Oncogene (2006) 25, 6026–6031

www.nature.com/onc

SHORT COMMUNICATION

E-cadherin is a novel transcriptional target of the KLF6 tumor suppressor

A DiFeo[1], G Narla[1,2], O Camacho-Vanegas[1], H Nishio[3], SL Rose[4], RE Buller[4,6], SL Friedman[2], MJ Walsh[3] and JA Martignetti[1,3,5]

[1]Department of Human Genetics, The Mount Sinai School of Medicine, New York, NY, USA; [2]Department of Medicine, The Mount Sinai School of Medicine, New York, NY, USA; [3]Department of Pediatrics, The Mount Sinai School of Medicine, New York, NY, USA; [4]Holden Comprehensive Cancer, Division of Gynecologic Oncology, The University of Iowa Hospitals and Clinics, Iowa City, IA, USA and [5]Department of Oncological Sciences, The Mount Sinai School of Medicine, New York, NY, USA

The tumor suppressor KLF6 is a member of the Krüppel-like family of transcription factors, which has been implicated in the pathogenesis of several human carcinomas. Uncovering the transcriptional targets relevant for its tumorigenic properties, including cellular proliferation and invasion, will be essential to understanding possible mechanisms by which KLF6 and its antagonistic splice form, KLF6-SV1, regulate this development. To begin defining possible metastatic-related pathways, we analysed the effect of KLF6 dysregulation on a recognized suppressor of cellular invasion, E-cadherin. Targeted KLF6 reduction in an ovarian cancer cell line, SKOV-3, resulted in a 50% reduction of E-cadherin expression ($P<0.01$) and conversely, KLF6-SV1 silencing upregulated E-cadherin approximately fivefold ($P<0.0001$). These changes resulted from KLF6 directly transactivating the E-cadherin promoter as demonstrated by luciferase promoter assay and chromatin immunoprecipitation (ChIP). KLF6-mediated changes in E-cadherin levels were accompanied by downstream changes in both the subcellular localization of β-catenin and c-myc expression levels. Moreover, and consistent with these experimental findings, patient-derived epithelial ovarian tumors with low KLF6 and high KLF6-SV1 expression ratios had significantly decreased E-cadherin expression ($P<0.0001$). These combined findings highlight the E-cadherin pathway as a novel and functionally important mediator by which changes in KLF6 and KLF6-SV1 can directly alter ovarian tumor invasion and metastasis.
Oncogene (2006) **25,** 6026–6031. doi:10.1038/sj.onc.1209611; published online 15 May 2006

Keywords: KLF6; KLF6-SV1; E-cadherin; alternative splicing; ovarian cancer

The tumor suppressor KLF6 is a member of the Krüppel-like family of zinc-finger transcription factors, which regulate differentiation and development through their roles in growth-related signal transduction pathways, cell proliferation, apoptosis and angiogenesis (Bieker, 2001; Black *et al*., 2001). KLF6 inactivation was initially demonstrated in prostate cancer (Narla *et al*., 2001; Chen *et al*., 2003) and since then, additional studies have extended the range of human cancers associated with KLF6 inactivation including colorectal (Reeves *et al*., 2004), gastric (Cho *et al*., 2005), lung (Ito *et al*., 2004), hepatocellular (Tal-Kremer *et al*., 2004), nasopharyngeal (Chen *et al*., 2002), astrocytic glioma (Jeng and Hsu, 2003), as well as ovarian (DiFeo *et al*., in press). In addition, two converging lines of evidence suggest that KLF6 status can both define cancer susceptibility and long-term outcome. First, in a multi-institutional study of over 3400 men, we have recently shown that a specific germline KLF6 SNP, regardless of family history of disease, increases lifetime risk of prostrate cancer (Narla *et al*., 2005a). This single nucleotide polymorphism (SNP) increases KLF6 gene alternative splicing to yield the biologically active, growth-promoting isoform KLF6-SV1, which increases tumor cell proliferation, invasion and *in vivo* tumor growth (Narla *et al*., 2005a, b). Second, multiple gene expression studies in different cancers have linked decreased KLF6 expression with tumor recurrence, aggressive behavior, poor clinical outcomes and chemotherapeutic resistance (Singh *et al*., 2002; Glinsky *et al*., 2004; Kettunen *et al*., 2004; MacLeod *et al*., 2005; Stanbrough *et al*., 2006).

One mechanism by which KLF6 suppresses cellular proliferation is through upregulation of p21 in a p53-independent manner in a number of cellular contexts (Narla *et al*., 2001). Tumor-derived KLF6 mutations can abrogate its growth-suppressive effects, at least in part, through a loss of ability to transactivate the p21 gene (Narla *et al*., 2001; Kremer-Tal *et al*., 2004; Reeves *et al*., 2004). Additional pathways by which KLF6 regulates cancer development and progression are emerging and include inhibition of key oncogenic signaling pathways, such as the cyclin-dependent kinase complex CDK4-cyclinD1 (Benzeno *et al*., 2004) and c-jun oncoprotein (Slavin *et al*., 2004) and through an uncharacterized, p53-independent induction of apoptosis (Ito *et al*., 2004). Identifying additional

Correspondence: Dr JA Martignetti, Department of Human Genetics, Mount Sinai School of Medicine, 1425 Madison Ave, Box 1498, New York, NY 10029, USA.
E-mail: john.martignetti@mssm.edu
[6]Current address: Oncology Medicine Development Centre, Glaxo-SmithKline, Collegeville, PA, USA
Received 6 December 2005; revised 13 March 2006; accepted 13 March 2006; published online 15 May 2006

transcriptional and translational pathways regulated by KLF6 and KLF6-SV1 should therefore provide important insights into KLF6/KLF6-SV1-mediated tumorigenic mechanisms.

We recently demonstrated that inhibition of either KLF6 or KLF6-SV1 has dramatic and opposite effects on both ovarian cancer cell growth and *in vivo* tumor dissemination (DiFeo *et al*., manuscript in revision). Specifically, stably silencing KLF6 in SKOV-3 ovarian cancer cells resulted in rapid tumor growth, marked ascites and intraperitoneal dissemination in an *in vivo* mouse model. No viable tumors formed when KLF6-SV1 was silenced before tumor cell implantation. To explore possible underlying molecular mechanisms related to the invasive phenotypic changes, we analysed the effect of KLF6 dysregulation on a recognized suppressor of cellular invasion, E-cadherin. Reduced E-cadherin expression is a critical molecular event contributing to dysregulated cell adhesion, triggering cancer invasion and metastasis (Naora and Montell, 2005). Additionally, E-cadherin loss may result in β-catenin nuclear localization and subsequent upregulation of the transcriptional activities of TCF/LEF DNA-binding factors (Stockinger *et al*., 2001; Conacci-Sorrell *et al*., 2003). In turn, nuclear β-catenin upregulation activates growth-promoting genes, such as c-myc (Stockinger *et al*., 2001). Thus, resulting downstream changes in β-catenin subcellular localization could potentially affect two critical cancer-promoting processes, invasion and proliferation.

First, using SKOV-3 cells, which express low levels of both KLF6 and KLF6-SV1, we stably expressed siRNA to luciferase (si-luc), KLF6 (si-KLF6) and KLF6-SV1 (si-SV1) and examined the effects on E-cadherin expression by quantitative reverse transcription–polymerase chain reation (qtRT–PCR). Strikingly, E-cadherin expression and protein levels were reduced $\sim$50% in si-KLF6 cells ($P<0.01$) and upregulated approximately fivefold in si-SV1 cells ($P<0.0001$) (Figure 1). This effect on E-cadherin expression was not restricted to SKOV-3 cells, and in fact, was present in virtually all cell lines tested from multiple tissue origins including MCF10A (normal mammary epithelial), 293T (embryonic kidney), Hep3B (hepatoma), HCT116 (colon carcinoma), HuH7 (hepatoma), MDA-435 (breast carcinoma) and MDA-453 (breast carcinoma) (data not shown).

Therefore, we next sought to determine whether these differences were a result of direct transcriptional regulation by KLF6. The Kruppel-like family of transcription factors (KLFs) and the related Sp1-like proteins regulate a large number of genes through their direct binding of GC-box promoter elements (Kaczynski *et al*., 2003). Therefore, we generated a series of deletion constructs in the E-cadherin promoter sequentially eliminating five putative GC-box elements that were clustered within four upstream sites (Figure 2). Although the -1000 and -230 nt E-cadherin deletion constructs maintained strong transactivation of the reporter, this effect was lost in the -103 and -37 constructs. These findings strongly suggested that either

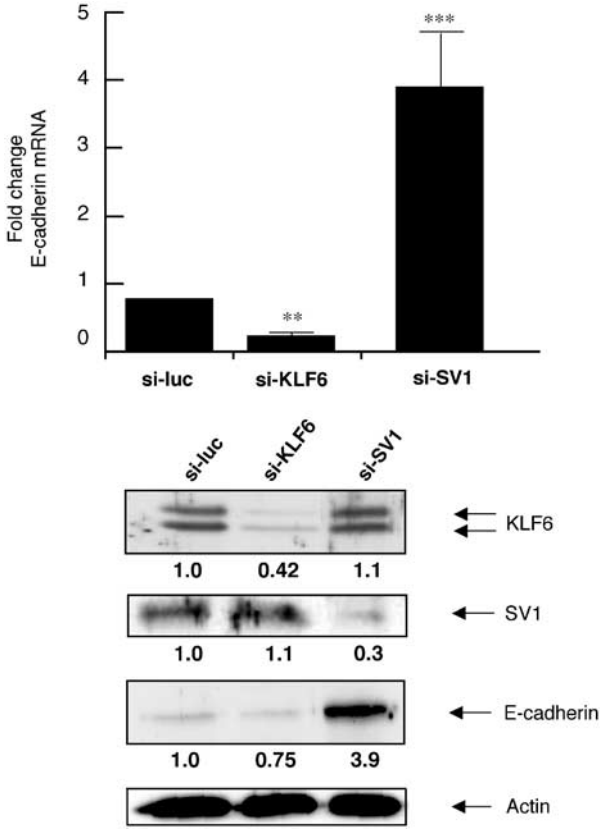


Figure 1 KLF6 and KLF6-SV1 affect E-cadherin expression levels. Quantitative reverse transcription–polymerase chain reaction (RT-PCR) of si-KLF6 and si-SV1 SKOV3 stable cell lines demonstrated that both result in significant changes to E-cadherin levels. Stable cell lines were generated by co-transfection of the pSUPER-si-Luc, pSUPER-si-KLF6 and pSUPER-si-SV1 with a puromycin expression plasmid (Narla *et al*., 2005a, b) and selected with 2 μg/ml of puromycin. Polyclonal pools of each short interfering RNA (siRNA)-infected cell line were collected and the levels of KLF6 and KLF6-SV1 were determined by qRT-PCR and Western blot. RNA isolation, reverse transcription and quantification was performed as previously described (Narla *et al*., 2005b) using E-cadherin-forward: 5′-CAA AGT GGG CAC AGA TGG TGT G-3′ and reverse: 5′-CTG CTT GGA TTC CAG AAA CGG-3′ and GAPDH forward: 5′-CAA TGA CCC CTT CAT TGA CC-3′ and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) reverse: 5′-GAT CTC GCT CCT GGA AGA TG-3′ primers. Expression levels were calculated by normalizing each cDNA to GAPDH and then using this normalized value to calculate fold change. All experiments were performed at least three times and in triplicate. Statistical significance was determined by one-way ANOVA using a Bonferroni correction. For Western blot analysis, cell extracts were harvested in RIPA buffer (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Equal amounts of protein (50 μg; BioRad, Hercules, CA, USA) DC Protein quantification assay, BioRad) were loaded, separated by polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes and probed with KLF6 (SC-7158) actin (SC-1616) and E-cadherin (SC-507) antibodies (Santa Cruz Biotechnology). Enhanced chemiluminescent immunoblot images were analyzed by scanning densitometry and quantified (BIOQUANT NOVA imaging system). Values were expressed as fold change relative to control and normalized to actin.

the -150 and/or -117 GC-box elements, both contained within Site #3, were necessary and sufficient *in vitro* for full KLF6-mediated activation ($P<0.0001$). The

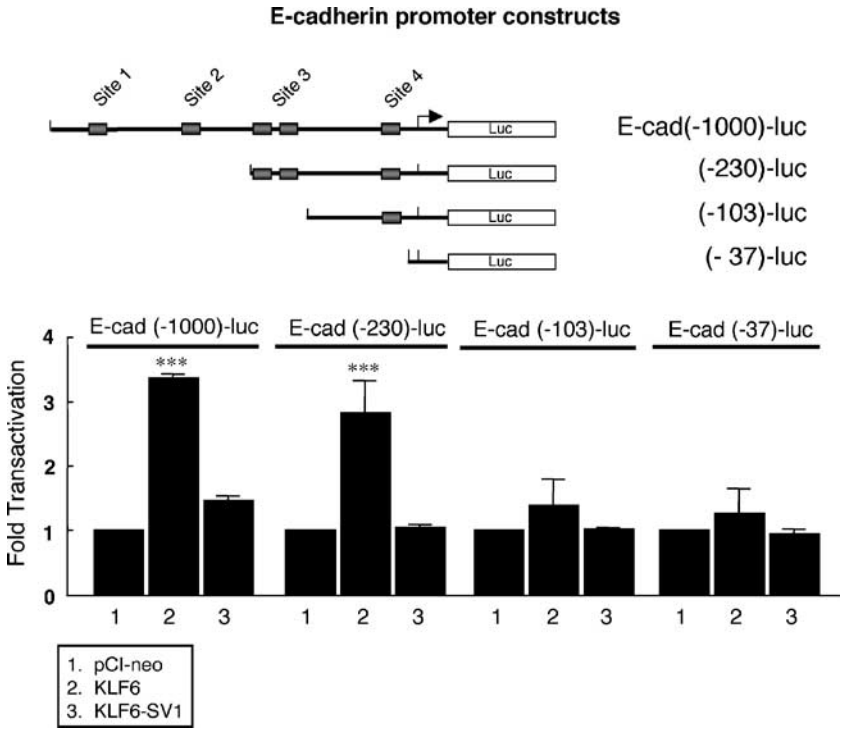


Figure 2 Deletion mapping of the E-cadherin promoter. Luciferase transactivation assays were performed in 293T cells 24 h after transfection of 1.5 μg E-cadherin promoter constructs using 100,000 cells/well in 12-well dishes with either 1.5 μg pCI-neo-KLF6, pCI-neo-KLF6-SV1 or pCIneo-empty vector. The TK promoter-Renilla Luciferase construct (Promega, Madison, WI, USA), 2 ng, was used to normalize each experiment. Luciferase activity was determined for each deletion construct by co-transfection with KLF6 and KLF6-SV1 cDNA expression vectors, and compared to pCiNeo. KLF6 co-transfection with the full-length E-cadherin-luciferase construct (E-cad(-1000)-luc) increased luciferase expression $\sim$3-fold ($P \leqslant 0.0001$).

specificity of these findings was reinforced by the finding that the -37 GC-rich region was insufficient for increasing basal transcription levels by KLF6, unlike previous results demonstrating WT1-mediated transactivation through this motif (Hosono *et al*., 2000) (Figure 2). As predicted, KLF6-SV1 failed to transactivate any of the constructs, as it lacks a DNA-binding domain and is localized to the cytoplasm (Narla *et al*., 2005a) (Figure 2). One explanation for KLF6-SV1-mediated changes in E-cadherin expression (Figure 1) may be owing to a dominant-negative interaction with endogenous KLF6, as suggested previously (Narla *et al*., 2005a, b).

To establish a direct interaction between KLF6 and the native E-cadherin promoter *in vivo*, ChIP analysis was performed. In HeLa cells transfected with a mammalian KLF6 expression vector, KLF6 protein was shown to occupy the GC box elements contained within Sites 3 (-150 and -117 nt) and 4 (-53 nt) (Figure 3). Both these sites are contained within the region required for maximal transcriptional transactivation using the E-cadherin promoter–reporter constructs. (Figure 2). The two most upstream sites failed to demonstrate a detectable interaction; also consistent with the promoter–reporter assays. An additional confirmation of KLF6 binding selectivity was demonstrated by lysine acetylation. Chromatin immunoprecipitation analysis demonstrated that the level of lysine acetylation of histone H3 at lysine 9 (K9), an epigenetic modification directly associated with gene activation, clearly overlaps with KLF6 binding site occupation (Figure 3).

As E-cadherin is a main binding partner of β-catenin and plays a pivotal role in its stabilization, localization and subsequent upregulation of the transcriptional activities of TCF/LEF DNA-binding factors (Stockinger *et al*., 2001; Conacci-Sorrell *et al*., 2003), we next investigated the effect of KLF6-mediated E-cadherin changes on β-catenin subcellular localization. Two complementary methods were used. First, cytosolic β-catenin protein levels were measured following stepwise -separation of cytosolic and nuclear fractions. KLF6 downregulation decreased cytosolic β-catenin fivefold (Figure 4a). Conversely, KLF6-SV1 silencing upregulated cytosolic β-catenin 2.5-fold and there was a concomitant upregulation of nuclear KLF6 (Figure 4a). Second, differences in β-catenin nuclear and cytosolic translocation were also measured by immunocytochemistry. Consistent with cell fractionation findings, si-KLF6 cells displayed twice as many cells with nuclear β-catenin expression ($P < 0.01$) (Figure 4b), whereas it was half as frequent in si-SV1 stable cell lines ($P < 0.01$) (Figure 4b).

We also assayed c-myc expression as a functional end point marker of β-catenin-LEF/TCF activity. In si-SV1 cells, *c-myc* was reduced $\approx$70% (Figure 4a), whereas in si-KLF6 cells, *c-myc* was not increased. This blunted response possibly reflects already highly increased c-myc

expression in certain ovarian cancer cells (Berchuck and Charney, 1997).

Having established the role of KLF6 on E-cadherin expression in multiple cell lines, we next sought to further the physiologic relevance of these findings by examining patient-derived ovarian tumor samples.

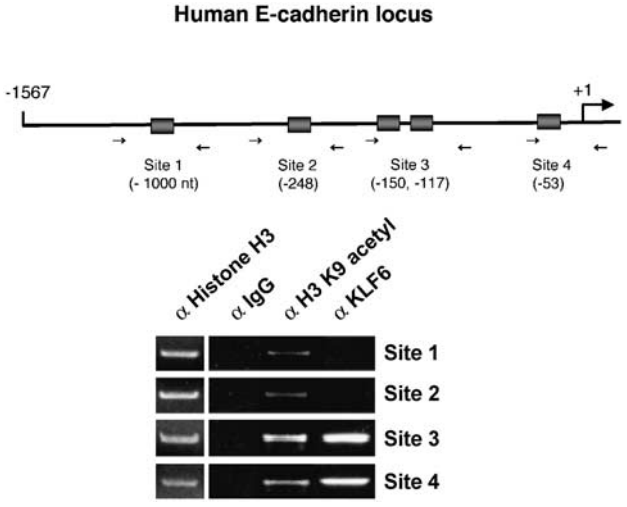
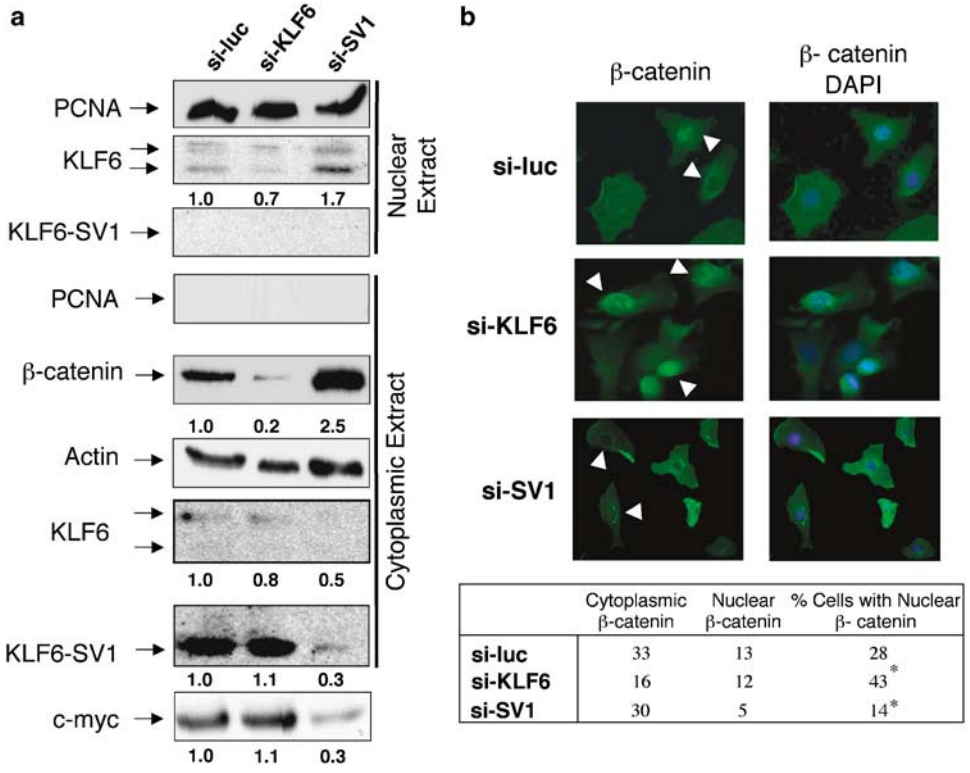


Figure 3 The human E-cadherin promoter and *in vivo* KLF6 binding sites. Polymerase chain reaction primers used for chromatin immunoprecipitation (ChIP) within the E-cadherin locus are indicated as Sites 1, 2, 3 and 4. Rectangles denote relative positions of putative KLF6 target sites. Sites 3 and 4 are only 64 nt apart rendering signal discrimination ambiguous. Chromatin immunoprecipitation analysis was performed as previously described (Ananthanarayanan *et al*., 2004) using antisera against histone H3 and anti-acetyl (K9) histone H3 (Upstate Biotech, Lake Placid, NY, USA). Oligonucleotide primers used: Site 1: forward 5′-GAC TACAGG CGC CCA CCA CCA-3′, reverse 5′-TGT GGG ACT CCC ATA CAA TTA AAA-3′; site 2: forward 5′-GCC CCG ACT TGT CTC TCT ACA A-3′, reverse 5′-TGG AGA TGG GGT CTC ACT CTT TC-3′; Site 3: forward 5′-GTC TTA GTG AGC CAC CGG CGG G-3′, reverse 5′-GTT CAC CTG CCG GCC ACA GCC-3′; Site 4: forward 5′-GCG GTA CGG GGG GCG GT-3′, reverse 5′-ACG CCG AGC GAG GGC AGG CG-3′.



	Cytoplasmic β-catenin	Nuclear β-catenin	% Cells with Nuclear β- catenin
si-luc	33	13	28
si-KLF6	16	12	43*
si-SV1	30	5	14*

Figure 4 *β*-Catenin subcellular localization. (**a**) Subcellular fractionation was used to examine the effect of KLF6 and KLF6-SV1 on *β*-catenin localization. Stepwise separation and preparation of respective SKOV3 stable siRNA cell line cytoplasmic and nuclear extracts was performed (NE-PER kit, Pierce Biotechnology, Rockford, IL, USA). Upon extraction of the two fractions, Western blots were then performed using *β*-catenin, proliferation cell nuclear antigen (nuclear protein marker) and actin (cytoplasmic protein marker) antibodies. In si-KLF6 cells, decreased E-cadherin expression is accompanied by an 80% decrease in *β*-catenin cytoplasmic expression without c-myc level changes. In si-KLF6-SV1 cells, increased E-cadherin is paralleled by a 2.5-fold increase in cytoplasmic *β*-catenin and a 70% decrease in c-myc. (**b**) Changes in *β*-catenin subcellular localization were confirmed by immunocytochemical analysis. SKOV3 stable cell lines were cultured at equal cell density on growth-promoting coverslips (Fisher Scientific, Pittsburgh, PA, USA). After 24 h, cells were washed twice with cold PBS, fixed with ice-cold methanol then acetone and blocked with 5% bovine serum albumin/PBS before incubation with mouse *β*-catenin monoclonal antibody (1:250) (Santa Cruz Biotechnologies, SC-7963) and signal detected with Alexa flour 488 conjugated anti-mouse secondary antibody (Molecular Probes). For nuclear staining, cells were incubated with DAPI (1:5000) (Invitrogen, Carlsbad, CA, USA), washed with PBS 4 × and then mounted on cover slides. Cells with either nuclear or cytoplasmic *β*-catenin were counted from five high-power fields and the percentage of nuclear *β*-catenin was determined.

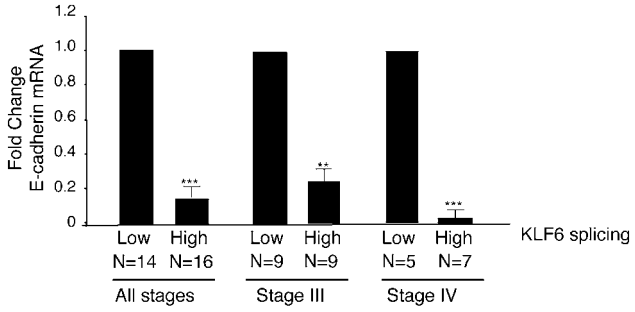


Figure 5 E-cadherin levels correlate with KLF6-SV1/KLF6 ratios. Tumor specimens were collected and analysed under IRB approval. Histologic diagnosis was validated by pathology review at the University of Iowa Institutional Gynecological Oncology Tumor Board and staged in accordance with the International Federation of Gynecology and Obstetrics (FIGO) surgical staging system. Tumor samples were snap-frozen at the time of surgery in liquid nitrogen. KLF6-SV1/KLF6 splicing ratio was approximated by quantitative reverse transcription–polymerase chain reaction (qtRT-PCR) using the following PCR primers on an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA): KLF6 forward: 5′-CGG ACG CAC ACA GGA GAA AA-3′ and reverse: 5′-CGG TGT GCT TTC GGA AGT G-3′; total KLF6 forward: 5′-CTG CCG TCT CTG GAG GAG T-3′ and reverse: 5′-TCC ACA GAT CTT CCT GGC TGT C-3′. Fold change was calculated by dividing the fold changes in total KLF6 (KLF6 + alternatively spliced KLF6 transcripts) by that in KLF6 alone. Expression levels were normalized and statistical significance determined as described in legend to Figure 1.

We recently demonstrated that changes in KLF6 and KLF6-SV1 expression levels are present in a majority of ovarian tumors and can affect tumor growth, invasion and dissemination (DiFeo *et al*., in press). Samples were binned according to their KLF6-SV1/KLF6 expression pattern. Samples with decreased KLF6 and increased KLF6-SV1 expression have a high KLF6-SV1/KLF6 ratio. As the expression of KLF6 increases or KLF6-SV1 decreases, the KLF6-SV1/KLF6 ratio decreases. In accordance with these findings, papillary serous tumors with a high KLF6-SV1/KLF6 ratio expression pattern ($n = 16$) were associated with an approximately 80% reduction in E-cadherin expression compared to those samples with low levels ($n = 14$) ($P < 0.0002$) (Figure 5). As E-cadherin decreases are believed to contribute to EOC development and progression (Frixen *et al*., 1991; Vleminckx *et al*., 1991), it is particularly relevant that stage IV tumors with a high KLF6-SV1/KLF6 splicing ratio had minimal E-cadherin expression ($P < 0.0001$). Similarly, stage III tumors with high KLF6-SV1/KLF6 splicing ratio had approximately 80% lower E-cadherin levels ($P < 0.0004$) (Figure 5).

Taken together, our findings define E-cadherin as a novel KLF6 transcriptional target and suggest that KLF6-SV1/KLF6 regulation of this critical suppressor of cellular invasion is one mechanism by which KLF6-SV1/KLF6 contributes to ovarian cancer pathogenesis. KLF6 transcriptionally regulates a number of essential proliferation and differentiation genes, including $p21^{(CIP1/Waf1)}$ (Narla *et al*., 2001), transforming growth factor-β1, type I and II receptors (Kim *et al*., 1998) and the insulin-like growth factor I receptor (Rubinstein *et al*., 2004). Our combined findings, both in cultured cells and patient-derived ovarian cancer samples, now suggest that changes in tumor proliferation and invasion may, in part, be functionally linked through the action of KLF6 and KLF6-SV1 on E-cadherin. Indeed, loss of E-cadherin expression is proposed to be a central event in enhancing metastasis from ovarian tumors (Frixen *et al*., 1991; Vleminckx *et al*., 1991) and is associated with decreased patient survival (Gabbert *et al*., 1996). In turn, increased nuclear β-catenin may activate growth promoting genes as demonstrated by c-myc upregulation in cultured cells and si-KLF6 tumor xenografts (DiFeo *et al*., in press). As previously demonstrated in a mouse mammary epithelial cell system, β-catenin mislocalization ultimately affects cellular proliferation (Stockinger *et al*., 2001). Interestingly, changes in KLF6-SV1 levels which decrease cellular proliferation also upregulate E-cadherin expression and result in β-catenin's cytoplasmic sequestration. In summary, these findings define E-cadherin regulation by KLF6 and KLF6-SV1 as a potentially critical pathway linking together KLF6 and KLF6-SV1's dual effects on both proliferation and invasion in ovarian cancer.

Acknowledgements

This research was financially supported by a Grant, JAM: DAMD17-03-10129.

References

Ananthanarayanan M, Li S, Balasubramaniyan N, Suchy FJ, Walsh MJ. (2004). *J Biol Chem* **279**: 54348–54357.
Benzeno S, Narla G, Allina J, Cheng GZ, Reeves HL, Banck MS *et al*. (2004). *Cancer Res* **64**: 3885–3891.
Berchuck A, Charney M. (1997). *Biochem Pharmacol* **54**: 541–544.
Bieker JJ. (2001). *J Biol Chem* **276**: 34355–34358.
Black AR, Black JD, Azizkhan-Clifford J. (2001). *J Cell Physiol* **188**: 143–160.
Chen C, Hyytinen ER, Sun X, Helin HJ, Koivisto PA, Frierson HF *et al*. (2003). *Am J Pathol* **162**: 1349–1354.
Chen HK, Liu XQ, Lin J, Chen TY, Feng QS, Zeng YX *et al*. (2002). *Ai Zheng* **21**: 1047–1050.
Cho YG, Kim CJ, Park CH, Yang YM, Kim SY, Nam SW *et al*. (2005). *Oncogene* **24**: 4588–4590.
Conacci-Sorrell M, Simcha I, Ben-Yedidia T, Blechman J, Savagner P, Ben-Ze'ev A. (2003). *J Cell Biology* **163**: 847–857.
DiFeo A, Narla G, Hirsnfeld J, Camacho-Vanegas O, Narla J, Rose S *et al*. (2006). *Clin Cancer Res* (in press).
Frixen UH, Behrens J, Sachs M, Eberle G, Voss B, Warda A *et al*. (1991). *J Cell Biol* **113**: 173–185.
Gabbert HE, Mueller W, Schneiders A, Meier S, Moll R, Birchmeier W *et al*. (1996). *Int J Cancer* **69**: 184–189.
Glinsky GV, Glinskii AB, Stephenson AJ, Hoffman RM, Gerald WI. (2004). *J Clin Invest* **113**: 913–923.
Hosono S, Gross I, English MA, Hajra KM, Fearon ER, Licht JD *et al*. (2000). *J Biol Chem* **275**: 10943–10953.
Ito G, Uchiyama M, Kondo M, Mori S, Usami N, Maeda O *et al*. (2004). *Cancer Res* **64**: 3838–3843.

Jeng YM, Hsu HC. (2003). *Int J Cancer* **105**: 625–629.
Kaczynski J, Cook T, Urrutia R. (2003). *Genome Biol* **4**: 206.
Kettunen E, Anttila S, Seppanen JK, Karjalainen A, Edgren H, Lindstrom I *et al*. (2004). *Cancer Genet Cytogenet* **149**: 98–106.
Kim Y, Ratziu V, Choi SG, Lalazar A, Theiss G, Dang Q *et al*. (1998). *J Biol Chem* **273**: 33750–33758.
Kremer-Tal S, Reeves HL, Narla G, Tnung SN, Schwartz M, DiFeo A *et al*. (2004). *Hepatology* **40**: 1047–1052.
Macleod K, Mullen P, Sewell J, Rabiasz G, Lawrie S, Miller E *et al*. (2005). *Cancer Res* **65**: 6789–6800.
Naora H, Montell DJ. (2005). *Nat Rev Cancer* **5**: 355–366.
Narla G, DiFeo A, Reeves HL, Schaid DJ, Hirshfeld J, Hod E *et al*. (2005a). *Cancer Res* **65**: 1213–1222.
Narla G, DiFeo A, Yao S, Banno A, Hod E, Reeves HL *et al*. (2005b). *Cancer Res* **65**: 5761–5768.
Narla G, Heath KE, Reeves HL, Li D, Giono LE, Kimmelman AC *et al*. (2001). *Science* **294**: 2563–2566.
Reeves HL, Narla G, Ogunbiyi O, Haq AI, Katz A, Benzeno S *et al*. (2004). *Gastroenterology* **126**: 1090–1103.
Rubinstein M, Idelman G, Plymate SR, Narla G, Friedman SL, Werner H *et al*. (2004). *Endocrinology* **145**: 3769–3777.
Singh D, Febbo PG, Ross K, Jackson DG, Manola J, Ladd C *et al*. (2002). *Cancer Cell* **2**: 203–209.
Slavin DA, Koritschoner NP, Prieto CC, Lopez-Diaz FJ, Chatton B, Bocco JL *et al*. (2004). *Oncogene* **23**: 8196–8205.
Stanbrough M, Bubley GJ, Ross K, Golub TR, Rubin MA, Penning TM *et al*. (2006). *Cancer Res* **66**: 2815–2825.
Stockinger A, Eger A, Wolf J, Beug H, Foisner R. (2001). *J Cell Biol* **154**: 1185–1196.
Tal-Kremer S, Reeves HL, Narla G, Thung SN, Schwartz M, DiFeo A *et al*. (2004). *Hepatology* **40**: 1047–1052.
Vleminckx K, Vakaet Jr L, Mareel M, Fiers W, van Roy F *et al*. (1991). *Cell* **66**: 107–119.