DEDD Interacts with PI3KC3 to Activate Autophagy and Attenuate Epithelial–Mesenchymal Transition in Human Breast Cancer

Qi Lv, Wei Wang, Jianfei Xue, Fang Hua, Rong Mu, Heng Lin, Jun Yan, Xiaoxi Lv, Xiaoguang Chen, and Zhuo-Wei Hu

Abstract

Epithelial-to-mesenchymal transition (EMT), a crucial developmental program, contributes to cancer invasion and metastasis. In this study, we show that death-effector domain-containing DNA-binding protein (DEDD) attenuates EMT and acts as an endogenous suppressor of tumor growth and metastasis. We found that expression levels of DEDD were conversely correlated with poor prognosis in patients with breast and colon cancer. Both in vitro and in vivo, overexpression of DEDD attenuated the invasive phenotype of highly metastatic cells, whereas silencing of DEDD promoted the invasion of nonmetastatic cells. Via direct interaction with the class III PI3-kinase (PI3KC3)/Beclin1, DEDD activated autophagy and induced the degradation of Snail and Twist, two master regulators of EMT. The DEDD–PI3KC3 interaction led to stabilization of PI3KC3, which further contributed to autophagy and the degradation of Snail and Twist. Together, our findings highlight a novel mechanism in which the intracellular signaling protein DEDD functions as an endogenous tumor suppressor. DEDD expression therefore may represent a prognostic marker and potential therapeutic target for the prevention and treatment of cancer metastasis. Cancer Res; 72(13): 3238–50. ©2012 AACR.

Introduction

Metastasis is the spread of cancer cells to other parts of the body and metastatic tumor is primarily responsible for most cancer deaths (1). Despite the mechanism of tumor metastasis remains most enigmatic in the field of cancer biology, new concepts and mechanistic insights into the process of tumor metastasis have emerged from recent advances (2, 3). For instance, the epithelial-to-mesenchymal transition (EMT), an essential cell-biological program during embryonic development, plays a critical role in the regulation of tumor progression and metastasis (4–6). In contrast to the temporally and spatially precise regulation of EMT during development, EMT in tumor progression and metastasis may bedroved by autonomous oncogenic activation or inactivation of signaling molecules with or without additional stimulation (7). Moreover, recent studies show that the activation of EMT can promote noncancer stem cells (CSC) to enter into a CSCs-like state; cell obtaining self-renewal and tumor-initiating ability (8–10). Hence, once undergoing EMT, cancer cells acquiring invasive properties can detach from primary tumor tissue and enter the surrounding stroma and circulation, creating a favorable tissue microenvironment for the cancer cell colonization in distant tissue (11, 12).

The death-effector domain-containing DNA-binding protein (DEDD) is a member of family of death effector domain-containing proteins that comprise 7 proteins including FADD, c-FLIP, Caspase-8/10, and DEDD1/2 (13). DEDD plays an important role in CD95-mediated apoptosis in response to the activation of TNF-α through activating caspase-3 in cytoplasm or caspase-6 in nucleus as a scaffold protein (14). Recent studies found that DEDD can suppress the activity of Cdk1/cyclin B1 complexes and keep S6K1 activity, suggesting that DEDD participates in the regulation of cell cycle and inhibits cell mitosis (15–17). Although other members of DED-containing proteins such as FADD have been implicated in a diverse array of diseases such as Huntington’s disease, Type II diabetes, autoimmune disease, arthritis, and cancer (18), there is no direct evidence to show whether DEDD involves in the regulation of tumor development, growth, invasion, and metastasis. Using a yeast 2-hybrid screening system, we have recently identified a number of molecules, including DEDD and TRB3, physically interacting with TGF-β1 signal molecule SMAD3 to attenuate or enhance TGF-β1/Smad3-mediated cancer cell invasion or EMT (19, 20).

In this study, we therefore hypothesized that DEDD might play a role in the regulation of tumor metastasis through regulation of EMT. We found that the expression levels of...
DEDD correlate conversely with the poor prognosis of breast and colon cancers. Having discovered the functional association of DEDD expression with cancer progression and metastasis, we study the potential mechanism responsible for the DEDD-mediated antimetastatic effect. Our studies suggest that DEDD can function as an endogenous suppressor of tumor growth and metastasis through regulating the stability of critical autophagy modulators to maintain autophagy activation and promote the degradation of master EMT inducers.

Materials and Methods

Cell culture
Breast cancer and other cancer cell lines were purchased from the American Type Culture Collection (ATCC) and maintained as described (21–24). 293ET cells were obtained from the Cell Culture Center of Peking Union Medical College (PUMC, China). All of the cell lines were authenticated by the PUMC using the AmpF/STR Identifier Kit (Applied Biosystems) and the ATCC fingerprint database. The last cell authentication was tested at December 2011.

Plasmids
Flag-DEDD was a gift from Dr. Marcus E. Peter, University of Chicago, USA (25). HA-FADD was a gift from Dr. Sug Hyung Lee, The Catholic University of Korea, South Korea (26). The constructs pcDNA6-HA-PEKC3, pCMV6-Entry-Flag-Beclin1, pGEX-4T-1-DEDD, pCMV2-Flag-Snail/Twist, and pGL6-E-cadherin-luc were generated by PCR and confirmed by sequencing.

DEDD-silencing cells
Three shRNAs were designed on the basis of the DEDD sequence (NM_032998) identified with siRNA Target Finder (Ambion). Stable cell lines were selected by hygromycin. A single clone was amplified by a dilution cloning technique.

DEDD-overexpressing cells
Human DEDD cDNA was cloned into the pCDNA 3.1 expression vector (Invitrogen) and transfected into MDA-MB-231 cells. Stable cell lines were selected by G418. A single clone was amplified by a dilution cloning technique.

Tissue microarray slides and immunohistochemistry
Human breast or colon cancer tissue microarray slides (US Biomax) were detected as previously described (27).

Chemoinvasion assay
Assays were conducted following previous report (28) as in Supplementary Methods.

Soft agar assay
Soft agar assay were carried out as previously described (29).

Analysis of metastasis
Female BALB/C-nu mice were injected with MCF-7 cells or MDA-MB-231 cells according to a previous study (30). Nine weeks after injection, all mice were killed and the number of surface metastases per lung was determined under a dissecting microscope.

Analysis of tumor growth
Female BALB/C-nu mice were injected with MCF-7 cells or MDA-MB-231 cells into the 4th mammary fat pad from flank. Tumor growth was monitored externally using vernier calipers for 48 to 52 days. More details are provided in Supplementary Methods.

Results

DEDD expression negatively correlates with poor prognosis of breast and colon cancers
To determine the potential role of DEDD in the regulation of tumor growth and metastasis, relationship between the expression levels of DEDD and the metastatic grade of cancers was determined using cancer tissue microarrays with known clinical follow-up records, containing 60 cases ductal breast carcinoma specimens (Fig. 1A, top) and 60 cases colon adenocarcinoma specimens (Fig. 1B, top) were analyzed. Tissues were scored and classified (low or high DEDD staining) on the basis of the intensity of DEDD labeling and percentage of DEDD-positive tumor cells. Among these specimens, Kaplan–Meier survival analysis of ductal breast carcinoma specimens and colon adenocarcinoma specimens revealed a correlation between the lower DEDD expression and reduced overall survival times (P < 0.05; Fig. 1A and B, bottom). In addition, the expression of DEDD was examined in 6 breast epithelial cancer cell lines, including 3 nonmetastatic cancer cell lines and 3 metastatic cancer cell lines (Fig. 1C). The expression of DEDD could be detected in metastatic and nonmetastatic cancer cell lines but the metastatic cancer cells expressed much lower level of DEDD than the nonmetastatic cancer cells did. The identical results were also observed in several cancer cell lines derived from liver, colon, pancreas, and prostate cancers (Fig. 1D). These data suggest that the expression level of DEDD negatively correlates with the metastatic phenotypes of tumor and may be used as a prognosis marker for these cancers.

DEDD inhibits metastatic phenotypes of cancer cells
We investigated whether the expression level of DEDD regulated invasive phenotypes of breast cancer cells by expressing short hairpin RNAs (shRNA) to knock down DEDD expression in nonmetastatic MCF-7 cells or by stably overexpressing DEDD in metastatic MDA-MB-231 cells. The shRNAs from 3 different DEDD sequences (shRNA1, shRNA2, or shRNA3) expressed in MCF-7 cells reduced DEDD expression by 50%, 90%, and 84%, respectively (Supplementary Fig. S1A), compared with the cells expressing a control shRNA whose sequence did not match any known human gene. Overexpressing DEDD in MDA-MB-231 by transfecting pCDNA3.1-DEDD significantly increased the expression of DEDD compared with the cells transfected with pCDNA3.1-control vector (Supplementary Fig. S1B).
Silencing DEDD expression in nonmetastatic MCF-7 cells resulted in the acquisition of invasive ability as indicated by Transwell invasion assay (Fig. 2A). Consistently, depletion of DEDD stimulated colony formation of these cells in soft agar, indicating that silencing DEDD increases anchorage-dependent growth (Fig. 2B). Moreover, knocking down of DEDD increased the proliferation (Supplementary Fig. S2A) and inhibited the TNF-α–induced apoptosis in MCF-7 cells (Supplementary Fig. S2B). Major differences in cell morphology had been observed between cells expressing control shRNA and cells expressing DEDD shRNAs grown on Matrigel. The former exhibited a cobble-stone-like morphology, the border of cells

Figure 1. The expression levels of DEDD conversely correlate with the poor prognosis of breast and colon cancers. A and B, DEDD levels in breast (A) and colon (B) tumor tissues (top; scale bars, 40 μm) and Kaplan–Meier plots (bottom) of overall survival of 60 patients with ductal breast carcinomas and colon adenocarcinomas stratified by DEDD expression levels. Tissues were scored as low or high DEDD staining for all tumor cells. C and D, DEDD levels were detected by immunoblotting (IB) in the nonmetastatic or metastatic breast cancer cell lines (C) and other tissue-derived cancer cell lines (D). Data are representative of 3 independent assays with identical results.
and Matrigel was sharp; although the latter exhibited a cobblestone-like morphology too, the border of cells showed a spike-like structure, data suggesting that MCF-7 cells silencing DEDD acquire a metastatic phenotype (Fig. 2C, left). Because DEDD shRNA2 was more efficient to silence DEDD expression, it was chosen for the subsequent studies. In contrast, overexpressing DEDD significantly reduced the invasive capacity of MDA-MB-231 cells as indicated in Transwell invasion assay (Fig. 2A), and prevented colony formation of these cells in soft agar (Fig. 2B). Overexpressing DEDD also decreased proliferation but promoted apoptosis of MDA-MB-231 cells (Supplementary Fig. S2A and S2B). Moreover, cells expressing empty vector exhibited a spindle-like fibroblastic morphology but DEDD-overexpressing cells had a cobblestone-like morphology as showed by 3-dimensional Matrigel culture (Fig. 2C, right). Interestingly, prolonged growth time or increased cell number did not enhance the invasive capacity of MCF-7-control shRNA cells or MDA-MB-231-DEDD cells.

Figure 2. Ectopic expression of DEDD inhibits but depletion of DEDD promotes tumor cell migration, invasion, and the anchorage-independent growth. A, the invasive properties of DEDD shRNA MCF-7 and pcDNA3.1-DEDD MDA-MB-231 cells together with their controls were analyzed by Transwell invasion assay (magnification, ×200). Data are presented as mean ± SEM of 3 independent assays. B, representative photographs indicating soft agar colony formation were taken at 20 days after culture of the indicated cells (top) and summary of 3 independent assays (bottom) are presented as mean ± SEM. C, Data are phase-contrast micrographs of MCF-7 (left, magnification, ×400) and MDA-MB-231 cells (right, magnification, ×100) cultured on Matrigel. **, P < 0.01.
(Supplementary Fig. S2C and S2D), indicating that the regulatory effect of DEDD expression level on the invasive capacity is not a simple growth-dependent phenomenon.

**DEDD inhibits tumor metastasis and growth**

We next evaluated the in vivo effects of DEDD-changing expression on tumor metastasis and growth. Control shRNA MCF-7 and DEDD shRNA MCF-7 cells were injected into the lateral tail vein of 6-week-old athymic mice. Nine weeks after injection, injection of mice with DEDD shRNA MCF-7 cells but not with control shRNA MCF-7 cells resulted in the formation of large metastatic nodules in the lungs. In contrast, the number and volume of metastatic nodules in the lung of mice injected with MDA-MB-231-DEDD cells were significantly reduced as compared with that injected with MDA-MB-231-control vector cells (Fig. 3A and B). These results indicate that expression level of DEDD correlates inversely with the metastatic ability of breast cancer cells.

To determine the effect of DEDD expression level on tumor growth, the DEDD-silencing or DEDD-overexpressing breast cancer cells were implanted under the 4th mammary fat pads of athymic nude mice. Comparing with mice implanted with...
MDA-MB-231-control vector cells, which formed large tumors within 52 days (Fig. 3C), the mice implanted with the MDA-MB-231-DEDD cells showed a significant reduction in the tumor growth, even no tumors were observed in some mice. Also, silencing DEDD significantly promoted the tumor growth in mice implanted with MCF-7 cancer cells containing DEDD shRNA compared with mice implanted with MCF-7 cancer cells containing control shRNA (Fig. 3D). These results indicate that the expression level of DEDD correlates negatively with the tumor growth of breast cancer.

**DEDD inhibits EMT by downregulating the expression of Snail and Twist**

EMT, a process by which tumor-associated epithelial cells obtain mesenchymal features and result in a reduced cell–cell contact and increased motility, has a critical role in metastasis (10, 11). To assess the potential roles of DEDD in the regulation of EMT, we examined the expression of EMT-associated markers in the presence or absence of DEDD expression. We found that DEDD shRNA MCF-7 cells showed spindle-like fibroblastic morphology, one of the main characteristics of EMT (data not shown). The expression of epithelial marker E-cadherin was significantly reduced in these cells (Fig. 4A and B, left). In contrast, the expression of mesenchymal markers Vimentin and α-SMA was dramatically upregulated in these cells (Fig. 4A and B, left). However, overexpression of DEDD in MDA-MB-231 cells led to an increase in the expression of epithelial marker E-cadherin and a decrease in the expression of mesenchymal markers vimentin and α-SMA (Fig. 4A and B, right). To verify the universal effects of DEDD on EMT, DEDD was knocked-down in a nonmetastatic colon cancer cell line SW480 by DEDD shRNA. We found that the expression of E-cadherin was...
significantly reduced but the expression of Vimentin was increased in SW480 DEDD shRNA cells (Supplementary Fig. S3A). Taken together, our studies indicate that DEDD is a negative EMT modulator.

A number of factors, particularly transcriptional factors Snail and Twist, play critical role in the regulation of EMT (31–34). We examined the expression of Snail and Twist in the presence or absence of DEDD. The expression of Snail and Twist was very low in the control shRNA MCF-7 cells. Silencing DEDD in MCF-7 cells significantly upregulated the expression of Snail and Twist (Fig. 4C). In contrast, overexpression of DEDD significantly decreased the expression of Snail and Twist in MDA-MB-231 cells (Fig. 4C). However, DEDD–knocking down or DEDD-overexpressing cancer cells did not change the amounts of Snail and Twist mRNAs compared with their controls as indicated by quantitative PCR analysis (Fig. 4D). Because Snail and Twist negatively regulate the expression of epithelial marker E-cadherin, the upregulated Snail and Twist expression in DEDD-silencing cells resulted in a suppressive promoter activity of E-cadherin as indicated by luciferase reporter assays (Fig. 4E). In contrast, the downregulated expression of Snail and Twist in DEDD-overexpressing cells resulted in an enhanced promoter activity of E-cadherin in compared with the control vector cells (Fig. 4E).

**DEDD induces the degradation of Snail and Twist by activating the autophagy–lysosome degradation system**

Because the expression level of DEDD did not significantly change the expression of Snail/Twist mRNAs (Fig. 4D), we examined the potential effect of DEDD-changing expression on the stability of Snail and Twist. The pCMV2-Flag-snail or pCMV2-Flag-twist expression vector was transiently transfected into the MCF-7-control shRNA or MCF-7-DEDD shRNA cells. pEGFP-N1 vector expressing EGFP protein was used as control to normalize the experimental variation between different time points. We found that the half-life of Flag-Snail was 5.7 hours in MCF-7-control shRNA cells but the degradation of Flag-Snail was restrained and the half-life was significantly prolonged to 10 hours in the MCF-7-DEDD shRNA cells (Fig. 5A). Similarly, depletion of DEDD inhibited the degradation of Twist and the half-life of Twist was extended from 6.2 hours in MCF-7-control shRNA cells to 9.7 hours in MCF-7-DEDD shRNA cells (Supplementary Fig. S4A).

Previous work indicated the degradation of Snail and Twist depends on the ubiquitin-proteasome degradation pathway (35, 36), we thus examined if the inhibition of proteasome changed the DEDD-mediated degradation of Snail/Twist. Flag-Snail was transfected into MCF-7-control shRNA or MCF-7-DEDD shRNA cells. The cells were then treated with protein synthesis inhibitor cycloheximide and proteasome inhibitor MG132 to block de novo synthesis and the ubiquitin-proteasome degradation of Snail. We found that the Flag-Snails degradation in MCF-7-control shRNA cells was much rapid than that in MCF-7-DEDD shRNA cells (Fig. 5B). The identical result was also observed in MCF-7 cells transfected with Flag-Twist (Supplementary Fig. S4B). However, treatment of cells with proteasome inhibitor MG132 did not change Snail/Twist degradation. These data indicate that the DEDD-mediated degradation of Snail/Twist does not depend on the ubiquitin-proteasome system.

Autophagy is another intracellular degradation system for the majority of proteins and some organelles (37). To verify whether autophagy-lysosome system participated in the regulation of DEDD-mediated degradation of Snail/Twist, GFP-LC3 and Flag-Snail or Flag-Twist were cotransfected into MCF-7 cells, and the cellular localization of LC3 and Snail/Twist was observed by immunofluorescence. We found that LC3 was colocalized with Snail/Twist in the presence or absence protein synthesis inhibitor cycloheximide (Fig. 5C; Supplementary Fig. S4C). Furthermore, inhibiting autophagy by 3-MA could significantly attenuate the degradation of Snail/Twist because a similar degradation rate was observed in MCF-7-control shRNA and MCF-7-DEDD shRNA cells (Fig. 5D; Supplementary Fig. S4D). Thus, activation of the autophagy-lysosome system participates in the degradation of Snail/Twist, and this process is DEDD dependent.

**DEDD activates autophagy modulating the expression of PI3KC3**

Because we had found that DEDD-mediated degradation of Snail/Twist, we examined if altering the expression level of DEDD directly activate autophagic signal molecules. We found that silencing or overexpressing DEDD did not change the expression of autophagic signal molecules such as Akt, p-Akt, p-mTOR in MCF-7 or MDA-MB-231 cells (Fig. 6A). However, the expression of class III PI-3-kinase (PI3KC3) was suppressed in MCF-7-DEDD shRNA cells compared with MCF-7-control shRNA cells. In contrast, the expression of PI3KC3 was increased in DEDD-overexpressing MDA-MB-231 cells compared with the cells with the empty vector. Also, Beclin1 expression was upregulated in DEDD-overexpressing MDA-MB-231 cells (Fig. 6A).

LC3 and p62/SQSTM are 2 indicators reflecting the effectual autophagy flux (38). We found that alternating DEDD expression did not change the expression level of LC3 I/I. However, silencing DEDD in MCF-7 cells significantly increased the expression and aggregation of p62 but overexpressing DEDD in MDA-MB-231 cells reduced the expression and aggregation of p62 (Fig. 6A and B). These results suggest that the enhanced expression of DEDD induces but the reduced expression of DEDD attenuates autophagy activation.

PI3KC3 and Beclin1 are 2 critical components of the PI3KC3 core complex, which plays a central role in the activation of autophagy (39, 40). To examine the effect of PI3KC3 and Beclin1 on the DEDD-mediated EMT, different concentrations of PI3KC3-siRNA pools or Beclin1-siRNA pools were transfected into MCF-7-control shRNA cells. The different amounts of pCDNA6-HA-PI3KC3 or pCMV6-Flag-Beclin1 expressing vector were also transfected into MCF-7-DEDD shRNA cells. We found that silencing of PI3KC3 or Beclin1 could increase the expression of Snail and Twist, downregulate the expression of E-cadherin, and inhibit the degradation of p62 in a concentration-dependent manner (Fig. 6C and D, left). In contrast, the overexpression of PI3KC3 or Beclin1 could decrease the expression of Snail/Twist, increase the expression of E-cadherin, and the degradation of p62 in a concentration-
Figure 5. Depletion of DEDD stabilizes Snail through attenuating the autophagy-lysosome degradation system. A and B, MCF-7 cells were cotransfected with pCMV2-Flag-Snail and pEGFP-N1 vector. Cells were treated with 20 μmol/L of cycloheximide (CHX; A) or CHX plus 10 μmol/L of MG132 (B) for the indicated times. Flag-Snail and EGFP were detected by immunoblotting (IB) with indicated antibodies. C, LC3-GFP (green) and Flag-snail (red) were cotransfected into the MCF-7 cells containing control shRNA and incubated for 36 hours. The cells were treated with 20 μmol/L of CHX for the indicated times. The cell nuclei were stained with DAPI (scale bar, 35 μm). The arrows indicate the colocalization of LC3 and Snail. D, cells were handled as described in A and treated with 20 μmol/L of CHX and 10 mmol/L of 3-methyladenine (3-MA) for the indicated times. Cell extracts were prepared and immunoblotting was carried out as described in A. DAPI, 4',6-diamidino-2-phenylindole.
dependent manner (Fig. 6C and D, right). These data indicate that PI3KC3 and Beclin1 participate in the DEDD-mediated regulation of EMT by promoting the autophagy-associated degradation of Snail and Twist.

**DEDD stabilizes PI3KC3 by interacting with PI3KC3/Beclin1**

The function or activity of PI3KC3/Beclin1 complex can be regulated by several signal molecules such as UVRAG, Bcl-2, and Rubicon (39, 41). We wondered if DEDD interacted directly with the complex to regulate autophagy. We found that DEDD interacted physically with PI3KC3 and Beclin1, as showed by co-precipitated of Flag-DEDD with endogenous PI3KC3 or Beclin1 (Fig. 7A), which was confirmed by glutathione S-transferase pull-down assay (Fig. 7B), and by immunoprecipitation of endogenous DEDD with PI3KC3 or Beclin1 (Fig. 7C). To determine whether the interaction of DEDD with PI3KC3 and Beclin1 have effect on the formation of core complex, the interaction of PI3KC3 with Beclin1 was determined by co-immunoprecipitation in...
MDA-MB-231 cells containing empty or DEDD-expressing vector. Overexpression of DEDD significantly enhanced the interaction between PI3KC3 and Beclin1 (Fig. 7D). We further found that silencing DEDD promoted the degradation of PI3KC3 and the half-life of PI3KC3 was reduced from 9.2 to 3.3 hours (Fig. 7E). A similar result was also observed in SW480 control shRNA and DEDD shRNA cells (Supplementary Fig. S3B). However, silencing DEDD did not significantly change the half-life of Beclin1 (data not shown). Taken together, DEDD supports the stability of PI3KC3 through interacting with PI3KC3/Beclin1.

Discussion

Our recent work indicates that DEDD attenuates TGF-β1/Smad3 signaling through interacting with transcription factor Smad3 to suppress the TGF-β1/Smad3-mediated invasion in cancer cells (19). In this study, we have showed that the expression level of DEDD in breast and colon cancers correlates conversely with the poor prognosis of breast and colon cancers: the lower is DEDD expression in breast and colon cancers, the higher is the cancer-induced death rate because the expression level of DEDD decides the metastatic phenotypes of the cancer cells, including migration, invasion, growth, and metastasis.
PI3KC3, leading to an attenuation of autophagy activity and the expression promotes the degradation and inactivation of the Snail and Twist, 2 master inducers of EMT, to attenuate the leading to the autophagy-lysosome-dependent degradation of PI3KC3 through physically interacting with this protein, accumulation and activation of Snail and Twist in these cancer cells promote the activation of the EMT process. Indeed, restoring DEDD expression in metastatic MAD-MB-231 breast cells results in a loss of metastatic phenotype whereas knockdown of DEDD expression in the nonmetastatic MCF-7 breast cells results in the acquisition of the most central traits of the CSCs: invasive/metastatic and tumor-initiating ability (1–3, 7). Thus, our studies indicate that DEDD is critically involved in the negative regulation of cancer progression and metastasis through maintaining autophagy activity to suppress the EMT (summarized in Fig. 7F).

Metastasis is a complex and multistep process, which has to be divided into 2 phases, namely, physical translocation of a tumor cell from the primary tumor to a distant tissue to seed and colonization of disseminated tumor cell in the tissue (4–6). Recent researches indicate that EMT is not only involved in the 2-phase events in cancer to promote metastasis, but also participates in the regulation of tumor progression (42). The molecular and cellular mechanisms for the regulation of EMT have been emerged from these studies and, particularly, many positive inducers and signal pathways for the induction of the EMT have been identified (5, 42). For example, a mechanistic linkage between the Snail/Twist-induced EMT and CSC-like behaviors has been indicated for the understanding of prometastatic role of this EMT inducer because these cancer cells on the EMT process acquire CSC-like traits (7). Our current studies provide evidence to show that the alteration in the expression levels of DEDD regulates the EMT and results in loss or acquisition of metastatic phenotypes, thereby determining the process of tumor progression and metastasis. Interestingly, a very recent study by Mori and colleagues report that DEDD deficiency results in a defect in uterine decidualization and infertile because of reducing Akt expression, indicating that DEDD is indispensable for the establishment of an adequate uterine environment to support early pregnancy in mice (43). This finding is consistent and support to the fact that DEDD is involved in the regulation of embryos developmental programs including EMT (19). However, it needs to point out, previous work indicated that DEDD can enter nucleus to inhibit rDNA transcription (25), regulate cell cycle, and inhibit cell mitosis to reduce cell and body size via modulation of rRNA synthesis (17), attenuate protein synthesis, and promote apoptosis (44, 45). These growth-impeding or apoptosis-promoting roles of DEDD may also contribute to the DEDD-induced antimetastatic role. Taken together, these studies support a notion that tumor metastatic ability may be an innate property shared by the bulk of cells present early in a developing tumor mass (1). Hence, further study is required to decipher the precise role and mechanism for antimetastatic roles of DEDD.

Our studies indicate that the alterations in the expression level of DEDD regulate the EMT through DEDD interacting with the PI3KC3/Beclin1 complex, which plays central role in the initiation and maturation of autophagosomes (39, 40). The DEDD’s effect on the stability of PI3KC3/Beclin1 complex seems to be achieved through DEDD interacting with PI3KC3 and stabilizes this protein. Indeed, we find that DEDD can interact with PI3KC3 or Beclin1, respectively. Ectopic expression of DEDD results in an increase in the expression of PI3KC3 and Beclin1 in MDA-MB-231 cancer cells, whereas knockdown of DEDD results in a decrease in the expression of PI3KC3 and Beclin1 in MCF-7 cancer cells. However, silencing DEDD expression results in a marked decrease in the degradation half-life of PI3KC3 but not Beclin1 (Fig. 7), indicating that the DEDD–PI3KC3 interaction plays a crucial role in maintaining the stability of the PI3KC3/Beclin1 complex. Thus, alterations in the expression of PI3KC3 result in either activation or inactivation of autophagy-dependent degradation of Snail/Twist and other positive EMT markers. Despite we do not know the precise mechanism how DEDD interacts with PI3KC3 to maintain the stability of this protein and why loss of DEDD expression promotes the degradation of PI3KC3, recent work indicate that DEDD induces a diversity of cell biologic activity through interacting with several key molecules to attenuate or support the stability of these proteins in the cells (19, 46). Thus, a key question to be addressed is: what program is responsible for shutdown or turn-on of DEDD expression under the physiologic or pathologic situations.

Although targeting the activity of E-cadherin repressors such as Snail/Twist to attenuate EMT may be promising therapeutic strategy against tumor metastasis and progression, these EMT inducers are transcription factors and difficult to access or target (5). Our discovery, DEDD interacting with PI3KC3/Beclin1 to promote the autophagy-dependent degradation of Snail and Twist, may provide mechanistic insights into developing novel therapeutic strategy against tumor metastasis and progression. Currently, a wide range of selective PI3K inhibitors has been tested in preclinical studies and some have entered clinical trials in oncology. Unveiling the Vps34 structure (47) and discovery of selective PI3KC3 inhibitor (48) may shed the new light on the perspective anticancer targets. However, because of the complexity of PI3K signaling pathways, developing an effective anticancer therapy may be difficult. Particularly, the potential therapeutic effects of pharmacologic Vps34 modulation may well be context-dependent, and thus there could perhaps be a need for biomarkers for patient selection. For this perception, our current finding may provide mechanistic insights into design of chemical tools and potential drugs to activate or antagonize this key molecule involved in vesicular trafficking and autophagy.

In summary, our current studies show that DEDD is an endogenous suppressor of tumor growth and metastasis in human breast cancer and the expression level of DEDD decides the phenotypes of cancer cells in migration, invasion, growth, metastasis, and maybe resistance to the chemotherapy because it negatively regulates the EMT. As tumor metastasis...
is a devastating disease, our findings suggest that the expression level of DEDD can be detected and used as the prognostic marker of cancers and therapeutic target for the prevention and treatment of cancer progression and metastasis. Therefore, additional insight into the regulatory role of DEDD-PI3KC3 interaction in autophagy and EMT may ultimately serve as an entry point to translate modifiers of these interactions into clinical endpoints.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: Q. Lv, Z.-W. Hu
Development of methodology: Q. Lv, Z.-W. Hu
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Q. Lv, W. Wang, J. Xue, F. Hua, R. Mu, H. Lin, J. Yan, X. Lv

References

Analysis and interpretation of data (e.g., statistical analysis, biosististics, computational analysis): Q. Lv, J. Xue, Z.-W. Hu
Writing, review, and/or revision of the manuscript: Q. Lv, F. Hua, X. Chen, Z.-W. Hu
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Q. Lv
Study supervision: Z.-W. Hu

Grant Support
This work was supported by grants from National Major Basic Research Program of China (973 #2006CB503808), National Natural Science Foundation of China (81103056, 30973357, 8110195), and Creation of Major New Drugs (2009ZX09301-003-12, 2009ZX09301-003-9-1).

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Received November 28, 2011; revised March 16, 2012; accepted April 6, 2012; published OnlineFirst June 19, 2012.

www.aacajournals.org Cancer Res; 72(13) July 1, 2012 3249

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