

The Triangulating Stapling Technique for Cervical Esophagogastric Anastomosis After Esophagectomy

YASUSHI TOH, YOSHIHISA SAKAGUCHI, OSAMU IKEDA, EISUKE ADACHI, KIPPEI OHGAKI, YOICHI YAMASHITA, EIJI OKI, KAZUHITO MINAMI, and TAKESHI OKAMURA

Department of Gastroenterological Surgery, National Kyushu Cancer Center, 3-1-1 Notame, Minami-ku, Fukuoka 811-1395, Japan

Abstract

Purpose. To evaluate the safety and feasibility of the triangulating stapling technique (TST) for cervical esophagogastric anastomosis after esophagectomy (CEGA).

Methods. The subjects were 123 patients who underwent transthoracic esophagectomy with three-field lymph node dissection and reconstruction with a 3.5-cm wide gastric tube, for thoracic esophageal cancer. We performed the TST for CEGA in 33 patients operated on after December, 2006 (TST group) and hand-sewn anastomosis in 90 patients operated on between 2002 and 2006 (HSA group).

Results. In the TST group, CEGA was performed in an end-to-end fashion using three linear staplers. The first anastomosis was applied to the posterior walls of the remnant esophagus and gastric tube in an inverted fashion. The second and the third anastomoses were done in an everted fashion to make the anterior wall. The end-to-end HSA was performed with interrupted sutures using 4-0 absorbable material. Anastomotic leakage occurred in only 1 (3.0%) of the 33 TST patients, but in 13 (14.4%) of the 90 HSA patients ($P = 0.07$). The frequency of anastomotic stenosis was 9.1% and 25.6% in the TST and HSA groups, respectively ($P < 0.05$).

Conclusions. Cervical esophagogastric anastomosis using TST may reduce the frequency of anastomotic leakage and stenosis. This technique is a safe and reliable alternative for CEGA after esophagectomy.

Key words Linear stapler · Triangulating stapling technique · Esophagogastric anastomosis · Anastomotic leakage · Anastomotic stenosis

Introduction

The most frequent and serious postoperative complications of esophagectomy are respiratory failure and anastomotic leakage, both of which are associated with high mortality.¹⁻³ Anastomotic leakage is thought to be caused by ischemia of the gastric or colon conduit and by the methods of anastomosis and surgical techniques used.^{2,4,5} The methods used for esophagogastric anastomosis after esophagectomy vary among institutes, although many reports have addressed this issue. In Japan, the standard surgical procedure for thoracic esophageal cancers is transthoracic esophagectomy with extended cervical, mediastinal, and abdominal lymph node dissection, known as the “three field lymph node dissection” (3FLND). This procedure includes cervical esophagogastric anastomosis (CEGA) and CEGA failure occurs more frequently than intrathoracic esophagogastric anastomosis failure.⁶ The leakage of CEGA after transthoracic esophagectomy is associated with more serious sequelae, such as empyema and sepsis, than after transhiatal esophagectomy without thoracotomy.⁷ Thus, a reliable and safe anastomotic method is necessary to prevent this life-threatening complication of esophagectomy.

Postoperative stenosis at the CEGA is also a critical postoperative complication, which compromises the patient's quality of life by causing anastomotic stenosis-related dysphagia.^{4,8} These patients require repeated endoscopic dilation of the stenosis. Furthermore, anastomotic leakage often leads to anastomotic stenosis.^{9,10} Therefore, we must develop an anastomotic method that reduces the risk of anastomotic stenosis and leakage.

The triangulating stapling technique (TST) was first used for a colorectal anastomosis by Venkatesh et al. in 1993¹¹ and has been utilized mainly for colo-colonic anastomoses.¹² The TST seems to be a safe, reliable, and easy technique for reconstruction of the alimentary

tract, which reduces the occurrence of both postoperative anastomotic leakage and stenosis.^{11,12} However, to our knowledge only one study has been published on esophagogastric anastomosis after esophagectomy, based on the results of only 12 cases.¹³ We conducted this study to evaluate the application of the TST for CEGA after transthoracic esophagectomy with 3FLND for thoracic esophageal cancers, in comparison with hand-sewn anastomosis (HSA).

Patients and Methods

Patients

Between January 2002 and April 2008, 175 patients underwent surgery for esophageal cancer in the National Kyushu Cancer Center, Japan. All cancers were pathologically diagnosed as squamous cell carcinoma. Among these patients, 122 with a thoracic esophageal cancer underwent transthoracic esophagectomy with 3FLND,

performed via a right-side thoracotomy. The alimentary tract was reconstructed using a gastric tube made of the greater curvature of the stomach. CEGA was performed by hand sewing until September 2006 (HSA group; $n = 90$), and by the TST after October 2006 (TST group; $n = 33$) through a retrosternal or posterior mediastinal route. The TST group included one patient with CEGA performed after a bypass operation. All of the operations were performed by a single surgeon (Y.T.) or by other surgeons under the direction of Y.T.

Surgical Technique

A gastric tube, 3.5 cm wide, was made along the greater curvature of the stomach using linear staplers (GIA Universal 60-3.5, Tyco Healthcare, Mansfield, MA, USA) with seromuscular sutures added along the staple line. This narrow gastric conduit was long enough to be pulled up to the cervix through a retrosternal or posterior mediastinal route and had adequate blood supply to its tip.¹⁴ The width of 3.5 cm was considered suitable

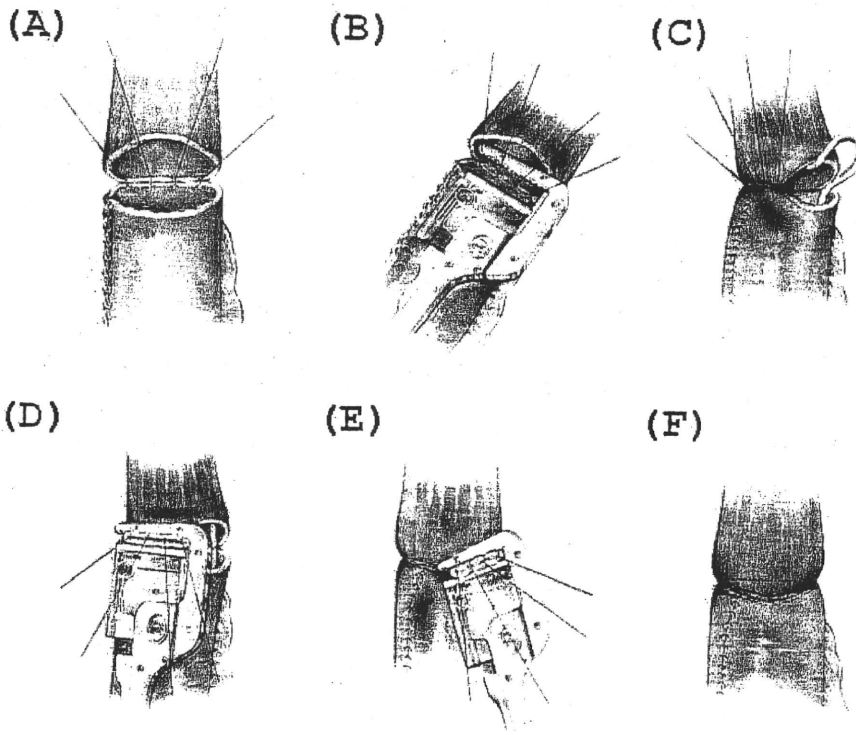


Fig. 1A-F. Triangulating stapling technique for cervical esophagogastric anastomosis using three linear staplers. **A** Three or four suspension sutures are placed through all layers of the posterior wall of the remnant esophagus and gastric tube. **B** The first anastomosis is done using a linear stapler, pulling up the suspension sutures to securely anastomose all layers of the posterior wall in an inverted fashion. **C** Before the second anastomosis of the anterior wall, a nasogastric tube is inserted and advanced downward into the gastric tube. **D** A linear stapler is applied to create the second side of the anas-

tomosis on the anterior wall. It is important that the second suture intersects the first staple line of the posterior wall. **E** Note that the linear staple line of the gastric tube is positioned at the center of the second side of the triangle. The third anastomosis is done in the same manner, using suspension sutures. **F** The end-to-end anastomosis using triangulating stapling technique is completed. Note that the three staple lines are mutually intersected. The two sides of the anterior wall are stapled in an everted fashion

for end-to-end cervical anastomosis between the remnant esophagus and the gastric tube. For the TST group, an end-to-end CEGA was performed using three linear staplers (TA30-3.5, Tyco Healthcare; Fig. 1). The first instrumental anastomosis was applied to about two-thirds of the posterior wall of the remnant esophagus and the gastric tube in an inverted fashion. The first linear stapler was applied to the esophagus and gastric tube after three or four suspension sutures in the whole layer were added to secure the first anastomosis. Great care was taken to securely suture the whole layer by pulling up the suspension sutures. After firing the first linear stapler, the remaining walls of the esophagus and gastric tube were resected sharply and the stapler was released. Next, suspension sutures were secured to both ends of the staple line on the sutured posterior wall. To make the anterior wall of the reconstructed tract, the second and the third anastomosis were performed in the same manner using the second and third linear staples; however, these were done in an everted, rather than in an inverted fashion. When performing the TST, it is critical that the staple lines are securely intersected mutually and that the longitudinal staple line of the gastric conduit is positioned at the center of the suture line to the right of the anterior wall. Thus, the triangle-shaped end-to-end anastomosis was completed between the remnant esophagus and the gastric tube in the cervical region (Fig. 2). Before the third stapling, a 14-F or 16-F nasogastric tube was inserted by the anesthetist and advanced downward into the gastric tube for postoperative monitoring.

End-to-end HSA for CEGA was performed interruptedly using 4-0 absorbable monofilament sutures in a single layer for the anterior wall and in two layers for the posterior wall. In both the TST and HSA groups, a leakage test was done just after completion of the anastomosis, by inflating the reconstructed tract with dyed saline. A closed suction drain was placed in the anastomotic region.

Definitions of Anastomotic Leakage and Stenosis

The condition of the draining fluid and cervical wound as well as the patients' general condition, determined by body temperature, pulse rate, C-reactive protein value, and white blood cell count, were monitored to detect any sign of anastomotic leakage. Anastomotic leakage was classified into the following three categories: minor leakage, defined as purulent and/or salivary discharge from a cervical drain without the need for surgical intervention, which resolved in fewer than 14 days; moderate leakage, defined as purulent and/or salivary discharge from a cervical drain without the need for surgical intervention, which took more than 15 days to heal; and severe leakage, defined as purulent and/or salivary dis-

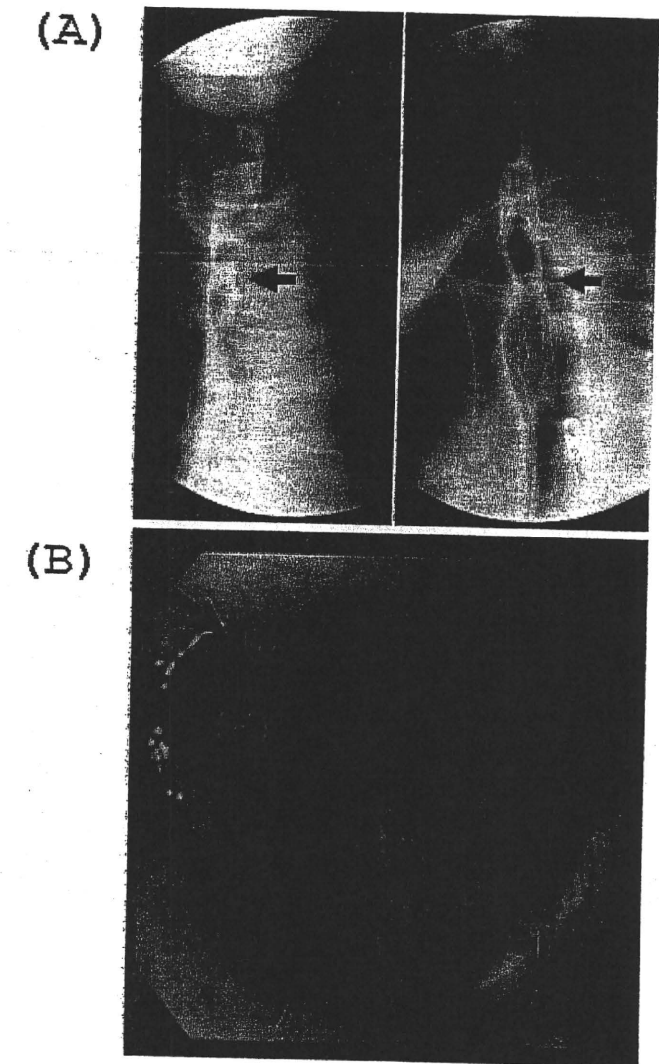


Fig. 2A,B. Demographic presentation of the triangulating stapling technique for cervical esophagogastric anastomosis. **A** Contrast-swallow on postoperative day 7 shows no anastomotic leakage. The passage of contrast medium is good and the anastomosis is sufficiently wide. **B** Endoscopic view of the anastomotic site on postoperative day 14. The triangle-shaped lumen is wide and three corners can be seen. Two of the three sides of the triangle are everted (*ev*) and there are no mucosal defects. One of the three sides is anastomosed in an inverted (*inv*) fashion. The three corners of the triangle are indicated by arrows

charge from a cervical drain, requiring surgical intervention for adequate drainage or repair. Anastomotic stenosis was defined as any stenosis in the anastomotic region, which required either endoscopic balloon dilation or bougienage at least once.

Statistical Analysis

Statistical analyses were performed using Student's *t*-test or the chi-square test, with the StatView software

program (SAS Institute, Cary, NC, USA). A *P* value of less than 0.05 was considered significant.

Results

The clinical characteristics of the patients in the HSA and TST groups are shown in Table 1. There were no significant differences in the patients' backgrounds between the two groups. There were no significant differences in the route of esophageal reconstruction, other operative procedures, operation time, or estimated blood loss between the groups. According to change in policy in our institute, after 2004, patients were extubated immediately after the operation. Thus, 40 patients from the HSA group and all of those from the TST group were managed without any mechanical ventilation.

The frequencies of anastomotic leakage and anastomotic stenosis are shown in Table 2. Anastomotic

leakage occurred in only 1 (3.0%) of the 33 TST patients, but in 12 (13.3%) of the 90 HSA patients. Of the 13 cases of leakage in the HSA group, 2 (16.6%) and 8 (66.7%) were minor and moderate, respectively, and all resolved after drainage from a cervical drain inserted during the original surgery. However, 2 of these 13 patients required surgical intervention. In the TST group, there was only one minor leakage, which healed after 13 days of drainage. This occurred in the third TST group patient and the following 30 had no leakage. Anastomotic leakage tended to occur more frequently in the HSA group than in TST group, although the difference was not significant (*P* = 0.09). Anastomotic stenosis was observed in 24 (26.7%) patients from the HSA group and in 3 (9.1%) from the TST group, representing a significant difference (*P* < 0.05). The average number of dilation procedures was 3.1 in the HSA group and 3.6 in the TST group.

Table 1. Clinical characteristics of the patients who underwent hand-sewn or triangular stapling for cervical esophago-gastric anastomosis

	HSA group (n = 90)	TST group (n = 33)	<i>P</i> value
Age (years), mean ± SD	62.9 ± 7.7	63.0 ± 7.9	NS
Sex Male:female	74:16	26:7	NS
Comorbidity			
Cardiac	18 (20.0%)	6 (18.2%)	NS
Pulmonary	4 (4.4%)	4 (12.1%)	NS
Renal	1 (1.1%)	1 (3.0%)	NS
Liver	4 (4.4%)	0	NS
Others	13 (14.4%)	4 (12.1%)	NS
p Stage ^a 0/1/2/3/4 ^a	5/24/30/18/9	3/5/14/3/7 ^b	NS
Neoadjuvant CRT -/+	64/26	26/7	NS

HSA, hand-sewn anastomosis; TST, triangular stapling technique; CRT, chemoradiotherapy; SD, standard deviation; NS, not significant

^aThe p Stage was based on the Japan Esophageal Society²³

^bOne bypass operation was excluded

Table 2. Postoperative data after hand-sewn vs triangular stapling for cervical esophago-gastric anastomosis

	HSA group (n = 90)	TST group (n = 33)	<i>P</i> value
Postop. complications			
Anastomotic leakage	12 (13.3%)	1 (3.0%)	NS
Minor ^a	2 (2.2%)	1 (3.0%)	
Moderate ^a	8 (8.9%)	0	
Severe ^a	2 (2.2%)	0	
Pulmonary	14 (15.6%)	4 (12.1%)	NS
Cardiac	3 (3.3%)	2 (6.1%)	NS
Renal/liver	5 (5.6%)	2 (6.1%)	NS
Others	10 (11.1%)	5 (15.2%)	NS
Anastomotic stenosis	24 (26.7%)	3 (9.1%)	<0.05
Operative mortality	1 (1.1%)	1 (3.0%)	NS

HSA, hand-sewn anastomosis; TST, triangular stapling technique; Postop., postoperative; NS, not significant

^aSee text for definition of leakage

Discussion

The risk of leakage at the anastomosis between the remaining esophagus and its replacement conduit after esophagectomy is highest among all types of gastrointestinal anastomoses.^{3,7,15} Moreover, it is associated with high mortality, once it occurs.¹⁻³ Much effort has been devoted to reducing the occurrence of anastomotic leakage, with some good results.^{10,16} However, the rate of anastomotic leakage after esophagectomy still exceeds 10% in the most series.^{13,17-20} Anastomotic leakage is reported to be much higher after transthoracic esophagectomy with extended 3FLND, because of the excessive surgical stress.¹⁵ Anastomotic leakage is attributed to ischemia of the gastric or colon conduit, and to technical problems, including the methods of anastomosis and surgeon's experience.^{2,4,5} Many surgeons prefer to perform anastomosis using a circular stapler than to using a hand-sewn anastomosis, because the stapling technique is simpler and easier,¹⁷ whereas many institutes use hand-sewn anastomosis for CEGA and achieve excellent results.¹⁶ Beitler et al.¹⁸ reviewed the results of four randomized and seven nonrandomized trials comparing circular stapled and hand-sewn esophagogastric anastomoses and concluded that both methods resulted in equivalent rates of anastomotic leakage.

The TST was originally designed for colorectal anastomosis and has been utilized mainly for colo-colonic anastomosis. It seems to be a safe, reliable, and easy technique in reconstruction of the alimentary tract, which reduces the occurrence of postoperative anastomotic leakage and stenosis.^{11,12} Furukawa et al.¹³ reported their experience of performing the TST for CEGA after esophagectomy in 12 patients. Their anastomotic leakage rate was greatly reduced to 8.3%, in comparison with 27% and 25% after hand-sewn anastomosis and circular stapled anastomosis, respectively. This rate is consistent with that in the current study. The one case of anastomotic leakage after TST in the current series involved the third among 33 patients, and this leakage resolved with conservative treatment within 1 week. Although the exact cause was unknown, the technique of TST might not have been established. No primary anastomotic leakage occurred in the subsequent 30 patients, although there was one case of leakage caused by direct spread of a mediastinal abscess and pyothorax to the anastomotic site in the retrosternal space.

The TST is also a time-saving modality. Furukawa et al.¹³ reported reducing the time to perform CEGA by using the TST (24 min vs 54 min for hand-sewn anastomosis and 35 min for the circular stapler method). Although we did not record the exact time required for CEGA by the TST, it is known that the TST for CEGA

can be completed within 30 min: approximately half the time required for hand-sewn anastomosis. This advantage may be an important contributor to the safety of esophagectomy because CEGA is the final step in all esophagectomies with lymph node dissection, which require extended surgical time and much concentration.

An additional advantage of the TST is that the CEGA can be made end-to-end in a safe, simple, and stable manner, using instruments. An end-to-end anastomosis of the alimentary tract is better than an end-to-side or side-to-side anastomosis. Although an end-to-end anastomosis can also be achieved with the hand-sewn method, the technical stability and the time it takes are problematic, and it requires extremely skilled surgeons. The circular stapling method is also a time-saving and stable technique for this anastomosis. However, CEGA using a circular stapler results in an end-to-side anastomosis, which may be associated with serious blood perfusion problems at the tip of the gastric conduit because the vascular net of the gastric tube wall is blocked by the stapling itself,²¹ leading to anastomotic failure. Triangulating stapling does not disturb the blood supply to the tip of gastric tube.

Anastomotic stenosis is a critical postoperative complication of esophageal surgery because it can result in life-threatening aspiration pneumonia and at the very least, nutritional disorders. This complication occurs in 16% and 27%-31% of patients who undergo hand-sewn and circular stapled CEGA, respectively.¹⁸ Even higher rates of 32% and 45%, respectively, have been reported by others.¹³ Therefore, anastomotic stenosis at the CEGA is more common in stapled anastomoses than in hand-sewn anastomoses.^{18,19} This is because the mucosa is not directly approximated in a circular stapled anastomosis, leaving a circular mucosal defect, which can lead to stenosis formation.^{9,13,22} The TST approximates the mucosa in two of the three sides of the triangle because two of the three anastomoses are made in an everted fashion, resulting in accurate mucosa-to-mucosa apposition, which is considered good for anastomotic healing. This may explain the lower rates of anastomotic stenosis in this (9.1%) and other (8.3%) series.¹³

In conclusion, the TST is a good alternative method for CEGA for the following reasons: the anastomotic leakage rate is low; the anastomotic stenosis rate is low; an end-to-end anastomosis is possible; the mucosal defect is less than with circular stapling, causing less circular ulcer formation; and the technique is simple, even for inexperienced surgeons. Finally, the medical costs for three linear staplers are equivalent to, or less than, those for a circular stapler.

References

1. Muller JM, Erasmi H, Stelzner M, Zieren U, Fichlmaier H. Surgical therapy of oesophageal carcinoma. *Br J Surg* 1990;77:845-57.
2. Urschel JD. Esophagogastric anastomotic leaks complicating esophagectomy: a review. *Am J Surg* 1995;169:634-40.
3. Alanezi K, Urschel JD. Mortality secondary to esophageal anastomotic leak. *Ann Thorac Cardiovasc Surg* 2004;10:71-5.
4. Ercan S, Rice TW, Murthy SC, Rybicki LA, Blackstone EH. Does esophagogastric anastomotic technique influence the outcome of patients with esophageal cancer? *J Thorac Cardiovasc Surg* 2005;129:623-31.
5. Mitchell JD. Anastomotic leak after esophagectomy. *Thorac Surg Clin* 2006;16:1-9.
6. Hsu HH, Chen JS, Huang PM, Lee JM, Lee YC. Comparison of manual and mechanical cervical esophagogastric anastomosis after esophageal resection for squamous cell carcinoma: a prospective randomized controlled trial. *Eur J Cardiothorac Surg* 2004;25:1097-101.
7. Korst RJ, Port JL, Lee PC, Altorki NK. Intrathoracic manifestations of cervical anastomotic leaks after transthoracic esophagectomy for carcinoma. *Ann Thorac Surg* 2005;80:1185-90.
8. Santos RS, Raftopoulos Y, Singh D, DeHoyos A, Fernando HC, Keenan RJ, et al. Utility of total mechanical stapled cervical esophagogastric anastomosis after esophagectomy: a comparison to conventional anastomotic techniques. *Surgery* 2004;136:917-25.
9. Wong J, Cheung H, Lui R, Fan YW, Smith A, Siu KF. Esophagogastric anastomosis performed with a stapler: the occurrence of leakage and stricture. *Surgery* 1987;101:408-15.
10. Orringer MB, Marshall B, Iannettoni MD. Eliminating the cervical esophagogastric anastomotic leak with a side-to-side stapled anastomosis. *J Thorac Cardiovasc Surg* 2000;119:277-88.
11. Venkatesh KS, Morrison N, Larson DM, Ramanujam P. Triangulating stapling technique: an alternative approach to colorectal anastomosis. *Dis Colon Rectum* 1993;36:73-6.
12. Fukunaga Y, Higashino M, Tanimura S, Osugi H. Triangulating stapling technique for reconstruction after colectomy. *Hepatogastroenterology* 2007;54:414-7.
13. Furukawa Y, Hanyu N, Hirai K, Ushigome T, Kawasaki N, Toyama Y, et al. Usefulness of automatic triangular anastomosis for esophageal cancer surgery using a linear stapler (TA-30). *Ann Thorac Cardiovasc Surg* 2005;11:80-6.
14. Sugimachi K, Ikeda M, Kai H, et al. Assessment of the blood flow in various gastric tubes for esophageal substitutes. *J Surg Res* 1982;33:463-8.
15. Fujita H, Kakegawa T, Yamana H, Shima I, Toh Y, Tomita Y, et al. Mortality and morbidity rates, postoperative course, quality of life, and prognosis after extended radical lymphadenectomy for esophageal cancer. Comparison of three-field lymphadenectomy with two-field lymphadenectomy. *Ann Surg* 1995;222:654-62.
16. Zhu ZJ, Zhao YF, Chen LQ, Hu Y, Liu LX, Wang Y, et al. Clinical application of layered anastomosis during esophagogastric anastomosis. *World J Surg* 2008;32:583-8.
17. Lee Y, Fujita H, Yamana H, Kakegawa T. Factors affecting leakage following esophageal anastomosis. *Surg Today* 1994;24:24-9.
18. Beitler AL, Urschel JD. Comparison of stapled and hand-sewn esophagogastric anastomoses. *Am J Surg* 1998;175:337-40.
19. Okuyama M, Motoyama S, Suzuki H, Saito R, Maruyama K, Ogawa J. Hand-sewn cervical anastomosis versus stapled intrathoracic anastomosis after esophagectomy for middle or lower thoracic esophageal cancer: a prospective randomized controlled study. *Surg Today* 2007;37:947-52.
20. Sarela AI, Tolan DJ, Harris K, Dexter SP, Sue-Ling HM. Anastomotic leakage after esophagectomy for cancer: a mortality-free experience. *J Am Coll Surg* 2008;206:516-23.
21. Tabira Y, Sakaguchi T, Kuhara H, Teshima K, Tanaka M, Kawasuji M. The width of a gastric tube has no impact on outcome after esophagectomy. *Am J Surg* 2004;187:417-21.
22. Law S, Fok M, Chu KM, Wong J. Comparison of hand-sewn and stapled esophagogastric anastomosis after esophageal resection for cancer: a prospective randomized controlled trial. *Ann Surg* 1997;226:169-73.
23. The Japanese Esophageal Society. Guide lines for the clinical and pathological studies on carcinoma of the esophagus. 10th ed. Tokyo: Kanehara; 2007.

The role of the *MTA* family and their encoded proteins in human cancers: molecular functions and clinical implications

Yasushi Toh · Garth L. Nicolson

Received: 3 October 2008 / Accepted: 12 December 2008 / Published online: 31 December 2008
© Springer Science+Business Media B.V. 2008

Abstract *MTA* (metastasis-associated gene) is a newly discovered family of cancer progression-related genes and their encoded products. *MTA1*, the first gene found in this family, has been repeatedly reported to be overexpressed along with its protein product MTA1 in a wide range of human cancers. In addition, the expression of *MTA1/MTA1* correlates with the clinicopathological properties (malignant properties) of human cancers. *MTA* proteins are transcriptional co-repressors that function in histone deacetylation and are involved in the NuRD complex, which contains nucleosome remodeling and histone deacetylating molecules. MTA1 expression correlates with tumor formation in the mammary gland. In addition, MTA1 converts breast cancer cells to a more aggressive phenotype by repression of the estrogen receptor (ER) α trans-activation function through deacetylation of the chromatin in the ER-responsive element of ER-responsive genes. Furthermore, MTA1 plays an essential role in c-MYC-mediated cell transformation. Another member of this family, MTA3, is induced by estrogen and represses the expression of the transcriptional repressor Snail, a master regulator of “epithelial to mesenchymal transitions”, resulting in the expression of the cell adhesion molecule E-cadherin and maintenance of a differentiated, normal epithelial phenotype in breast cells. In addition, tumor suppressor p53 protein is deacetylated and inactivated by both MTA1 and MTA2, leading to inhibition

of growth arrest and apoptosis. Moreover, a hypoxia-inducible factor-1 α (HIF-1 α) is also deacetylated and stabilized by MTA1, resulting in angiogenesis. Thus, *MTA* proteins, especially MTA1, represent a possible set of master co-regulatory molecules involved in the carcinogenesis and progression of various malignant tumors. *MTA* proteins are proposed to be important new tools for clinical application in cancer diagnosis and treatment.

Keywords Metastasis-associated gene 1 (MTA1) · Chromatin remodeling · Histone deacetylation · Gene expression · Protein modification · Cancer progression · Metastasis

Abbreviations

MTA	Metastasis-associated gene/protein
HDAC	Histone deacetylase
NuRD	Nucleosome remodeling and histone deacetylation
ER	Estrogen receptor
HIF	Hypoxia-inducible factor

Introduction

Recent advances in molecular biology have resulted in the discovery of a wide variety of new molecules involved in carcinogenesis and cancer progression. Although additional molecules related to cancer will be identified in the future, the existing and new molecules must fulfill two major requirements in order to be clinically useful as molecular targets for the diagnosis and treatment of human cancers. The first is that abnormalities in expression or structure of molecules of interest and their clinical relevance must be definitely demonstrated in human cancers by

Y. Toh (✉)
Department of Gastroenterological Surgery, National Kyushu
Cancer Center, 3-1-1 Notame, Minami-ku,
Fukuoka 811-1395, Japan
e-mail: ytoh@nk-cc.go.jp

G. L. Nicolson
Department of Molecular Pathology, The Institute for Molecular
Medicine, Huntington Beach, CA, USA

independent studies. The second is the underlying molecular mechanisms necessary for the molecules to exert their functions in carcinogenesis or cancer progression must be determined.

Among a number of cancer-related genes and molecules that have been discovered in the last few years, we identified a candidate metastasis-associated gene by use of a differential cDNA screening method. Thus, we identified a gene that was abundantly overexpressed in highly metastatic rat mammary adenocarcinoma cell lines compared to poorly metastatic cell lines [1, 2]. When this gene was sequenced, it was revealed as a completely novel gene without any homologous or related genes in the database. The rat gene was named *mta1* (metastasis-associated gene 1). A homologous gene was also expressed in human cancer cell lines [1], and its human cDNA counterpart, *MTA1*, was cloned by our group in 2000 [3]. Using surgically resected human tissues, we showed that high levels of *MTA1* mRNA expression were clinicopathologically correlated to the invasive and growth properties of gastrointestinal cancers, including esophageal, gastric and colorectal cancers [4, 5]. Subsequently, several reports from independent research groups followed our observations and showed similar correlations between *MTA1* expression and the malignant potentials of human cancers.

Several genes related to *MTA1* have now been identified, indicating *MTA1* consists of a gene family, which we now call the “*MTA* family”. Further studies on molecular biological and biochemical properties of the *MTA* family have shown that the gene products of the main members of the family (*MTA1*, *MTA2*, and *MTA3*) are tightly associated in a protein complex called NuRD (nucleosome remodeling and histone deacetylation), which has transcriptional regulatory function via histone deacetylation and chromatin remodeling. At the moment, the *MTA* family has attracted widespread attention as one of the key molecules that plays an indispensable role in the genesis and progression of a wide variety of cancers [6–8]. In this brief review, we will examine the significance of the expression of *MTA* family members in human cancers and the important molecular mechanisms that are currently known by which *MTA* proteins exert their functions. Finally, future directions for clinical applications of this protein family for the diagnosis and treatment of human cancers will be discussed.

Members of the *MTA* family and their protein structures

At present, the *MTA* proteins represent a family of gene products encoded by three distinct genes (*MTA1*, *MTA2*, and *MTA3*) and six reported isoforms (*MTA1*, *MTA1s*, *MTA1-ZG29p*, *MTA2*, *MTA3*, and *MTA3L*). The molecular masses

of the gene products of *MTA1*, *MTA2*, and *MTA3* are approximately 80, 70, and 65 kDa, respectively. The nucleotide and protein alignment homologies and the phylogenetic comparative analyses are discussed elsewhere [8, 9].

Except for ZG-29p, the *MTA* family sequences contain several common domain structures [10]. One of these, the BAH (bromo-adjacent homology) domain is involved in protein–protein interactions. Another, the SANT (SWI, ADA2, N-CoR, TFIIB-B) domain shares a high degree of homology with the DNA-binding domain of the Myb-related proteins, suggesting that this domain may be involved in DNA-binding. The ELM (egl-27 and *MTA1* homology) domain has an unknown function [11]. *MTA* family members also contain a highly conserved GATA-type zinc finger motif, which indicates a direct interaction with DNA [3]. *MTA1* has two src-homology (SH)-binding motifs at its C-terminal region, which are known to be important in signal transduction involving many kinase, adaptor and scaffolding proteins [1, 10]. Similar SH2- and SH3-binding domains are also found in *MTA2* and *MTA3*. These common domain structures clearly show that the *MTA* family is involved in protein–protein and DNA-binding interactions, indicating possible functions in signal transduction and transcriptional regulation.

MTA proteins contain basic nuclear localization signals [1, 10]. They also localize in the nucleus in many cancer cells [4, 8]. However, *MTA1* localizes to both the cytoplasm and nucleus in some tumors [12–14]. *MTA3* also localizes to the nucleus, but it has no apparent nuclear localization signal [15]. *MTA1s*, a short splice-variant of *MTA1*, is predominantly localized in the cytoplasm [16].

The expression of *MTA* proteins in various cancers and its clinicopathological and biological relevance

Clinicopathological relevance of the increased *MTA1* expression in human cancer tissues

Since the first report by us showing that the up-regulation of *MTA1* expression was significantly correlated to the malignant properties of human gastric and colorectal cancers [4], many researchers have been investigating the expression levels of *MTA* family members, especially *MTA1*, in various human cancers. This has revealed that the expression levels of *MTA* family members have clinicopathological significance (The data are summarized in Table 1).

Breast cancer

MTA1 was identified as a candidate progression molecule that was associated with breast cancer metastasis [1, 2] and

growth (the antisense RNA of *MTA1* inhibited the growth of highly metastatic breast cancer cell lines [3]). The involvement of *MTA1* in the carcinogenesis or progression of human breast cancer was also shown by other data using clinical samples. For example, Martin et al. [17] mapped the

chromosomal locus 14q that might be responsible for axillary lymph node metastasis in human breast cancers by comparing the rate of loss of heterozygosity between node-positive and -negative breast cancers. They found that the *MTA1* gene was contained in that gene locus, suggesting that

Table 1 Clinicopathological implications of the increased *MTA1* expression in various human cancer tissues

Type of cancer	Method	Clinicopathological implications	Reference
<i>Breast cancer</i>	LOH	Higher LN meta.	[17]
	IHC	Earlier recurrence	[18]
	IHC	Higher tumor grade Higher MVD (angiogenesis)	[19]
<i>Gastrointestinal cancer</i>			
Esophageal	RT-PCR	Deeper adventitial invasion Higher LN meta.	[5]
	IHC	Deeper adventitial invasion Higher LN meta. More advanced stage Poorer prognosis	[21]
Gastric	RT-PCR	Deeper serosal invasion Higher LN meta.	[4]
Colorectal	RT-PCR	Deeper wall invasion Higher LN meta.	[4]
	RT-PCR	Higher expression in cancer tissue	[20]
<i>Carcinoid</i>			
Gastric	RT-PCR	Deeper tumor invasion	[25]
Small intestine	RT-PCR	Malignant carcinoid More liver and LN meta.	[22]
Appendiceal	RT-PCR	Significant increase in malignant tumors	[26]
Pancreatic	IHC	Poorer prognosis (in combination with HDAC1)	[28]
Hepatocellular	RT-PCR	Shorter disease-free survival	[29]
	IHC	Larger tumor size More vascular invasion	[12]
	IHC	More microvascular invasion Higher recurrence rate Poorer survival	[30]
<i>Other cancers</i>			
NSCLC	RT-PCR	Larger tumor size Higher LN meta.	[31]
Thymoma	RT-PCR	More advanced stage	[33]
Ovarian	RT-PCR	Higher LN meta.	[32]
	IHC	More advanced stage Higher FIGO staging	[34]
Prostate	IHC	Metastatic prostate ca.	[35]
Lymphoma	Microarray	Highest expression in diffuse B-cell lymphoma	[36]
HNSCC	Microarray	Higher LN meta.	[37]
	IHC	Higher LN meta. More advanced stage	[38]
		Deeper wall invasion	

NSCLC non-small cell lung cancer, *HNSCC* head and neck squamous cell carcinoma, *IHC* immunohistochemistry, *LOH* loss of heterozygosity, *RT-PCR* reverse transcription-polymerase chain reaction, *MVD* microvessel density, *LN meta.* lymph node metastasis

MTA1 is a strong candidate for a breast cancer metastasis-promoting gene. Furthermore, using immunohistochemistry they examined the *MTA1* protein expression in primary human breast cancer samples and demonstrated that node-negative breast cancers with overexpression of *MTA1* protein had a higher risk of disease relapse similar to node-positive tumors. Thus, the overexpression of *MTA1* may be a useful predictor of early disease relapse [18].

Jang et al. [19] also showed that *MTA1* overexpression was closely associated with higher tumor grade and high intratumoral microvessel density in surgically resected human breast cancers, suggesting that *MTA1* may be a useful predictor of an aggressive phenotype and a possible angiogenesis-promoting molecule in breast cancer.

Gastrointestinal cancer

By using a reverse-transcription polymerase chain reaction (RT-PCR) method, we demonstrated that the higher expression of *MTA1* mRNA in surgically resected human gastric and colorectal cancer specimens compared to the paired normal counterpart tissues was significantly correlated to the depth of cancer invasion and lymph node metastasis [4]. This study was the first to demonstrate the clinical relevance of *MTA1* expression to the malignant potentials of human cancers. Higher expression of *MTA1* mRNA was also shown in colorectal cancers compared to the normal counterpart tissue by another group [20].

Using a RT-PCR method, we found that human esophageal squamous cell cancers overexpressed *MTA1* mRNA. The overexpressing cancer cells showed significantly higher frequencies of adventitial invasion and lymph node metastasis and tended to have a higher rate of lymphatic involvement [5]. Using immunohistochemistry, we further examined the protein expression level of *MTA1* in human esophageal squamous cell cancers and reconfirmed the results obtained by RT-PCR [21]. In this study, we also demonstrated that *MTA1* was a predictor of poor prognosis after surgery [21].

In another observation, Kidd et al. [22] showed that it was useful to examine the expression of *MTA1* mRNA and *MTA1* protein in order to determine the malignant potential and the propensity to metastasize of small intestinal carcinoid (enterochromaffin cell) tumors. When compared to nonmetastatic primary tumors, the expression of *MTA1* was increased in malignant small intestinal carcinoids and in metastases to liver and lymph nodes [22–24]. This same group further reported that *MTA1* was a good candidate genetic molecular marker to discriminate gastric carcinoids from other gastric neoplasms [25] as well as malignant appendiceal carcinoids from benign tissue [26]. In these studies, *MTA1* was thought to be a good marker to define the malignancy of carcinoid tumors.

In addition to cancers of the gastrointestinal tract, the involvement of *MTA1* overexpression in carcinogenesis and cancer progression was shown in other gastrointestinal tumors, such as pancreatic cancers and hepatocellular carcinomas. Iguchi et al. [27] examined *MTA1* mRNA expression in pancreatic cancer cell lines and resected pancreatic cancer tissues and found that increased levels of *MTA1* mRNA expression might be involved in the progression of pancreatic cancer. Recently, Miyake et al. [28] showed the expression level of *MTA1* protein correlated with poorer prognosis of pancreatic cancer patients.

The possible association of *MTA1* expression with the malignant properties of hepatocellular carcinomas (HCC) was first reported by Hamatsu et al. [29]. In this study, *MTA1* mRNA level was assessed by RT-PCR in resected human HCC tissues, and its high expression predicted a lower disease-free survival rate after curative hepatectomy for HCC. Using immunohistochemistry, Moon et al. [12] examined *MTA1* protein expression in resected human HCC specimens. They showed that overexpression of *MTA1* was associated with HCC growth and vascular invasion and that nuclear localization of estrogen receptor (ER) α inversely correlated with *MTA1* expression, suggesting that *MTA1* was involved in negative regulatory mechanisms. Ryu et al. [30] reported that *MTA1* was closely associated with microvascular invasion, frequent postoperative recurrence, and poor prognosis in patients with HCC, especially in those with hepatitis B virus (HBV)-associated HCC.

Other cancers

The relationship between *MTA1* expression and malignant properties, such as invasion and metastasis, has been investigated in many other carcinomas and sarcomas. High expression of *MTA1* mRNA was correlated clinicopathologically with lymph node metastasis of human non-small cell lung cancers [31] and ovarian cancers [32], and to the advanced stage and invasiveness of thymomas [33]. Dannenmann et al. [34] reported that overexpression of *MTA1* protein in ovarian cancer was significantly correlated to more advanced stage and higher FIGO staging. The potential role of *MTA1* protein expression has also been suggested in the progression of human endometrial carcinomas [14]. In prostate cancers, Hofer et al. [35] showed that metastatic prostate tumors demonstrated significantly higher intensities of *MTA1* protein expression and higher percentages of tissue cores staining positive for *MTA1* than in clinically localized prostate cancers or benign prostate tissues. The high expression of *MTA1* in diffuse B-cell lymphomas was also reported in human cases [36].

Using DNA microarray analysis, Roepman et al. [37] investigated gene expression patterns in lymph node

metastases of head and neck squamous cell carcinomas. They showed that the *MTA1* gene was the only single gene that showed consistently changed expression between numbers of matched pairs of primary tumor and lymph node metastases. Recently, further evidence was reported showing that overexpression of MTA1 protein in oral squamous cell carcinoma correlated to higher lymph node metastasis, deeper wall invasion and more advanced stage [38].

Biological relevance of MTA proteins to carcinogenesis and cancer progression

In addition to the clinicopathological evidences mentioned above, the biological relevance of MTA proteins to carcinogenesis and cancer progression has been made much clearer by the following important experiments.

The direct evidence to show the association of MTA1 expression with breast cancer malignant properties was first obtained by Mazumdar et al. in 2001 [39]. They demonstrated that forced expression of the MTA1 protein in breast cancer cell line MCF-7 was accompanied by enhancement of the ability of cells to invade an artificial matrix and to grow in an anchorage-independent manner. They also showed that the enhancement was associated with the interaction between MTA1 protein and histone deacetylase, resulting in a repression of ER α -mediated transcription (This will be discussed in more detail later).

The above study was extended by further experiments by the same group where they showed direct in vivo evidence of the involvement of MTA1 in the carcinogenesis of breast cancer in an animal model [10, 40]. This group established transgenic mice that overexpressed MTA1 protein. The MTA1-transgenic mice showed an inappropriate development of mammary glands, and the mice eventually developed hyperplastic nodules and mammary tumors, including adenocarcinomas. Most interestingly, MTA1-transgenic mice were accompanied by high incidence of spontaneous B cell lymphomas, including diffuse large B cell lymphomas [13, 41].

The clinicopathological correlation of MTA1 overexpression with squamous cell carcinomas was reinforced by the experimental results of Mahoney et al. [42]. They transfected *MTA1* cDNA into immortalized human keratinocytes and clearly showed that forced expression of *MTA1* contributed to several aspects of enhanced metastatic behavior, including increased migration, invasion and survival in the anchorage independent state of the immortalized keratinocytes. Furthermore, Qian et al. [43] inhibited *MTA1* expression by RNA interference (RNAi) in a human esophageal squamous cell carcinoma cell line and showed the significant inhibition of in vitro invasion and migration properties of the cancer cells.

Direct evidence showing the role of MTA1 in the progression of pancreatic cancer was provided by Hofer et al. [44]. They transfected *MTA1* cDNA into the pancreatic cell line PANC-1 and demonstrated that enhanced expression of MTA1 promoted the acquisition of an invasive and metastatic phenotype and that it enhanced the malignant potentials of pancreatic adenocarcinomas by modulation of the cytoskeleton via IQGAP1.

Molecular mechanisms of the MTA family, especially MTA1, in carcinogenesis and cancer progression

As mentioned above, it was demonstrated by different approaches and by different laboratories that MTA1 overexpression was closely correlated with carcinogenesis and cancer progression of a wide range of cancers originating in disparate organs and tissues. This strongly indicates that MTA1 may be one of the important key molecules in the cancer progression field. Thus, it will be absolutely necessary to clarify the molecular mechanisms in which MTA family members exert their functions for the clinical utilization of MTA proteins for diagnosis or treatment of human cancers. Here, we introduce the several important functions of MTA proteins that have been clarified, especially those that are concerned with carcinogenesis and cancer progression.

Nucleosome remodeling and histone deacetylation complex and transcriptional regulation

The first notion about the molecular and biochemical functions of MTA1 was accidentally obtained by four independent groups in 1998–1999 [9, 45–48]. In these studies, two disparate chromatin modifying activities, ATP-dependent nucleosome remodeling activity and histone deacetylation, were functionally and physically linked in the same protein complex. This complex has been named the NuRD (Nucleosome remodeling and histone deacetylation), and it contains histone deacetylase (HDAC) 1, HDAC2, the histone binding proteins RbAp46/48 and the dermatomyositis-specific autoantigen Mi-2, which has been shown to have transcription repressing activity. Xue et al. [46] reported that MTA1 protein was found in the NuRD complex, and it had strong transcription repressing activity. Subsequently, Zhang et al. [47] reported that a protein similar to MTA1 (named MTA2) was also a component of the NuRD complex and that MTA2 is highly expressed in rapidly dividing cells. Later, MTA3 was identified as an estrogen-inducible gene product that forms a distinct NuRD complex [15]. We also reported the physical interaction between MTA1 and HDAC1 [49] (Fig. 1).

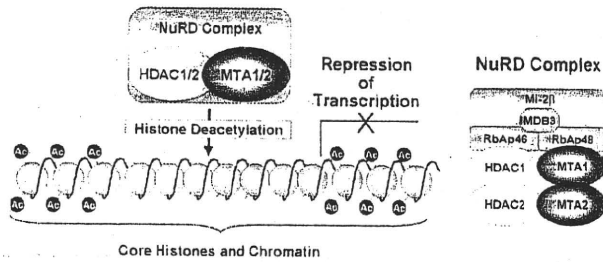


Fig. 1 The fundamental function of MTA proteins. The fundamental function of MTA proteins is chromatin remodeling and histone deacetylation, resulting in repression of transcription. MTA proteins are included in the protein complex named NuRD, which also contains histone deacetylases (HDAC1 and 2), major DNA binding protein 3 (MDB3), histone binding proteins RbAp46/48 and the dermatomyositis-specific autoantigen Mi-2, and has strong transcription repressing activities

Thus, the fundamental functions of the MTA family members appear to be exerted through a NuRD complex that has chromatin remodeling and histone deacetylating properties (There is also deacetylating property of non-histone proteins in the NuRD complex). In addition, the MTA-NuRD complex shows transcriptional repression activities [6–8, 10, 50]. Although all MTA family proteins are found in NuRD complexes, these proteins form distinct NuRD complexes that are thought to target different sets of promoters [9].

Repression of the transactivating function of ER α by MTA proteins

Although the involvement of MTA proteins in NuRD complexes suggested that such complexes might function in chromatin remodeling and histone deacetylation, a direct target of MTA proteins was first identified by Mazumdar et al. in 2000 [39]. MTA1 was identified as a molecule induced by a growth factor, heregulin-beta1 (HRG), which is a natural ligand of the human epidermal growth factor receptors HER3 and HER4 that can also transactivate HER2 (c-erbB-2) in human breast cancer cell lines. They showed that MTA1 directly interacted with the ligand-binding domain of ER α and that HRG stimulated the association of MTA1 and HDAC2 on the chromatin of an ER-responsive element (ERE) in the promoters of the estrogen responsive genes, such as pS2 and c-myc. This explains the phenomenon that activation of HRG/HER2 pathway in ER-positive breast cancers results in the suppression of ER α functions, resulting in more invasive and aggressive phenotypes observed in ER-negative breast cancers [51]. The repressive function of MTA1 on ER α is mediated through histone deacetylation by HDAC1 and HDAC2, suggesting that MTA1 has a potent corepressor function on the transactivation function of ER α through histone deacetylation (Fig. 2a). MTA2 has also been shown

to physically interact with ER α and to repress its transactivating function. Furthermore, overexpression of MTA2 rendered cells unresponsive to estrogen and suppressed estrogen-induced colony formation in breast cancer cells [52] (Fig. 2a, b).

Recently, Khaleque et al. [53] showed that MTA1 binds to a heat shock factor 1 (HSF1), the transcriptional activator of the heat shock genes, in vitro and in human breast carcinoma samples. They demonstrated that HSF1-MTA1 complex formation was strongly induced by HRG and that the complex was incorporated into the NuRD complex and participated in repression of estrogen-dependent transcription in breast cancer cell treated with HRG.

Following the report by Mazumdar et al. [39], the same research group reported that several molecules, such as ménages a trios 1 (MAT1), MTA1-interacting coactivator (MICoA) and nuclear receptor interacting factor 3 (NRIF3), all interact with MTA1 and repress the transactivation function of ER α [8]. These three MTA1-binding proteins themselves have coactivator properties upon ER α transactivation. Talukder et al. [54] identified MAT1, an assembly and targeting ring finger factor for cyclin-dependent kinase-activating kinase (CAK), as a MTA1-binding protein. The interactions between CAK and MTA1 apparently regulate the transactivation activity of ER α in a CAK-dependent manner in breast cancer cells. In contrast, MICoA-mediated ER α transactivation functions are opposed by MTA1 through the recruitment of HDACs [55]. Furthermore, the interactions between MTA1 and NRIF3, an estrogen-inducible gene, may be important in modulating the sensitivity of breast cancer cells to estrogen [56]. Singh et al. [57] identified another MTA1-binding partner, Lim-only protein 4 (LMO4). LMO4 was found to be a component of the MTA1 corepressor complex, and its overexpression repressed ER α transactivation functions in a HDAC-dependent manner, proposed to result in the acquisition of the ER α -negative phenotype and increased aggressiveness in breast cancer cells.

A short form of MTA1 protein was subsequently identified and named MTA1s (Fig. 2a) [16]. MTA1s is a splice-variant of MTA1 and contains an ER-binding motif (nuclear binding motif) without any nuclear localization signals at the C-terminus. This protein localizes in the cytoplasm where it sequesters ER α , resulting in the prevention of ligand-induced nuclear translocation of ER α and of stimulation of the malignant phenotype of breast cancer cells. This suggests that the regulation of the cellular localization of ER α by MTA1s may represent a mechanism for redirecting nuclear receptor signaling by nuclear exclusion. MTA1s has also been shown to associate with casein kinase I-gamma2, which is an estrogen-responsive kinase [58].

MTA3 is the latest addition to the MTA family. It was identified as an estrogen-dependent component of the

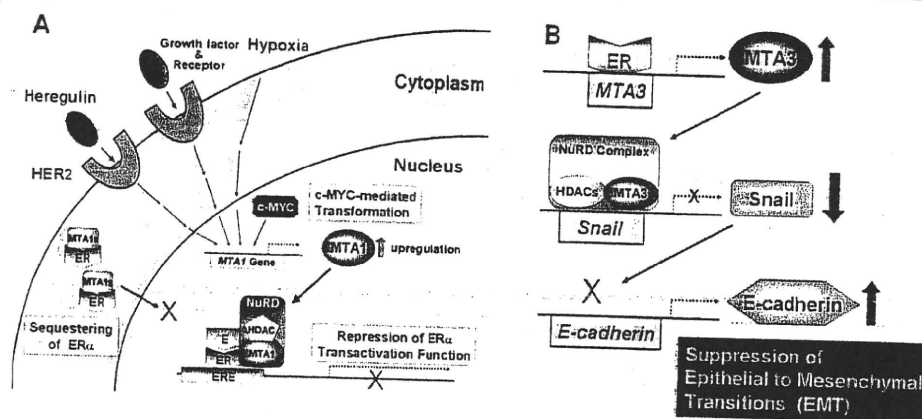


Fig. 2 Roles of MTA family in the carcinogenesis and cancer progression. Schematic presentation of the main functions of MTA family proteins. **a** MTA1 protein is included in NuRD complex that represses the transactivation function of estrogen receptor (ER) α , rendering breast cancer cells more phenotypically aggressive. MTA1 protein in NuRD complexes is one of the first downstream targets of c-MYC function, and it is essential for the transformation potential of c-MYC. MTA1s is a splice-variant of MTA1 that localizes in the

cytoplasm where it sequesters $ER\alpha$, resulting in the prevention of the ligand-induced nuclear translocation of $ER\alpha$ and stimulation of the development of the malignant phenotype of breast cancer cells. **b** MTA3 protein induced by estrogen represses the expression of the transcriptional repressor Snail, a master regulator of “epithelial to mesenchymal transitions”, resulting in the expression of the cell adhesion molecule E-cadherin and maintenance of a differentiated, normal epithelial status in breast cells

Mi-2/NuRD transcriptional corepressor in breast epithelial cells [15]. The absence of MTA3 as well as the absence of ER results in an aberrantly increased expression of the transcriptional repressor Snail, a master regulator of epithelial-to-mesenchymal transitions (EMT). This increased expression of Snail results in reduction of the cell adhesion molecule E-cadherin expression and subsequently changes in epithelial architecture and invasive growth (Fig. 2b). MTA3 is a transcriptional target of $ER\alpha$, and in the presence of estrogen, $ER\alpha$ directly binds to the MTA3 promoter at the SP1 site in close proximity of the ERE half-site, resulting in stimulation of MTA3 transcription [59, 60]. Thus, MTA3 functions to maintain a differentiated, normal epithelial status in breast cells, which is in stark contrast to MTA1 or MTA1s. Any potential up-regulation of MTA1 may repress MTA3 expression through repression of the $ER\alpha$ function, leading to up-regulation of Snail, down-regulation of E-cadherin, promotion of EMT and consequently an increase in metastatic potential in breast cancer cells. In fact, Mishra et al. [59] reported that MTA3 gene expression was regulated by the endogenous MTA1 and the knockdown of MTA1 resulted in a significant increase in both basal and estrogen-induced promoter activity of the MTA3 gene. Furthermore, Fujita et al. [60] revealed that a transient forced expression of MTA1 lead to loss of MTA3 protein in breast cancer cell lines. Interestingly, the same phenomenon was also observed in ovarian cancer cell line, in which MTA1 overexpression resulted in down-regulation of E-cadherin and MTA3 expression and enhanced expression of the Snail and Slug [34].

The expression of MTA3 inhibits ductal branching in virgin and pregnant mammary glands in MTA3-transgenic mice [61]. This property is in contrast to MTA1-transgenic

mice, where the inappropriate development of mammary glands results in the development of hyperplastic nodules and mammary tumors, including adenocarcinomas and lymphomas [8, 40]. MTA3 also represses Wnt4 transcription and Wnt4 secretion, inhibiting Wnt-target genes in mammary epithelial cells. This repression of Wnt4 transcription was found to be mediated through a MTA3-NuRD complex, which interacts with the Wnt4-containing chromatin in an HDAC-dependent manner [61].

Although the fundamental functions of MTA proteins are exerted via transcriptional repression by histone deacetylation, a transcriptional activating function has also been demonstrated. Gururaj et al. [62, 63] showed that Breast Cancer Amplified Sequence (BCAS) 3, a gene amplified and overexpressed in breast cancers, was a chromatin target of MTA1, and the transcription of BCAS3 was stimulated by MTA1. This suggested that MTA1 has a transcriptional coactivator function in addition to a corepressor function. A similar finding has also been suggested for mouse Mta2 protein [64].

Deacetylation of non-histone proteins by the MTA family

The protein targets for deacetylation by HDAC via NuRD complexes containing MTA proteins are not only the chromatin histones but also other non-histone proteins. The tumor suppressor gene p53 product was the first non-histone protein that was reported to be deacetylated by MTA protein-containing NuRD complexes. Luo et al. [65] found that the deacetylation of p53 was mediated by a HDAC1 complex containing MTA2 protein. This MTA2-associated NuRD complex interacted with p53 in vitro and

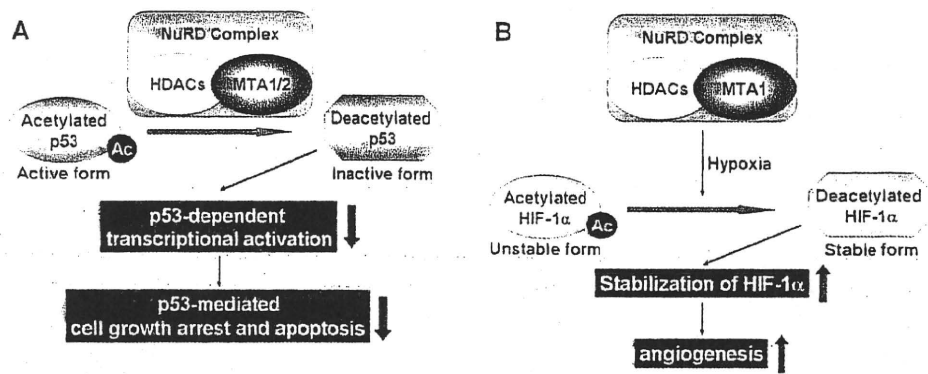


Fig. 3 Roles of MTA family in the carcinogenesis and cancer progression. Deacetylation of non-histone proteins by MTA family proteins. **a** Tumor suppressor p53 protein is deacetylated and inactivated by both MTA1 and MTA2 proteins in NuRD complexes,

resulting in inhibition of growth arrest and apoptosis. **b** A hypoxia-inducible factor-1 α (HIF-1 α) is also deacetylated and stabilized by MTA1 protein, leading to angiogenesis

in vivo and reduced significantly the steady-state levels of acetylated p53. Deacetylation of p53 results in an increase of its own degradation through MDM2 and a reduction in p53-dependent transcriptional activation. This eventually leads to the repression of the normal p53 function that mediates cell growth arrest and apoptosis. The same phenomenon was observed between p53 and MTA1. HDAC1/MTA1 complexes possessed deacetylation activity against p53 protein in human non-small cell carcinoma and human hepatoma cells, and the complexes were found to inhibit p53-induced apoptosis by attenuating the transactivation function of p53 [66] (Fig. 3a, b).

Another important non-histone protein that is deacetylated by HDAC1/MTA1 complexes is hypoxia-inducible factor (HIF)-1 α , a key regulator of angiogenic factors [67] (Fig. 3b). The expression of MTA1 is strongly induced under hypoxic conditions in breast cancer cell lines, and MTA1 overexpression enhanced the transcriptional activity and stability of HIF-1 α protein. MTA1 physically binds to HIF-1 α and deacetylates it by increasing the expression of HDAC1, leading to the stabilization of HIF-1 α . These results indicated evidence for positive cross-talk between MTA1 and HIF-1 α , which is mediated by HDAC1 recruitment. They also indicated the existence of a close connection between MTA1-associated metastasis and HIF-1 α -induced tumor angiogenesis. Furthermore, Moon et al. [68] showed that MTA1 increased the transcriptional activity of HIF-1 α and the expression of vascular endothelial growth factor (VEGF), a target molecule of HIF-1 α . Conditioned medium collected from MTA1 transfectants increased angiogenesis in vitro and in vivo. This functional link between HIF-1 α and MTA1 has been demonstrated in clinical samples of pancreatic carcinoma. Using immunohistochemistry and surgically resected pancreatic carcinomas, Miyake et al. [28] examined the expression of HIF-1 α , HDAC1 and MTA1 proteins and suggested that

HIF-1 α expression, which is associated with a poor prognosis in patients with pancreatic cancers, might be regulated by HDAC1/MTA1 complexes. The contribution of MTA1 protein to tumor angiogenesis was also demonstrated in human breast cancers. Using immunohistochemistry, Jang et al. [19] examined MTA1 protein expression and intra-tumoral microvessel density (MVD) in clinical samples of breast cancer and showed that MTA1 expression was significantly correlated with higher tumor grade and higher tumor MVD. The relationship between MTA1 expression and MVD was also observed in HBV-associated HCC [30]. Recently, Yoo et al. [69] experimentally demonstrated that HBV-X (HBx) protein strongly induced the expressions of MTA1 and HDAC1, resulting in those physical link to HIF-1 α . This suggests that positive crosstalk between HBx and MTA1/HDAC1 complex occurs and may be important in stabilizing HIF-1 α , which could play a critical role in angiogenesis and metastasis of HBV-associated HCC [69].

The protein members of NuRD complexes, including MTA1 and MTA2 proteins are co-immunoprecipitated with the ataxia teleangiectasia mutated (ATM)- and Rad3-related protein (ATR) [70]. ATR is a phosphatidylinositol-kinase-related kinase that has been implicated in the response of human cells to multiple forms of DNA damage and may play a role in the DNA replication checkpoint. This fact suggests that MTA proteins may contribute to the regulation of DNA checkpoints.

Other possible functions of MTA proteins in cancer

There are other reports suggesting the possible roles of MTA proteins in carcinogenesis and cancer progression. Among them, the most important may be the relationship of MTA1 protein with c-MYC oncoprotein (Fig. 2a). By expression profiling, Zhang et al. [71] identified MTA1 protein as a

target of the c-MYC protein in primary human cancer cells and showed that c-MYC binds to the genomic MTA1 locus and recruits transcriptional coactivators. They also presented data suggesting that MTA1 protein in NuRD complexes was one of the first downstream targets of c-MYC function, essential for the transformation potential of c-MYC, because reduction of MTA1 expression by a short hairpin RNA blocked the ability of c-MYC to transform mammalian cells [71]. There are little data at present concerning the relationship between MTA1 and other important oncogene products such as c-JUN and c-FOS.

As mentioned above, Kumar's group established transgenic mice that overexpressed MTA1 protein and found that the MTA1-transgenic mice showed inappropriate development of mammary glands. These mice also developed hyperplastic nodules and mammary tumors [40]. In this study, the underlying molecular mechanisms were also examined, and the results suggested that MTA1 dysregulation in mammary epithelium and cancer cells triggered down-regulation of the progesterone receptor-B isoform and up-regulation of the progesterone receptor-A isoform, resulting in the up-regulation of the progesterone receptor-A target genes Bcl-XL and cyclin D1 in mammary glands of virgin mice. It would be extremely intriguing and important to examine the HIF-1 α /VEGF expressions and angiogenesis in various organs of the MTA1-transgenic mice, although there are no data concerning these questions at present.

Recently, Molli et al. [72] reported that MTA1/NuRD complexes negatively regulated BRCA1 transcription by physically associating with ERE of the BRCA1 promoter in an ER α -dependent manner and that this repressive effect of MTA1 on BRCA1 expression resulted in an abnormal centrosome number and chromosomal instability. The relationship of MTA proteins with tumor suppressor genes other than p53 and BRCA1 remains to be determined.

Our group showed by the yeast two-hybrid system that mouse Mta1 protein physically linked to endophilin 3 and that the binding of those proteins was made between the SH 3-binding domain of Mta1 protein and the SH-3 domain of endophilin 3 [73]. This suggested that MTA1 protein might be involved in the regulation of endocytosis mediated by endophilin 3.

MTA proteins as new molecular targets: clinical implications

On the basis of the available data discussed briefly in this review, it is very likely that MTA proteins have important and critical roles in the genesis and progression of a wide variety of cancers [74]. MTA1 protein can be thought of as a master co-regulatory molecule, strongly and clearly

suggesting the possibility that MTA1 protein (or its gene) could be an excellent molecular target for cancer therapy as well as its use in cancer diagnosis/prognosis. Although studies are not yet available which show the "clinical" efficacy of targeting MTA proteins, several experiments have shown that MTA1 protein (or its gene) could be a molecular target for cancer therapy.

The first studies that suggested the possibility of targeting MTA1 were reported by Nawa et al. [3] and Nicolson et al. [74]. They used antisense phosphorothioate oligonucleotides against *MTA1* mRNA and found a growth inhibitory effect on human metastatic breast cancer cell lines. Since these reports, others have shown that inhibition of MTA1 expression can result in the inhibition of the malignant phenotypes of various cancers, as mentioned below.

Various techniques have been used to regulate MTA1 expression. Using RNAi, Qian et al. [43] inhibited MTA1 expression in a human esophageal squamous cell carcinoma cell line and demonstrated significant inhibition of in vitro invasion and migration properties of the cancer cells. The same group further examined the therapeutic value of MTA1 levels in malignant melanoma cells and demonstrated that down-regulation of MTA1 by RNAi successfully suppressed the growth in vitro and experimental metastasis of mouse B16F10 melanoma cells in vivo, suggesting a promising use of the *MTA1* gene as a target for cancer gene therapy [75].

MTA1s may also be a useful target in the treatment of breast cancer. MTA1s functions as a repressor of ER α transcriptional activity by binding and sequestering the ER α in the cytoplasm [16]. MTA1s has a unique C-terminal 33-amino acid region containing a nuclear receptor-box motif that mediates the interaction of MTA1s and ER α . Singh et al. [76] showed that the MTA1s peptide containing this motif could effectively repress the ER α transactivation function, estrogen-induced proliferation and anchorage-independent growth of the human breast cancer cell line MCF-7. Using an animal model, they also showed the effect of MTA1s peptide in blocking the tumor progression of MCF-7 overexpressing ER α .

There is a good possibility that MTA1 will be a target of immunotherapy. In a review on a model for immunotherapy using a vector, disabled infectious single cycle-herpes simplex virus (DISC-HSV), Assudani et al. [77] proposed that MTA1 is a promising antigen for tumor rejection, because it is greatly overexpressed in many different tumors and is only expressed at lower levels in normal tissues. Their initial studies demonstrated the presence of immunogenic MHC class I-restricted peptides of MTA1. Furthermore, MTA1 was identified as a SEREX antigen, and hence it is likely to be capable of inducing a T-cell response in cancer patients [78].

Conclusions and future directions

This review has focused on the clinical and biological significance of the newly emerging gene family named MTA, paying particular attention to its relevance to carcinogenesis and cancer progression, such as invasion and metastasis. The fundamental roles of MTA proteins are thought to be transcriptional corepressors that function through histone deacetylation via NuRD complexes, which contain chromatin remodeling and histone deacetylating molecules. Repression of ER α transactivation function by MTA1 protein through deacetylation of ERE chromatin of the ER-responsive genes has been the most extensively investigated, and the data clearly demonstrate that MTA1 expression results in tumor formation in mammary glands and renders breast cancer cells phenotypically more aggressive. In addition, MTA proteins deacetylate non-histone proteins. For example, the tumor suppressor p53 protein is deacetylated and inactivated by both MTA1 and MTA2 proteins, resulting in inhibition of growth arrest and apoptosis. HIF-1 α is also deacetylated and stabilized by MTA1, leading to angiogenesis. Considering the many reports showing the clinical relevance of the expression of *MTA1* mRNA and its encoded protein in a wide variety of human cancers as well as definitive studies showing the molecular and biochemical mechanisms of MTA protein actions, it is likely that MTA proteins, especially MTA1, represent master co-regulatory molecules involved in the carcinogenesis and progression of various malignant tumors. Ultimately this will lead to clinical applications of MTA proteins as a new class of molecular targets for cancer therapy. For example, inhibition of MTA1 expression or function may enhance the chemosensitivity of cancer cells by restoring tumor suppressor function of p53, or it may inhibit tumor angiogenesis by destabilizing the angiogenesis promoting function of HIF-1 α . Moreover, inhibitors of MTA proteins may cooperate with HDAC inhibitors, which are now expected to be a new class of anticancer agents. MTA1 will also be clinically useful for the prediction of the malignant potentials of various human cancers, such as esophageal, gastric and colorectal cancers. Thus, evaluating the expression levels of MTA proteins in individual cases of various cancers may provide clinicians with important clues to prognosis and anticancer therapy.

It will be important to understand the physiological functions and underlying mechanisms of MTA proteins in normal cells, because MTA proteins are also expressed in normal cells and tissues, although at lower levels than found in cancer cells. Physiological roles of MTA1 reported are the followings: (1) MTA1 is thought to play a crucial role in postnatal testis development and spermatogenesis [79, 80], (2) The expression level of MTA1 decreases in mouse brain

in age-dependent manner, which influences the estrogen-mediated signaling pathway during aging [81], (3) MTA1 protein is a direct stimulator of rhodopsin expression [82], (4) MTA1 stimulates hepatic proliferation in vivo and hepatocyte differentiation in vitro [83]. Furthermore, *Caenorhabditis elegans* has MTA1 homologues, *egl-27* and *egr-1*, which are related to embryonic patterning [11, 84] and NURD complex including *egr-1* antagonizes vulval development of *C. elegans*, which is induced by Ras signal transduction pathway [85]. Thus, understanding the physiological functions of MTA proteins will be absolutely necessary to understand the pathological functions of MTA proteins in human cancers. It will be also important to understand MTA1's roles in tissue maintenance via HIF-1 α /VEGF expressions against hypoxic condition.

In conclusion, MTA proteins, especially MTA1, are undoubtedly excellent candidates for therapy and diagnosis/prognosis of human cancers and should be intensively studied for their possible clinical applications.

References

- Toh Y, Pencil SD, Nicolson GL (1994) A novel candidate metastasis-associated gene, *mta1*, differentially expressed in highly metastatic mammary adenocarcinoma cell lines. cDNA cloning, expression, and protein analyses. *J Biol Chem* 269: 22958–22963
- Toh Y, Pencil SD, Nicolson GL (1995) Analysis of the complete sequence of the novel metastasis-associated candidate gene, *mta1*, differentially expressed in mammary adenocarcinoma and breast cancer cell lines. *Gene* 159:97–104. doi:10.1016/0378-1119(94)00410-T
- Nawa A, Nishimori K, Lin P et al (2000) Tumor metastasis-associated human MTA1 gene: its deduced protein sequence, localization, and association with breast cancer cell proliferation using antisense phosphorothioate oligonucleotides. *J Cell Biochem* 79:202–212. doi:10.1002/1097-4644(20001101)79:2<202::AID-JCB40>3.0.CO;2-L
- Toh Y, Oki E, Oda S et al (1997) Overexpression of the MTA1 gene in gastrointestinal carcinomas: correlation with invasion and metastasis. *Int J Cancer* 74:459–463. doi:10.1002/(SICI)1097-0215(19970822)74:4<459::AID-IJC18>3.0.CO;2-4
- Toh Y, Kuwano H, Mori M et al (1999) Overexpression of metastasis-associated MTA1 mRNA in invasive oesophageal carcinomas. *Br J Cancer* 79:1723–1726. doi:10.1038/sj.bjc.6690274
- Kumar R, Wang RA, Bagheri-Yarmand R (2003) Emerging roles of MTA family members in human cancers. *Semin Oncol* 30:30–37. doi:10.1053/j.seminoncol.2003.08.005
- Manavathi B, Kumar R (2007) Metastasis tumor antigens, an emerging family of multifaceted master coregulators. *J Biol Chem* 282:1529–1533. doi:10.1074/jbc.R600029200
- Manavathi B, Singh K, Kumar R (2007) MTA family of coregulators in nuclear receptor biology and pathology. *Nucl Recept Signal* 5:e010
- Bowen NJ, Fujita N, Kajita M, Wade PA (2004) Mi-2/NuRD: multiple complexes for many purposes. *Biochim Biophys Acta* 1677:52–57

10. Singh RR, Kumar R (2007) MTA family of transcriptional metaregulators in mammary gland morphogenesis and breast cancer. *J Mammary Gland Biol Neoplasia* 12:115–125. doi:10.1007/s10911-007-9043-7
11. Solari F, Bateman A, Ahringer J (1999) The *Caenorhabditis elegans* genes *egl-27* and *egr-1* are similar to MTA1, a member of a chromatin regulatory complex, and are redundantly required for embryonic patterning. *Development* 126:2483–2494
12. Moon WS, Chang K, Tarnawski AS (2004) Overexpression of metastatic tumor antigen 1 in hepatocellular carcinoma: relationship to vascular invasion and estrogen receptor- α . *Hum Pathol* 35:424–429. doi:10.1016/j.humpath.2003.11.007
13. Bagheri-Yarmand R, Balasenthil S, Gururaj AE et al (2007) Metastasis-associated protein 1 transgenic mice: a new model of spontaneous B-cell lymphomas. *Cancer Res* 67:7062–7067. doi:10.1158/0008-5472.CAN-07-0748
14. Balasenthil S, Broaddus RR, Kumar R (2006) Expression of metastasis-associated protein 1 (MTA1) in benign endometrium and endometrial adenocarcinomas. *Hum Pathol* 37:656–661. doi:10.1016/j.humpath.2006.01.024
15. Fujita N, Jaye DL, Kajita M, Geigerman C, Moreno CS, Wade PA (2003) MTA3, a Mi-2/NuRD complex subunit, regulates an invasive growth pathway in breast cancer. *Cell* 113:207–219. doi:10.1016/S0092-8674(03)00234-4
16. Kumar R, Wang RA, Mazumdar A et al (2002) A naturally occurring MTA1 variant sequesters oestrogen receptor- α in the cytoplasm. *Nature* 418:654–657. doi:10.1038/nature00889
17. Martin MD, Fischbach K, Osborne CK, Mohsin SK, Allred DC, O'Connell P (2001) Loss of heterozygosity events impeding breast cancer metastasis contains the MTA1 gene. *Cancer Res* 61:3578–3580
18. Martin MD, Hilsenbeck SG, Mohsin SK et al (2006) Breast tumors that overexpress nuclear metastasis-associated 1 (MTA1) protein have high recurrence risks but enhanced responses to systemic therapies. *Breast Cancer Res Treat* 95:7–12. doi:10.1007/s10549-005-9016-8
19. Jang KS, Paik SS, Chung H, Oh YH, Kong G (2006) MTA1 overexpression correlates significantly with tumor grade and angiogenesis in human breast cancers. *Cancer Sci* 97:374–379. doi:10.1111/j.1349-7006.2006.00186.x
20. Giannini R, Cavallini A (2005) Expression analysis of a subset of coregulators and three nuclear receptors in human colorectal carcinoma. *Anticancer Res* 25:4287–4292
21. Toh Y, Ohga T, Endo K et al (2004) Expression of the metastasis-associated MTA1 protein and its relationship to deacetylation of the histone H4 in esophageal squamous cell carcinomas. *Int J Cancer* 110:362–367. doi:10.1002/ijc.20154
22. Kidd M, Modlin IM, Mane SM, Camp RL, Eick G, Latich I (2006) The role of genetic markers—NAP1L1, MAGE-D2, and MTA1—in defining small-intestinal carcinoid neoplasia. *Ann Surg Oncol* 13:253–262. doi:10.1245/ASO.2006.12.011
23. Modlin IM, Kidd M, Pfragner R, Eick GN, Champaneria MC (2006) The functional characterization of normal and neoplastic human enterochromaffin cells. *J Clin Endocrinol Metab* 91:2340–2348. doi:10.1210/jc.2006-0110
24. Kidd M, Modlin IM, Pfragner R et al (2007) Small bowel carcinoid (enterochromaffin cell) neoplasia exhibits transforming growth factor- β -mediated regulatory abnormalities including up-regulation of C-Myc and MTA1. *Cancer* 109(12):2420–2431. doi:10.1002/cncr.22725
25. Kidd M, Modlin IM, Mane SM et al (2006) Utility of molecular genetic signatures in the delineation of gastric neoplasia. *Cancer* 106:1480–1488. doi:10.1002/cncr.21758
26. Modlin IM, Kidd M, Latich I et al (2006) Genetic differentiation of appendiceal tumor malignancy: a guide for the perplexed. *Ann Surg* 244:52–60. doi:10.1097/01.sla.0000217617.06782.d5
27. Iguchi H, Imura G, Toh Y, Ogata Y (2000) Expression of MTA1, a metastasis-associated gene with histone deacetylase activity in pancreatic cancer. *Int J Oncol* 16:1211–1214
28. Miyake K, Yoshizumi T, Imura S et al (2008) Expression of hypoxia-inducible factor-1 α , histone deacetylase 1, and metastasis-associated protein 1 in pancreatic carcinoma: correlation with poor prognosis with possible regulation. *Pancreas* 36:e1–e9. doi:10.1097/MPA.0b013e3181675010
29. Hamatsu T, Rikimaru T, Yamashita Y et al (2003) The role of MTA1 gene expression in human hepatocellular carcinoma. *Oncol Rep* 10:599–604
30. Ryu SH, Chung YH, Lee H et al (2008) Metastatic tumor antigen 1 is closely associated with frequent postoperative recurrence and poor survival in patients with hepatocellular carcinoma. *Hepatology* 47:929–936. doi:10.1002/hep.22124
31. Sasaki H, Moriyama S, Nakashima Y et al (2002) Expression of the MTA1 mRNA in advanced lung cancer. *Lung Cancer* 35:149–154. doi:10.1016/S0169-5002(01)00329-4
32. Yi S, Guangqi H, Guoli H (2003) The association of the expression of MTA1, nm23H1 with the invasion, metastasis of ovarian carcinoma. *Chin Med Sci J* 18:87–92
33. Sasaki H, Yukiue H, Kobayashi Y et al (2001) Expression of the MTA1 mRNA in thymoma patients. *Cancer Lett* 174:159–163. doi:10.1016/S0304-3835(01)00704-2
34. Dannenmann C, Shabani N, Friese K, Jeschke U, Mylonas I, Bruning A (2008) The metastasis-associated gene MTA1 is upregulated in advanced ovarian cancer, represses ER β , and enhances expression of oncogenic cytokine GRO. *Cancer Biol Ther* 7:1460–1467
35. Hofer MD, Kuefer R, Varambally S et al (2004) The role of metastasis-associated protein 1 in prostate cancer progression. *Cancer Res* 64:825–829. doi:10.1158/0008-5472.CAN-03-2755
36. Hofer MD, Tapia C, Browne TJ, Mirlacher M, Sauter G, Rubin MA (2006) Comprehensive analysis of the expression of the metastasis-associated gene 1 in human neoplastic tissue. *Arch Pathol Lab Med* 130:989–996
37. Roepman P, de Jager A, Groot Koerkamp MJ, Kummer JA, Slootweg PJ, Holstege FC (2006) Maintenance of head and neck tumor gene expression profiles upon lymph node metastasis. *Cancer Res* 66:11110–11114. doi:10.1158/0008-5472.CAN-06-3161
38. Kawasaki G, Yanamoto S, Yoshitomi I, Yamada S, Mizuno A (2008) Overexpression of metastasis-associated MTA1 in oral squamous cell carcinomas: correlation with metastasis and invasion. *Int J Oral Maxillofac Surg* 37:1039–1046. doi:10.1016/j.ijom.2008.05.020
39. Mazumdar A, Wang RA, Mishra SK et al (2001) Transcriptional repression of oestrogen receptor by metastasis-associated protein 1 corepressor. *Nat Cell Biol* 3:30–37. doi:10.1038/35050532
40. Bagheri-Yarmand R, Talukder AH, Wang RA, Vadlamudi RK, Kumar R (2004) Metastasis-associated protein 1 deregulation causes inappropriate mammary gland development and tumorigenesis. *Development* 131:3469–3479. doi:10.1242/dev.01213
41. Balasenthil S, Gururaj AE, Talukder AH et al (2007) Identification of Pax5 as a target of MTA1 in B-cell lymphomas. *Cancer Res* 67:7132–7138. doi:10.1158/0008-5472.CAN-07-0750
42. Mahoney MG, Simpson A, Jost M et al (2002) Metastasis-associated protein (MTA)1 enhances migration, invasion, and anchorage-independent survival of immortalized human keratinocytes. *Oncogene* 21:2161–2170. doi:10.1038/sj.onc.1205277
43. Qian H, Lu N, Xue L et al (2005) Reduced MTA1 expression by RNAi inhibits in vitro invasion and migration of esophageal squamous cell carcinoma cell line. *Clin Exp Metastasis* 22:653–662. doi:10.1007/s10585-006-9005-2
44. Hofer MD, Menke A, Genze F, Gierschik P, Giehl K (2004) Expression of MTA1 promotes motility and invasiveness of PANC-1 pancreatic carcinoma cells. *Br J Cancer* 90:455–462. doi:10.1038/sj.bjc.6601535

45. Tong JK, Hassig CA, Schnitzler GR, Kingston RE, Schreiber SL (1998) Chromatin deacetylation by an ATP-dependent nucleosome remodelling complex. *Nature* 395:917–921. doi:10.1038/27699
46. Xue Y, Wong J, Moreno GT, Young MK, Cote J, Wang W (1998) NURD, a novel complex with both ATP-dependent chromatin-remodeling and histone deacetylase activities. *Mol Cell* 2:851–861. doi:10.1016/S1097-2765(00)80299-3
47. Zhang Y, Ng HH, Erdjument-Bromage H, Tempst P, Bird A, Reinberg D (1999) Analysis of the NuRD subunits reveals a histone deacetylase core complex and a connection with DNA methylation. *Genes Dev* 13:1924–1935. doi:10.1101/gad.13.15.1924
48. Wade PA, Geggion A, Jones PL, Ballestar E, Aubry F, Wolffe AP (1999) Mi-2 complex couples DNA methylation to chromatin remodelling and histone deacetylation. *Nat Genet* 23:62–66
49. Toh Y, Kuninaka S, Endo K et al (2000) Molecular analysis of a candidate metastasis-associated gene, MTA1: possible interaction with histone deacetylase 1. *J Exp Clin Cancer Res* 19:105–111
50. Fearon ER (2003) Connecting estrogen receptor function, transcriptional repression, and E-cadherin expression in breast cancer. *Cancer Cell* 3:307–310. doi:10.1016/S1535-6108(03)00087-4
51. Tang CK, Perez C, Grunt T, Waibel C, Cho C, Lupu R (1996) Involvement of heregulin-beta2 in the acquisition of the hormone-independent phenotype of breast cancer cells. *Cancer Res* 56:3350–3358
52. Cui Y, Niu A, Pestell R et al (2006) Metastasis-associated protein 2 is a repressor of estrogen receptor alpha whose overexpression leads to estrogen-independent growth of human breast cancer cells. *Mol Endocrinol* 20:2020–2235. doi:10.1210/me.2005-0063
53. Khaleque MA, Bharti A, Gong J et al (2008) Heat shock factor 1 represses estrogen-dependent transcription through association with MTA1. *Oncogene* 27:1886–1893. doi:10.1038/sj.onc.1210834
54. Talukder AH, Mishra SK, Mandal M et al (2003) MTA1 interacts with MAT1, a cyclin-dependent kinase-activating kinase complex ring finger factor, and regulates estrogen receptor transactivation functions. *J Biol Chem* 278:11676–11685. doi:10.1074/jbc.M209570200
55. Mishra SK, Mazumdar A, Vadlamudi RK et al (2003) MICOA, a novel metastasis-associated protein 1 (MTA1) interacting protein coactivator, regulates estrogen receptor-alpha transactivation functions. *J Biol Chem* 278:19209–19219. doi:10.1074/jbc.M301968200
56. Talukder AH, Gururaj A, Mishra SK, Vadlamudi RK, Kumar R (2004) Metastasis-associated protein 1 interacts with NRIF3, an estrogen-inducible nuclear receptor coregulator. *Mol Cell Biol* 24:6581–6591. doi:10.1128/MCB.24.15.6581-6591.2004
57. Singh RR, Barnes CJ, Talukder AH, Fuqua SA, Kumar R (2005) Negative regulation of estrogen receptor alpha transactivation functions by LIM domain only 4 protein. *Cancer Res* 65:10594–10601. doi:10.1158/0008-5472.CAN-05-2268
58. Mishra SK, Yang Z, Mazumdar A, Talukder AH, Larose L, Kumar R (2004) Metastatic tumor antigen 1 short form (MTA1s) associates with casein kinase I-gamma2, an estrogen-responsive kinase. *Oncogene* 23:4422–4429. doi:10.1038/sj.onc.1207569
59. Mishra SK, Talukder AH, Gururaj AE et al (2004) Upstream determinants of estrogen receptor-alpha regulation of metastatic tumor antigen 3 pathway. *J Biol Chem* 279:32709–32715. doi:10.1074/jbc.M402942200
60. Fujita N, Kajita M, Taysavang P, Wade PA (2004) Hormonal regulation of metastasis-associated protein 3 transcription in breast cancer cells. *Mol Endocrinol* 18:2937–2949. doi:10.1210/me.2004-0258
61. Zhang H, Singh RR, Talukder AH, Kumar R (2006) Metastatic tumor antigen 3 is a direct corepressor of the Wnt4 pathway. *Genes Dev* 20:2943–2948. doi:10.1101/gad.1461706
62. Gururaj AE, Singh RR, Rayala SK et al (2006) MTA1, a transcriptional activator of breast cancer amplified sequence 3. *Proc Natl Acad Sci USA* 103:6670–6675. doi:10.1073/pnas.0601989103
63. Gururaj AE, Holm C, Landberg G, Kumar R (2006) Breast cancer-amplified sequence 3, a target of metastasis-associated protein 1, contributes to tamoxifen resistance in premenopausal patients with breast cancer. *Cell Cycle* 5:1407–1410
64. Matsusue K, Takiguchi S, Toh Y, Kono A (2001) Characterization of mouse metastasis-associated gene 2: genomic structure, nuclear localization signal, and alternative potentials as transcriptional activator and repressor. *DNA Cell Biol* 20:603–611. doi:10.1089/104454901753340596
65. Luo J, Su F, Chen D, Shiloh A, Gu W (2000) Deacetylation of p53 modulates its effect on cell growth and apoptosis. *Nature* 408:377–381. doi:10.1038/35042612
66. Moon HE, Cheon H, Lee MS (2007) Metastasis-associated protein 1 inhibits p53-induced apoptosis. *Oncol Rep* 18:1311–1314
67. Yoo YG, Kong G, Lee MO (2006) Metastasis-associated protein 1 enhances stability of hypoxia-inducible factor-1alpha protein by recruiting histone deacetylase 1. *EMBO J* 25:1231–1241. doi:10.1038/sj.emboj.7601025
68. Moon HE, Cheon H, Chun KH et al (2006) Metastasis-associated protein 1 enhances angiogenesis by stabilization of HIF-1alpha. *Oncol Rep* 16:929–935
69. Yoo YG, Na TY, Seo HW et al (2008) Hepatitis B virus X protein induces the expression of MTA1 and HDAC1, which enhances hypoxia signaling in hepatocellular carcinoma cells. *Oncogene* 27:3405–3413. doi:10.1038/sj.onc.1211000
70. Schmidt DR, Schreiber SL (1999) Molecular association between ATR and two components of the nucleosome remodeling and deacetylating complex, HDAC2 and CHD4. *Biochemistry* 38:14711–14717. doi:10.1021/bi991614n
71. Zhang XY, DeSalle LM, Patel JH et al (2005) Metastasis-associated protein 1 (MTA1) is an essential downstream effector of the c-MYC oncoprotein. *Proc Natl Acad Sci USA* 102:13968–13973. doi:10.1073/pnas.0502330102
72. Molli PR, Singh RR, Lee SW, Kumar R (2008) MTA1-mediated transcriptional repression of BRCA1 tumor suppressor gene. *Oncogene* 27:1971–1980. doi:10.1038/sj.onc.1210839
73. Aramaki Y, Ogawa K, Toh Y et al (2005) Direct interaction between metastasis-associated protein 1 and endophilin 3. *FEBS Lett* 579:3731–3736. doi:10.1016/j.febslet.2005.05.069
74. Nicolson GL, Nawa A, Toh Y, Taniguchi S, Nishimori K, Moustafa A (2003) Tumor metastasis-associated human MTA1 gene and its MTA1 protein product: role in epithelial cancer cell invasion, proliferation and nuclear regulation. *Clin Exp Metastasis* 20:19–24. doi:10.1023/A:1022534217769
75. Qian H, Yu J, Li Y et al (2007) RNA interference against metastasis-associated gene 1 inhibited metastasis of B16F10 melanoma cell in C57BL/6 model. *Biol Cell* 99:573–581. doi:10.1042/BC20060130
76. Singh RR, Kaluarachchi K, Chen M et al (2006) Solution structure and antiestrogenic activity of the unique C-terminal, NR-box motif-containing region of MTA1s. *J Biol Chem* 281:25612–25621. doi:10.1074/jbc.M604444200
77. Assudani DP, Ahmad M, Li G, Rees RC, Ali SA (2006) Immunotherapeutic potential of DISC-HSV and OX40L in cancer. *Cancer Immunol Immunother* 55:104–111. doi:10.1007/s00262-005-0004-y
78. Li G, Miles A, Line A, Rees RC (2004) Identification of tumor antigens by serological analysis of cDNA expression cloning.

- Cancer Immunol Immunother 53:139–143. doi:10.1007/s00262-003-0471-y
79. Li W, Zhang J, Liu X, Xu R, Zhang Y (2007) Correlation of appearance of metastasis-associated protein1 (Mta1) with spermatogenesis in developing mouse testis. *Cell Tissue Res* 329:351–362. doi:10.1007/s00441-007-0412-8
80. Li W, Liu XP, Xu RJ, Zhang YQ (2007) Immunolocalization assessment of metastasis-associated protein 1 in human and mouse mature testes and its association with spermatogenesis. *Asian J Androl* 9:345–352. doi:10.1111/j.1745-7262.2007.00245.x
81. Thakur MK, Ghosh S (2008) Interaction of estrogen receptor alpha transactivation domain with MTA1 decreases in old mouse brain. *J Mol Neurosci*. doi:10.1007/s12031-008-9131-1
82. Manavathi B, Peng S, Rayala SK et al (2007) Repression of Six3 by a corepressor regulates rhodopsin expression. *Proc Natl Acad Sci USA* 104:13128–13133. doi:10.1073/pnas.0705878104
83. Li W, Zhu H, Bao W et al (2008) Involvement of metastasis tumor antigen 1 in hepatic regeneration and proliferation. *Cell Physiol Biochem* 22:315–326. doi:10.1159/000149810
84. Chen Z, Han M (2001) Role of *C. elegans* lin-40 MTA in vulval fate specification and morphogenesis. *Development* 128:4911–4921
85. Solari F, Ahringer J (2000) NURD-complex genes antagonise Ras-induced vulval development in *Caenorhabditis elegans*. *Curr Biol* 10:223–226. doi:10.1016/S0960-9822(00)00343-2

ORIGINAL ARTICLE

Yasushi Toh · Eiji Oki · Kazuhito Minami
Takeshi Okamura

Evaluation of the feasibility and safety of immediate extubation after esophagectomy with extended radical three-field lymph node dissection for thoracic esophageal cancers

Received: January 8, 2009 / Accepted: May 2, 2009

Abstract

Background. No standard procedure exists in respiratory management, including mechanical ventilation, which is commonly administered, after thoracic esophagectomy for esophageal cancer.

Methods. Various perioperative clinical parameters and complications were retrospectively compared between the patients who underwent mechanical ventilation (MV group, $n = 38$) and those who were extubated immediately (immediate extubation: IE group, $n = 75$), following transthoracic esophagectomy with three-field lymph node dissection (3FLND) for thoracic esophageal cancers.

Results. There were no significant differences between the two groups in the early postoperative clinical course. The frequencies of postoperative complications were 39% and 47% in the IE and MV groups, respectively, and pulmonary complications tended to occur more frequently in the MV group (23.7%) than in the IE group (12.0%). Mobilization of the patients was significantly earlier in the IE group than in the MV group ($P < 0.0001$).

Conclusions. IE is feasible and safe even after transthoracic esophagectomy with 3FLND. To avoid the possible disadvantages of MV after surgery, IE can be a standard protocol for postoperative management after transthoracic esophagectomy with radical lymph node dissection.

Key words Esophageal cancer · Esophagectomy with three-field lymph node dissection · Immediate extubation · Mechanical ventilation · Postoperative complications

Introduction

In spite of recent advances in perioperative managements of radical esophagectomy for thoracic esophageal cancers,

morbidity and mortality remain high, even in centers that perform a high volume of esophagectomies [1–5], because (1) patients with esophageal cancers frequently have some underlying complications before surgery, including malnutrition caused by dysphagia, (2) many of the patients are elderly and tend to have significant comorbidities, and (3) resection of thoracic esophageal cancers inflicts considerable physiological stress. Especially, as the extent of the surgical stress of transthoracic esophagectomy with cervical, mediastinal, and abdominal (so-called three-field) lymph node dissection (3FLND) is thought to be extremely great [6–8], careful perioperative management is mandatory. However, no standard protocol for this management has yet been established.

Respiratory complications are the primary cause of morbidity and mortality after esophageal surgery [9,10]. Adult respiratory distress syndrome (ARDS), a major contributor to respiratory complications after esophagectomy, causes a high postoperative death rate [11,12]. To avoid this severe complication, the patients are commonly managed under artificial mechanical ventilation (MV) for one or several days after surgery in many institutes, on the assumption that this may minimize the occurrence of respiratory complications. However, the duration and intensity of MV for patients after esophagectomy vary among institutes, and thus no optimal procedure for respiratory management has yet been established. In addition, there is little clear evidence for the necessity of MV itself.

On the other hand, there is increasing evidence that MV is potentially harmful even for a short period [13–15]. Ventilator-induced pulmonary edema can cause pulmonary changes similar to ARDS, as often occurs in patients after transthoracic esophagectomy, because one-lung ventilation is required during a thoracotomy that is associated with the development of microbarotrauma in the dependent lung and with organ reperfusion injury in the collapsed lung [11,16,17]. Thus, latent damage might already exist in the lungs at the end of transthoracic esophagectomy and MV continued after esophagectomy itself may therefore be harmful rather than useful.

Y. Toh (✉) · E. Oki · K. Minami · T. Okamura
Department of Gastroenterological Surgery, National Kyushu
Cancer Center, 3-1-1 Notame, Minami-ku, Fukuoka 811-1395, Japan
Tel. +81-92-541-3231; Fax +81-92-542-8503
e-mail: yttoh@nk-cc.go.jp