCR reactions was performed using the LightCycler™ system (Roche Applied Science, Indianapolis, IN) and SYBR-Green I dye (Roche Diagnostics). Monitoring was performed according to the manufacturer's instructions, as described previously (23,24). In brief, a master mixture was prepared on ice, containing 1 μl of cDNA, 2 μl of DNA Master SYBR-Green I mix, 50 ng of primers and 2.4 μl of 25 mM $MgCl_2$. The final volume was adjusted to 20 μl with water. After the reaction mixture was loaded into the glass capillary tube, quantitative RT-PCR was performed with the following cycling conditions: initial denaturation at 95˚C for 10 min, followed by 32 cycles of 95˚C for 10 sec, annealing at 60˚C for 10 sec and extension at 72˚C for 10 sec. After amplification, products were subjected to a temperature gradient from 68˚C to 95˚C at 0.2˚C/sec, under continuous fluorescence monitoring, to produce a melting curve of the products.

Data analysis. Expression levels of *MATS1* and *GAPDH* mRNA were determined by comparing with cDNA transcribed from Human Universal Reference total RNA (Clontech, Palo Alto, CA). After proportional baseline adjustment, the fit point method was employed to determine the cycle in which the log-linear signal was first distinguishable from the baseline. This cycle number was used as the crossing-point value. A standard curve was produced by measuring the crossing point of each standard value and plotting it against the logarithmic value of concentration. Concentrations of the unknown samples were calculated by plotting their crossing points against the standard curve and dividing by *GAPDH* content. *GAPDH* expression confirmed no differences between tumor and normal tissue.

Immunohistochemistry. Immunohistochemical studies for MATS1 were performed on formalin-fixed, paraffin-embedded surgical sections obtained from patients with colorectal cancer. Tissue sections were deparaffinised, soaked in 0.01 M sodium citrate buffer and boiled in a microwave for 5 min at 500 W to retrieve cell antigens. Primary antibodies against MATS1 (mouse monoclonal antibody provided by Professor Zhi-Chun Lai, Department of Biology, Department of Biochemistry and Molecular Biology, Pennsylvania State University) were incubated overnight at 4˚C. Tissue sections were immunohistochemically stained using the streptavidin-biotin peroxidase method (LSAB + system - HRP; Dako, Cat #K0690, Kyoto, Japan). All sections were counter-stained with hematoxylin.

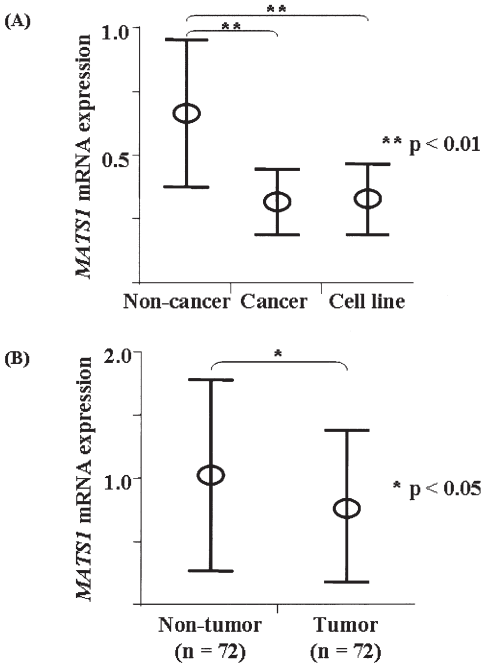

Figure 2. *MATS1* mRNA in LMD samples, cell lines and clinical samples. Correction values of *MATS1* mRNA expression were calculated by dividing *MATS1* amounts by the amounts of an endogenous reference (*GAPDH*) in the same samples. (A) Mean expression levels of *MATS1* in cancerous samples and cell lines were significantly lower than levels in non-cancerous samples ($p<0.01$, Student's t-test). (B) Mean expression levels of *MATS1* in tumor tissue specimens were significantly lower than levels in non-tumor tissue specimens ($p<0.05$, Student's t-test).

Statistics. For continuous variables, data was expressed as mean ± standard deviation. The relationship between *MATS1* mRNA expression and clinicopathological factors was analyzed using the chi-square test and Student's t-test. Surviving curves were plotted according to the Kaplan-Meier method and the generalized log-rank test was applied to compare the survival curve. All tests were analyzed using JMP software (SAS Institute Inc., Cary, NC, USA) and the findings were considered significant when the p-value was <0.05.

Results

Expression of MATS1 mRNA in clinical LMD samples, tissue specimens and cell lines. We determined levels of *MATS1* mRNA expression by comparing with a human universal reference quantifying standard, which expressed human *MATS1* sufficiently. Mean expression levels of *MATS1* in the cancerous portion of 8 patients' samples were significantly lower than levels in the non-cancerous part of the samples (Fig. 2A; $p<0.01$, Student's t-test). *MATS1* mRNA expression was also significantly lower in 8 human colon cancer cell lines. The mean expression level of *MATS1* mRNA in tumor tissue specimens was significantly lower than that of non-tumor tissue (Fig. 2B; $p<0.05$, Student's t-test). No differences in mRNA expression level were found between non-tumor tissue and non-cancerous part ($p=0.16$).

MATS1 mRNA expression and clinicopathological characteristics. To investigate the clinical significance of *MATS1* expression, the tumor (T)/normal (N) ratio of *MATS1* mRNA expression was calculated in each case. We apportioned all 72 patients into two groups as follows: 25 cases into the high *MATS1* expression group (T/N>1) and 47 cases into the low *MATS1* expression group (T/N<1). The association between clinicopathological features and *MATS1* mRNA expression is summarized in Table I (chi-square test). In the low *MATS1* expression group, incidences of large tumor size, serosal invasion and positive lymphatic invasion were significantly increased compared to the high *MATS1* expression group ($p<0.05$, $p<0.01$ and $p<0.05$, respectively). Also, the incidence of liver metastasis in the low *MATS1* expression group was significantly higher than that in the high *MATS1* expression

Table I. *MATS1* mRNA expression and clinicopathological factors.

Factors	Low expression (n=47)	High expression (n=25)	p value
Sex (male:female)	29:18	15:10	0.89
Age (mean ± SD)	67.1±10.2	68.6±9.8	0.66
Histological grade			
Well	11	9	0.25
Moderate	34	16	
Poor	2	0	
Tumor size			
Small (<30 mm)	5	8	<0.05
Large (>31 mm)	42	17	
Serosal invasion			
Absent	9	13	<0.01
Present	38	12	
Lymphatic permeation			
Absent	27	20	<0.05
Present	20	5	
Venous permeation			
Absent	29	18	0.38
Present	18	7	
Lymph node metastasis			
Absent	26	17	0.29
Present	21	8	
Liver metastasis			
Absent	36	24	<0.05
Present	11	1	
Peritoneal dissemination			
Absent	46	25	0.35
Present	1	0	
Dukes stage			
A	6	12	<0.01
B	16	5	
C	13	7	
D	12	1	

Table II. Relation of liver metastasis with *MATS1* expression and venous permeation.

	Venous permeation			*MATS1* expression		
Liver metastasis	Absent (n=47)	Present (n=25)	p value	Low (n=47)	High (n=25)	p value
Absent (n=60)	41	19		36	24	
Present (n=12)	6	6	0.23	11	1	<0.05

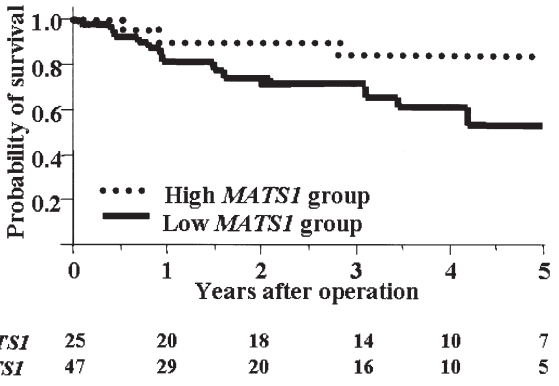

Figure 3. Kaplan-Meier overall survival curves for the low *MATS1* expression group and the high *MATS1* expression group. The difference in overall survival rate between the two groups was not significant (p=0.06, log-rank test).

group (p<0.05). On the other hand, venous permeation was not associated with liver metastasis (p=0.23; Table II). There was no significant difference in lymph node metastasis, however, there was a significant association (p<0.01) among Dukes A group and Dukes B, C and D groups. Other pathological variables, such as sex, age, histological grade, peritoneal dissemination and venous permeation, were not significantly different between the two groups. The 5-year actuarial disease-specific survival ratios in the low *MATS1* expression group and the high *MATS1* expression group were 53.5% and 84.4%, respectively. Moreover, prognosis of the low *MATS1* expression group was poor compared with the high *MATS1* expression group in the Kaplan-Meier survival curve (Fig. 3; p=0.06, log-rank test).

Immunohistochemistry of MATS1 expression. Expression of MATS1 protein was evaluated by immunohistochemistry of resected colorectal tissue specimens, using an anti-MATS1 antibody. Intracellular expression of MATS1 protein was remarkably stronger in non-cancerous colorectal cells than in the cancer cells (Fig. 4).

Discussion

Disruption of the MST2 - SAV1 - LATS1 - MATS1 pathway is associated with cancer development. With respect to *LATS1*, Hisaoka *et al* studied human soft tissue sarcoma and reported that 7 of 50 tumors showed low or no expression of *LATS1* due to hypermethylation of the promoter region in 6 tumors and a point mutation in the other (14). Jiang *et al* studied 88 human astrocytomas and showed hypermethylation of the promoter region in 56 cases (17), while Takahashi *et al* reported hypermethylation of the promoter region in 60% of breast cancers. Cases with low or no expression of *LATS1* mRNA showed larger tumor size, positive lymph node metastasis, or negative estrogen receptor and/or progesterone receptor. In addition, low expression of the gene was associated with patient prognosis and found to be an independent prognostic factor by multivariate analysis (15). Asaka *et al* studied lymph node-positive breast cancer, with or without recurrence, by cDNA microarray analysis and determined that *LATS1* gene expression was suppressed in tumors with recurrence (16). With respect to the *MATS1* gene, only one study has been published, which reports the absence of *MATS1* expression in some human skin melanomas or mouse mammary gland carcinomas. This was due to a three nucleotide deletion in the sixth or seventh codon for human melanomas or a 38-bp insertion, immediately downstream of the fifth codon, that caused a premature termination of the *MATS1* gene in mouse carcinomas (1).

Our current study determined that *MATS1* gene expression was significantly suppressed in human colorectal cancer tissue compared to normal tissue. When mRNA expression levels in tumors and normal tissue were calculated, a statistically significant inverse correlation was found between the size of tumor and *MATS1* expression. Therefore, we speculated that diminished expression of MATS1 in normal colorectal tissue suppressed LATS1 expression. This might cause disruption of the normal cell cycle and apoptotic mechanism and could accelerate carcinogenesis or promote proliferation of cancer cells.

According to the clinicopathological variables (Table I), the group of tumors with low *MATS1* expression tended to invade deeper into the wall and permeated lymphatic vessels more frequently than the group of tumors with high *MATS1* expression. *MATS1* expression was unrelated to the incidence of lymph node metastasis. However, the increased incidence of serosal invasion in cases with the low *MATS1* expression determined the Dukes clinical stage. Therefore, low expression of *MATS1* in human colorectal cancer was considered intimately associated with tumor invasion.

Cases with low *MATS1* expression tended to indicate a poor prognosis compared to cases with high *MATS1* expression. This may be because the number of cases with liver metastases at the time of the operation was larger in the low *MATS1* expression group. Several reports have suggested that venous invasion of colorectal cancer is profoundly related to liver metastasis or prognosis (25-31). However, in our study, low

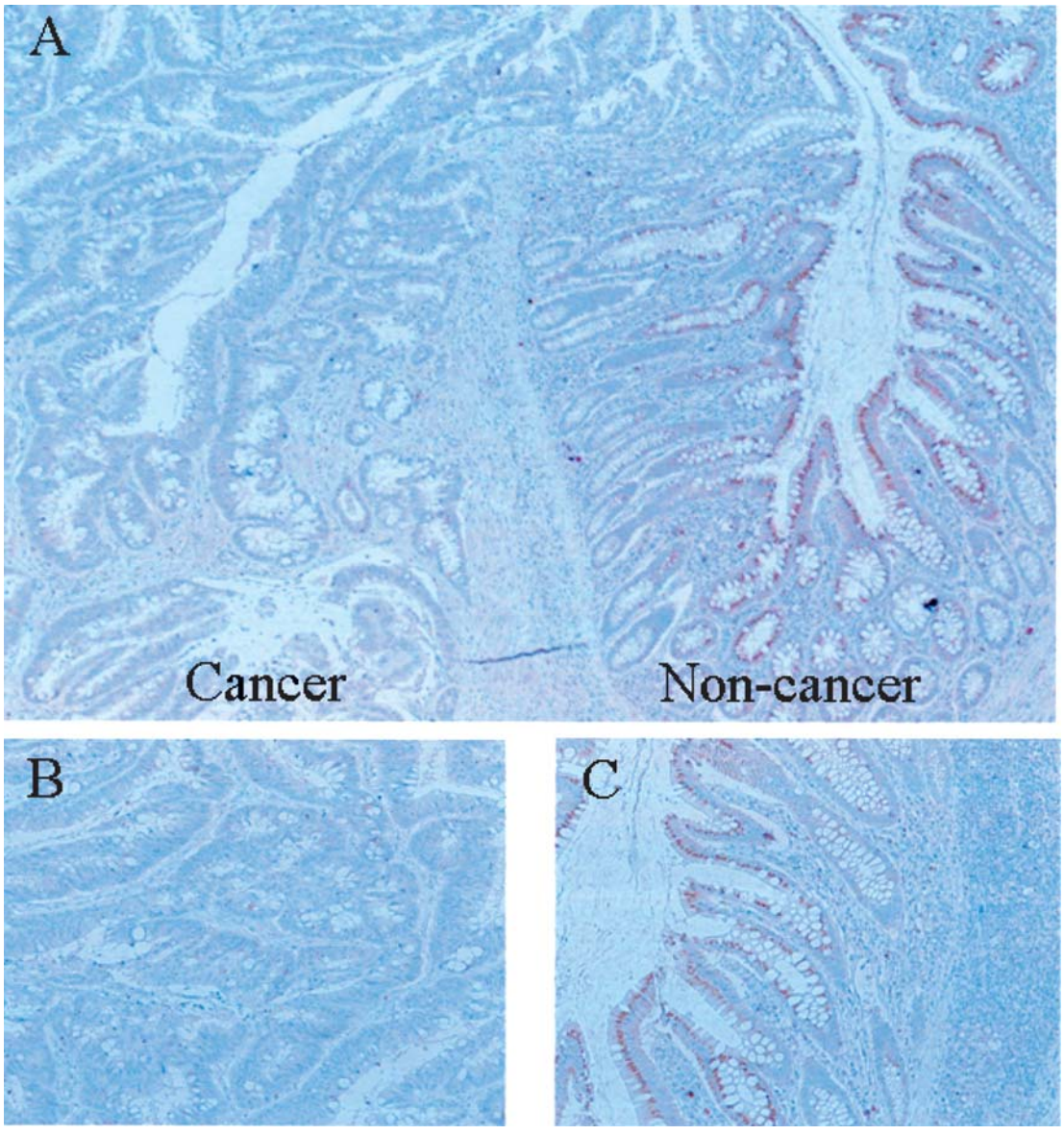

Figure 4. A representative example of MATS1 protein expression in colorectal cancer. Immunohistochemical staining with an anti-MATS1 antibody in colorectal cancer cells (A, left; B) and non-cancer cells (A, right; C). MATS1 protein was predominantly expressed in non-cancer cells (A, low power field, x40; B and C, high power field, x100).

MATS1 expression was associated more with liver metastasis than positive venous invasion (Table II). Therefore, low *MATS1* expression in the primary tumor may be a good indicator of liver metastasis. As liver metastasis is one of the critical indicators that predicts a poor prognosis of colorectal cancer cases (32), surgical resection of liver tumors is effective for extending the survival period of those patients (33,34). In other words, once the magnitude of MATS1 expression has been established, we can predict the likelihood of liver metastasis and treat patients accordingly.

In conclusion, the current study identifies and determines the clinical significance of MATS1 expression in human colorectal cancer cases. We found that MATS1 expression was diminished in malignant tissues compared to surrounding normal tissues and that cases of low *MATS1* expression were significantly associated with increased tumor size, depth of tumor invasion, and incidences of lymphatic permeation. In addition, low expression of *MATS1* may be a critical indicator for the prediction of liver metastasis in primary colorectal cancer cases.

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References

1. Lai ZC, Wei X, Shimizu T, *et al*: Control of cell proliferation and apoptosis by mob as tumor suppressor, mats. Cell 120: 675-685, 2005.
2. Hergovich A, Schmitz D and Hemmings BA: The human tumour suppressor LATS1 is activated by human MOB1 at the membrane. Biochem Biophys Res Commun 345: 50-58, 2006.
3. Bothos J, Tuttle RL, Ottey M, Luca FC and Halazonetis TD: Human LATS1 is a mitotic exit network kinase. Cancer Res 65: 6568-6575, 2005.
4. Hay BA and Guo M: Coupling cell growth, proliferation, and death. Hippo weighs in. Dev Cell 5: 361-363, 2003.
5. Ryoo HD and Steller H: Hippo and its mission for growth control. Nat Cell Biol 5: 853-855, 2003.
6. Rothenberg ME and Jan YN: Cell biology: the hippo hypothesis. Nature 425: 469-470, 2003.
7. Yang X, Yu K, Hao Y, *et al*: LATS1 tumour suppressor affects cytokinesis by inhibiting LIMK1. Nat Cell Biol 6: 609-617, 2004.
8. Tao W, Zhang S, Turenchalk GS, *et al*: Human homologue of the Drosophila melanogaster lats tumour suppressor modulates CDC2 activity. Nat Genet 21: 177-181, 1999.
9. Xu T, Wang W, Zhang S, Stewart RA and Yu W: Identifying tumor suppressors in genetic mosaics: the Drosophila lats gene encodes a putative protein kinase. Development 121: 1053-1063, 1995.
10. Yang X, Li DM, Chen W and Xu T: Human homologue of Drosophila lats, LATS1, negatively regulates growth by inducing G(2)/M arrest or apoptosis. Oncogene 20: 6516-6523, 2001.
11. Kuninaka S, Nomura M, Hirota T, *et al*: The tumor suppressor WARTS activates the Omi/HtrA2-dependent pathway of cell death. Oncogene 24: 5287-5298, 2005.
12. Chan EH, Nousiainen M, Chalamalasetty RB, Schafer A, Nigg EA and Sillje HH: The Ste20-like kinase Mst2 activates the human large tumor suppressor kinase Lats1. Oncogene 24: 2076-2086, 2005.
13. Hansen LL, Jensen LL, Dimitrakakis C, *et al*: Allelic imbalance in selected chromosomal regions in ovarian cancer. Cancer Genet Cytogenet 139: 1-8, 2002.
14. Hisaoka M, Tanaka A and Hashimoto H: Molecular alterations of h-warts/LATS1 tumor suppressor in human soft tissue sarcoma. Lab Invest 82: 1427-1435, 2002.
15. Takahashi Y, Miyoshi Y, Takahata C, *et al*: Down-regulation of LATS1 and LATS2 mRNA expression by promoter hypermethylation and its association with biologically aggressive phenotype in human breast cancers. Clin Cancer Res 11: 1380-1385, 2005.
16. Asaka S, Fujimoto T, Akaishi J, Ogawa K and Onda M: Genetic prognostic index influences patient outcome for node-positive breast cancer. Surg Today 36: 793-801, 2006.
17. Jiang Z, Li X, Hu J, *et al*: Promoter hypermethylation-mediated down-regulation of LATS1 and LATS2 in human astrocytoma. Neurosci Res 56: 450-458, 2006.
18. Japanese Society for Cancer of the Colon and Rectum. General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum and Anus. 6th edition. Kanehara, Tokyo, 1998.
19. Nishida K, Mine S, Utsunomiya T, *et al*: Global analysis of altered gene expressions during the process of esophageal squamous cell carcinogenesis in the rat: a study combined with a laser microdissection and a cDNA microarray. Cancer Res 65: 401-409, 2005.
20. Utsunomiya T, Okamoto M, Hashimoto M, *et al*: A gene-expression signature can quantify the degree of hepatic fibrosis in the rat. J Hepatol 41: 399-406, 2004.
21. Utsunomiya T, Hara Y, Kataoka A, *et al*: Cystatin-like metastasis-associated protein mRNA expression in human colorectal cancer is associated with both liver metastasis and patient survival. Clin Cancer Res 8: 2591-2594, 2002.
22. Utsunomiya T, Inoue H, Taguchi K, Shimada M, Sugimachi K and Mori M: G protein gamma 7 expression as a new clinicopathological marker in patients with intrahepatic cholangiocarcinoma. Arch Surg 137: 181-185, 2002.
23. Masuda TA, Inoue H, Nishida K, *et al*: Cyclin-dependent kinase 1 gene expression is associated with poor prognosis in gastric carcinoma. Clin Cancer Res 9: 5693-5698, 2003.
24. Ogawa K, Utsunomiya T, Mimori K, *et al*: Clinical significance of elongation factor-1 delta mRNA expression in oesophageal carcinoma. Br J Cancer 91: 282-286, 2004.
25. Minsky B and Mies C: The clinical significance of vascular invasion in colorectal cancer. Dis Colon Rectum 32: 794-803, 1989.
26. Minsky BD, Mies C, Recht A, Rich TA and Chaffey JT: Resectable adenocarcinoma of the rectosigmoid and rectum. II. The influence of blood vessel invasion. Cancer 61: 1417-1424, 1988.
27. Krasna MJ, Flancbaum L, Cody RP, Shneibaum S and Ben Ari G: Vascular and neural invasion in colorectal carcinoma. Incidence and prognostic significance. Cancer 61: 1018-1023, 1988.
28. Talbot IC, Ritchie S, Leighton MH, Hughes AO, Bussey HJ and Morson BC: Spread of rectal cancer within veins. Histologic features and clinical significance. Am J Surg 141: 15-17, 1981.
29. Talbot IC, Ritchie S, Leighton M, Hughes AO, Bussey HJ and Morson BC: Invasion of veins by carcinoma of rectum: method of detection, histological features and significance. Histopathology 5: 141-163, 1981.
30. Talbot IC, Ritchie S, Leighton MH, Hughes AO, Bussey HJ and Morson BC: The clinical significance of invasion of veins by rectal cancer. Br J Surg 67: 439-442, 1980.
31. Lui KK, Enjoji M and Inokuchi K: Venous permeation of colorectal carcinoma. Jpn J Surg 10: 284-289, 1980.
32. Taylor I: Liver metastases from colorectal cancer: lessons from past and present clinical studies. Br J Surg 83: 456-460, 1996.
33. Scheele J, Stangl R and Altendorf-Hofmann A: Hepatic metastases from colorectal carcinoma: impact of surgical resection on the natural history. Br J Surg 77: 1241-1246, 1990.
34. Doci R, Gennari L, Bignami P, Montalto F, Morabito A and Bozzetti F: One hundred patients with hepatic metastases from colorectal cancer treated by resection: analysis of prognostic determinants. Br J Surg 78: 797-801, 1991.