Inhibitory effect of a TGFβ receptor type-I inhibitor, Ki26894, on invasiveness of scirrhou s gastric cancer cells

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BACKGROUND: Gastric cancer cells frequently metastasise, partly because of their highly invasive nature. Transforming growth factor-β (TGF-β) receptor signalling is closely associated with the invasion of cancer cells. The aim of this study was to clarify the effect of a TGF-β receptor (TβR) phosphorylation inhibitor on the invasiveness of gastric cancer cells.

METHODS: Four gastric cancer cell lines, including two scirrhous-type cell lines and two non-scirrhous-type cell lines, were used. A TβR type I (TβR-I) kinase inhibitor, Ki26894, inhibits the phosphorylation of Smad2 at an ATP-binding site of TβR-I. We investigated the expression levels of TβR and phospho-Smad2, and the effects of TGF-β in the presence or absence of Ki26894 on Smad2 phosphorylation, invasion, migration, epithelial-to-mesenchymal transition (EMT), Ras homologue gene family member A (RhoA), ZO-2, myosin, and E-cadherin expression of gastric cancer cells.

RESULTS: TβR-I, TβR-II, and phospho-Smad2 expressions were found in scirrhous gastric cancer cells, but not in non-scirrhous gastric cancer cells. Ki26894 decreased Smad2 phosphorylation induced by TGF-β1 in scirrhous gastric cancer cells. Transforming growth factor-β1 upregulated the invasion, migration, and EMT ability of scirrhous gastric cancer cells. Transforming growth factor-β1 significantly upregulated the activity of RhoA and myosin phosphorylation, whereas TGF-β1 decreased ZO-2 and E-cadherin expression in scirrhous gastric cancer cells. Interestingly, Ki26894 inhibited these characteristics in scirrhous gastric cancer cells. In contrast, non-scirrhous gastric cancer cells were not affected by TGF-β1 or Ki26894 treatment.

CONCLUSION: A TβR-I kinase inhibitor decreases the invasiveness and EMT of scirrhous gastric cancer cells. Ki26894 is therefore considered to be a promising therapeutic compound for the metastasis of scirrhous gastric carcinoma.

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Effect of a novel T/R-I inhibitor Ki26894 on the invasiveness of gastric cancer.

MATERIALS AND METHODS

Compounds
A small synthetic molecule that interrupts the phosphorylation of Smad2/Smad3 by T/R-I, namely, Ki26894, was synthesised by Kirin Brewery Company (Gunma, Japan) as previously reported (Ehata et al, 2007). Ki26894 was dissolved in PBS (Nikken Bio, Kyoto, Japan), stored at 4°C, and used within 5 days. Transforming growth factor-β1 was purchased from R&D Systems (Minneapolis, MN, USA).

Cell lines
OCUM-2MLN (Fujihara et al, 1999) and OCUM-12 (Qiu et al, 2009) were derived from scirrhous gastric carcinomas. MKN-45 (Motoyama et al, 1986) and MKN-74 (Motoyama et al, 1986) were derived from non-scirrhous gastric carcinomas. The culture medium consisted of DMEM (Nikken Bio.) with the addition of 10% heat-inactivated fetal bovine serum (FBS; Equitech-Bio, Kerrville, TX, USA), 100 IU ml⁻¹ penicillin (ICN Biomedicals, Costa Mesa, CA, USA), 100 µg ml⁻¹ streptomycin (ICN Biomedicals), and 0.5 mM sodium pyruvate (Cambrex, Walkersville, MD, USA). Cells were cultured at 37°C in a humidified atmosphere containing 5% CO₂ in the air.

Morphological findings
Cancer cells were also cultured with TGF-β1 (10 ng ml⁻¹) and/or Ki26894 (0.1, 0.3, 1, 3, or 10 µM). Cell morphology was observed microscopically, 24, 48, and 72 h after addition.

Wound healing assay
In vitro wound healing ability was measured by the method described in the study by Borensztajn et al (2008), with some modifications. Gastric cancer cells were cultured in six-well plates. After the cells reached semi-confluence, a wound was created in the cell monolayer using a pipette tip. Cancer cells were cultured in serum-free DMEM, along with TGF-β1 (10 ng ml⁻¹) and/or Ki26894 (10 µM). Four scratched fields were randomly chosen and the number of cell migrations was counted from pictures 24 h after treatment.

Invasion assay
In vitro invasiveness was measured by the method described in the study by Albini et al (1987), with some modifications. We used chemotaxis chambers with a 12 µm-pore membrane filter (Kubota, Osaka, Japan) coated with 50 µg of matrigel in a 24-well culture plate. Gastric cancer cells (2 × 10⁵ cells per chamber) were seeded, and TGF-β1 (a final concentration of 0 or 10 ng ml⁻¹) and/or Ki26894 (a final concentration of 0, 0.1, 1, or 10 µM) were added to the upper chambers. After 72 h incubation, cells that had not moved to the lower wells were removed from the upper face of the filters using cotton swabs, and the cells that had moved to the lower surface of the filter were stained with haematoxyl. Cancer cells that invaded through a filter coated with matrigel to the lower surface of the membrane were manually counted under a microscope at ×200 magnification. The mean of six fields was calculated as the sample value. The culture was performed in triplicate.

Immunohistochemistry
BALB/c nude female mice, aged 4 weeks (Nihon CLEA, Tokyo, Japan), were used in the studies. All experiments were performed according to the standard guidelines for animal experiments of Osaka City University Medical School. The expression of T/R-I, T/R-II, and phosphorylated Smad2 of xenografts was examined. Xenografts were established by injecting each cell (1 × 10⁶) into the flanks of nude mice. Four weeks after inoculation, mice were killed, and the xenografted tumours were washed in PBS and fixed in 10% formalin for paraffin sectioning. Immunohistochemical determination of T/R-I, T/R-II, and phosphorylated Smad2 was carried out according to the manufacturer’s instructions. In brief, slides were deparaffinised, and were heated for 10 min at 105°C by autoclave in Target Retrieval Solution (Dako, Carpinteria, CA, USA). The sections were then incubated with 3% hydrogen peroxide to block endogenous peroxidase activity. The specimens were incubated with anti-T/R-I antibody (Lab vision, Fremont, CA, USA; 1:100), anti-T/R-II antibody (Lab vision; 1:100), and anti-phospho-Smad2 antibody (Chemicon International, Temecula, CA, USA; 1:2000) overnight at 4°C. The sections were incubated with biotinylated goat anti-rabbit immunoglobulin G for 30 min. The slides were treated with streptavidin-peroxidase reagent, and were incubated in PBS diaminobenzidine and 1% hydrogen peroxide v/v, followed by counterstaining with Mayer’s haematoxylin.

Western blot analysis
The inhibition by Ki26894 of the phosphorylation of Smad2 and its migration ability in gastric cancer cells were examined by western blotting. Briefly, cell lines were cultured in DMEM with 2% FBS for 2 days. The culture was rinsed with PBS and incubated in serum-free DMEM with reagent (TGF-β1: a final concentration of 0 or 10 ng ml⁻¹; Ki26894: a final concentration of 0, 0.1, 0.3, 1, 3, or 10 µM) for 60 min. The cells were lysed in a lysis buffer. Aliquots containing 30 µg of total protein were subjected to SDS–PAGE, and the protein bands were transferred to a polyvinylidene difluoride membrane (Amersham, Aylesbury, UK). The membrane was kept in TBS-T (10 mM TBS and 0.05% Tween 20) supplemented with 5% non-fat milk or 5% bovine albumin (Sigma, St Louis, MO, USA) at room temperature for 1 h. Next, the membrane was placed in a TBS-T solution containing each primary antibody. The membrane was placed in TBS-T solution containing the primary antibody, phospho-Smad2 (Cell Signaling Tec, Danvers, CO, USA, Ser 465/467; 1:1000), Smad2/3 (Cell Signaling Tec; 1:1000), ZO-2 (Cell Signaling Tec; 1:1000), phospho-myosin light chain-2 (Cell Signaling Tec, Thr18/Ser19; 1:1000, E-cadherin (Cell Signaling Tec; 1:1000), or β-actin (Cell Signaling Tec; 1:1000), and allowed to react at 4°C overnight for western blotting. Next, each antibody was washed three times with TBS-T for 10 min, and a peroxidase-labelled secondary antibody (Amersham) reactive with the primary antibody was added. The membrane was placed in the TBS-T solution, kept at room temperature for 1 h, and then washed. Bands were detected using an enhanced chemiluminescence system (Amersham). An immunoblot analysis was performed twice. PANC-1, a pancreas cancer cell line, was used as the positive control of Smad2 (Romero et al, 2008; Horiguchi et al, 2009).

Ras homologue gene family member A activation assay
Activated Ras homologue gene family member A (RhoA) proteins were measured with a RhoA activation G-LISA (Absorbance Based) assay kit (Cytokeleton, Denver, CO, USA) according to the manufacturer’s instructions. The activation of RhoA by TGF-β1 and the inhibition by Ki26894 in gastric cancer cells were examined as follows. Cell lines were cultured in DMEM with 2% FBS. The culture was rinsed with PBS and incubated in serum-free
DMEM with reagent (TGF-β1: a final concentration of 0 or 10 ng ml⁻¹; Ki26894: a final concentration of 0 or 10 μM) for 10 min. Activation of RhoA analysis was performed for the third time.

Statistical analysis
Comparisons among data sets were made with the Kruskal-Wallis one-way ANOVA by ranks, followed by Dunn’s multiple comparison test. A difference was considered significant when the P-value was 0.05 or less.

RESULTS
Expression of TβR-I, TβR-II, and phospho-Smad2 in gastric cancer cells
Figure 1A shows the expression level of TβR-I, TβR-II, and phospho-Smad2 of gastric cancer cells in xenografted tumours. TβR-I and TβR-II were immunolocalised at the membrane and cytoplasm, respectively, of gastric cancer cells. High expression levels of TβRI and TβRII were found in scirrhous gastric cancer cells (OCUM-2MLN and OCUM-12), but not in non-scirrhous gastric cancer cells (MKN-45 and MKN-74). Phospho-Smad2 was expressed in the nucleus of scirrhous gastric cancer cells. The overexpression of phospho-Smad2 was observed in scirrhous gastric cancer cells, but not in non-scirrhous gastric cancer cells.

Effects of Ki26894 on Smad2 phosphorylation in gastric cancer cells
To determine whether the small-molecule compound, Ki26894, inhibits TGF-β signalling, the effect of Ki26894 on TGF-β-induced Smad2 phosphorylation was examined in gastric cancer cells. Smad2 phosphorylation was increased by TGF-β1 (10 ng ml⁻¹) in scirrhous gastric cancer cell lines, OCUM-2MLN and OCUM-12. Smad2 phosphorylation was decreased in a dose-dependent manner by Ki26894 from 3 to 10 μM. In contrast, Smad2 phosphorylation was not detected with TGF-β1 treatment in non-scirrhous gastric cancer cell lines, MKN-45 and MKN-74 (Figure 1B, 1C).

Ki26894 reduces the migration of scirrhous gastric cancer cells induced by TGF-β
Figure 2A is a representative phase-contrast photograph of OCUM-2MLN cells. The number of migrating OCUM-2MLN cells was increased by TGF-β1 (10 ng ml⁻¹) and was decreased in the presence of Ki26894 (10 μM). The migrating ability of OCUM-12 cells was increased in the presence of TGF-β1 (Supplementary Movie 1), compared with the control (Supplementary Movie 2). Transforming growth factor-β1 significantly stimulated the migration of OCUM-2MLN (P < 0.001) and OCUM-12 cells (P = 0.004). Ki26894 significantly inhibited the migration-stimulating ability of TGF-β1 in both OCUM-2MLN (P < 0.001) and OCUM-12 cells (P = 0.006). In contrast, neither TGF-β1 nor Ki26894 affected migration by the non-scirrhous gastric cancer cell lines used (Figure 2B).

Effect of Ki26894 on scirrhous gastric cancer cell invasion
Figure 3A is a representative phase-contrast photograph of OCUM-12 cells that have invaded into a 12-μm-pore membrane filter. The number of OCUM-12 cells displaying EMT was significantly increased in the presence of TGF-β1 when compared with the control. The migration-stimulating activity of TGF-β1 was
Effects of TGF-β1 and Ki26894 on the morphological characteristics of gastric cancer cells

Epithelial-to-mesenchymal transition was found in both scirrhous gastric cancer cell lines (OCUM-12 and OCUM-2MLN) in culture with the addition of TGF-β1. An increase in the number of attached and spreading cells was found after the addition of TGF-β1, whereas most of the OCUM-2MLN and OCUM-12 cells without TGF-β1 treatment were still round. OCUM-12 cells exhibited loss of cell–cell adhesion and spindle-shaped cells. However, Ki26894 (10 μM) inhibited these morphological changes associated with EMT in scirrhous gastric cancer cells. In contrast, non-scirrhous gastric cancer cell lines (MKN-45 and MKN-74) did not display these morphological changes after the addition of TGF-β1 (Figure 4).

Effects of Ki26894 on cellular signalling pathways exhibited during migration

Transforming growth factor-β1 significantly upregulated the active form of RhoA in scirrhous gastric cancer cell lines, OCUM-2MLN (P = 0.008) and OCUM-12 (P = 0.007), when compared with control. However, Ki26894 significantly inhibited the active RhoA induced by TGF-β1 in scirrhous gastric cancer cell lines, OCUM-2MLN (P = 0.047) and OCUM-12 (P = 0.029). In contrast, active RhoA was not affected by TGF-β1 or Ki26894 in non-scirrhous gastric cancer cell lines (Figure 5A). Transforming growth factor-β1 (10 ng ml⁻¹) decreased ZO-2 and E-cadherin expression in scirrhous gastric cancer cells and increased myosin light chain-2 phosphorylation (p-myosin), but not in non-scirrhous gastric cancer cells. In addition, Ki26894 increased ZO-2 and E-cadherin expression and inhibited p-myosin in scirrhous gastric cancer cells (Figure 5B).

DISCUSSION

In this study, scirrhous gastric cancer cells expressed T/R-I and T/R-II. Furthermore, TGF-β1 stimulated the phosphorylation of Smad2 in scirrhous gastric cancer cells as previously reported (Komuro et al, 2009), but not in non-scirrhous gastric cancer cells, suggesting that TGF-β signalling is active in scirrhous gastric cancer, but not in non-scirrhous gastric cancer.

Transforming growth factor-β1 stimulated EMT in scirrhous gastric cancer cells, but not in non-scirrhous gastric cancer cells. Epithelial-to-mesenchymal transition is characterised by spindle-shaped cells with a reduction in epithelial cell markers of cell–cell adhesion molecules (Thiery, 2002; Jung et al, 2006; Revenu and Gilmour, 2009). The expression levels of cell–cell adhesion
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molecules, ZO-2 and E-cadherin, were downregulated by TGF-β1 in scirrhous gastric cancer cells. Moreover, TβR signalling activates RhoA, which mediates the upregulation of myosin light chain-2 phosphorylation (Shook and Keller, 2003; Fan et al, 2007) and downregulation of cell–cell tight junctions (Takaishi et al, 1997; Yamazaki et al, 2008). Transforming growth factor-β1 significantly upregulated active RhoA of scirrhous gastric cancer cells. Ras homologue gene family member A might regulate EMT and migrating ability in scirrhous gastric cancer cells. Transforming growth factor-β1 significantly upregulated active RhoA and myosin light chain-2 phosphorylation (Shook and Keller, 2003; Ikeguchi et al, 2009). Previous studies have reported that the phenotypes of metastatic cancer cells are associated with migration ability (Jung et al, 2006; Revenu and Gilmour, 2009). The different response in TGF-β/TβR signalling between scirrhous and non-scirrhous types might explain the poorer prognosis of scirrhous-type gastric cancers compared with non-scirrhous-types of gastric cancers.

Transforming growth factor-β is produced not only by scirrhous gastric cancer cells (Yoshida et al, 1989; Niki et al, 2000) but also by cancer-associated fibroblasts (Mizoi et al, 1993; Inoue et al, 1997; Zeisberg et al, 2007). Our preliminary study recognised that both scirrhous gastric cancer cell lines and gastric fibroblasts produced TGF-β (Yashiro et al, 1996b; Inoue et al, 1997). These
findings suggest that the invasive capacity induced by TGF-β may be influenced in both an autocrine and paracrine manner. Tumour cells in scirrhous carcinoma produce more TGF-β, which is a key mediator of fibroblast activation, than those in non-sicrhous carcinoma (Yoshida et al., 1989; Mahara et al., 1994). Scirrhous gastric carcinoma is characterised by cancer cell infiltration accompanied by extensive stromal fibrosis (Yashiro et al., 1996a). The typical histological findings of rapid infiltration with fibrosis might indicate that the surrounding stromal fibroblasts contribute to cancer progression more intensely in scirrhous gastric cancer than in non-sicrhous gastric cancer.

Specific small molecules designed to inhibit the effects of TGF-β at the level of signalling receptors may be a promising strategy for patients with advanced gastric carcinoma. In this study, a TβR-I inhibitor, Ki26894, displayed inhibitory activity against phosphorylation of Smad2 in scirrhous gastric cancer, suggesting that the small-molecule compound Ki26894 is a potent TβR kinase inhibitor. Migration and invasion stimulated by TGF-β were significantly inhibited by Ki26894 in scirrhous gastric cancer cells. In contrast, the migration and invasion by non-sicrhous cancer cells were not affected by TGF-β1 or by Ki26894. Cell motility and invasion are critical metastatic events in cancer progression (Ehata et al., 2007). Ki26894, a small molecule designed to inhibit TβR, might be a promising therapeutic agent for antagonising the metastatic phenotypes seen through autocrine TGF-β/TβR signalling in scirrhous gastric cancer. We previously reported that the TβR inhibitor, A-77, decreased the expression of integrins in cancer cells, which resulted in a decreased adhesion of scirrhous gastric cancer cells to peritoneum (Kawajiri et al., 2008). Furthermore, Ki26894 inhibited the upregulation of active RhoA and phosphomyosin and inhibited the downregulation of E-cadherin and ZO-2 expression in scirrhous gastric cancer cells. Invasion by scirrhous gastric cancer cells may be inhibited by a TβR inhibitor through regulation of RhoA, myosin, E-cadherin, and ZO-2 expression. These findings suggested that inhibition of the biological effects of TGF-β might affect the adhesion, migration, and invasion of cancer cells and may be an attractive strategy for prevention of distant metastasis by scirrhous gastric cancers.

In conclusion, TGFβ signalling stimulated the EMT and invasion ability of scirrhous gastric cancer cells through regulation of RhoA, myosin, ZO-2, and E-cadherin. Ki26894, which inhibits TβR-I phosphorylation, significantly inhibited invasion by scirrhous gastric cancer cells. The TβR might be a promising target molecule for the treatment of scirrhous gastric carcinoma.
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REFERENCES

Albiní A, Iwamoto Y, Kleinman HK, Martin GR, Aaronson SA, Kozlowski JM, McEwan RN (1987) A rapid in vitro assay for quantitating the invasive potential of tumor cells. Cancer Res 47: 3239 – 3245
Borensztajn K, Stieksma J, Nijmeijer S, Reitsma PH, Peppelenbosch MP, Spek CA (2008) Factor Xa stimulates proinflammatory and profibrotic responses in fibroblasts via protease-activated receptor-2 activation. Am J Pathol 172: 309 – 320
Chang YW, Bean RR, Jakobi R (2009) Targeting RhoA/Rho kinase and p21-activated kinase signaling to prevent cancer development and progression. Recent Patents on Anti-Cancer Drug Discovery 4: 110 – 124
Ehata S, Hanyu A, Fujime M, Katsuno Y, Fukunaga E, Goto K, Ishikawa T, Nomura K, Yokoo H, Shimizu T, Ogata E, Miyazono K, Shimizu K, Imamura T, Miyazono K, Saitoh M (2007) Ki26894, a novel transforming growth factor-beta type I receptor kinase inhibitor, inhibits in vitro invasion and in vivo bone metastasis of a human breast cancer cell line. Cancer Sci 98: 127 – 133
Fan L, Sebe A, Peterfi Z, Masszi A, Thirone AC, Rotstein OD, Nakano H, McCulloch CA, Szaszi K, Mucsi I, Kapus A (2007) Cell contact-dependent regulation of epithelial-myofibroblast transition via the rho-rho kinase-phospho-myosin pathway. Mol Biol Cell 18: 1083 – 1097
Fujihara T, Yashiro M, Inoue T, Sawada T, Kato Y, Ohira M, Nishiguchi Y, Ishikawa T, Sowa M, Chung KH (1999) Decrease in ICAM-1 expression on gastric cancer cells is correlated with lymph node metastasis. Gastric Cancer 2: 221 – 225
Heldin CH, Miyazono K, ten Dijke P (1997) TGF-beta signalling from cell membrane to nucleus through SMAD proteins. Nature 390: 465 – 471
Horiguchi K, Shirakihara T, Nakano A, Imamura T, Miyazono K, Saitoh M (2009) Role of Ras signaling in the induction of snail by transforming growth factor-beta. J Biol Chem 284: 245 – 253
Ikeguchi M, Miyake T, Matsuura T, Yamamoto M, Fukumoto Y, Yamada Y, Fukuda K, Saito H, Tatebe S, Tsujitani S (2009) Recent results of therapy for scirrhous gastric cancer. Surg Today 39: 290 – 294
Inoue T, Chung YS, Yashiro M, Nishimura S, Hasuma T, Otani S, Sowa M (1997) Transforming growth factor-beta1 and hepatocyte growth factor produced by gastric fibroblasts stimulate the invasiveness of scirrhous gastric cancer cells. Jpn J Cancer Res 88: 152 – 159
Jung AC, Ribeiro C, Michaut L, Certa U, Affolter M (2006) Polychaetoid/ZO-1 is required for cell specification and rearrangement during Drosophila tracheal morphogenesis. Curr Biol 16: 1224 – 1231

Figure 5 Effects of Ki26894 on cellular migration signals in gastric cancer cells. (A) ELISA of RhoA. Transforming growth factor-beta (10 ng ml⁻¹) significantly upregulated the active form of RhoA in scirrhous gastric cancer cell lines, OCUM-2MLN and OCUM-12, and Ki26894 (10 μM) significantly inhibited this effect. In contrast, the active form of RhoA was not increased by TGF-beta or by Ki26894 in non-scirrhous cancer cell lines, MKN-45 and MKN-74. (B) Transforming growth factor-beta (10 ng ml⁻¹) increased myosin light chain-2 phosphorylation (p-myosin) and decreased ZO-2 and E-cadherin expression in scirrhous gastric cancer cell lines, but not in non-scirrhous gastric cancer cell lines. Ki26894 (10 μM) decreased p-myosin, and increased ZO-2 and E-cadherin expression in scirrhous gastric cancer cell lines.
Kano MR, Bae Y, Iwata C, Morishita Y, Yashiro M, Oka M, Fuji T, Komuro A, Kiyono K, Kaminishi M, Hirakawa K, Ouchi Y, Nishiyama N, Kataoka K, Miyazono K (2007) Improvement of cancer-targeting therapy, using nanocarriers for intractable solid tumors by inhibition of TGF-beta signaling. Proc Natl Acad Sci USA 104: 3460 – 3465

Kawajiri H, Yashiro M, Oshino O, Nakamura K, Tendo M, Takemura S, Node M, Hamashima Y, Kajimoto T, Sawada T, Ohira M, Hirakawa K (2008) A novel transforming growth factor beta receptor kinase inhibitor, A-77, prevents the peritoneal dissemination of scirrhous gastric carcinoma. Clin Cancer Res 14: 2850 – 2860

Kinugasa S, Abe S, Tachibana M, Hishikawa Y, Yoshinuma H, Monden N, Dhar DK, Nagasue N, Nagaka S (1998) Overexpression of transforming growth factor-beta1 in scirrhous carcinoma of the stomach correlates with decreased survival. Oncology 55: 582 – 587

Komuro A, Yashiro M, Iwata C, Morishita Y, Johansson E, Matsumoto Y, Watanabe A, Aburatani H, Miyoshi H, Kiyono K, Shiraiz YT, Suzuki HI, Hirakawa K, Kano MR, Miyazono K (2009) Diffuse-type gastric carcinoma: progression, angiogenesis, and transforming growth factor beta signaling. J Natl Cancer Inst 101: 592 – 604

Kunisaki C, Shimada H, Nomura M, Matsuda G, Otsuka Y, Ono H, Akiyama H (2005) Therapeutic strategy for scirrhous type gastric cancer. Hepatogastroenterology 52: 314 – 318

Lauren P (1965) The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histoclinical classification. Acta Pathol Microbiol Scand 64: 31 – 49

Mahara K, Kato J, Terui T, Takimoto B, Horimoto M, Murakami T, Migi Y, Watanabe N, Kohgo Y, Niitsu Y (1994) Transforming growth factor beta 1 secreted from scirrhous gastric cancer cells is associated with excess collagen deposition in the tissue. Br J Cancer 69: 777 – 783

Massague J (2008) TGFbeta in cancer. Cell 134: 215 – 230

Mizoi T, Ohtani H, Miyazono K, Miyazawa M, Matsuno S, Nagura H (1993) Immunoelectron microscopic localization of transforming growth factor beta 1 and latent transforming growth factor beta 1 binding protein in human gastrointestinal carcinomas: qualitative difference between cancer cells and stromal cells. Cancer Res 53: 183 – 190

Motoyama T, Hojo H, Watanabe H (1986) Comparison of seven cell lines derived from human gastric carcinomas. Acta Pathol Jpn 36: 65 – 83

Nakazawa K, Yashiro M, Hirakawa K (2003) Keratinocyte growth factor produced by gastric fibroblasts specifically stimulates proliferation of cancer cells from scirrhous gastric carcinoma. Cancer Res 63: 8848 – 8852

Niki M, Toyoda M, Nomura E, Shinhara H, Nakamura M, Nishiguchi K, Tanigawa N (2000) Expression of transforming growth factor beta (TGF-beta) may contribute, in part, to the variations in histogenesis and the prevalence of peritoneal dissemination in human gastric carcinoma. Gastric Cancer 3: 187 – 192

Otsuji E, Kurui Y, Okamoto K, Ochiai T, Ichikawa D, Hagiwara A, Yamagishi H (2004) Outcome of surgical treatment for patients with scirrhous carcinoma of the stomach. Am J Surg 188: 327 – 332

Qiu H, Yashiro M, Shint o O, Matsu zaki T, Hirakawa K (2009) DNA methyltransferase inhibitor 5-aza-CdR enhances the radiosensitivity of gastric cancer cells. Cancer Sci 100: 181 – 188

Revenu C, Gilmour D (2009) EMT 2.0: shaping epithelia through collective migration. Curr Opin Genet Dev 19: 338 – 342

Romero D, Iglesias M, Vary CP, Quintanilla M (2008) Functional blockade of Smad4 leads to a decrease in beta-catenin levels and signaling activity in human pancreatic carcinoma cells. Carcinogenesis 29: 1070 – 1076

Saito H, Tsujitani S, Oka S, Kondo A, Ikeguchi M, Maeta M, Kaibara N (2000) An elevated serum level of transforming growth factor-beta 1 (TGF-beta 1) significantly correlated with lymph node metastasis and poor prognosis in patients with gastric carcinoma. Anticancer Res 20: 4489 – 4493

Shook D, Keller R (2003) Mechanisms, mechanics and function of epithelial-mesenchymal transitions in early development. Mech Dev 120: 1351 – 1383

Takaiishi K, Sasaki T, Kotani H, Nishio S, Takai Y (1997) Regulation of cell-cell adhesion by rac and rho small G proteins in MDCK cells. J Cell Biol 139: 1047 – 1059

Tateishi M, Kusaba I, Masuda H, Tanaka T, Matsumata S, Sugimachi K (2000) The progression of invasiveness regarding the role of transforming growth factor beta receptor type II in gastric cancer. Eur J Surg Oncol 26: 377 – 380

Thiery JP (2002) Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer 2: 442 – 454

Yamazaki Y, Umeda K, Wada M, Nada S, Okada M, Tsukita S, Tsukita S (2008) ZO-1- and ZO-2-dependent integration of myosin-2 to epithelial zonula adherens. Mol Biol Cell 19: 3801 – 3811

Yang L, Moses HL (2008) Transforming growth factor beta: tumor suppressor or promoter? Are host immune cells the answer? Cancer Res 68: 9107 – 9111

Yashiro M, Chung YS, Kubo T, Hato F, Sowa M (1996a) Differential responses of scirrhous and well-differentiated gastric cancer cells to orthotopic fibroblasts. Br J Cancer 74: 1096 – 1103

Yashiro M, Chung YS, Nishimura S, Inoue T, Sowa M (1996b) Fibrosis in the peritoneum induced by scirrhous gastric cancer cells may act as ‘soil’ for peritoneal dissemination. Cancer 77: 1668 – 1675

Yokota T, Kunii Y, Teshima S, Yamada Y, Saito T, Takahashi M, Kikuchi S, Yamauchi H (1999) Clinicopathologic prognostic features in patients with gastric cancer associated with esophageal or duodenal invasion. Ups J Med Sci 104: 217 – 229

Yoshida K, Yokozaki H, Niimoto M, Ito H, Ito M, Tahara E (1989) Expression of TGF-beta and procollagen type I and type III in human gastric carcinomas. Int J Cancer 44: 394 – 398

Zeisberg EM, Potenta S, Xie L, Zeisberg M, Kalluri R (2007) Discovery of endothelial to mesenchymal transition as a source for carcinoma-associated fibroblasts. Cancer Res 67: 10123 – 10128