

Dominant-negative E-cadherin inhibits the invasiveness of inflammatory breast cancer cells in vitro

Hui-Ming Dong · Gang Liu · Yi-Feng Hou · Jiong Wu ·
Jin-Song Lu · Jian-Min Luo · Zhen-Zhou Shen · Zhi-Ming Shao

Received: 1 February 2006 / Accepted: 20 June 2006 / Published online: 24 August 2006
© Springer-Verlag 2006

Abstract E-cadherin is a transmembrane glycoprotein which mediates epithelial cell-to-cell adhesion function as a tumor suppressor and frequently loss of expression in a wide spectrum of human cancer. However, recent studies demonstrated that E-cadherin was always over-expressed in inflammatory breast cancer (IBC) specimen and cell lines, which is a clinical extreme malignancy of breast cancer. It is hypothesized that the gain and not the loss of the E-cadherin axis contributes to the IBC unique phenotype. To test this assumption, we generated dominant negative mutant E-cadherin high-expression inflammatory breast cancer cells by introduced dominant negative mutant E-cadherin (H-2kd-E-cad) cDNA into human IBC SUM149 cells. Our studies demonstrated that the ability of invasion of SUM149 cells was significantly inhibited by H-2kd-E-cad via down-regulation of MMP-1 and MMP-9 expression. The underlying signal pathway of MAPK phosphorylated Erk 1/2(P44/42) in H-2kd-E-cad-transfected SUM149 cells was significantly down-regulated compared to parental and mock contrast. Our studies provided further functional evidence as the gain of E-cadherin expression dedicated to the IBC malignant phenotype and the blockage of

MAPK/Erk activation maybe a promising therapeutic target.

Keywords E-cadherin · Inflammatory breast cancer · MMP · MAPK

Introduction

The cadherin adhesion system plays an important role during early embryogenesis and in association with the actin cytoskeleton, contributes to morphogenesis and maintenance of tissue architecture in the adult organism (Takeichi 1988; Yap 1998). Importantly, cadherin adhesion receptors have also been shown to serve as tumor invasion suppressors, since loss of adhesion in epithelial carcinoma cells contributes to tumor formation and to the progression of metastasis (Birchmeier and Behrens 1994; Takeichi 1993; Tlsty 1998). In addition, recent findings implicate E-cadherin as an authentic tumor suppressor in both human and mice systems. However, some studies indicated that E-cadherin may contribute to tumor metastasis in inflammatory breast carcinoma (IBC). Inflammatory breast cancer is one of the most aggressive types of human breast cancer tending to exhibit axillary nodal metastases, a high incidence of local and systemic recurrence, and distant metastases at early stage. Recent experimental and clinical studies consistently showed that E-cadherin was expressed highly in inflammatory breast cancer (Alpaugh et al. 1999; Klier et al. 2001). While down-regulated of E-cadherin in IBC tissue xenograft animal model definitely decrease tumorigenity and lymphovascular emboli formation (Tomlinson et al. 2001). It is interesting to know whether the role of E-cadherin in IBC is

Hui-Ming Dong and Gang Liu contributed equally to this work.

H.-M. Dong · G. Liu · Y.-F. Hou · J. Wu · J.-S. Lu ·
J.-M. Luo · Z.-Z. Shen · Z.-M. Shao (✉)
Department of Breast Surgery, Breast Cancer Institute,
Cancer Hospital/Cancer Institute, Fudan University,
270 Dong'An Road, 200032 Shanghai,
People's Republic of China
e-mail: zhimingshao@yahoo.com

idiographic than other carcinomas. So we seek further functional evidence to elucidating this phenomenon and investigate whether E-cadherin truly contributes to the invasion and metastasis in IBC? The use of a dominant-negative mutant cadherin is a powerful way to elucidate the function of cadherin-catenin complexes and to define the molecular mechanisms involved in cadherin functions. It has been proposed that dominant-negative mutant cadherins may act either by competing with the endogenous cadherins for homophilic binding or by binding to catenins, thus depleting catenins available to bind with endogenous cadherins (Amagai et al. 1995; Levine et al. 1994; Zhu and Watt 1996). To understand more detail of the mechanisms by which E-cadherin in regulating cell behavior in IBC, we transfected a dominant-negative mutant E-cadherin into SUM149 cells, a human inflammatory breast cancer cell line expressing E-cadherin. This chimera consisted of the extracellular domain of class I major histocompatibility (MHC) antigen H-2kd and the transmembrane and cytoplasmic domains of mouse E-cadherin (H-2kd-E-cad) (Vizirianakis et al. 2002; Zhu and Watt 1996). Our studies demonstrated that the ability of invasion of SUM149 cells was significantly inhibited by H-2kd-E-cad via down-regulation of MMP-1 and MMP-9 expression. The MAPK Erk1/2 signal pathway was involved in the regulation of MMPs.

Materials and methods

Reagents and cell lines

Dulbecco's modified Eagle's medium, Ham's F-12 medium (DMEM/F12), and fetal bovine serum (FBS) were obtained from Life Technologies, Inc., USA. The plasmid pcDNA3.1 (-) *Myc/His* encoding H-2kd-E-cad dominant-negative E-cadherin construct was kindly provided by Vizirianakis et al. (2002) (Thessaloniki, Greece). Human inflammatory breast cancer cell line SUM149 was a kindly gift from Professor Stephen P. Ethier (van Golen et al. 1999) (Michigan, USA). Cells were routinely cultured in DMEM/F12 medium supplemented with 10% FBS. The cultures were incubated at 37°C in a humidified 5% CO₂ atmosphere.

Antibodies

The following antibodies were used: G-10 (mouse anti-E-cadherin Ab), E-5 (mouse anti- β -catenin Ab), and H-297 (rabbit anti- α -catenin polyclonal antibody) are products of Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Fluorescein isothiocyanate (FITC)-

conjugated mouse anti-H-2kd^d (product of eBioscience). P44/42 MAP kinase antibody, phospho-P44/42 MAP kinase antibody, P38 MAP kinase antibody and phospho-P38 MAP kinase antibody (products of Cell Signal Technology, Inc., USA)

Stable transfection of dominant-negative E-cadherin

The dominant negative mutant E-cadherin cDNA construct (pKSH-2UMTC) encodes 66-kDa chimeric protein which consists of the extracellular part of mouse MHC class I antigen H-2kd [297 amino acids (aa)] linked to a small part of the extracellular region (16 aa) and the entire transmembrane and cytoplasmic domains (175 aa) of mouse E-cadherin (H-2kd-E-cad) (Ozawa et al. 1990). For stable transfection of H-2kd-E-cad dominant-negative E-cadherin construct, SUM149 cells were transfected with the H-2kd-E-cad construct by Lipofectamine2000-mediated transfection (Invitrogen Life Technologies, Inc., Rockville, MD, USA) according to the manufacturer's instructions. G418-resistant colonies were selected in medium with G418 (200 μ g/ml) and expanded. The H-2kd-E-cad-expressing colonies were identified by RT-PCR and Flow cytometry analysis which used fluorescein isothiocyanate (FITC)-conjugated mouse anti-H-2kd. In this study, the clone in which H-2kd-E-cad construct was successfully transfected was named SUM149/ H-2kd-E-cad (two positive clones were selected, named clone1 and clone2, respectively). The one only transfected with G418-resistant gene was named SUM149/mock (mock).

Expression analysis of H-2kd-E-cad transfectants

RNA isolation and RT-PCR analysis Total RNA was isolated from cells using Trizol reagent (Life Technologies, Inc., USA), according to the manufacturer's instructions. In all, 1 μ g of total RNA was converted to cDNA using an avian myeloblastosis virus reverse transcription system (Promega, Madison, WI, USA). A measure of 2.5 μ l out of each RT reaction mixture was applied to 25 μ l of PCR mixture, containing 2.5 U AmpliTaq DNA polymerase (Promega), 1.5 mmol/l MgCl₂, 1 \times Taq buffer and 0.2 mmol/l 4 deoxynucleotide triphosphates (dNTPs). The specific primers of H-2 kd used in the experiment were H-2kd up 5'-ATT-CGCTGAGGTATTTTCGT-3' and H-2kd down 5'-CGCCCTTGCTCTGGTTGT-3' 264 bp; In total, 30 cycles of PCR were carried out with the program of 45 s at 94°C, 30 s at 58°C and 1 min at 72°C. Part of the PCR products were electrophoresed on 1.5% agarose gel and imaged on an Alpha Image 950 documentation system (Alpha Innotech, San Leandro, CA, USA).

Densitometry of images was performed using NIH Image version 1.62. Mouse liver tissues were used as positive control of H-2kd. Total mRNA samples without reverse transcription were used as negative control.

Flow cytometry analysis

Sub-confluent SUM149/ H-2kd-E-cad cells were detached with trypsin/EDTA, and the single-cell suspensions were suspended in medium and incubated with an optimal concentration of anti-mouse H-2kd FITC-conjugated antibody in wash buffer [2% normal goat serum in phosphate-buffered saline (PBS)] for 1 h on ice. The cells were then washed three times, cells were analyzed in a FACscan flow cytometer (Becton-Dickinson Immunocytometry Systems, Palo Alto, CA). SUM149 Cells stained with anti-mouse H-2kd FITC-conjugated antibody or unstained served as controls.

Western blot

Cells were washed twice with ice-cold PBS and scraped into 1 ml of ice-cold NP40 lysis buffer (10 mM Tris-

HCl (pH 8.0), 150 mM NaCl, 1% (v/v) NP40, 1 mM EDTA, 50 mM NaF, 5 mM Na PPI, 1 mM phenylmethylsulfonyl fluoride, 1 mg/ml leupeptin and 1 mg/ml pepstatin A). Cells were then sonicated for 5 s at 5 W. Insoluble debris was removed by centrifugation at 1,000 g for 15 min. Total proteins (50 µg) were analysed by SDS-PAGE. Western blot, using primary monoclonal or polyclonal antibodies was performed according to standard protocols (Shao et al. 1998). In all, 7.5% polyacrylamide gels were used to detect E-cadherin, β-catenin, α-catenin proteins. 12% polyacrylamide gels were used to detect P44/42, P38. The blots were probed with primary antibody and then incubated with a horseradish-peroxidase-labeled secondary antibody. Normalization of protein loading was done with β-actin. Blot quantitation was done with a molecular dynamics laser densitometer (Model PSD) and the Image QuantVersion 1 software.

In vitro assays of invasion

Invasion experiments were conducted with a Matrigel invasion chamber (BD Labware, Bedford, MA, USA)

Table 1 primers for RT-PCR and their annealing temperatures

Cyclin A	Up	5'-TCCATGTCAGTGCTGAGAGGA-3'	45 bp	58°C
	down	5'-GAAGGTCCATGAGACAAGGC-3'		
Cyclin E	Up	5'-AATAGAGAGGAAGTCTGG-3'	443 bp	52°C
	down	5'-AGATATGCAACC TGCATG-3'		
Cyclin D1	Up	5'-CTGGCCATGAACTACCTGGA-3'	482 bp	51°C
	down	5'-GTCACACTTGATCACTCTGG-3'		
P 21	Up	5'-TGTCCTCAGAACCCATG-3'	208 bp	60°C
	down	5'-TGGAAGGTAGAGCTTGG-3'		
c-myc	Up	5'- GCTGCCAAGAGGGTCA -3'	357 bp	55°C
	down	5'- CGCACAAGAGTTCCGTAG-3'		
Rho A	Up	5'-ATCTGGGTGCCTTGTCT-3'	426 bp	55°C
	Down	5'-AAAGCCCTGAAGTGGTG-3'		
Cdc42	Up	5'-CGATGGTGCTGTTGGT-3'	311 bp	57°C
	Down	5'-CCAACAAGCAAGAAAGGA-3'		
Rac1	Up	5'-CATCAAGTGTGTGGTGGTGGG-3'	448 bp	58°C
	Down	5'-TTACAGCACCAATCTCCTTA G-3'		
MMP-1	Up	5'-TTCATTTCTGTTTTCTGGCC-3'	462 bp	52°C
	down	5'-ATTTTCTCTGCAGTTGAACC-3'		
MMP-2	Up	5'-CAGGCTCTTCTCCTTTCACAAC-3'	398 bp	55°C
	down	5'-AAGCCACGGCTTGGTTTTCTC-3'		
MMP-3	Up	5'-CAGTTTGCTCAGCCTATCC-3'	314 bp	58°C
	down	5'-CCTGTATGTAAGGTGGGTTT-3'		
MMP-7	Up	5'-GTTTAG,AAGCCAAACTCAAGG-3'3'	232 bp	55°C
	down	5'-CTTTGACACTAATCGATCCAC-3'		
MMP-9	Up	5'-GGCGCTCATGTACCCTATGT-3'-		
E-cadherin	Up	5'-AGCCATGGGCCCTTGGAG-3'	653 bp	60°C
	Down	5'-CCAGAGGCTCTGTACCTTC-3'		
β-catenin	Up	5'-AAGGTCTGAGGAGCAGCTTC-3'	668 bp	60°C
	Down	5'-TGGCCATAACTGCAGCCTT-3'		
VEGF	Up	5'-CTACCTCCACCATGCCAAGT-3'	311 bp	60°C
	Down	5'-TCTCTCTATGTGCTGGCCT-3'		
GADPH	Up	5'-GGGAGCCAAAAGGGTCATCATCTC-3'	353 bp	60°C
	Down	5'-CCATGCCAGTGAGCTTCCCGTTC-3'		

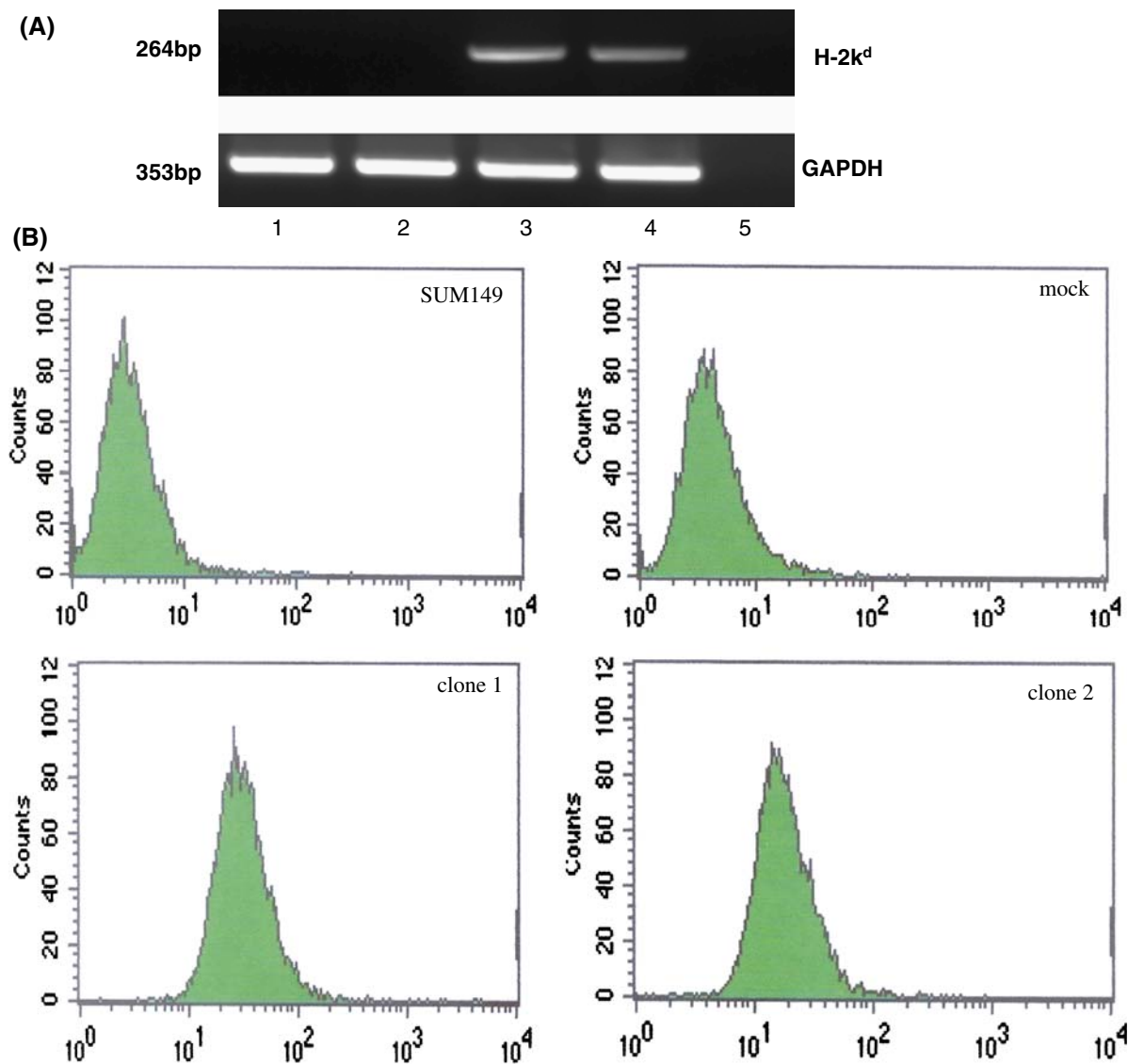


Fig. 1 **a** Expression of H-2k^d was detected by RT-PCR. *Lane 1* SUM149, *lane 2* SUM149-Mock, *lane 3* clone 1: SUM149/H-2k^d1, *lane 4* clone 2: SUM149/H-2k^d2, *lane 5* negative control (total

mRNA sample of SUM149/H-2k^d1 without reverse transcription). **b** Expression of H-2k^d was detected by flow cytometry analysis using FITC-conjugated mouse anti-H-2k^d antibody

(Albini 1998). Each well insert was layered with 120 ml of 1:3 mixture Matrigel:MEM (1,400 mgMatrigel/cm²). An amount of 10⁵ cells were added to the top of this Matrigel layer. The wells were incubated at 37°C for 36 h. Invasion was assessed by counting the cells that had traveled across the filter and were attached to the bottom side of the filter. Then, the filters were fixed in 10% formalin and stained with H E. Cells which had invaded through the Matrigel and reached the lower surface of the filter were counted under a light microscope, at a magnification of 200. Five fields should be counted for each sample. The invasive ability was calculated as following: %invasion = total no. of invading cells (lower well sample)/total number of cells seeded (upper well sample).

RT-PCR for MMPs, cyclin D1, cyclin A, cyclin E, P21, c-myc, RhoA, cdc42 Rac1

We had described the procedure above. Here we just listed specific primers and their annealing temperatures (Table 1).

Gelatin zymography

For gelatin zymography, cell cultures were maintained to 80% confluency in 10% FBS DMEM/F12. Then cells were incubated under serum-free conditions for 24 h. At the time of medium collection, the cells were counted for the purpose of adjusting the volume of the medium to the cell number. MMP9

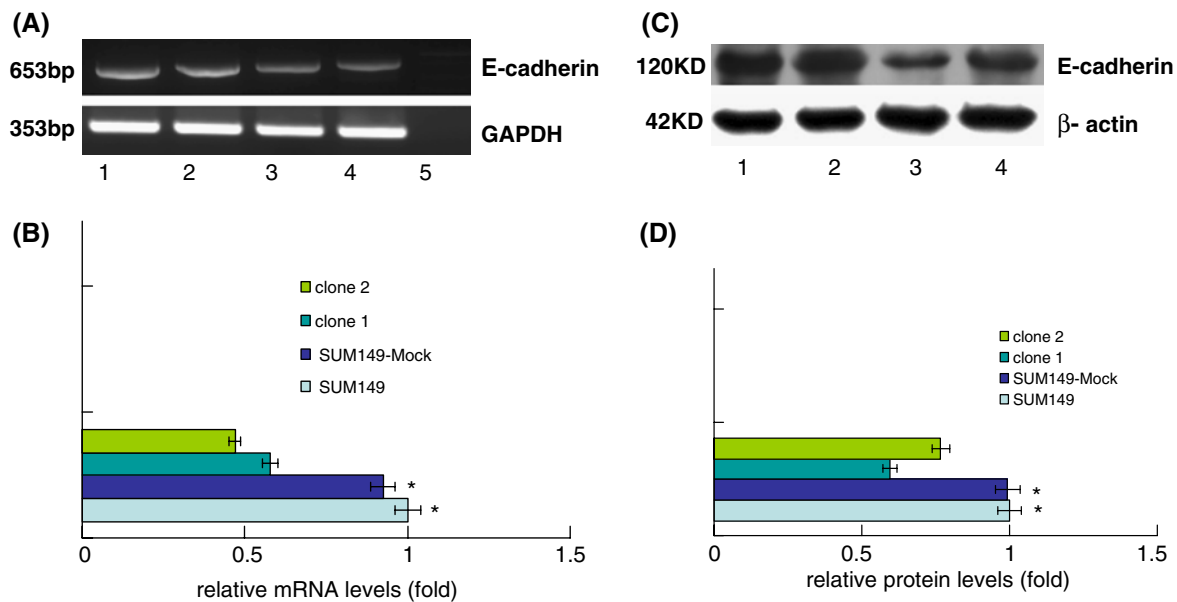


Fig. 2 a. Expression of E-cadherin was detected by RT-PCR. Lane 1 SUM149, lane 2 SUM149-Mock, lane 3 clone 1: SUM149/H-2kd1, lane 4 clone 2: SUM149/H-2kd2, lane 5 negative control (total mRNA sample of SUM149/H-2kd1 without reverse transcription). **b** Representative quantitation of three independent experiments. The values are expressed relative to respective controls, which are given an arbitrary value of 1. The error bars represent the standard errors (* $P < 0.05$).

c Protein levels of E-cadherin were detected by Western blot in SUM149 (Lane 1) SUM149-Mock (lane 2) SUM149/H-2kd1 (lane 3), SUM149/H-2kd² (lane 4). **d** Representative quantitation of three independent experiments. The values are expressed relative to respective controls, which are given an arbitrary value of 1. The error bars represent the standard errors (* $P < 0.05$)

activity was assessed using 10% zymogram gels (BioRad, Richmond, CA, USA). After electrophoresis, gels were rinsed in 2.5% Triton X-100 for 30 min and then enzyme degradation was performed at 37°C for 40 h in 100 mmol/l Tris (pH 7.5), 5 mmol/l CaCl₂ and 0.04% NaN₃. The gels were stained with 2.5% Coomassie Blue and air-dried. Densitometry was used to analyze relative MMP9 activity with NIH Image version 1.62.

Statistical analysis

Analysis of variance and Student's *t*-test were used to determine the statistical significance of differences among experimental groups.

Results

Stable transfection of dominant negative E-cadherin cDNA in SUM149 cells

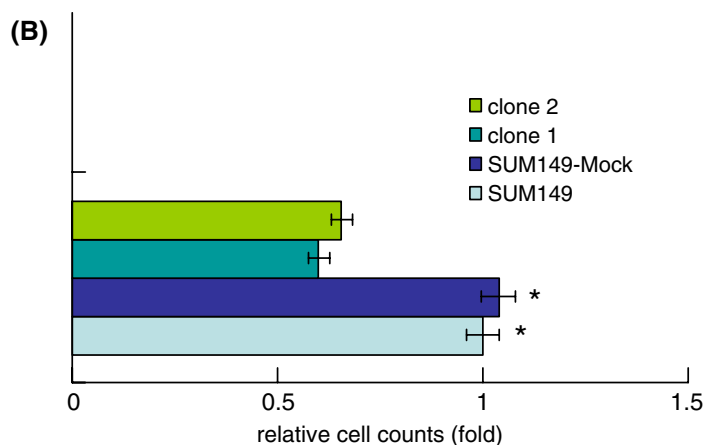
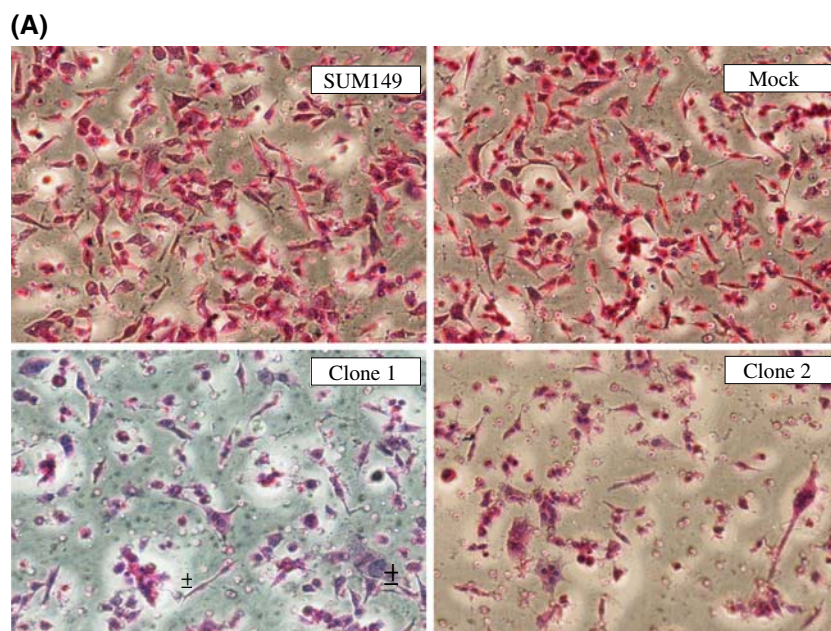
Stable transfectants of the H-2kd-E-cad dominant-negative E-cadherin construct SUM149 cells were established. The construct produces a chimeric protein of 66 kDa that consists of the transmembrane and cyto-

plasmic domains of mouse E-cadherin and the extracellular domain of mouse major histocompatibility antigen H-2kd (Vizirianakis et al. 2002; Zhu and Watt 1996). As a control, SUM149 cells were also transfected with the empty vector pcDNA3.1 (-) Myc/His DNA only (mock). We performed RT-PCR and Flow cytometry analysis, which used fluorescein isothiocyanate (FITC)-conjugated mouse anti-H-2kd antibody. Several randomized selected G418-resistant clones were tested for the integration of the H-2kd-E-cad dominant-negative E-cadherin expression vector into the transfectants. As shown in Fig. 1, two transfectants (clone 1 and clone 2) expressed both H-2kd mRNA and protein which were confirmed by flow cytometry analysis using FITC-conjugated mouse anti-H-2kd antibody.

E-cadherin expression in H-2kd-E-cad-transfected SUM149 cells

We examined the expression of E-cadherin in SUM 149 cells, clone 1, clone 2 and mock cells. As shown in Fig. 2, E-cadherin expression of the two transfected clones were significant decreased both in mRNA and protein levels, compared with parental SUM 149 cells and mock cells ($P < 0.05$)

Fig. 3 a The in vitro invasion assay using the classic Transwell. **b** The quantitation of the invasion ability. The data are representative three independent experiments. The values are expressed relative to respective controls. (* $P < 0.05$)



Down-regulation of E-cadherin expression reduces the invasion and modulation of relevant effector molecules in vitro

It was of interest to investigate whether E-cadherin expression might affect the ability of SUM149 cells' motility. To address this issue, we performed invasion study using the standard established Transwell in vitro assay. As shown in Fig. 3 H-2kd-E-cad-transfected SUM149 clones resulted in a significant reduction of invasion ability compared to mock-transfected and parental cells ($p < 0.05$) To further elucidate the potential mechanisms, we examined the MMPs expression both in mRNA and protein levels. By RT-PCR, H-2kd-E-cad-transfected SUM149 cells constitutively expressed low levels of MMP-9 and MMP-1 mRNA, compared to mock-transfected and wild-type cells (Fig. 4a, b). By direct gelatin zymography, H-2kd-E-

cad-transfected SUM149 cells constitutively excrete low levels of MMP-9, comparatively to the mock-transfected and parental controls (Fig. 4c) ($P < 0.05$). However, we did not find any significant differences of MMP-2, MMP-3 and MMP-7 expression at mRNA level between H-2kd-E-cad-transfected and the mock-transfected and parental controls (Data not shown). Since the MMP-9 was significantly decreased in H-2kd-E-cad-transfected SUM149 cells, we further decided to examine several signal pathways including Erk1/2 (extracellular regulated kinase) and AKT which may involve the MMP-9 regulation. As shown in Fig. 5, the phosphorylated Erk1/2 in H-2kd-E-cad-transfected SUM149 cells was significantly down-regulated when compared to the mock-transfected and wild-type cells ($P < 0.05$). However, we did not find any significant difference of P38 expression between H-2kd-E-cad-transfected and controls (data not shown). Also we

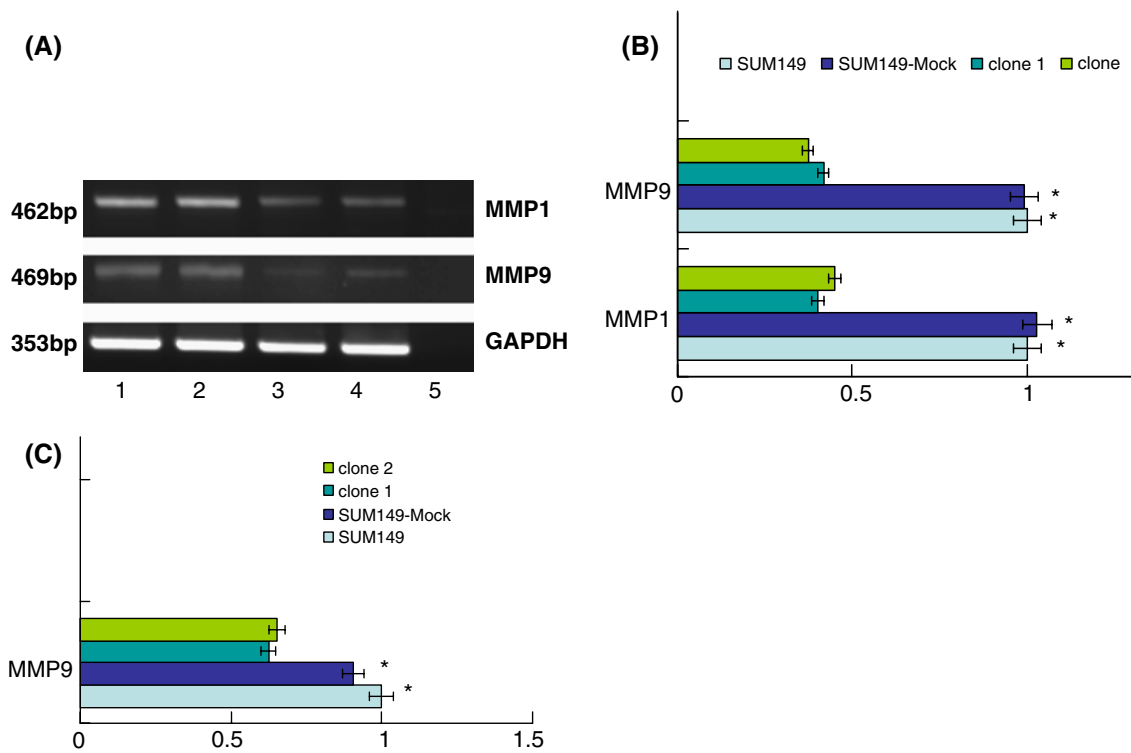


Fig. 4 **a** The expression levels of MMPs was detected by RT-PCR. Lane 1 SUM149, lane 2 SUM149-Mock, lane 3 clone 1: SUM149/H-2kd1, lane 4 clone 2: SUM149/H-2kd2, lane 5 negative control (total mRNA sample of SUM149/H-2kd1 without reverse transcription). **b** Representative quantitation of three independent experiments. The values are expressed relative to respective controls, which are given an arbitrary value of 1. The

error bars represent the standard errors. ($*P < 0.05$) **c** The quantitation of the activity levels of MMP-9 were detected by gelatin zymography. The data are representative three independent experiments. The values are expressed relative to respective controls, which are given an arbitrary value of 1. The error bars represent the standard errors ($*P < 0.05$)

didn't find any regulation of AKT pathway (data not shown). Ets-1 is one of MMP-9 upstream transcriptional factor; we also examined the expression of Est-1 mRNA among H-2kd-E-cad-transfected and control. However, we did not found significant difference of Est-1 mRNA among H-2kd-E-cad-transfected SUM149 cells, mock-transfected and wild-type cells (Data not shown). It has been reported that the pathway of RhoA, cdc42 Rac1 involved in regulation of MMP-9. We examined the expressions of these factors by RT-PCR, and no differences were found among H-2kd-E-cad-transfected SUM149 cells, mock-transfected and wild-type cells (Data not shown). Furthermore, we didn't find significant difference of N-cadherin and P-cadherin expression among H-2kd-E-cad-transfected SUM149 cells, mock-transfected and wild-type cells (Data not shown).

Cells proliferation and relevant effector molecules modulation

To investigate whether E-cadherin expression could modulate the proliferation of SUM149 cells, we used

MTT assay to assess the growth rate of H-2kd-E-cad-transfected SUM149 cells, mock-transfected and wild-type cells. No significant difference was observed among these groups. We also examined the cyclin D1, cyclin A, cyclin E, P21 and c-myc mRNA expression among these groups, no obvious difference was found (data not shown).

Discussion

E-cadherin is a transmembrane glycoprotein mediates calcium-dependent intercellular adhesion specifically involved in epithelial cell to cell adhesion (Takeichi 1988). It has been widely acknowledged that diminished E-cadherin expression related to the acquisition of invasiveness both in experimental tumors and advanced-stage carcinomas among various kinds of human cancer, including ductal carcinomas of the breast (Christofori and Semb 1999; Frixen et al. 1991; Perl et al. 1998; Vleminckx et al. 1991). Several studies have shown that E-cadherin expression is significantly reduced in high-grade, estrogen receptor (ER)-negative,

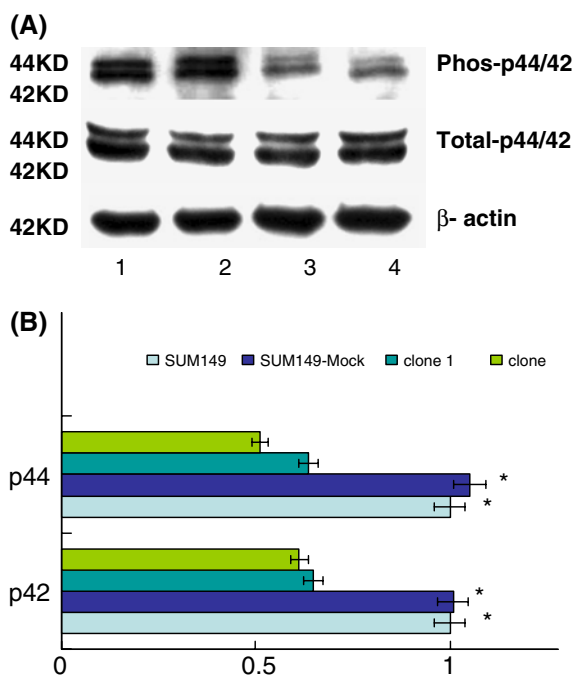


Fig. 5 **a** Protein levels of P44/42 were detected by Western blot. Lane 1 SUM149, lane 2, SUM149-Mock, lane 3 clone 1: SUM149/H-2kd1, lane 4 clone 2: SUM149/H-2kd2. **b** Representative quantitation of three independent experiments. The values are expressed relative to respective controls, which are given an arbitrary value of 1. The error bars represent the standard errors ($*P < 0.05$)

and metastatic breast carcinomas (Hunt et al. 1997; Siitonen et al. 1996). However, some recent studies indicated E-cadherin was prominently over-expressed in inflammatory breast cancer (IBC) compared with stage-matched non-IBC. IBC constitutes less than 10% of all breast cancers as a distinct and aggressive form of locally advanced malignant breast disease. Alpaugh et al. used the first established human transplantable inflammatory breast carcinoma xenograft (MARY-X) in scid/nude mice and verified that E-cadherin is not only 10–20 fold over-expressed but is part of an intact and amplified axis of adhesion. Furthermore, H-2Kd-E-cad-transfected MARY-X spheroids were only weakly tumorigenic and did not form lymphovascular emboli (Alpaugh et al. 1999; Tomlinson et al. 2001). It is an interesting issue whether E-cadherin contributes to IBC phenotype? We utilized stable expression dominant negative E-cadherin mutant transfected SUM149 cells to address this phenomenon and explore the underlying pivotal component. Invasion assay in vitro showed that the invasion ability of the transfected SUM149 cells was obviously inhibited compared to mock-transfected or wild-type SUM149 cells. However, no difference of proliferation index was found between the H-2kd-E-cadherin transfected SUM149

cells and the controls. Our results supported that E-cadherin contributes to the IBC SUM149 cell phenotype and may play an important role in the process of invasion in SUM149 cells.

The degradation of basement membrane and extracellular matrix (ECM) involves the participation of proteolytic enzymes. Matrix metalloproteinases (MMPs) constitute a multi-gene family of over 25 secreted and cell surface enzymes that are able to degrade almost all ECM components. MMP activity is controlled by specific inhibitors named TIMPs (tissue inhibitors of matrix metalloproteinases) (Chang and Werb 2001; Sternlicht and Werb 2001). The over-expression of MMPs, leading to a disruption of the balance between MMPs and TIMPs, has been extensively reported in various carcinoma types. In that way, MMPs have been implicated in early as well as in late stages of cancer progression, in particular in cell growth, invasion, angiogenesis, and metastasis (Chambers and Matrisian 1997; Curran and Murray 1999; Egeblad and Werb 2002; Nawrocki et al. 1997; Park et al. 2004). MMP over-expression is also associated with the acquisition of invasiveness by tumor cells in vitro. In the present study, we demonstrated that the expression of MMP1 and MMP9 in H-2kd-E-cadherin transfected SUM149 cells were significantly down-regulated when compared to the mock-transfected or wild-type cells, which may be responsible for the reduction of invasion ability of H-2kd-E-cadherin transfected SUM149 cells.

There are some data suggesting that the inappropriate expression of non epithelial cadherins, such as N-cadherin and cadherin-11, either in collaboration with or even instead of E-cadherin loss, may play an important role in the invasive and metastatic phenotype of cancer cells. Concomitant up-regulation of N-cadherin and/or cadherin-11 and down-regulation of E-cadherin has been termed cadherin switching. Cadherin switching has been demonstrated in primary tumors, including prostate carcinomas and gastric signet-ring cell carcinomas (Shibata et al. 1996a; Tomita et al. 2000), as well as in several carcinoma-derived cell lines (Islam et al. 1996; Giroldi et al. 1999; Pishvaian et al. 1999; Bussemakers et al. 2000). Interestingly, we did not find any significant differences of α - or β -catenin expression between the H-2kd-E-cadherin transfected SUM149 cells and the controls both at the mRNA and protein levels.

It has been reported that MAPK signal pathway involved in MMPs' expression regulation (Holvoet et al. 2003; Yao et al. 2001). Our studies further demonstrated that the activated phosphor-Erk1/2 (P44/42) expression was remarkably decreased in H-2kd-E-cadherin transfected SUM149 cells compared to the control cells, while the phosphor-P38 level has no

change. Although signal transduction pathways from E-cadherin to RhoA, Rac1, and Cdc42 have been identified recently (Braga 2002; Fukata and Kaibuchi 2001). Our study did not find any differences of these factors at the mRNA levels between the transfected and the controls.

In conclusion, our study firstly provided the functional evidence that E-cadherin contribute to the invasiveness of SUM149 cells and the Erk1/2 signal pathway was involved in the regulation of MMPs. Further investigation is urgently needed to elucidate the underlying molecular spectra and regulators dedicating to the extraordinary biological characteristic of this extreme malignancy.

Acknowledgments We thank Dr. Ioannis S. Vizirianakis and Professor Stephen P. Ethier for the plasmid pcDNA3.1(-)Myc/His and the SUM149 cell line This research was supported in part by grants from the Outstanding Young Investigator Award of National Natural Science Foundation of China (No. 30025015), National Natural Science Foundation of China (30371580) and National Key Project of China (No. 2001BA703BO5), and the Grant from Shanghai Science and Technology Committee (03J14019).

References

- Albini A (1998) Tumor, endothelial cell invasion of basement membranes. The matrigel chemoinvasion assay as a tool for dissecting molecular mechanisms. *Pathol Oncol Res* 4:230–241
- Alpaugh ML, Tomlinson JS, Shao ZM, Barsky SH (1999) A novel human xenograft model of inflammatory breast cancer. *Cancer Res* 59:5079–5084
- Amagai M, Fujimori T, Masunaga T, Shimizu H, Nishikawa T, Shimizu N et al (1995) Delayed assembly of desmosomes in keratinocytes with disrupted classic-cadherin-mediated cell adhesion by a dominant negative mutant. *J Invest Dermatol* 104:27–32
- Birchmeier W, Behrens J (1994) Cadherin expression in carcinomas: role in the formation of cell junctions and the prevention of invasiveness. *Biochim Biophys Acta* 1198:11–26
- Braga VM (2002) Cell–cell adhesion and signalling. *Curr Opin Cell Biol* 14:546–556
- Bussemakers MJ, Van Bokhoven A, Tomita K, Jansen CF, Schalken JA (2000) Complex cadherin expression in human prostate cancer cells. *Int J Cancer* 85:446–450
- Chambers AF, Matrisian LM (1997) Changing views of the role of matrix metalloproteinases in metastasis. *J Natl Cancer Inst* 89:1260–1270
- Chang C, Werb Z (2001) The many faces of metalloproteases: cell growth, invasion, angiogenesis and metastasis. *Trends Cell Biol* 11:S37–43
- Christofori G, Semb H (1999) The role of the cell-adhesion molecule E-cadherin as a tumour-suppressor gene. *Trends Biochem Sci* 24:73–76
- Curran S, Murray GI (1999) Matrix metalloproteinases in tumour invasion and metastasis. *J Pathol* 189:300–308
- Egeblad M, Werb Z (2002) New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer* 2:161–174
- Frixen UH, Behrens J, Sachs M, Eberle G, Voss B, Warda A et al (1991) E-cadherin-mediated cell–cell adhesion prevents invasiveness of human carcinoma cells. *J Cell Biol* 113:173–185
- Fukata M, Kaibuchi K (2001) Rho-family GTPases in cadherin-mediated cell–cell adhesion. *Nat Rev Mol Cell Biol* 2:887–897
- Giroldi LA, Bringuier PP, Shimazui T, Jansen K, Schalken JA (1999) Changes in cadherin–catenin complexes in the progression of human bladder carcinoma. *Int J Cancer* 82:70–76
- Hunt NC, Douglas-Jones AG, Jasani B, Morgan JM, Pignatelli M (1997) Loss of E-cadherin expression associated with lymph node metastases in small breast carcinomas. *Virchows Arch* 430:285–289
- Islam S, Carey TE, Wolf GT, Wheelock MJ, Johnson KR (1996) Expression of N-cadherin by human squamous carcinoma cells induces a scattered fibroblastic phenotype with disrupted cell–cell adhesion. *J Cell Biol* 135:1643–1654
- Kleer CG, van Golen KL, Braun T, Merajver SD (2001) Persistent E-cadherin expression in inflammatory breast cancer. *Mod Pathol* 14:458–464
- Levine E, Lee CH, Kintner C, Gumbiner BM (1994) Selective disruption of E-cadherin function in early xenopus embryos by a dominant negative mutant. *Development* 120:901–909
- Nawrocki B, Polette M, Marchand V, Monteau M, Gillery P, Tournier JM et al (1997) Expression of matrix metalloproteinases and their inhibitors in human bronchopulmonary carcinomas: quantitative and morphological analyses. *Int J Cancer* 72:556–564
- Ozawa M, Ringwald M, Kemler R (1990) Uvomorulin–catenin complex formation is regulated by a specific domain in the cytoplasmic region of the cell adhesion molecule. *Proc Natl Acad Sci USA* 87:4246–450
- Park CH, Lee MJ, Ahn J, Kim S, Kim HH, Kim KH et al (2004) Heat shock-induced matrix metalloproteinase (MMP)-1 and MMP-3 are mediated through ERK and JNK activation and via an autocrine interleukin-6 loop. *J Invest Dermatol* 123:1012–1019
- Pishvaian MJ, Feltes CM, Thompson P, Bussemakers MJ, Schalken JA, Byers SW (1999) Cadherin-11 is expressed in invasive breast cancer cell lines. *Cancer Res* 59:947–952
- Shao ZM, Nguyen M, Alpaugh ML, O’Connell JT, Barsky SH (1998) The human myoepithelial cell exerts antiproliferative effects on breast carcinoma cells characterized by p21WAF1/CIP1 induction, G2/M arrest, and apoptosis. *Exp Cell Res* 241:394–403
- Shibata T, Ochiai A, Gotoh M, Machinami R, Hirohashi S (1996) Simultaneous expression of cadherin-11 in signet-ring cell carcinoma and stromal cells of diffuse-type gastric cancer. *Cancer Lett* 99:147–153
- Siitonen SM, Kononen JT, Helin HJ, Rantala IS, Holli KA, Isola JJ (1996) Reduced E-cadherin expression is associated with invasiveness and unfavorable prognosis in breast cancer. *Am J Clin Pathol* 105:394–402
- Sternlicht MD, Werb Z (2001) How matrix metalloproteinases regulate cell behavior. *Annu Rev Cell Dev Biol* 17:463–516
- Takeichi M (1993) Cadherins in cancer: implications for invasion and metastasis. *Curr Opin Cell Biol* 5:806–811
- Takeichi M (1988) The cadherins: cell–cell adhesion molecules controlling animal morphogenesis. *Development* 102:639–655
- Tlsty TD (1998) Cell-adhesion-dependent influences on genomic instability and carcinogenesis. *Curr Opin Cell Biol* 10:647–653
- Tomita K, van Bokhoven A, van Leenders GJ, Ruijter ET, Jansen CF, Bussemakers MJ, Schalken JA (2000) Cadherin switching in human prostate cancer progression. *Cancer Res* 60:3650–3654

- Tomlinson JS, Alpaugh ML, Barsky SH (2001) An intact overexpressed E-cadherin/alpha,beta-catenin axis characterizes the lymphovascular emboli of inflammatory breast carcinoma. *Cancer Res* 61:5231–5241
- van Golen KL, Davies S, Wu ZF, Wang Y, Bucana CD, Root H et al (1999) A novel putative low-affinity insulin-like growth factor-binding protein, LIBC (lost in inflammatory breast cancer), and RhoC GTPase correlate with the inflammatory breast cancer phenotype. *Clin Cancer Res* 5:2511–219
- Vizirianakis IS, Chen YQ, Katak SS, Tsiftoglou AS, Kramer RH (2002) Dominant-negative E-cadherin alters adhesion and reverses contact inhibition of growth in breast carcinoma cells. *Int J Oncol* 21:135–144
- Vleminckx K, Vakaet L Jr, Mareel M, Fiers W, van Roy F (1991) Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. *Cell* 66:107–119
- Yao J, Xiong S, Klos K, Nguyen N, Grijalva R, Li P et al (2001) Multiple signaling pathways involved in activation of matrix metalloproteinase-9 (MMP-9) by heregulin-beta1 in human breast cancer cells. *Oncogene* 20:8066–8074
- Yap AS (1998) The morphogenetic role of cadherin cell adhesion molecules in human cancer: a thematic review. *Cancer Invest* 16:252–261
- Zhu AJ, Watt FM (1996) Expression of a dominant negative cadherin mutant inhibits proliferation and stimulates terminal differentiation of human epidermal keratinocytes. *J Cell Sci* 109(Pt 13):3013–3023