Clinical significance of altering epithelial-mesenchymal transition (EMT) in metastatic lymph nodes of gastric cancer

著者	大久保 啓史
journal or publication title	Gastric Cancer
volume	20
number	5
page range	802-810
year	2017
ファイル（説明）	博士論文全文 博士論文要旨 最終試験結果の要旨 論文審査の要旨
別言語のタイトル	胃癌の転移リンパ節における、EMT変化が与える臨床的意義
学位授与番号	17701甲総研第517号
URL	http://hdl.handle.net/10232/00030797

doi: 10.1007/s10120-017-0705-x

Original Article

Title: Clinical significance of altering epithelial-mesenchymal transition (EMT) in metastatic lymph nodes of gastric cancer

Authors and affiliations:

Keishi Okubo, MD[1]; Yoshikazu Uenosono, MD, PhD[2]; Takaaki Arigami, MD, PhD[1,2]; Shigehiro Yanagita, MD, PhD[1]; Daisuke Matsushita, MD[1]; Takashi Kijima, MD,[1]; Masahiko Amatatsu, MD,[1]; Yasuto Uchikado MD, PhD[1];Yuko Kijima, MD, PhD[1]; Kosei Maemura, MD, PhD[1]; and Shoji Natsugoe, MD, PhD[1,2]

[1]Department of Digestive Surgery, Breast and Thyroid Surgery, Field of Oncology,

[2]Molecular Frontier Surgery, Course of Advanced Therapeutics, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan

Corresponding Author: Keishi Okubo, MD

Department of Digestive Surgery, Breast and Thyroid Surgery, Field of Oncology, Course of Advanced Therapeutics, Kagoshima University Graduate School of Medical and Dental Sciences, 8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan

Tel.: +81-99-275-5361; Fax: +81-99-265-7426

E-mail: ok0627@m2.kufm.kagoshima-u.ac.jp

A short running head: EMT in lymph nodes of gastric cancer

The word count of the article: 2795

Abstract

Background: E-cadherin, N-cadherin, and Snail are epithelial-mesenchymal transition (EMT)-inducible genes. Previous studies demonstrated that the expression of EMT markers in the primary tumor sites of gastric cancer correlates with tumor progression and prognosis. However, the clinical significance of the expression of these EMT markers in metastatic lymph nodes remains unclear. In the present study, we investigated the expression of these EMT markers in the primary tumor sites and metastatic lymph nodes.

Methods: Immunohistochemistry was used to investigate the expression of E-cadherin, N-cadherin, and Snail in 89 primary tumors and 511 metastatic lymph nodes obtained from patients with gastric cancer.

Results: The weak expression of E-cadherin in tumors and lymph nodes increased with more lymph node metastasis and in more undifferentiated tumors. The strong expression of N-cadherin in lymph nodes correlated with more lymph nodes metastasis, an advanced stage and poor prognosis. The weak expression of Snail in tumors correlated with lymphatic invasion. The strong expression of Snail in lymph nodes correlated with more lymph node metastasis and an advanced stage. The strong expression of Snail in tumors and its weak expression in lymph nodes correlated with more lymph node

metastasis, an advanced stage, and poor prognosis.

Conclusions: The expression of N-cadherin in metastatic lymph nodes is useful for predicting the prognosis of patients with gastric cancer. The Snail switch, namely, the positive-to-negative conversion of the Snail status, between primary tumors and lymph node metastasis may be important for confirming EMT and mesenchymal-to-epithelial transition (MET).

Mini-abstract

The expression of N-cadherin in metastatic lymph nodes is useful for predicting the prognosis of patients with gastric cancer. The Snail switch may be important for confirming EMT and MET.

Key words: Epithelial-mesenchymal transition (EMT), Gastric cancer, Metastatic lymph nodes, Snail switch

Introduction

Gastric cancer is one of the most common malignancies and patients with advanced gastric cancer have a poor prognosis[1]. Recent studies clearly demonstrated that epithelial-mesenchymal transition (EMT), a developmental process in which epithelial cells reduce intercellular adhesion and myofibroblastic features, plays an important role in tumor progression and metastasis [2-5]. Significant changes occur during EMT, including the down-regulation of epithelial markers such as E-cadherin and up-regulation of mesenchymal markers including N-cadherin[6-9]. A switch in cadherin from the loss of E-cadherin to gain of N-cadherin is part of the EMT process.

Snail, SLUG, and TWIST are some of the transcription factors that govern EMT[3]. Snail was previously reported to be important during EMT in several carcinomas, including non-small cell lung carcinomas, ovarian carcinomas, urothelial carcinomas, esophageal squamous cell carcinomas, and gastric adenocarcinomas [10-14]. Natsugoe et al. [13] and Na Ri Shin et al.[14] reported that the overexpression of Snail in the main tumors of esophageal squamous cell carcinoma and gastric cancer was associated with a poor prognosis. A recent meta-analysis conducted by Chen et al. revealed Snail protein expression in gastric cancer[15]. Their findings indicated that the overexpression of Snail is associated with more lymph node metastasis (LNM) and an advanced stage.

Snail family proteins are core EMT regulatory factors that play essential roles in developmental and disease processes and have been associated with metastasis in carcinomas[16-23]. The overexpression of Snail in different epithelial cells has been shown to strongly induce conversion to a fibroblastic phenotype at the same time that E-cadherin expression is lost, and invasive and migratory properties are acquired[16]. Previous studies found that E-cadherin, N-cadherin, and Snail family proteins play a role in tumor progression in primary gastric cancer [14, 24-27]. However, the expression of these markers in LNM during EMT remains to be clarified. The aim of the present study was to examine the clinical significance of E-cadherin, N-cadherin, and Snail expression in the primary tumors and LNM of gastric cancer.

Materials and methods

Patients and specimens

Subjects comprised 89 patients with gastric cancer who underwent gastrectomy with lymph node dissection between 2005 and 2012 at Kagoshima University Hospital, Kagoshima, Japan. All 511 metastatic lymph nodes were examined in the present study. There were 60 males and 29 females with a median age of 67.1 years (range 33-89). None of the patients received preoperative chemotherapy. Clinicopathological findings

were based on the criteria of the tumor node metastasis (TNM) classification of the International Union against Cancer. The number of patients in each pT was as follows: 20 in pT1, 5 in pT2, 33 in pT3, and 31 in pT4. All patients had LNM: 28 in pN1, 26 in pN2, and 35 in pN3. Postoperative follow-up data were obtained from all patients, with a median follow-up period of 49.6 months (range, 3-157 months).

The Ethics Committee at Kagoshima University approved this study and all patients provided written informed consent for the use of their information.

Immunohistochemical staining and evaluation

All resected specimens were fixed in 10% formaldehyde and routinely embedded in paraffin, and 3-μm-thick sections were prepared for immunohistochemistry. Sections were soaked in methanol with 3% H_2O_2 for 30 min to block endogenous peroxidase activity. Sections were incubated with an anti-E-cadherin monoclonal antibody (1:100; NCH-38; Dako, Tokyo, Japan), anti-N-cadherin monoclonal antibody (1:50; 6G11; Dako, Tokyo, Japan), or anti-Snail polyclonal antibody (1:500; ab85936; Abcam, Tokyo, Japan) at 4°C overnight. E-cadherin, N-cadherin, and Snail expression in cancer tissue was visualized using the avidin biotinylated peroxidase method.

Immunohistochemical evaluations were performed by 2 independent investigators

(K.O. and Y.U.). In order to assess the expression of E-cadherin, N-cadherin, and Snail, 10 fields (within the tumor and at the invasive front) were selected and expression in 1000 tumor cells (100 cells/field) was examined using high-power (×200) microscopy. Regarding E-cadherin, more than 60% of tumor cell staining was considered to reflect the preserved expression of E-cadherin, whereas 60% or less indicated reduced expression. The positive expression of N-cadherin and Snail was defined as detectable immunoreactivity in more than 5% and 75% of cancer cells, respectively. These cut-off values for immunohistochemical evaluations of E-cadherin[28], N-cadherin[25], and Snail[14] expressions were set based on previously published papers.

In LNM, all 511 metastatic lymph nodes were evaluated using the same methods as that described for the primary tumors. E-cadherin-positive cases were defined by the positive expression in all LNM. N-cadherin- and Snail-positive cases were defined by more than one lymph node showing positive expression because the expression of N-cadherin and Snail was only detected in a few LNM.

Statistical Analysis

Statistical analyses of group differences were performed using the χ^2 test and *t*-test. The Kaplan-Meier method was used for a survival analysis, and differences in survival

were examined using the Log-rank test. Prognostic factors were assessed using univariate and multivariate analyses (Cox's proportional hazard regression model). All statistical calculations were performed using SAS statistical software (SAS Institute. Inc., Cary, NC). A *P* value of < 0.05 was considered significant.

Results

Expression of E-cadherin, N-cadherin, and Snail

The expression of E-cadherin was observed on the cell membranes of cancer cells, indicating preserved expression, in 50.5% of primary tumors (45 out of 89) and 51.6% of LNM (46 out of 89) (Fig. 1a, b). The expression of N-cadherin was observed on the cell membranes of cancer cells in 31.4% (28 out of 89) of primary tumors and LNM (Fig. 1c, d). The expression of Snail was observed in the nuclei of cancer cells in 48.3% of primary tumors (43 out of 89) and 51.7% of LNM (46 out of 89) (Fig. 1e, f).

Relationships between primary tumors and lymph nodes metastasis of E-cadherin, N-cadherin, and Snail Expression

We evaluated each expression in all 511 metastatic lymph nodes and primary tumors. The strong expression of E-cadherin in lymph nodes were recognized 41.6% (142/341)

in preserved E-cadherin expression in primary tumors. The weak expression of N-cadherin in lymph nodes were recognized 66.6% (272/408) in reduced N-cadherin expression in primary tumors (Supplementary Table 1).

Relationships between E-cadherin, N-cadherin, and Snail expression and Clinicopathological Factors

The weak expression of E-cadherin in primary tumors and lymph nodes increased with more LNM (primary tumor: P=0.027, lymph node: P=0.003) and in more undifferentiated tumors (primary tumor: P<0.0001, lymph node: P=0.015) (Table 1, 2). The strong expression of N-cadherin in primary tumors was associated with more LNM (P=0.061) (Table 1). The strong expression of N-cadherin in lymph nodes correlated with more LNM and lymphatic invasion and an advanced stage (P=0.004, P=0.004, and P=0.015, respectively) (Table 2). The strong expression of Snail in primary tumors correlated with lymphatic invasion (P=0.001) (Table 1). The weak expression of Snail in lymph nodes correlated with more LNM (P=0.002), and an advanced stage (P=0.048) (Table 2).

In lymph nodes, a correlation was observed between the expression of E-cadherin and N-cadherin. The weak expression of E-cadherin correlated with the strong expression of

N-cadherin in lymph nodes (Table 3) (P=0.012). In most LNM cases, the expression of E-cadherin was weak, while that of N-cadherin was high in primary tumors and lymph nodes. These expression patterns of E-cadherin and N-cadherin have emerged as one of the most common indicators of the onset of EMT. On the other hand, in most LNM cases, the expression of Snail was strong in primary tumors and weak in lymph nodes. These expression patterns in primary tumors corresponded to the onset of EMT, whereas those in lymph nodes did not.

Relationships between E-cadherin, N-cadherin, and Snail expression and Prognosis

No significant differences were observed in 5-year overall survival (OS) between patients with primary tumors and lymph nodes expressing E-cadherin (Supplementary Figure 1A, 1B). Furthermore, the expression of N-cadherin in primary tumors did not correlate with 5-year OS (Supplementary Figure 2). However, a correlation was found between the expression of N-cadherin in lymph nodes and 5-year OS (P=0.0029) (Figure 2). No correlation was observed between the expression of Snail in primary tumors and lymph nodes and 5-year OS (Supplementary Figure 3A, 3B). In LNM, the reduced expression of E-cadherin and preserved expression of N-cadherin, reflecting the EMT status, correlated with a poor prognosis (P=0.041) (Supplementary Figure 4).

Relationship between the Snail Switch and Clinicopathological Factors

We defined the positive-to-negative conversion of the Snail status in primary tumors and lymph nodes as the Snail switch and evaluated its clinicopathological and prognostic significance. Patients with the Snail switch showed positive Snail expression in primary tumors and negative expression in lymph nodes. The metastatic lymph nodes of Snail switch were accounted for 17.0% (87/511) of all metastatic lymph nodes (Supplementary Table 1).

Patients with the Snail switch accounted for 21.3% of all patients (19 out of 89) (Table 4A). Patients with the Snail switch had more LNM (P=0.0009) and lymphatic invasion (P=0.002) and an advanced stage (P=0.038). N-cadherin expression levels in patients with the Snail switch were significantly high in primary tumors and LNM (Table 4B). Furthermore, the Snail switch correlated with a poor OS (P=0.0002) (Figure 3).

Discussion

EMT is a process through which epithelial cells are converted into mesenchymal cells and are changed such as the loss of cell-cell adhesion, loss of cell polarity, and gain of migration and invasion. The EMT process has been correlated with the presence of

LNM, distant metastases, and a poor prognosis. Although previous studies only examined main tumors, we herein showed EMT in main tumors and metastatic nodes. Significant changes generally occur during EMT, including the down-regulation of epithelial markers such as E-cadherin and up-regulation of mesenchymal markers including N-cadherin. We previously examined the relationship of E-cadherin with Slug, and N-cadherin in patients with gastric cancer. Uchikado et al. reported that patients with weaker E-cadherin expression or positive Slug expression had poor clinical outcomes[24]. Kamikihara et al. found that neo N-cadherin expression may be a useful prognostic marker independent of E-cadherin[25].

In the present study, the expression of E-cadherin and N-cadherin in main tumors was consistent with previous findings. However, their expression in primary tumors did not correlate with prognosis. The reason for this may be that all patients in this series had LNM; no non-LNM cases were included. In LNM, the weaker expression of E-cadherin and preserved expression of N-cadherin correlated with a poor prognosis. Therefore, EMT, namely the weaker expression of E-cadherin and preserved expression of N-cadherin, may have been induced in LNM. The evaluation of EMT-related markers in lymph node metastasis may be more useful than in primary tumors.

Markiewicz et al. reported that the expression levels of TWIST1, SNAIL, and SLUG

were significantly higher in LNM than in primary tumors. Furthermore, the negative-to-positive conversion of the Snail status correlated with worse survival in breast cancer[29]. However, this is in contrast to our results for the conversion of the Snail status. The weaker expression of Snail in the lymph nodes was associated with LNM and stage, but did not correlate with OS, although the Snail switch, which is the positive-to-negative conversion of the Snail status, is associated with LNM, stage, and lymphatic invasion. The Snail switch correlated with a poor prognosis. Snail switch is new approach to understand of EMT system. When cancer cells transfer from metastatic lymph nodes to another lymph nodes, ordinarily Snail expression in metastatic lymph nodes might be positive. Because it is possible that Snail operate to reduce E-cadherin expression and to increase N-cadherin expression in metastatic lymph nodes. Although our result was that Snail expression reduced in metastatic lymph nodes. It seems unlikely that it caused of EMT, it is possible that it caused of MET in metastatic lymph nodes. In Snail switch cases, N-cadherin expression levels were significantly high in primary tumors and LNM but E-cadherin expression levels were not high (Table 4B). We think that because E-cadherin expression levels in metastatic lymph nodes were reduced by EMT after MET caused by the function of Snail. Therefore, Snail may reduce the expression of E-cadherin during development and tumor progression in the

gastrointestinal tract. Snail may also be down-regulated in lymph nodes in order to adhere to metastatic sites. But it is unclearly what mechanism of EMT in metastatic lymph nodes.

Keun Hur et al. analyzed the expression and methylation status of miRNA-200 family members in primary colorectal cancer and liver metastasis. The expression of the ZEB1 genes was significantly weaker in liver metastasis than in the corresponding primary tumors. Metastasized liver cells become hypomethylated at the miR-200c locus, which initiates the mesenchymal-to-epithelial transition (MET) process[30]. Kurashige et al. indicated that miRNA-200 inhibits the expression of ZEB2 and enhances that of E-cadherin in gastric cancer[31]. Their findings suggest that miR-200 negatively regulates EMT and, thus, may reduce the risk of metastasis in gastric cancer. Saito et al. reported a relationship between long non-coding RNA activated by TGF-β (lnc RNA-ATB) and the expression of ZEB1 and miR-200 in gastric cancer[32]. Joke et al. showed that ZEB family members, similar to Snail gene family members, also bind to the E-box in the E-cadherin gene promoter through their two zinc finger domains[33]. Thus, the expression of Snail may be the same as that of ZEB1 with zinc finger family. Our results indicate that the expression of Snail was reduced by the hypomethylation of miR-200 in LNM.

In the present study, most LNM cases were associated with the reduced expression of

E-cadherin and preserved expression of N-cadherin. Therefore, EMT appears to be induced in tumors for metastasis and MET in lymph nodes to adhere to metastatic sites through the down-regulation of Snail. EMT is then induced in lymph nodes in order to metastasize to the surrounding lymph nodes. However, EMT in lymph nodes may be associated with another factor other than Snail.

A recent study reported that EMT may be associated with “Cancer Stem Cells (CSCs)” and this is sufficient to induce stemness and tumorigenicity. Ryu et al. performed immunohistochemistry for EMT-related proteins including Snail, ZEB-1, E-cadherin, vimentin, and β-catenin as well as the CSC marker CD44 in 276 consecutive primary gastric cancers and 54 matched LNM. They showed that the gastric CSC marker CD44 correlated with the expression of EMT-activating transcription factors[34]. Moreover, in the gastric epithelium, stem cells at the base of the pyloric gastric glands were found to be reliant on an active and dynamically regulated Wnt pathway[35, 36]. Further studies are needed in order to elucidate the relationship between the Wnt pathway, Notch pathway, and CSCs.

In the present study, we only performed immunohistochemistry to examine protein expression. Therefore, we were unable to identify biological processes occurring in lymph nodes similar to miR-200. However, the Snail switch between the primary tumor

and LNM may be important for confirming EMT and MET.

The major advantage of this study is that it is first report investigated EMT in metastatic lymph node. However, this study sample size is too small to get stable or repeatable results. Consequently, further studies are required to investigate the EMT and MET in LNM.

Conclusions

The reduced expression of E-cadherin and preserved expression of N-cadherin play key roles in EMT. The expression of N-cadherin in LNM is useful for predicting prognoses in patients with gastric cancer. The positive-to-negative conversion of the Snail status correlated with LNM and a poor prognosis. The Snail switch may be important for confirming EMT and MET. Further studies are needed in order to elucidate the biological processes occurring in LNM.

Compliance with ethical standards

Conflict of interest

None of the authors has any financial conflicts of interest regarding the study.

Ethical standards

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. The Ethics Committee of Kagoshima University and all patients provided written informed consent to the use of their information.

REFERENCES

1. Boyle P. Global burden of cancer. Lancet 1997;349 Suppl 2:SII23-6.
2. Kalluri R and Weinberg RA. The basics of epithelial-mesenchymal transition. The Journal of clinical investigation 2009;119:1420-8.
3. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell 2009;139:871-90.
4. Ksiazkiewicz M, Markiewicz A, Zaczek A. Epithelial-mesenchymal transition: a hallmark in metastasis formation linking circulating tumor cells and cancer stem cells. Pathobiology : journal of immunopathology, molecular and cellular biology 2012;79:195-208.
5. Shook D and Keller R. Mechanisms, mechanics and function of epithelial-mesenchymal transitions in early development. Mechanisms of development 2003;120:1351-83.
6. Thiery JP and Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. Nature reviews Molecular cell biology 2006;7:131-42.
7. Kang Y and Massague J. Epithelial-mesenchymal transitions: twist in development and metastasis. Cell 2004;118:277-9.
8. Yang J and Weinberg RA. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. Developmental cell 2008;14:818-29.
9. Yook JI, Li XY, Ota I, Hu C, Kim HS, Kim NH. et al. A Wnt-Axin2-GSK3beta cascade regulates Snail1 activity in breast cancer cells. Nature cell biology 2006;8:1398-406.

10. Jin H, Yu Y, Zhang T, Zhou X, Zhou J, Jia L, et al. Snail is critical for tumor growth and metastasis of ovarian carcinoma. International journal of cancer Journal international du cancer 2010;126:2102-11.
11. Yanagawa J, Walser TC, Zhu LK, Hong L, Fishbein MC, Mah V, et al. Snail promotes CXCR2 ligand-dependent tumor progression in non-small cell lung carcinoma. Clinical cancer research : an official journal of the American Association for Cancer Research 2009;15:6820-9.
12. Kosaka T, Kikuchi E, Mikami S, Miyajima A, Shirotake S, Ishida M, et al. Expression of snail in upper urinary tract urothelial carcinoma: prognostic significance and implications for tumor invasion. Clinical cancer research : an official journal of the American Association for Cancer Research 2010;16:5814-23.
13. Natsugoe S, Uchikado Y, Okumura H, Matsumoto M, Setoyama T, Tamotsu K, et al. Snail plays a key role in E-cadherin-preserved esophageal squamous cell carcinoma. Oncology reports 2007;17:517-23.
14. Shin NR, Jeong EH, Choi CI, Moon HJ, Kwon CH, Chu IS,et al. Overexpression of Snail is associated with lymph node metastasis and poor prognosis in patients with gastric cancer. BMC cancer 2012;12:521.
15. Chen X, Li J, Hu L, Yang W, Lu L,Jin H, et al. The clinical significance of snail protein expression in gastric cancer: a meta-analysis. Human genomics 2016;10 Suppl 2:22.
16. Cano A, Perez-Moreno MA, Rodrigo I, Locascio A, Blanco MJ, del Barrio MG, et al. The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. Nature cell biology 2000;2:76-83.
17. Batlle E, Sancho E, Franci C, Dominguez D, Monfar M, Baulida J, et al. The transcription factor snail is a repressor of E-cadherin gene expression in epithelial tumour cells. Nature cell biology 2000;2:84-9.
18. Nieto MA. The snail superfamily of zinc-finger transcription factors. Nature reviews Molecular cell biology 2002;3:155-66.
19. Elloul S, Elstrand MB, Nesland JM, Trope C.G, Kvalheim G, Goldberg I, et al. Snail, Slug, and Smad-interacting protein 1 as novel parameters of disease aggressiveness in metastatic ovarian and breast carcinoma. Cancer 2005;103:1631-43.
20. Martin TA, Goyal A, Watkins G, Jiang W. Expression of the transcription factors snail, slug, and twist and their clinical significance in human breast cancer. Annals of surgical oncology 2005;12:488-96.
21. Moody SE, Perez D, Pan TC, Sarkisian CJ, Portocarreco CP, Sterner CJ, et al. The transcriptional repressor Snail promotes mammary tumor recurrence. Cancer cell 2005;8:197-209.

22. Soini Y, Tuhkanen H, Sironen R, Virtanen I, Kataja V, Auvinen P, et al. Transcription factors zeb1, twist and snai1 in breast carcinoma. BMC cancer 2011;11:73.
23. Toyama T, Zhang Z, Iwase H, Yamashita H, Ando Y, Hamaguchi M, et al. Low expression of the snail gene is a good prognostic factor in node-negative invasive ductal carcinomas. Japanese journal of clinical oncology 2006;36:357-63.
24. Uchikado Y, Okumura H, Ishigami S, Setoyama T, Matsumoto M, Owaki T, et al. Increased Slug and decreased E-cadherin expression is related to poor prognosis in patients with gastric cancer. Gastric cancer : official journal of the International Gastric Cancer Association and the Japanese Gastric Cancer Association 2011;14:41-9.
25. Kamikihara T, Ishigami S, Arigami T, Matsumoto M, Okumura H, Uchikado Y, et al. Clinical implications of N-cadherin expression in gastric cancer. Pathology international 2012;62:161-6.
26. Shino Y, Watanabe A, Yamada Y, Tanase M, Yamada T, Matsuda M, et al. Clinicopathologic evaluation of immunohistochemical E-cadherin expression in human gastric carcinomas. Cancer 1995;76:2193-201.
27. Gabbert HE, Mueller W, Schneiders A, Meier S, Moll R, Birchmeier W, et al. Prognostic value of E-cadherin expression in 413 gastric carcinomas. International journal of cancer Journal international du cancer 1996;69:184-9.
28. Otsuki S, Inokuchi M, Enjoji M, Ishikawa T, Takagi Y, Kato K, et al. Vimentin expression is associated with decreased survival in gastric cancer. Oncology reports 2011;25:1235-42.
29. Markiewicz A, Ahrends T, Welnicka-Jaskiewicz M, Seroczynska B, Skokowski J, Jaskiewicz J, et al. Expression of epithelial to mesenchymal transition-related markers in lymph node metastases as a surrogate for primary tumor metastatic potential in breast cancer. Journal of translational medicine 2012;10:226.
30. Hur K, Toiyama Y, Takahashi M, Balaguer F, Nagasaka T, Koike J, et al. MicroRNA-200c modulates epithelial-to-mesenchymal transition (EMT) in human colorectal cancer metastasis. Gut 2013;62:1315-26.
31. Kurashige J, Kamohara H, Watanabe M, Hiyoshi Y, Iwatsuki M, Tanaka Y, et al. MicroRNA-200b regulates cell proliferation, invasion, and migration by directly targeting ZEB2 in gastric carcinoma. Annals of surgical oncology 2012;19 Suppl 3:S656-64.
32. Saito T, Kurashige J, Nambara S, Komatsu H, Hirata H, Ueda M, et al. A Long Non-coding RNA Activated by Transforming Growth Factor-beta is an Independent Prognostic Marker of Gastric Cancer. Annals of surgical oncology 2015;22 Suppl 3:S915-22.
33. Comijn J, Berx G, Vermassen P, Verschueren K, van Grunsven L, Bruyneel E, et al. The two-handed E box binding zinc finger protein SIP1 downregulates E-cadherin and

induces invasion. Molecular cell 2001;7:1267-78.
34. Ryu HS, Park DJ, Kim HH, Kim WH, Lee HS. Combination of epithelial-mesenchymal transition and cancer stem cell-like phenotypes has independent prognostic value in gastric cancer. Human pathology 2012;43:520-8.
35. Clevers H. Wnt/beta-catenin signaling in development and disease. Cell 2006;127:469-80.
36. Barker N and Clevers H. Leucine-rich repeat-containing G-protein-coupled receptors as markers of adult stem cells. Gastroenterology 2010;138:1681-96.

FIGURE LEGENDS

Figure 1

Expression of E-cadherin in gastric cancer. E-cadherin expression was detected in the cell membranes of cancer cells. (a) Primary tumor, (b) lymph node. Expression of N-cadherin in gastric cancer. N-cadherin expression was detected in the cell membranes of cancer cells. (c) Primary tumor, (d) lymph node. Expression of Snail in gastric cancer. Snail expression was detected in the nuclei of cancer cells. (e) Primary tumor, (f) lymph node.

Figure 2

Postoperative 5-year survival curves of patients according to the expression of N-cadherin in lymph nodes. The preserved expression of N-cadherin in lymph nodes correlated with a poor prognosis (P=0.0029).

Figure 3

Postoperative 5-year survival curves of patients according to the Snail switch. The Snail

switch correlated with a poor OS (P=0.0002).

Supplementary Figure 1A, 1B

Postoperative 5-year survival curves of patients according to the expression of E-cadherin in primary tumors (A) and lymph nodes (B).

Supplementary Figure 2

Postoperative 5-year survival curves of patients according to the expression of N-cadherin in primary tumors.

Supplementary Figure 3A, 3B

Postoperative 5-year survival curves of patients according to the expression of Snail in primary tumors (A) and lymph nodes (B).

Supplementary Figure 4

In LNM, the reduced expression of E-cadherin and preserved expression of N-cadherin correlated with a poor prognosis (P=0.041).