The role of MMP and E-cadherin in the peritoneal metastasis of Gastric cancer

Thesis by

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The role of MMP and E-cadherin in the peritoneal metastasis of Gastric cancer

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E‑cadherin (E‑CD) germ‑line mutations have recently been described as a molecular basis of early‑onset familial gastric cancer. The loss of E‑cadherin expression or mutation of E‑

cadherin has been associated with accelerated tumorigenesis and progression. However, whether E-cadherin is involved the peritoneal metastasis in gastric cancer is not certain. To assess the role of E-cadherin (Ecad), the cell-cell adhesion protein, in peritoneal dissemination of gastric cancer, it was used human gastric cancer cell lines YCC-3 (Ecad-) and KATOIII (Ecad-) which are not expressed E-cadherin(Ecad). The full length E-cadherin was cloned into pCEP4 and transfected into two human gastric cancer cell line YCC-3 (Ecad-) and KATOIII (Ecad-) and it was isolated stable transfectant clones. Expression of E-cadherin in transfected cell lines was confirmed by RT-PCR and western blot analysis. To explore the peritoneal dissemination of gastric cancer, it was injected wild type cell line and transfectants expressing exogenous E-cadherin into the abdominal cavity of nude mice. These results demonstrated that over-expression of E-cadherin in human gastric cancer cell line YCC-3 (Ecad-) significantly decreased the peritoneal dissemination of gastric cancer. We are now studying the mechanism by which the E-cadherin is involved the peritoneal metastasis of gastric cancer.

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. Introduction

Cell–cell adhesion is an essential component of epithelial morphology and function. Epithelial cells adhere tightly to each other, and several specialized adhesive structures ensure the appropriate integrity and tensile strength of epithelial sheets. These adhesive structures are connected either to intermediate filaments (desmosomes)

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or to microfilaments (adherens junctions and tight junctions). This association with the cytoskeletal network is necessary for stable cell–cell adhesion and for the integration of cell–cell contacts[1]. Among the many types of cell-cell adhesion molecules, cadherins play a critical role in establishing adherens-type junctions by mediating Ca^{2+}-dependent cell-cell adhesion[2,3,4]. Cadherin-based cell-cell adhesion is critically involved in early embryonic morphogenesis, as exemplified by the early embryonic lethality of mice lacking E-cadherin, a prototype cadherin[5,6]. Cadherin-mediated cell-cell adhesion is accomplished by homophilic protein-protein interactions of extracellular cadherin domains in a zipper-like fashion. The intracellular domain of cadherins interacts with various proteins, collectively termed catenins, which assemble the cytoplasmic cell adhesion complex (CCC) that is critical for the formation of extracellular cell-cell adhesion. β-catenin and γ-catenin bind directly to α-catenin, which links the CCC to the actin cytoskeleton. p120[ctn] has been implicated both in cell-cell adhesion and in cell migration[12], and recent studies suggest that p120[ctn] promotes cell migration by recruiting and activating Rho-family GTPases[13].

It has long been known that cell-cell adhesion is dramatically changed during the development of malignant cancer. In particularly, in

most if not all cancers of epithelial origin, E-cadherin-mediated cell-cell adhesion is lost concomitantly with progression towards malignancy, and it has been proposed that the loss of E-cadherin-mediated cell-cell adhesion is a prerequisite for tumour cell invasion and metastasis formation[14]. Multiple mechanisms are found to underlie the loss of E-cadherin function during tumourigenesis: mutations or deletions of the E-cadherin gene itself, mutations in the β-catenin gene, transcriptional repression of the E-cadherin gene. Recent reports have highlighted that the DNA binding protein Snail acts as a strong repressor of E-cadherin gene expression in tumour cells, thus inducing tumour malignancy[15,16,17,18]. The observation that E-cadherin function is frequently lost in malignant cancers prompted an examination of the functional role of E-cadherin in tumour progression[,19,20,21]. Perhaps the strongest evidence in support of a causal role for cadherin alterations in cancer pathogenesis is the observation that germline mutations in the gene encoding E-cadherin strongly predispose affected individuals to diffuse-type gastric cancer[22,23,24,25]. In some kindreds segregating a germline *CDH1* mutation, individuals have been identified with colorectal[23,26], breast[24,25], and prostate cancers[22].

For a tumour cell to metastasize from the primary tumour to

other organs, it must locally degrade the extracellular matrix (ECM) components that are the physical barriers for cell migration. The ECM holds cells together and maintains the three-dimensional structure of the body[27]. It also plays critical roles in cell growth, differentiation, survival and motility[28]. The key enzymes responsible for ECM breakdown are matrix metalloproteinases (MMPs)[29]. ECM degradation by MMPs not only enhances tumour invasion, but also affects tumour cell behaviour and leads to cancer progression[28,29,30,31]. Recent studies on the gastric cancer have demonstrated that the expression of MMP-2 and MMP-9 which can degrade type IV collagen of basement membrane - the first barrier for cancer invasion are elevated in peritoneal disseminated metastasis of gastric cancer in association with low expression of E-cadherin[32, 33,34,35,36]. Furthermore, it has been suggested that the expression of MMP-7 is associated with the formation of peritoneal dissemination in gastric cancer[37].

To elucidate the role of E-cadherin in the formation of peritoneal dissemination in gastric cancer, stable human gastric cancer cell lines expressing human E-cadherin was used. We are now studying the mechanism by which the E-cadherin is involved the peritoneal metastasis of gastric cancer.

. Materials and Methods

1. Materials

Human gastric cancer cell lines MKN-28(80102), MKN-45(80103), NCI-N87 (CRL-5822), AGS (21739), SNU-216(00216), SNU-638(00638), SNU-668(00668), SNU-719(00719) was purchased from KCLB(Korean Cell Line Bank) and Human gastric cancer cell line KATO-III (30103) was purchased from ATCC(USA), and Human gastric cancer cell line YCC-3 was from Cancer Metastasis Research Center in University of Yonsei (Korea). Human E-cadherin cDNA was kindly provided by Dr. David L. Rimm (University of Yale, New Heaven, USA). Lipofectamine^{TM} 2000 was purchased from Invitrogen (USA). Hygromycine B was purchased Invitrogen (USA). Pro-Prep^{TM} Protein Extraction Solution was purchased iNtRON Biotechnology (Korea). Taq polymerase was purchased TaKaRa (Japan). Zymogram gel was purchased Invitrogen (USA). Monoclonal Anti-Human E-cadherin was purchased TaKaRa (Japan).

2. Cell culture and Western Blot.

Human gastric cancer cell line MKN-28, MKN-45, AGS, KATOIII, SNU-216, SNU-638, SNU-668, SNU-719 cell lines were maintained in

RPMI 1640 medium supplemented with 10% FBS, 100 mg/ml streptomycin sulfate, 100 units/ml penicillin G and 250 mg/ml amphotericin B under 5% CO_2 in air at 37° and NCI-N87 cell line was maintained in DMEM medium supplemented with 10% FBS, 100 mg/ml streptomycin sulfate, 100 units/ml penicillin G and 250 mg/ml amphotericin B under 5% CO_2 in air at 37° and YCC-3 cell line was maintained in MEM medium supplemented with 10% FBS, 100 mg/ml streptomycin sulfate, 100 units/ml penicillin G and 250 mg/ml amphotericin B under 5% CO_2 in air at 37°. 70% ~ 80% confluent cells in 100-mm culture plates were incubated at 37°C and were lysed in lysis buffer Pro-Prep^{TM} Protein Extraction Solution. Samples were sonicated 4 times for 5 second each, centrifuged at 10,000g at 4°C for 10 min and the supernatant was collected. The proteins were separated on 10% SDS–polyacrylamide gel and transferred to Immobilon-P membranes (Millipore). The blots were incubated with 5% dried milk powder in TBST (10 mM Tris, pH 8.0, 150 mM NaCl, 0.05% Tween 20; also used for all incubations and washing steps) for 30 min. Next, the blots were incubated for 1h with Monoclonal Anti-Human E-cadherin. The blots were subsequently incubated with anti mouse-IgG antibody(Amersham Life Science NA931). After washing, signals were visualized using the

Enhanced ChemiLuminescence detection system (ECL, Amersham).

3. RNA preparation and RT-PCR

The total cellular RNA from the Human gastric cancer cell lines was isolated by using LiCl RNA extraction buffer. For reverse transcription, 2 μg aliquot of the total RNA was primed with a hexadeoxyribonucleotide of the random primers (Gibco/BRL) and the first strand was synthesized using ImProm-II[TM] Reverse Transcription System(Promega) according to the manufacturer's protocol. The cDNA/mRNA hybrids were amplified with the sense and antisense primers by PCR. PCR amplification was performed for 25 cycles under the following conditions: (*a*) denaturing at 95°C for 1 min; and (*b*) polymerization at 72°C for 1 min. Each annealing condition for amplification of these cDNAs is included.

4. Zymograph

. Gelatin Zymography

NOVEX 4–16% zymogram gelatin gels (Invitrogen, Carlsbad, CA) were used to detect MMP-2 and MMP-9 enzymatic levels. Conditioned Serum Free media was loaded on a gel. The gel was run in Tris/glycine

SDS running buffer under nondenaturing conditions. The gels were washed twice in 2.5% (v/v) Triton X-100 (TX-100) for 30 min at room temperature to remove SDS. Zymograms were subsequently developed by incubation 72 h at 37°C in zymogram developing buffer [0.2 M NaCl, 5 mM CaCl$_2$, 1% Triton X-100 and 0.02% NaN$_3$ in 50 mM Tris-HCl (pH 7.4). Zymograms were stained with Coomassie R-250 for 30 minutes. And then gels were destained with destaining solution. Enzymatic activity was visualized as a clear band against a dark background of stained gelatin.

. Casein Zymography

NOVEX 4–16% zymogram blue casein gels (Invitrogen, Carlsbad, CA) were used to detect MMP-7 enzymatic levels. Conditioned Serum Free media was loaded on a gel. The gel was run in Tris/glycine SDS running buffer under nondenaturing conditions. The gels were washed twice in 2.5% (v/v) Triton X-100 (TX-100) for 30 min at room temperature to remove SDS. Zymograms were subsequently developed by incubation 72 h at 37°C in zymogram developing buffer [0.2 M NaCl, 5 mM CaCl$_2$, 1% Triton X-100 and 0.02% NaN$_3$ in 50 mM Tris-HCl (pH 7.4)]. Enzymatic activity was visualized as a clear band against a dark background of stained gelatin.

5. Stable cell lines

For establishing stable cell lines, Human gastric cancer cell line YCC-3 and KATOIII (2×10^6) were transfected with 4 μg of plasmid pCEP4-hEcad(Hygromycin B) using LipofectAmine[TM] reagent (Invitrogen, USA) according to the manufacturer's instructions. These transfectants were selected by supplementing the medium with 200 μg/ml of Hygromycin B(Invitrogen. USA). Single cell clones were isolated from the bulk transfectants by limited dilution or picking up cloning in 96-well plates, and characterized by Western blot analysis and RT-PCR as described above.

6. *In vivo* tumor growth assay

Male BALB/c-nu/nu mice weighing 20-30g (6-8 weeks old) were maintained in cages with controlled temperature (21 -23). After adapting to the environment for a week, they were used for the following experiments. A total of 1 X 10^7 cells of the YCC-3 cells and stable transfectant cells(YCC-3Ecad) inoculated into the peritoneal cavity of the nude mice. At 4 weeks after inoculation, the mice were sacrificed and the presence of disseminated nodules was evaluated.

Table1. PCR Primers

Gene	Primer	Sequence	bp
Human E-cadherin	5'	5' -TCCATTTCTTGGTCTACGCC-3'	361
	3'	5' -CACCTTCAGCCAACCTGTTT-3'	
α-catenin	5'	5' -GTCATTCAGTAGTCACCTCA-3'	301
	3'	5' -TTCTGACATCAAAATCTTCTGTC-3'	
β-catenin	5'	5' -AAGGTCTGAGGAGCAGCTTC-3'	668
	3'	5' -TGGACCATAACTGCAGCCTT-3'	
Plakoglobin (γ-catenin)	5'	5' -ATGGAGGTGATGAACCTGATGG-3'	284
	3'	5' -CCTGACACACCAGGGCACAT-3'	
MMP-2	5'	5' -CACCTACACCAAGAACTTCC-3'	515
	3'	5' -AACACAGCCTTCTCCTCCTG-3'	
MMP-7	5'	5' -CGACTCACCGTGCTGTGTGCT-3'	680
	3'	5' -TCAGAGGAATGTCCCATACCC-3'	
MMP-9	5'	5' -CCCTTCACTTTCCTGGGTAAG-3'	850
	3'	5' -CATCTTCCCCCTGCCACTCC-3'	
Mt1-MMP	5'	5 - CCCTATGCCTACATCCGTGA -3	572
	3'	5 - TCCATCCATCACTTGGTTAAT-3	
GAPDH	5'	5' -GAAGGTGAAGGTCGGAGTC-3'	226
	3'	5' -GAAGATGGTGATGGGATTTC- 3'	

. Results

1. Western blot analysis of E-cadherin in Human gastric cancer cell lines.

To identify the expression of the E-cadherin in Human gastric cancer cell lines, 10 human gastric cancer cell lines, MKN-28, MKN-45, NCI-N87, AGS, KATOIII, SNU-216, SNU-638, SNU-668, SNU-719, YCC-3 cells were used. To distinguish between the expression of E-cadherin and no expression of E-cadherin in 10 human gastric cancer cell lines, it was assayed by western blot analysis. It was shown that MKN-28 and NCI-N87 expressed E-cadherin at the expected molecular weight of 120 kDa[38]. In particular, it has been reported that the expression of E-cadherin in MKN-28 cell line was in normal form and should be used as positive control in this study[38]. As shown in Figure 1, MKN-28, NCI-N87, SNU-216 and SNU-719 cells highly expressed E-cadherin at 120 kDa. On the other hand, MKN-45, KATOIII, AGS, and SNU-638 cell lines slightly expressed E-cadherin while YCC-3 and SNU-668 cell lines did not expresse E-cadherin. In MKN-45, it was shown as one band at the expected molecular weight of 120 kDa and the second at 80 kDa, which is likely to represent the extracellular tryptic product of E-cadherin[41]. The lymph node metastatic model by gastric cancer cell line MKN-45 was

established by Matsuoka T et al.[42]. It can be associated with the peritoneal metastasis. In KATOIII, it has been reported that two protein bands reactive for E-cadherin were detected[41]. However, In this study, it was hard to find the expression of E-cadherin. In YCC-3, it was not expressed at the expected molecular weight of 120 kDa.
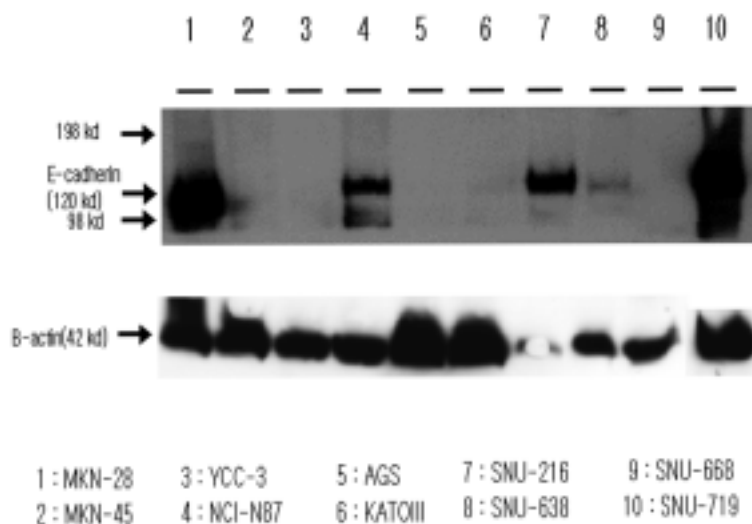

Fig. 1 Western blot analysis of E-cadherin in Human gastric cancer cell lines. 40 ug nuclear extracts from the cell lines were used in the assay.

2. RT-PCR analysis of E-cadherin, α-catenin, β-catenin, and γ-catenin in human gastric cancer cell line.

To explore the intactness of catenins (α-catenin, β-catenin and γ-

catenin) which consist of cadherin-catenin complex[7,8,9], the RT-PCR analysis of E-cadherin, α-catenin, β-catenin, and γ-catenin in 10 human gastric cancer cell lines were performed. E-cadherin mRNA was not expressed in YCC-3 cell line. However, other human gastric cancer cell lines expressed E-cadherin mRNA in various level. Among them, even though two gastric cancer cell lines MKN-45 and KATOIII showed the expression of E-cadherin mRNA, MKN-45 has 18-bp deletion in exon 6-intron 6 boundary in E-cadherin gene[39] and KATOIII cell line also has G to A base substitution of the last 3' nucleotide of exon 7 in E-cadherin gene[39]. Therefore, it might have an influence on the aberrant expression of E-cadherin in protein level[38]. All human gastric cancer cell lines expressed α-catenin while SNU-719 cell line showed small expression . In β-catenin, only NCI-N87 cell line showed weak expression, but others showed normal expression level. In γ-catenin, NCI-N87, SNU-216, and SNU-638 cell lines showed high expression level, but others expressed it normally. Through RT-PCR analysis of E-cadherin and catenines, MKN-45, YCC-3 and KATOIII cell lines which showed absence or aberrant expression E-cadherin(figure 1) and normal expression of α-catenin, β-catenin and γ-catenin normally were chosen for the identification of the role of E-cadherin.
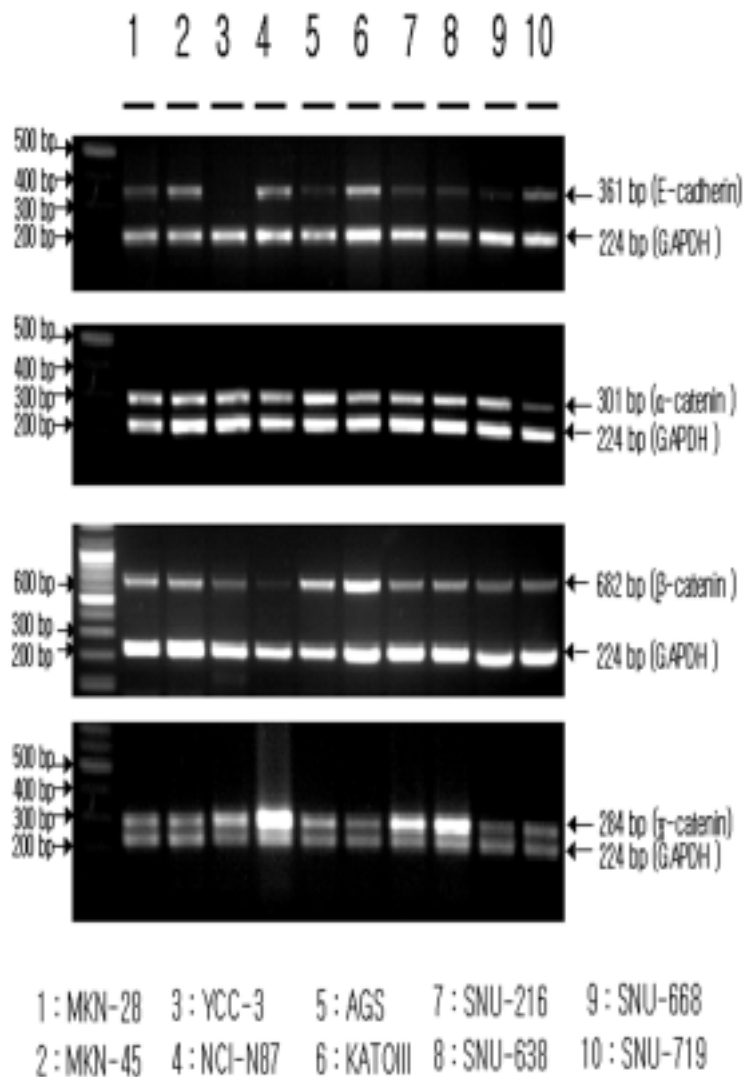
Fig 2. RT-PCR analysis of E-cadherin, α-catenin, β-catenin, and γ-catenin in Human gastric cancer cell lines. E-cadherin, α-catenin, β-catenin, and γ-catenin mRNA expressions in the 10 human gastric cancer cell lines. Total RNA samples were prepared by LiCl extraction buffer. Equal amounts of total RNA from individual samples (2 ug) were used for RT-PCR. After reverse transcription, specific primers for Human E-cadherin, α-catenin, β-catenin,

and γ-catenin were applied to the reaction system. Amplified genes were stained by ethidium bromide. M, molecular marker (100-bp ladder).

3. Expression of invasion candidate genes MMP-2, MMP-7, MMP-9 and Mt1-MMP in human gastric cancer cell lines.

It has been reported that invasion candidate genes MMP-2, MMP-7, MMP-9 and Mt1-MMP are associated with the E-cadherin expression in several papers[32,33,34,35,36,40]. To identify the expression of invasion candidate genes in 10 human gastric cancer cell lines, the direct protein activity was assayed by zymogram. According to its substrate, MMP-2 and MMP-9 are measured by gelatin zymograph and MMP-7 is measured by casein zymograph. All human gastric cancer cell lines expressed MMP-2 and MMP-9 in various levels while SNU-638 and SNU-668 showed the expression of MMP-7. However, it was difficult to identify MMP-7 expression through casein zymograph in human gastric cancer cell lines. In order to identify MMP-2, MMP-7, MMP-9 and Mt1-MMP mRNA levels in each cell lines, it was examined by RT-PCR using specific primers for them (Table 1). As shown in Figure 3 (B), mRNA expressions of MMP-2 and MMP-9 through RT-PCR by using specific primers for them have correlation with protein expression by zymogram. In MMP-7 mRNA level, YCC-3 cell line showed expression but MKN-45 and

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KATOIII showed no expression. In Mt1-MMP mRNA level, MKN-45 showed high expression while YCC-3 and KATOIII showed no expression.

A

| | 1 2 3 4 5 | 6 7 8 9 10 |

proMMP-9 (92 kd) →
active-MMP-9(83 kd) →
proMMP-2 (72 kd) →
active-MMP-2(66 kd) →

pro-MMP-7 (28kd) →
active-MMP-7 (19kd) →

1 : MKN-28   3 : YCC-3   5 : AGS   7 : SNU-216   9 : SNU-668
2 : MKN-45   4 : NCI-N87 6 : KATOIII 8 : SNU-638   10 : SNU-719

B

| | 1 2 3 4 5 6 7 8 9 10 |

500 bp→
200 bp→   ← MMP-2
           ← GAPDH

600 bp→
500 bp→   ← MMP-7
300 bp→
200 bp→   ← GAPDH

1000 bp→   ← MMP-9
500 bp→
200 bp→   ← GAPDH

600 bp→   ← Mt1-MMP
500 bp→
300 bp→   ← GAPDH
200 bp→

1 : MKN-28   3 : YCC-3   5 : AGS   7 : SNU-216   9 : SNU-668
2 : MKN-45   4 : NCI-N87 6 : KATOIII 8 : SNU-638   10 : SNU-719
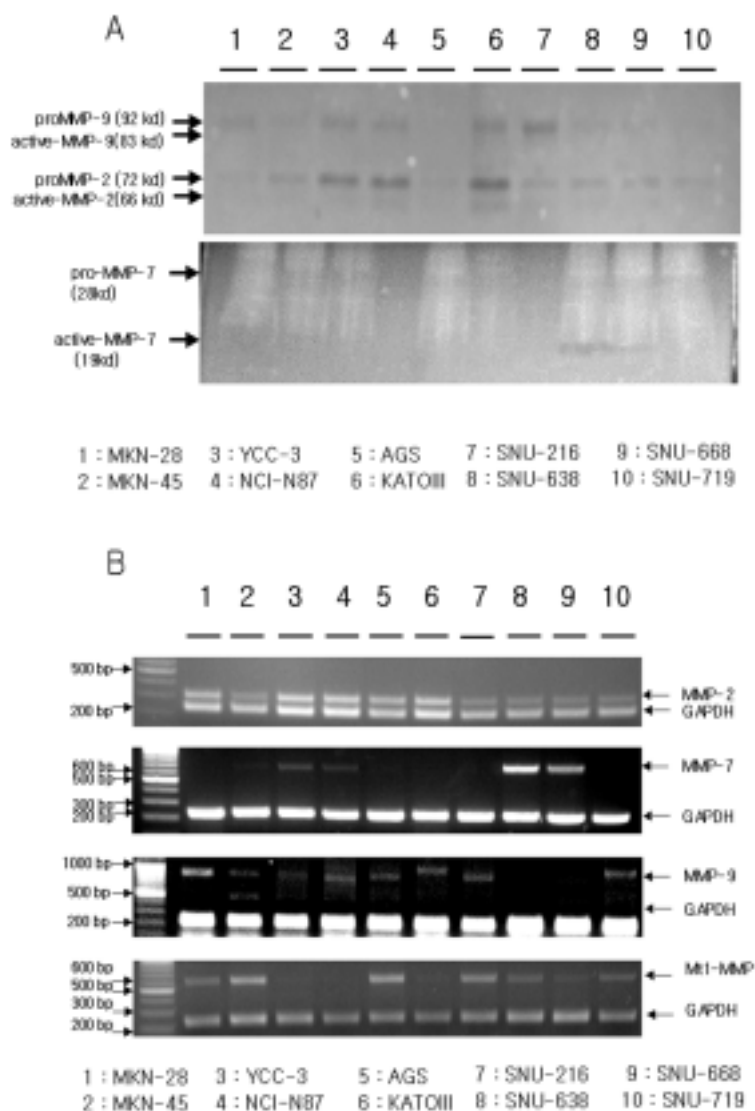
Fig 3. (A) Zymograph of conditioned media samples from human gastric

cancer cell lines. Conditioned media samples (20 µl/lane) were examined directly. Arrows: gelatinolytic activities at Mr 92, 83, 72, and 66 kDa, caseinolytic activities at Mr 19 kDa. **(B) RT-PCR analysis of invasion candidate genes MMP-2, MMP-7, MMP-9 and Mt1-MMP in human gastric cancer cell lines.** Total RNA samples were prepared by LiCl extraction buffer. Equal amounts of total RNA from individual samples (2 ug) were used for RT-PCR

4. Establishment of human E-Cadherin transfetant.

To identify the role of E-cadherin in the peritoneal metastasis in gastric cancer, it was constructed the stable cell lines YCC-3Ecad and KATOIIIEcad respectively. As shown in figure 4, the stable transfectants (YCC-3Ecad and KATOIIIEcad) were confirmed by western blot and RT-PCR. Lane 1 used positive control (MKN-28). Lane 2 is E-cadherin-negative parent cells. Lane 3 is stable transfectants. It has been reported that the effects of E-cadherin expression induce cell adhesion and decrease the cell proliferation in cancer cell line[40]. KATOIII cell line which is a suspension cell line and displays aberrant E-cadherin expression inclines to turn into a adhesion cell line (Figure 4B (*c*)) .
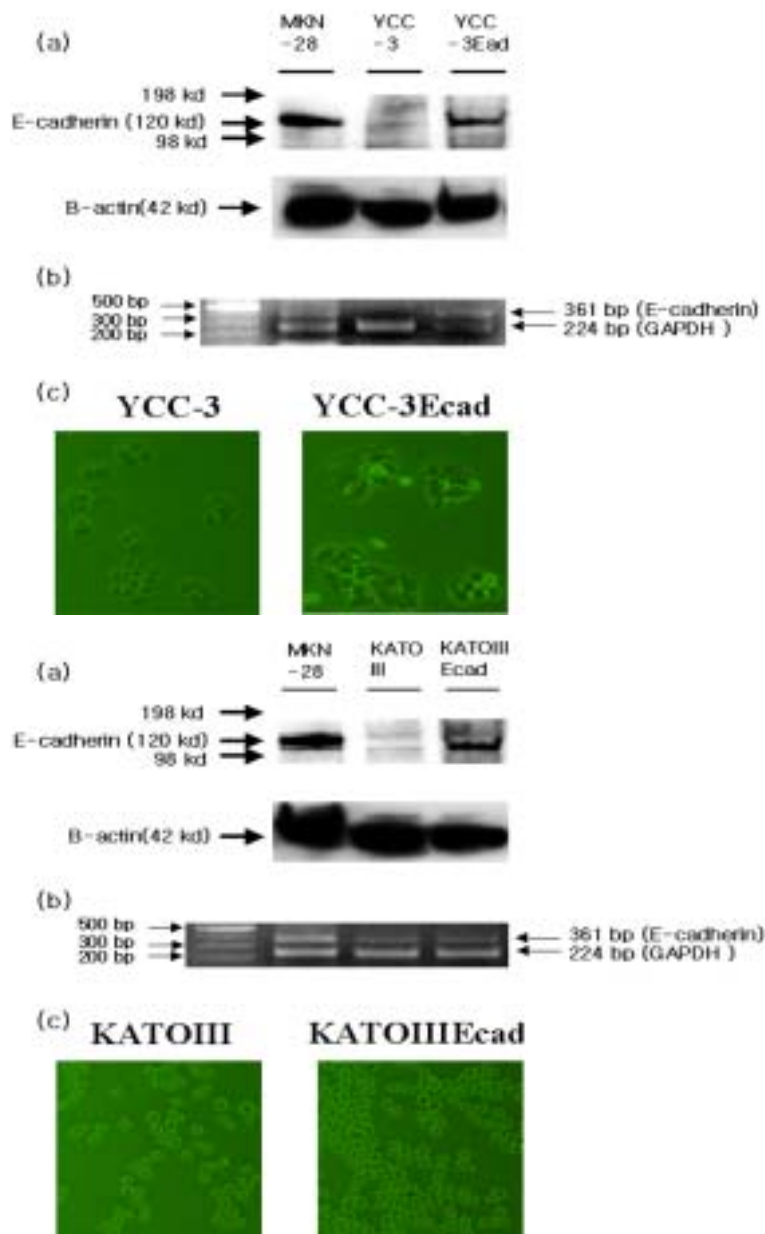
Fig 4. Restoration of E-cadherin expression in Human gastric cancer cell line YCC-3 and KATOIII cell lines. A.(*a,b*) Western blot analysis and RT-PCR analysis of E-cadherin in YCC-3 cell line (*c*) Morphology of YCC-3and YCC-3Ecad cell lines. **B**.(*a,b*) Western blot analysis and RT-PCR analysis of

E-cadherin in Kato3 cell line. (*c*) Morphology of KATOIII and KATOIIIEcad
cell lines.

5. Expression of invasion candidate genes MMP-2, MMP-7 and MMP-9 in stable transfectants YCC-3-Ecad and KATOIIIEcad.

To investigate whether E-cadherin is associated with invasion

candidate genes MMP-2, MMP-7, MMP-9, and Mt1-MMP, it was

studied by Zymogram and RT-PCR analysis. As shown in figure 5

(A), direct protein activity was measured by gelatin zymograph

which can assay MMP-2 and MMP-9 and casein zymograph which

can measure MMP-7. In MMP-2 and MMP-9 protein activity,

parent cell lines (YCC-3 and KATOIII) and stable transfectants

(YCC-3Ecad and KATOIIIEcad) showed no different expressions

them. On the other hand, in MMP-7 protein activity, between

parent cell line YCC-3 and stable transfectant YCC-3Ecad have

different expression of it. However, other parent cell line KATOIII

and stable transfectant KATOIIIEcad have no E-cadherin

expression. In order to check the expression of invasion candidate

genes in mRNA level, it was examined by RT-PCR analysis. As

shown in figure 5 (B), in MMP-7 mRNA level, YCC-3 and YCC-

3Ecad showed different expression of MMP7 while other invasion

candidate genes MMP-2, MMP-9, and Mt1-MMP showed no

difference in expression levels between parent cell lines (YCC-3 and KATOIII) and stable transfectants (YCC-3Ecad and KATOIIIEcad).

A

	YCC-3	YCC-3Ead	KATO III	KATOIII Ecad

pro-MMP-7 (28 kDa) →
active-MMP-7 (19 kDa) →

	YCC-3	YCC-3Ead	KATO III	KATOIII Ecad

proMMP-9 (92 kd) →
proMMP-2 (72 kd) →

B

	YCC-3	YCC-3Ead	KATO III	KATOIII Ecad

300 bp →
200 bp →
← MMP-2
← GAPDH

500 bp →
200 bp →
← MMP-7
← GAPDH

1000 bp →
200 bp →
← MMP-9
← GAPDH
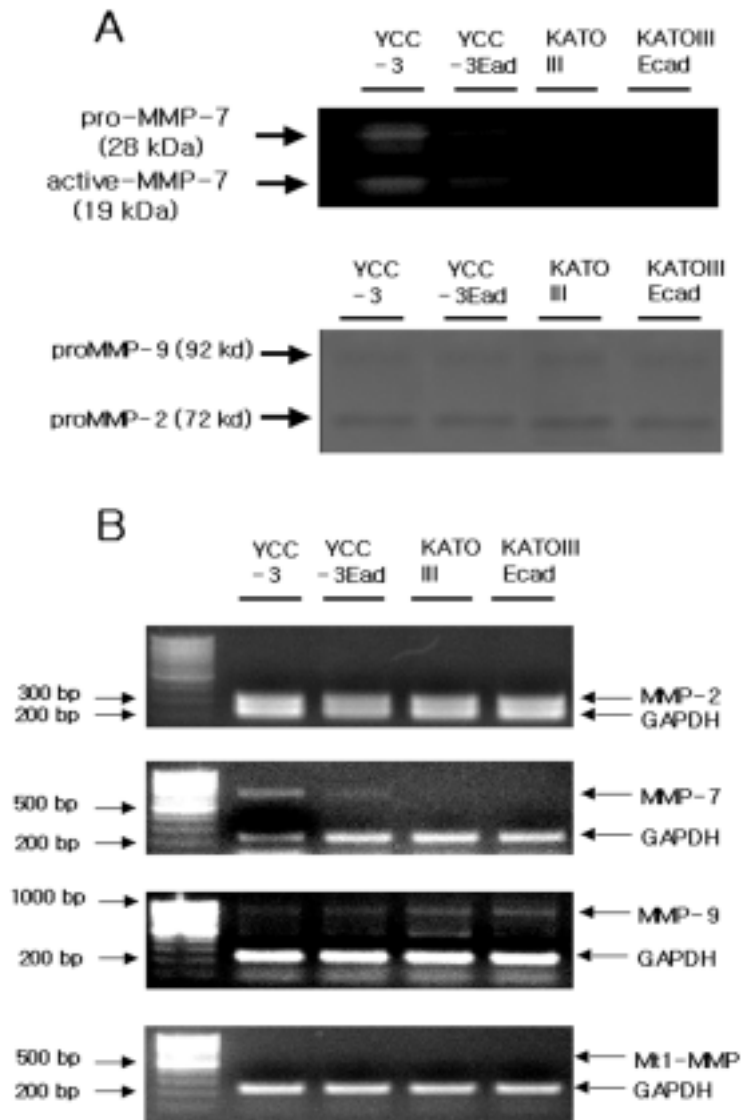
500 bp →
200 bp →
← Mt1-MMP
← GAPDH

Fig 5. Inhibition of MMP-7 expression but MMP-2, MMP-9 by E-cadherin in stable transfectants YCC-3-Ecad. A. Gelatin zymography and casein zymography of invasion candidate genes in protein level and B. RT-PCR analysis of Invasion candidate genes in mRNA level in stable transfectants YCC-3-Ecad and KATOIIIEcad.

6. Suppression of peritoneal metastasis by E-cadherin YCC3-Ecad transfectant

Stable transfectant (YCC-3Ecad) showed a significantly lower level of peritoneal dissemination than parent cell line(YCC-3) . The frequency and the degree of the dissemination was decreased in Human E-cadherin cDNA transfectants in comparison with E-cadherin-deficiency cell line after inoculation of the peritoneal cavity of the nude mice (n=4)(Figure 6)
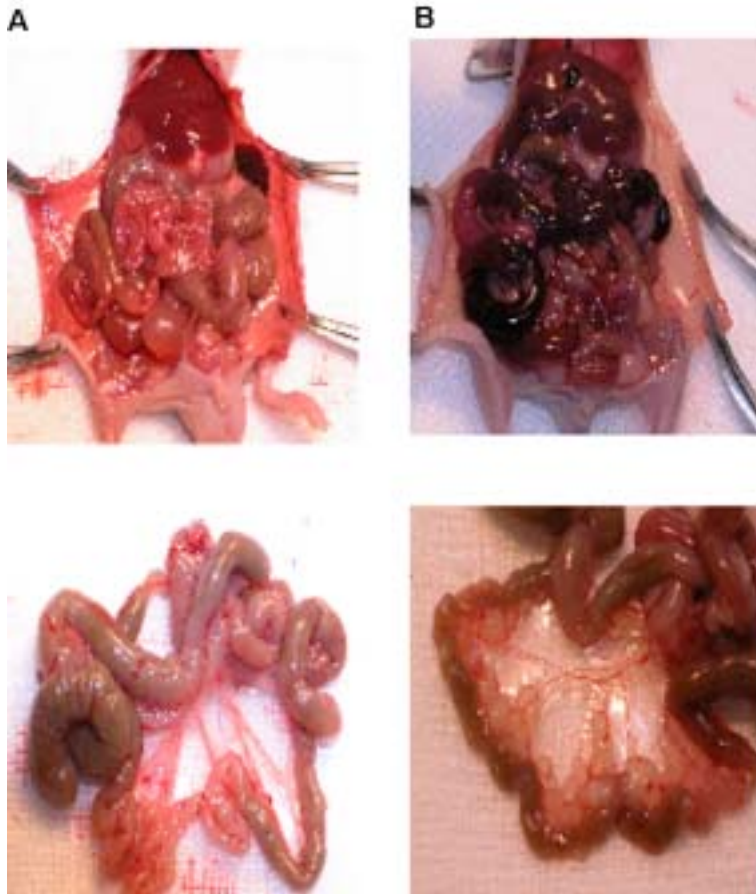
Fig 6. Human gastric cancer cell line YCC-3 and YCC-3Ecad transfectant was injected into the peritoneal cavity of BALB/c-nu/nu mice. Mice were sacrificed 4 weeks later. One of YCC-3Ecad transfectants injected group(A) showed no peritoneal metastases. In contrast, one of parent cell line YCC-3 injected group (B) showed massive peritoneal metastases.

. Discussion

E-cadherin is a cell adhesion molecule normally expressed at adherent junctions between epithelial cells. Recently, it has been reported that E-cadherin(Ecad) germ-line mutations were associated with the early-onset familial gastric cancer[22,23,24,25]. Many studies have demonstrated that the loss of E-cadherin expression or mutation of E-cadherin has been related with accelerated tumorigenesis and progression[22,23,24,25,26]. Although an exact mechanism on the peritoneal metastasis in gastric cancer regulated by E-cadherin is still unclear, some reports suggested that the decreased expression of E-cadherin attenuates the expression of protease and thus cancer cells can invade their environment[44,45,46,47].

10 human gastric cancer cell lines MKN-28, MKN-45, YCC-3, NCI-N87, AGS, KATOIII, SNU-216, SNU-638, SNU-668, SNU-719 were used in order to assess the role of E-cadherin (E-CD), the cell-cell adhesion protein, in peritoneal dissemination of gastric cancer, and was screened which human gastric cancer cell lines among them did not express E-cadherin but had intact catenins(α-catenin, β-catenin, and γ-catenin) using western blot analysis (figure 1) and RT-PCR analysis(figure 2). Many studies demonstrated that MKN-45 and KATOIII gastric cancer

cell lines had aberrant mRNA expression due to E-cadherin(Ecad) mutations while other intracellular molecules such as α-catenin, β-catenin, and γ-catenin connected with E-cadherin are intact in MKN-45 and KATOIII[38].

The overexpression of E-cadherin in human gastric cancer cell line YCC-3 and KATOIII altered the shape of the cell-cell interaction. Compared with parent cell lines, both YCC-3Ecad cell line and KATOIIIEcad cell line showed slightly tight cell-cell contacts and strong cell adhesion (Figure 4(A)(B)).

It was recently demonstrated that the down-regulation of E-cadherin enhances a migratory and invasive phenotype linked to invasion candidate genes such as MMP-2, MMP-7, MMP-9 and Mt1-MMP.[32,33,34,35,36,40]. Many studies demonstrated that the loss of E-cadherin showed increased levels of invasion candidate genes (MMPs), motility *in vitro*, and metastatic potential *in vivo*[32,33,34,35,36,40]. However, it remains to be clarified whether the E-cadherin (CDH1) and invasion candidate genes are associated with the peritoneal metastasis in gastric cancer. Miyaki et al. mentioned that a colon cancer cell line which was transfected with mouse E-cadherin cDNA demonstrated decreased invasiveness with concomitant reduction of 62 kDa gelatinase

expression[45]. Luo et al.   reported that the rat prostate cancer cell lines transfected with mouse E-cadherin cDNA were decreased in MMP-2 activity, thereby being less invasive than their parental cells[47].

The association of E-cadherin with invasion candidate genes in parent cell lines(YCC-3 and KATOIII) and stable transfectants (YCC-3Ecad and KATOIIIEcad) were examined by zymograph analysis and RT-PCR analysis(Figure 3 (A),(B)). The amount of MMP-2, MMP-9 and Mt1-MMP expression and their activities were almost identical among parent cell lines (YCC-3 and KATOIII) and stable transfectants (YCC-3Ecad and KATOIIIEcad) in RT-PCR and Zymogram. These results demonstrated that MMPs, at least MMP-2, MMP-9 and Mt1-MMP, did not affect the invasive phenotype of these cell lines. However, in MMP-7 level, there is a difference between parent cell line (YCC-3) and stable transfectant (YCC-3Ecad). The expression of MMP-7 might be inhibited by E-cadherin in stable transfectant (YCC-3Ecad). Furthermore, it was recently reported that MMP-7 may have a large role in the formation of peritoneal dissemination in gastric cancer[37]. These data strongly suggested that the loss of E-cadherin may be involved in the formation of the peritoneal dissemination in gastric cancer.

In conclusion, these results demonstrated that E-cadherain

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overexpression in YCC-3 gastric cancer cell line demonstrate the reduced potential of peritoneal metastasis. Some mechanisms by which E-cadherin is a metastasis-suppressor gene have been suggested, but further study should be continued.

. Conclusion

In the present study, it is investigated whether invasion candidate genes are associated with E-cadherin expression for the peritoneal metastasis in gastric cancer.

1. The effect of E-cadherin expression in KATOIIIEcad cell line displays the induction of early-time cell adhesion.

2. Human gastric cancer cell line KATOIII injected into the peritoneal cavity of BALB/c-nu/nu mice didn't induce any cancers in nude mice.

3. Invasion candidate gene MMP-7 expression was decreased by E-cadherin using Zymograph and RT-PCR in stable transfectant YCC-3Ecad cell line.

4. E-cadherin overexpression in YCC-3 gastric cancer cell line demonstrates the reduced potential of peritoneal metastasis.

5. Some mechanisms by which E-cadherin is one of the metastasis-suppressor gene, have been suggested, but further study should be continued.

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E- cadherin

(MMP)

(   )

Ca^{2+} cadherin   120

kDa   E- cadherin, P- cadherin, N- cadherin

         .   E- cadherin

                   (adherens junction)   .   E-

cahderin   ,   ,   3

                               cadherin   α- catenin, β-

catenin, γ- catenin   . E- cadherin

germline   mutation

. E- cadherin

(invasion)   (metastasis)   ,

E- cadherin

.   E- cadherin

E- cadherin

E- cadherin

(YCC- 3Ecad),   KATOIIIEcad)   western

blot   RT- PCR   . E- cadherin

(YCC- 3)   E- cadherin

(YCC- 3Ecad)   *In vitro*

In vivo

E-

cadherin   .

:   ,   ,   , E-

cadherin,