Expression profile of E-cadherin, estrogen receptors, and P53 in early-onset gastric cancers

Citation
Zhou, Fan, Yuanyuan Xu, Jiong Shi, Xing Lan, Xiaoping Zou, Lei Wang, and Qin Huang. 2016. “Expression profile of E-cadherin, estrogen receptors, and P53 in early-onset gastric cancers.” Cancer Medicine 5 (12): 3403-3411. doi:10.1002/cam4.931. http://dx.doi.org/10.1002/cam4.931.

Published Version
doi:10.1002/cam4.931

Permanent link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:30371040

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA

Share Your Story
The Harvard community has made this article openly available. Please share how this access benefits you. Submit a story.

Accessibility
Introduction

The incidence of gastric cancer varies between regions of the world, with more cases in eastern Asia [1]. Recently, the data of the National Cancer Institute’s Surveillance Epidemiology and End Results (SEER) program showed a significant rising incidence of EOGC (early-onset gastric cancer) in both female and male patients, but with a conspicuous female gender predominance [2, 3]. EOGC is a subtype of gastric cancer in patients younger than 45 years old. Approximately 10–20% of young gastric cancer patients have a positive family history [4], some of whom present with inherited gastric cancer predisposition syndromes. Although the underlying genetic events are not always known, EOGC may show CDH1 gene germline mutations [5–7], encoding an aberrant form of E-cadherin, a cardinal feature of hereditary diffuse gastric cancer (HDGC), as recently reviewed by Carneiro et al. [7]. However, CDH1 may partially explain EOGC [8], and more studies [9, 10] would suggest p53 as a candidate mutated gene in EOGC.

Abstract

Early-onset gastric cancer (EOGC) is predominant in females, diffuse histology, and hereditary pattern. Germline mutation of CDH1 and p53 has been reported previously and female dominance was speculated to be associated with estrogen and its receptors. Expression of E-cadherin, estrogen receptor α (ERα), estrogen receptor β (ERβ), and p53 in EOGC remains unclear, which was the focus of this study, to assess clinical significance of their expression in EOGC. The expression of E-cadherin, ERα, ERβ, and p53 in tumors and normal tissues from surgically resected EOGCs was assessed by immunohistochemistry (n = 139) and Western blot (n = 7) methods, respectively. The expression in tumor tissues was significantly higher for ERα, ERβ, and p53, but lower for E-cadherin, compared to uninvolved mucosa. Positive staining of ERβ and p53 was more frequently observed in younger patients with advanced TNM stages. For E-cadherin, significant correlation was observed between the immunopositivity and TNM stages IA+IB. P53-negative patients had significantly better outcomes than p53-positive patients. Significant association between expression of E-cadherin and histologic types was found in familial, but not in sporadic, EOGC. In conclusion, our results demonstrated E-cadherin may have a role in initiation of EOGC and positive ERβ and p53 expression may partially explained early-onset and tumor progression of EOGC.
frequently observed during the development of numerous human malignancies [13, 14]. Overexpression of p53 has been shown in numerous human tumors, and high levels of p53 protein have been correlated with malignant progression in colorectal tumors and lung carcinoma in advanced stages. In addition, overexpression of p53 has been shown to be independently related to poor prognosis in breast carcinoma [15, 16]. However, few studies have been conducted to assess p53 expression in EOGC [13].

In terms of gender differences in EOGC, most studies attributed the female predominance to possible roles of estrogen receptors in the pathogenesis of EOGC [17]. Since Tokunaga et al. [18] first reported estrogen receptor (ER) α expression in gastric cancer, a series of studies have been focused on the role of ERα in gastric cancer progression. In 1996, two forms of ERs, ERα and ERβ, were identified, but only ERβ, not ERα, was expressed in gastric cancer [19], whereas others show the evidence of expression for both ERα and ERβ receptor genes [19–21]. Recently, a large Chinese cohort study [21] shows the presence of ERα, ERβ, progesterone receptor (PR), and androgen receptor (AR) in both gastric cancer and noncancer tissues with predominant expression in ERβ and no prognostic significance for the expression.

Herein in this study, we investigated the expression and clinicopathological significance of E-cadherin, p53 in EOGC, and explored the role of ERα in gastric carcinoma in young Chinese patients treated at a single high-volume hospital in China. To our knowledge, this study was the largest sample study regarding the predictive significance of E-cadherin, p53, and estrogen receptors in EOGC.

**Materials and Methods**

**Patients and tissue samples**

EOGC patients younger than 40 years old at Nanjing Drum Tower Hospital, Jiangsu, China, from Jan 2004 to Dec 2014 were enrolled. Patients without enough tissue sample or necessary clinicopathological information, or loss to follow-up were excluded from the study. The study cohort was part of our previous study [22]. The paired formalin-fixed paraffin-embedded tissue blocks (tumor and nontumor in the same case) were retrieved and recut for immunohistochemistry. Proteins were extracted in frozen matched tumor and nontumor tissues from our biobank at this hospital. The study protocol was approved by the Medical Ethics Committee of the Nanjing Drum Tower Hospital. Informed consent was obtained from all individual participants included in this study.

**Immunohistochemistry**

Immunohistochemical (IHC) analysis for E-cadherin, ERα, ERβ, and p53 expression was performed on formalin-fixed, paraffin-embedded sections of surgical specimens. Briefly, sections were deparaffinized in xylene and rehydrated in gradient ethanol solutions up to distilled water. Endogenous peroxidase activity was blocked by 0.3% H2O2 in methanol for 20 min. The slides were immersed in 10 mM citric buffer (pH 6.0) with heating for 15 min for antigen retrieval. Nonspecific binding sites were blocked with 10% normal goat serum for 10 min. Then, sections were incubated in a humidified chamber overnight with primary antibody (listed as in Table S1). Immunostaining was visualized with DAB and hematoxylin counterstain.

Two experienced pathologists (JS, QH) independently reviewed the expression of the four antibodies without the knowledge of patients’ clinicopathological parameters. The scoring for E-cadherin and ERβ (expressed at a high level) was based on the area intensity score method (AIS) [23]. Intensity scores from 0 to 3, respectively, represented absent, weak, moderate, and strong positive immunostaining. The area scores from 0 to 4 were estimated for the proportion of positively stained neoplastic cells in the entire tumor on the slide, as 0 = <5%, 1 = 5–24%, 2 = 25–49%, 3 = 50–74%, and 4 = ≥75%, respectively. The overall AIS score was obtained by multiplication. For ERα and p53 immunostaining, a negative stain was defined as less than 10% positive neoplastic cells on the slide; otherwise the stain was classified to be positive. Overexpression of p53 generally reflects an underlying mutation(s) in the p53 gene, and manifests as positive immunostaining.

**Western blot analysis**

Target tissues were homogenized in the RIPA lysis buffer. The supernatant was used for Western blot analysis. Protein concentrations were determined using the BCA assay reagent. Thirty to sixty micrograms of protein lysates were separated on 6–12% sodium dodecyl sulfate-polyacrylamide gels and then transferred to the PVDF membranes (Millipore). TBST (TBS and 0.1% Tween-20) containing 5% nonfat milk or bovine serum albumin was used to block nonspecific binding for 2 h at room temperature. Then, the membranes were incubated with the primary antibodies against ERα, ERβ, E-cadherin, and p53 (detailed information is shown in Table S1). The membranes were rinsed three times with TBST for 10 min and reincubated for 1 h at room temperature in blocking buffer with each HRP-conjugated secondary antibody (1:5000), and then washed three times for 10 min each. Signals generated by enhanced chemiluminescence (Millipore) were recorded with a CCD camera (CLINX, Shanghai).
Statistical analysis

Difference in expression of E-cadherin, estrogen receptors, and p53 between gastric tumors and corresponding uninvolved mucosal tissues was compared by the Students’ t test or Wilcoxon matched-pairs signed-rank test where appropriate. Correlations were computed using the Spearman rank test. The associations between expression of E-cadherin, estrogen receptors, and p53 and clinicopathological characteristics were analyzed using the Chi-square test. The probability of survival was estimated by Kaplan–Meier method with a log-rank test. All P values were two sided and considered statistically significant if less than 0.05. Statistical analyses were performed by the SPSS 22.0 for Windows (SPSS, Chicago, IL).

Results

Protein expression

By immunohistochemistry performed in 139 EOGC tumors, expression of E-cadherin was absent in 36 (25.9%), aberrant in 43 (30.9%), and normal in 60 (43.2%) patients, significantly lower than those (2.2%, 18.7%, and 79.1%, respectively) in uninvolved mucosal tissue (P < 0.01) (Fig. 1, Table 1). In contrast, the expression of ERα (69, 49.6%), ERβ (122, 72.2%), and p53 (42, 33.8%) in tumor tissues was significantly higher than those (2.2%, 60.3%, and 4.3% for ERα, ERβ, and p53, respectively, detailed data for normal mucosal not shown) in uninvolved mucosal tissues (P < 0.01). In terms of location, staining of E-cadherin and p53 is consistent with previous studies, membranous and nuclei expression was demonstrated. Nuclei staining with anti-ERα antibody was seen. While for ERβ, EOGC was stained in both cytoplasmic and nuclei.

Western blotting in seven paired EOGC and uninvolved mucosal tissues showed the patterns of changes similar to those by immunohistochemistry (Fig. 2). Due to p53 mutation in gastric cancer, those mutated cases showed no signal in western blot.

Correlation with clinicopathological characteristics

As shown in Table 1, for E-cadherin, significant correlations were observed between the positive expression and TNM stages at IA+IB (P = 0.017). And interestingly, absent E-cadherin expression is significantly associated with lower lymphovascular invasion (LVI) (P = 0.007). Importantly, protein expression of ERβ and p53 was significantly associated with age and TNM stage, respectively. Positive staining of ERβ and p53 was significantly more frequently observed in younger patients with advanced TNM stages (P < 0.01). P53 expression is also significantly related to LVI, perineural
Table 1. Association between expression of E-cadherin, ERα, ERβ, and P53 and clinicopathological characteristics in early-onset gastric cancer.

| Clinicopathologic Characteristics | Cases (n = 139) | E-cadherin | ERα | ERβ | P53 |
|-----------------------------------|----------------|------------|-----|-----|-----|
| Gender                            |                | Absent     |     |     |     |
| M                                 | 52             | 14 (38.9)  | 18 (41.9) | 20 (33.3) | 0.663 |
| F                                 | 87             | 22 (61.1)  | 25 (58.1) | 40 (66.7) |     |
| Age                               | 33.8 ± 5.47    | 35.0 ± 4.01 | 32.5 ± 6.61 | 33.9 ± 5.22 | 0.126 |
| Family history                    | 34             | 8 (24.2)   | 14 (32.6) | 12 (20.7) | 0.393 |
| Size (cm)                         | 4.6 ± 2.82     | 4.39 ± 3.31 | 4.84 ± 2.40 | 4.51 ± 2.82 | 0.751 |
| Hp infection                      | 62             | 17 (48.6)  | 20 (50.0) | 25 (49.0) | 0.992 |
| Lauren’s classification           |                |            |     |     |     |
| Diffuse                           | 100            | 27 (84.4)  | 31 (79.5) | 42 (72.4) | 0.403 |
| Intestinal                        | 13             | 2 (6.3)    | 3 (7.7)   | 8 (13.8)  | 0.564 |
| Mixed                             | 16             | 3 (9.4)    | 5 (12.8)  | 8 (13.8)  | 0.888 |
| LVI                               | 80             | 16 (44.4)  | 33 (76.7) | 31 (51.7) | 0.007 |
| PNI                               | 90             | 19 (52.8)  | 32 (74.4) | 39 (65)   | 0.134 |
| LN                                | 94             | 19 (52.8)  | 33 (76.7) | 42 (70)   | 0.067 |
| Stage                             |                |            |     |     |     |
| IA+IB                             | 31             | 13 (36.1)  | 4 (9.3)   | 14 (23.3) | 0.017 |
| IIA+IIB                           | 31             | 7 (19.4)   | 13 (30.2) | 11 (18.3) | 0.321 |
| III                               | 65             | 12 (33.3)  | 22 (51.2) | 31 (51.7) | 0.172 |
| IV                                | 12             | 4 (11.1)   | 4 (9.3)   | 4 (6.7)   | 0.741 |

Bold values (P < 0.05) are statistically significant.

Hp, Helicobacter pylori; LVI, lymphovascular invasion; PNI, perineural invasion; LN, positive lymph node metastasis.
invasion (PNI), and lymph node metastasis \( (P < 0.001) \). No significant associations were found between expression of \( \text{ER} \alpha \) and clinicopathological features.

**Prognostic value**

In all 139 patients with postresection survival information, expression of E-cadherin, \( \text{ER} \alpha \), \( \text{ER} \beta \), and p53 was analyzed for prognostic values by the Kaplan–Meier method. P53-negative patients had a significantly better outcome than p53-positive patients \( (P = 0.005; \text{Fig. } 3\text{D}) \). However, expression of E-cadherin, \( \text{ER} \alpha \), and \( \text{ER} \beta \) showed no prognostic values in EOGC patients (Fig. 3A, B, and C). Univariate Cox regression (Table 2) showed that higher CA 72-4, CA 125, and CA 19-9 level, larger tumor size, positive resection margin, LVI, PNI, advanced staging,
Expression Profile of Young Gastric Cancer  

F. Zhou et al.

and positive p53 expression were related with worse prognosis of EOGC. While for multivariate analysis, only CA 72–4 (RR: 4.622, 95% CI: 1.487–14.369, P = 0.008), larger tumor size (RR: 1.139, 95% CI: 1.000–1.296, P = 0.05), positive resection margin (RR: 5.718, 95% CI: 1.797–18.189, P = 0.003), and stage IV (RR: 20.119, 95% CI: 1.486–272.465, P = 0.024) are independent prognostic factors in EOGC.

**Comparison in expression between tumor and uninvolved mucosal tissues**

Table 3 shows the Spearman correlations in expression of E-cadherin, ERα, ERβ, and p53 between cancer and adjacent uninvolved tissues. Significant correlation was identified for expression of only E-cadherin but not ERα, ERβ, and p53. However, the correlation coefficients of E-cadherin expression were so small (r = 0.261) that the correlation was extremely weak.

**Expression in diffuse and intestinal familiar and sporadic gastric cancers**

As shown in Table 4, expression of E-cadherin was significantly more frequent in intestinal and diffuse mixed EOGC tumors of familiar, (P < 0.05), but not sporadic EOGC cases. No significant associations were found between expression of ERα, ERβ, and p53 and different histology types of EOGC.

**Discussion**

Our study reveals that expression of E-cadherin, estrogen receptors, and p53 is significantly altered in EOGC. As expected, expression of E-cadherin was decreased in the diffuse-type familial EOGCs, whereas positive ERβ and p53 expression correlated with younger age and advanced TNM stages in EOGC, which has not been described previously. Independent prognostic factors in EOGC were higher CA 72–4 level, larger tumor size, positive resection margin, and stage IV cancer.

Although decreased expression of E-cadherin is known in the diffuse-type gastric cancer in general, aberrant expression of this gene is widely present in EOGC regardless of histological type [24], but also correlated significantly with the diffuse and mixed types in the familial gastric cancer (FGC) [25], suggesting a role of E-cadherin in FGC tumorigenesis. We showed that E-cadherin expression was not associated with gender, age, family history of cancer, tumor location, size, and Hp infection, except for stages IA+IB. This suggests that E-cadherin may play a critical part in the early-stage tumorigenesis of EOGC [26]. However, we also reported that absent E-cadherin expression was associated with lower LVI rate compared to aberrant and normal group. This phenomenon may be explained by mesenchymal to epithelium transition (MET)[27]. E-cadherin is important in cellular junction and maintenance of epithelial phenotype. When E-cadherin switched to N-cadherin, epithelial–mesenchymal transition

---

**Table 2. Univariate and Multivariate Analyses (Cox Regression) on Prognosis of Early-onset gastric cancer Patients.**

| Factors                  | Univariate analysis | Multivariate analysis |
|--------------------------|---------------------|-----------------------|
|                          | RR (95% CI)         | P                     | RR(95% CI)  | P       |
| Female                   | 1.340 (0.727–2.470) | 0.348                 |             |         |
| Age                      | 1.001 (0.950–1.055) | 0.713                 |             |         |
| Positive Family history  | 0.769 (0.366–1.617) | 0.489                 |             |         |
| Higher CA 72–4           | 3.185 (1.473–6.886) | 0.003                 | 4.622 (1.487–14.369) | 0.008 |
| Higher CA 125            | 3.701 (1.486–9.216) | 0.005                 |             |         |
| Higher CA 19–9           | 4.241 (2.016–8.918) | <0.001                |             |         |
| Larger Tumor size (cm)   | 1.430 (1.295–1.580) | <0.001                | 1.139 (1.000–1.296) | 0.05  |
| Positive resection Margin| 5.617 (2.831–11.143) | <0.001               | 5.718 (1.797–18.189) | 0.003 |
| Lymphovascular invasion  | 7.556 (2.960–19.288) | <0.001               |             |         |
| Perineural invasion      | 6.629 (2.583–17.013) | <0.001               |             |         |
| Staging I                |                      |                       |             |         |
| II                       | 2.722 (0.283–26.221) | 0.386                 |             |         |
| III                      | 22.488 (3.053–165.661) | 0.002               |             |         |
| IV                       | 69.400 (8.597–560.260) | <0.001              | 20.119 (1.486–272.465) | 0.024 |
| E-cadherin expression    | 1.018 (0.701–1.480) | 0.924                 |             |         |
| ERα expression           | 1.230 (0.682–2.216) | 0.492                 |             |         |
| ERβ expression           | 1.854 (0.663–5.185) | 0.239                 |             |         |
| P53 expression           | 2.269 (1.262–4.077) | 0.006                 |             |         |

RR, relative risk; CI, confidence interval.
(EMT) happens. After tumor cells spread to targeted region, disseminated tumor cells undergo MET. E-cadherin is regulated by both transcriptional and epigenetic mechanism. As our study was limited to the protein level of E-cadherin expression, further analysis at gene levels and epigenetic level may help reveal the molecular pathogenesis mechanisms for E-cadherin in a large sample Chinese EOGC cases [28].

p53 is one of the most important genes that are mutated in gastric cancer [29, 30]. In accordance with the previous reports [31], patients with p53-positive and HIF-1α-positive gastric cancer have worse prognosis, compared with those with double negative cancers. Our study on EOGC further suggests that p53-negative patients have a significantly better outcome than p53-positive patients, which is in disagreement with a most recent Turkish report because of the different study population and a high percentage of elderly patients investigated [32]. We show that overexpression of p53 is associated with younger age but advanced stage of EOGC, suggestive of an aggressive biology behavior. And positive expression was associated with worse prognosis. However, multivariate analysis suggested it was not an independent factor. A recently one study [33] from Korean reported that overexpression of p53 is less frequent in younger GC patients. The inconsistency may be explained by the selection of patients. We did not include older GC patients who are reported to have a high p53 mutation rate [9].

Relative to old gastric cancer patients, young patients have a female preponderance, a more frequent occurrence of the diffuse-type cancer, and less intestinal metaplasia [4, 24, 34]. This female gender predominance in EOGC is considered to be related to hormonal factors [35, 36]. While our results show an absence of a significant correlation between ERα expression and clinicopathologic parameters, ERβ expression is indeed correlated with younger age and advanced cancer stages. In conventional gastric cancer with a high proportion of elderly patients, expression of ERα correlates with poor overall survival, as an independent predictor of overall survival [20], which is not confirmed in EOGC, suggesting different

| Correlation          | \( r^1 \) | \( P \text{ value}^2 \) |
|----------------------|-----------|-------------------------|
| E-cad (T) vs. E-cad (NT) | 0.261     | 0.002                   |
| ERα (T) vs. ERα (NT)    | 0.051     | 0.554                   |
| ERβ (T) vs. ERβ (NT)    | 0.022     | 0.798                   |
| P53 (T) vs. P53 (NT)    | −0.03     | 0.722                   |
| ERα (T) vs. ERβ (T)     | −0.046    | 0.591                   |

E-cad, E-cadherin.

1Spearman rank correlation coefficients

2Spearman rank correlation test.

Table 3. Correlations among expression of E-cadherin, ERα, ERβ, and P53 in gastric cancer and adjacent nontumor tissue.

Table 4. Expression of E-cadherin, ERα, ERβ, and P53 in diffuse and intestinal FGC and SGC.

Table 5. Correlations among expression of E-cadherin, ERα, ERβ, and P53 in gastric cancer and adjacent nontumor tissue.
Expression Profile of Young Gastric Cancer

3. Bai, Y., and Z. S. Li. 2011. Endoscopic, clinicopathological features and prognosis of very young patients with gastric cancer. J. Gastroen. Hepatol. 26:1626–1629.

4. Kokkola, A., and P. Sipponen. 2001. Gastric carcinoma in young adults. Hepatogastroenterology 48:1552–1555.

5. Suriano, G., C. Oliveira, P. Ferreira, J. C. Machado, M. C. Bordin, O. De Wever, et al. 2003. Identification of CDH1 germline missense mutations associated with functional inactivation of the E-cadherin protein in young gastric cancer probands. Hum. Mol. Genet. 12:575–582.

6. Suriano, G., S. Yew, P. Ferreira, J. Senz, P. Kaurah, J. M. Ford, et al. 2005. Characterization of a recurrent germ line mutation of the E-cadherin gene: implications for genetic testing and clinical management. Clin. Cancer Res. 11:5401–5409.

7. Carneiro, F., C. Oliveira, G. Suriano, and R. Seruca. 2008. Molecular pathology of familial gastric cancer, with an emphasis on hereditary diffuse gastric cancer. J. Clin. Pathol. 61:25–30.

8. Sugimoto, S., H. Yamada, M. Takahashi, Y. Morohoshi, N. Yamaguchi, Y. Tsunoda, et al. 2013. Early-onset diffuse gastric cancer associated with a de novo large genomic deletion of CDH1 gene. Gastric Cancer 17:745–749.

9. Rugge, M., Y. H. Shiao, G. Busatto, M. Cassaro, C. Strobbe, V. M. Russo, et al. 2000. The p53 gene in patients under the age of 40 with gastric cancer: mutation rates are low but are associated with a cardiac location. Mol. Pathol. 53:207–210.

10. Oliveira, C., P. Ferreira, S. Nabais, L. Campos, A. Ferreira, L. Cirnes, et al. 2004. E-Cadherin (CDH1) and p53 rather than SMAD4 and Caspase-10 germline mutations contribute to genetic predisposition in Portuguese gastric cancer patients. Eur J. Cancer 40:1897–1903.

11. Sgambato, A., M. Migaldi, P. Locatana, L. Ventura, M. Criscuolo, C. Di Giacomo, et al. 2000. Loss of p27(Kip1) expression is a strong independent prognostic factor of reduced survival in NO gastric carcinomas. Cancer 89:2247–2257.

12. Kamp, W. M., P. Y. Wang, and P. M. Hwang. 2016. TP53 mutation, mitochondria and cancer. Curr. Opin. Genet. Dev. 38:16–22.

13. Muller, W., and F. Borchard. 1996. Prognostic influence of p53 expression in gastric cancer. J. Pathol. 178:255–258.

14. van den Berg, F. M., I. O. Baas, M. M. Polak, and G. J. Offerhaus. 1993. Detection of p53 overexpression in routinely paraffin-embedded tissue of human carcinomas using a novel target unmasking fluid. Am. J. Pathol. 142:381–385.

15. Maeda, T., Y. Nakanishi, Y. Hirono, F. Fujimoto, K. Enomoto, K. Sakurai, et al. 2016. Immunohistochemical co-expression status of cytookeratin 5/6, androgen receptor, and p53 as prognostic factors of adjuvant chemotherapy for triple negative breast cancer. Med. Mol. Morphol. 49:11–21.
16. Jiang, T., Y. Wang, F. Zhou, G. Gao, S. Ren, and C. Zhou. 2016. Prognostic value of high EZH2 expression in patients with different types of cancer: a systematic review with meta-analysis. Oncotarget 7:4584–4597.

17. Kim, J. H., Y. J. Boo, J. M. Park, S. S. Park, S. J. Kim, C. S. Kim, et al. 2008. Incidence and long-term outcome of young patients with gastric carcinoma according to sex: does hormonal status affect prognosis?. Arch. Surg. 143:1062–1067; discussion 7.

18. Tokunaga, A., K. Nishi, N. Matsukura, N. Tanaka, M. Onda, A. Shirata, et al. 1986. Estrogen and progesterone receptors in gastric cancer. Cancer 57:1376–1379.

19. Matsuyama, S., Y. Ohkura, H. Eguchi, Y. Kobayashi, K. Akagi, K. Uchida, et al. 2002. Estrogen receptor beta is expressed in human stomach adenocarcinoma. J. Cancer Res. Clin. Oncol. 128:319–324.

20. Xu, C. Y., J. L. Guo, Z. N. Jiang, S. D. Xie, J. G. Shen, J. Y. Shen, et al. 2010. Prognostic role of estrogen receptor alpha and estrogen receptor beta in gastric cancer. Ann. Surg. Oncol. 17:2503–2509.

21. Gan, L., J. He, X. Zhang, Y. J. Zhang, G. Z. Yu, Y. Chen, et al. 2012. Expression profile and prognostic role of sex hormone receptors in gastric cancer. BMC Cancer 12:566–576.

22. Zhou, F., J. Shi, C. Fang, X. Zou, and Q. Huang. 2016. Gastric Carcinomas in Young (Younger than 40 Years) Chinese Patients: clinicopathology, Family History, and Postresection Survival. Medicine (Baltimore) 95:e2873.

23. Kurzen, H., I. Munzing, and W. Hartschuh. 2003. Expression of desmosomal proteins in squamous cell carcinomas of the skin. J. Cutan. Pathol. 30:621–630.

24. Lim, S., H. S. Lee, H. S. Kim, Y. I. Kim, and W. H. Kim. 2003. Alteration of E-cadherin- mediated adhesion protein is common, but microsatellite instability is uncommon in young age gastric cancers. Histopathology 42:128–136.

25. Mikne, A. N. A., R. Carvalho, F. M. Morsink, A. R. Musler, W. W. J. de Leng, A. Ristimaki, et al. 2006. Early-onset gastric cancers have a different molecular expression profile than conventional gastric cancers. Modern Pathol. 19:564–572.

26. Peng, Z., C. X. Wang, E. H. Fang, G. B. Wang, and Q. Tong. 2014. Role of epithelial-mesenchymal transition in gastric cancer initiation and progression. World J. Gastroenterol. 20:5403–5410.

27. Gao, D., L. T. Vahdat, S. Wong, J. C. Chang, and V. Mittal. 2012. Microenvironmental regulation of epithelial-mesenchymal transitions in cancer. Cancer Res. 72:4883–4889.

28. Chan, A. C. O., K. M. Chu, S. K. Lam, B. C. Y. Wong, K. F. Kwok, S. Law, et al. 2003. Soluble E-cadherin is an independent pretherapeutic factor for long-term survival in gastric cancer. J. Clin. Oncol. 21:2288–2293.

29. Karim, S. 2014. Clinicopathological and p53 gene alteration comparison between young and older patients with gastric cancer. Asian Pac. J. Cancer Prev. 15:1375–1379.

30. Keller, G., H. Vogelsang, I. Becker, S. Plaschke, K. Ott, G. Suriano, et al. 2004. Germine mutations of the E-cadherin(CDH1) and TP53 genes, rather than of RUNX3 and HPP1, contribute to genetic predisposition in German gastric cancer patients. J. Med. Genet. 41:e89.

31. Sumiyoshi, Y., Y. Kakeji, A. Egashira, K. Mizokami, H. Orita, and Y. Maehara. 2006. Overexpression of hypoxia-inducible factor 1alpha and p53 is a marker for an unfavorable prognosis in gastric cancer. Clin. Cancer Res. 12:5112–5117.

32. ÇAlik, M., E. DemIrCl, E. Altun, I. ÇAlik, Ö. B. GÜNdöGDu, N. GÜrsan, et al. 2015. Clinicopathological importance of Ki-67, p27, and p53 expression in gastric cancer. Turk. J. Med. Sci. 45:118–128.

33. Seo, J. Y., E. H. Jin, H. J. Jo, H. Yoon, C. M. Shin, Y. S. Park, et al. 2015. Clinicopathologic and molecular features associated with patient age in gastric cancer. World J. Gastroenterol. 21:6905–6913.

34. Matley, P. J., D. M. Dent, M. V. Madden, and S. K. Price. 1988. Gastric-Carcinoma In Young-Adults. Ann. Surg. 208:593–596.

35. Derakhshan, M. H., S. Liptrot, J. Paul, I. L. Brown, D. Morrison, and K. E. L. McColl. 2009. Oesophageal and gastric intestinal-type adenocarcinomas show the same male predominance due to a 17 year delayed development in females. Gut 58:16–23.

36. Maeta, M., H. Yamashiro, A. Oka, S. Tsujitani, M. Ikeguchi, and N. Kaibara. 1995. Gastric cancer in the young, with special reference to 14 pregnancy-associated cases: analysis based on 2,325 consecutive cases of gastric cancer. J. Surg. Oncol. 58:191–195.

37. Rahman, M. S., and J. Cao. 2016. Estrogen receptors in gastric cancer: advances and perspectives. World J. Gastroenterol. 22:2475–2482.

Supporting Information
Additional supporting information may be found in the online version of this article:
Table S1. Antibodies used in this study.