

δ -Catenin Affects the Localization and Stability of p120-Catenin by Competitively Interacting with E-Cadherin

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E-cadherin is a member of the cadherin family of Ca^{2+} -dependent cell-cell adhesion molecules. p120-Catenin and δ -catenin are known to bind to similar juxtamembrane regions of E-cadherin, and p120-catenin is known to stabilize E-cadherin. However, the function of competition between p120-catenin and δ -catenin for E-cadherin has not been fully explained. In this report, we show that cells overexpressing δ -catenin contain less p120-catenin than control cells at the cell-cell interface and that this causes the re-localization of p120-catenin from the plasma membrane to the cytosol. We show that successful binding by one to E-cadherin adversely affects the stability of the other.

INTRODUCTION

E-cadherin is a Ca^{2+} -dependent cell-cell adhesion molecule and plays an essential role in the formation of adherens junctions and in the function of epithelial cells (Takeichi, 1995). It is known that cell-cell adhesion is reduced in human cancers during invasion and metastasis (Iozzo et al., 2009; Nollet et al., 1999). Immunohistochemical studies of many different human cancer tissues have also shown that levels of E-cadherin in tumors tissues are below normal (Shun et al., 1998). Furthermore, several studies have suggested that E-cadherin acts as a growth suppressor (St Croix et al., 1998).

The cytoplasmic tail of E-cadherin is associated with several proteins termed catenins. p120-Catenin and δ -catenin bind to the proximal region of the cytoplasmic tail of E-cadherin, termed the juxtamembrane domain (JMD) (Lu et al., 1999), which is separated from the β -catenin binding distal region of the cadherin cytoplasmic tail, termed the catenin binding domain (CBD). The E-cadherin/catenin complexes are associated with the actin cytoskeleton, and this linkage is essential to retain strong cell-cell adhesion (Aberle et al., 1994).

Although p120-catenin (unlike β -catenin) does not directly interact with α -catenin or the actin cytoskeleton, p120-catenin is a critical regulator of cadherin-mediated adhesive activity and cytoskeletal organization (Thoreson et al., 2000). Several stud-

ies have demonstrated that p120-catenin importantly regulates the stabilities of cadherins, and it has been reported that in the absence of p120-catenin, cadherins are destabilized and targeted for degradation (Ireton et al., 2002). Furthermore, several studies indicate that p120-catenin expression is frequently lost in cancers of the colon, bladder, gastric, breast, and prostate (Thoreson and Reynolds, 2002), and in many cases, the loss of p120-catenin is correlated with a poor prognosis, which suggests that p120-catenin is associated with cancer progression.

δ -Catenin belongs to the p120-catenin subfamily of armadillo proteins that possess 10 Arm repeats (Anastasiadis and Reynolds, 2000). It was initially identified due to its interaction with presenilin-1 (PS1) protein, which is prominently implicated in familial Alzheimer's disease (Zhou et al., 1997), and it was later shown that δ -catenin is a neuron-specific protein in brain (Ho et al., 2000; Lu et al., 1999). However, δ -catenin is overexpressed in human tumors, including prostate and breast cancers, which suggests that δ -catenin performs some function in epithelial cancer cells (Burger et al., 2002; Lu et al., 2005). Indeed, δ -catenin is known to interact and colocalize with E-cadherin, β -catenin and p120-catenin in epithelial cells (Lu et al., 1999).

Although initially, δ -catenin was exclusively found in brain, recent studies have demonstrated using complementary DNA (cDNA) micro-array assays, that δ -catenin transcript levels are elevated in prostate cancer (Burger et al., 2002). Furthermore, the overexpression of δ -catenin in human prostate cancer was found to be associated with a decrease in the immunoreactivities of E-cadherin and p120-catenin at the plasma membrane and with their redistributions (Lu et al., 2005). Taken together, these results suggest possible roles for δ -catenin in cancer and in the organization of adherens junctions. In this report, we demonstrated that p120-catenin and δ -catenin compete with each other for the interaction with E-cadherin and that successful binding by one to E-cadherin adversely affects the stability of the other.

MATERIALS AND METHODS

Plasmids and antibodies

The constructions of wild type (WT) in pEGFP-C1 have been

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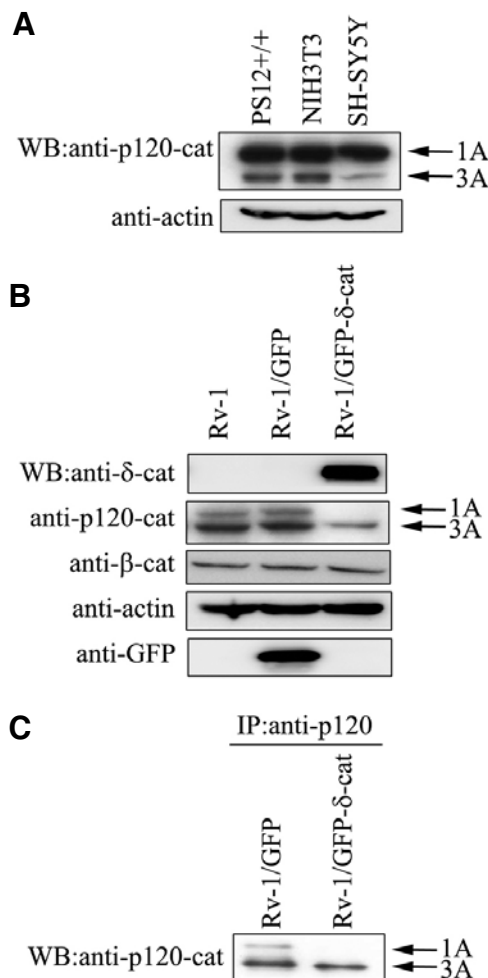


Fig. 1. Cells overexpressing δ -catenin contained less p120-catenin 1A and 3A than control cells. (A) The expression of p120-catenin isoforms in total cell lysates of PS-1/2 +/+, NIH3T3 and SH-SY5Y cells were analyzed by Western blotting using anti-p120-catenin Ab (top panel). The upper band indicates p120-catenin isoform 1A, and the bottom band indicates p120-catenin isoform 3A. Actin was used as a loading control (bottom panel). (B) Proteins were extracted with lysis buffer from parental CWR22Rv-1 human prostate cancer cells (Rv-1) or from CWR22Rv-1 human prostate cancer cells stably overexpressing either GFP- δ -catenin (Rv-1/GFP- δ -cat) or GFP (Rv-1/GFP). The expressions of different proteins in total cell lysates from these cells were analyzed using specific antibodies indicated on the left sides of panels. (C) Proteins from CWR22Rv-1/GFP- δ -catenin or CWR22Rv-1/GFP cells were immunoprecipitated with anti-p120-catenin Ab, and then immunoblotted with anti-p120-catenin Ab.

previously described (Kim et al., 2002), and the p120-catenin 1A in pEGFP-C1 construct was kindly provided by Panos Z. Anastasiadis.

The antibodies used were as follows: anti- δ -catenin (BD bioscience); anti-GFP (Sigma); anti- β -catenin (Sigma); anti-actin (Calbiochem); anti-E-cadherin (Santa Cruz biotechnology); and anti-p120-catenin (Zymed Laboratories).

Cell culture, transfection

CWR22Rv-1 human prostate cancer cell lines were maintained

in RPMI supplemented with 10% FBS and 1% penicillin/streptomycin at 37°C in a 5% CO₂ atmosphere. In addition, CWR22Rv-1/GFP- δ -catenin and CWR22Rv-1/GFP cells were treated with 125 μ g/ml G418 (Sigma). Cells were transfected using Lipofectamine Plus reagent (Invitrogen), according to the manufacturer's instructions.

Immunoblotting and immunoprecipitation

Transfected cells were harvested with lysis buffer (10% glycerol, 25 mM HEPES, 150 mM NaCl, 1 mM EDTA, 25 mM NaF, 1 mM Na₃VO₄, 1% NP40, 0.2 mM phenylmethylsulfonyl fluoride, and protease inhibitor mixture). Equal amounts of protein samples were then solubilized, boiled with SDS sample buffer for 2 min and separated by SDS-PAGE. Proteins were run on appropriate gels, transferred to hydrophobic polyvinylidene difluoride membranes (Millipore), and developed using ECL Western blotting detection reagents (Millipore). Immunoprecipitation was performed as previously described (Kim et al., 2005).

Cell fractionation

To separate cytoplasmic, nuclear, and membrane extracts, cell fractionation was performed as previously described (Ki et al., 2008).

RESULTS

Human prostate cancer cells overexpressing δ -catenin contained less p120-catenin than control cells

To investigate the effects of overexpressed δ -catenin in human prostate cancer cells, we made CWR22Rv-1 human prostate cancer cells stably expressing GFP- δ -catenin or empty GFP (a negative control). CWR22Rv-1/GFP- δ -catenin and CWR22Rv-1/GFP cells were confirmed by immunoblotting (Fig. 1B). It has been reported that most cells express multiple p120-catenin isoforms, and that macrophages and fibroblasts preferentially express p120-catenin 1A isoforms, but that epithelial cells express p120-catenin 3A isoforms (Mo and Reynolds, 1996). PS-1/2 +/+, NIH3T3 fibroblasts and SH-SY5Y neuroblastoma cells contain more abundant endogenous p120-catenin-1A than the catenin-3A isoforms, whereas CWR22Rv-1 human prostate cancer cells show the opposite pattern. Interestingly, we observed that CWR22Rv-1/GFP- δ -catenin cells contained significantly less of the p120-catenin 1A and 3A isoforms than control cells overexpressing empty GFP. To investigate whether δ -catenin overexpression also affects the level of β -catenin, we examined β -catenin levels. It was observed that the levels of β -catenin were not affected by δ -catenin overexpression (Fig. 1B). To confirm p120-catenin levels, immunoprecipitation was performed in CWR22Rv-1/GFP and CWR22Rv-1/GFP- δ -catenin cells using anti-p120-catenin antibody (Ab). As was expected, CWR22Rv-1/GFP- δ -catenin cells were found to contain less of the 1A and 3A isoforms of p120-catenin than control cells (Fig. 1C).

δ -Catenin overexpression caused p120-catenin re-localization from the plasma membrane to the cytosol

To test whether δ -catenin overexpression affects the localization of p120-catenin, we performed a cell fractionation assay. Total cell lysates and nuclear and membrane fractions of CWR22Rv-1/GFP- δ -catenin cells contained less p120-catenin than control cells overexpressing empty GFP. However, the cytosolic fraction of CWR22Rv-1/GFP- δ -catenin cells contained more p120-catenin than control cells (Fig. 2A), which suggested that p120-catenin is released from the cell-cell interface because of competition with δ -catenin and that it undergoes protein degradation in

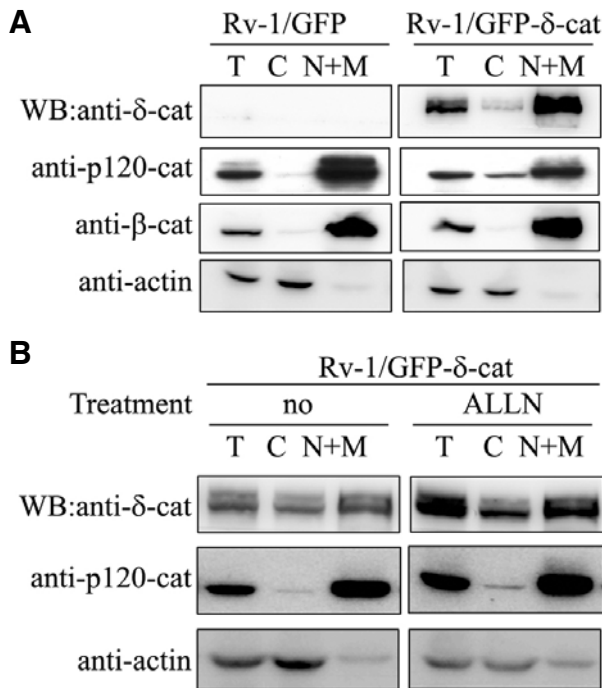


Fig. 2. δ -Catenin overexpression caused p120-catenin re-localization. (A) Cell fractionation assays were performed using CWR22Rv-1/GFP- δ -catenin and CWR22Rv-1/GFP cells. Letters indicate the following: T, total cell lysates; C, cytosolic fractions; N, nuclear fractions; and M, membrane fractions. (B) CWR22Rv-1/GFP- δ -catenin cells were treated with ALLN (5 μ M) for 4 h prior to cell fractionation analyses.

the cytosol. Furthermore, no significant difference in β -catenin levels was observed between CWR22Rv-1/GFP- δ -catenin and control cells (Fig. 2A). To determine whether the cytosolic fraction of p120-catenin is degraded in a proteasome-dependent manner, we used ALLN, a specific inhibitor of proteasome-dependent proteolysis. We observed that when ALLN was administered to CWR22Rv-1/GFP- δ -catenin cells it induced a little increase in the levels of cytosolic p120-catenin than in control cells (Fig. 2B). It seems to be no difference between two panels, but there are more increases in the level of p120-catenin comparing to the anti-actin band. Thus, we concluded that the overexpression of δ -catenin causes p120-catenin re-localization from the plasma membrane to the cytosol, where it undergoes proteasome-mediated degradation.

δ -catenin competed with p120-catenin in human prostate carcinoma cells

During our studies, we observed that δ -catenin overexpression reduced p120-catenin levels in human prostate carcinoma cells. To test whether p120-catenin overexpression affects δ -catenin levels, we transiently transfected p120-catenin and δ -catenin at different gene-dosage ratios into CWR22Rv-1/GFP cells. The results obtained showed that p120-catenin overexpression did in fact decrease δ -catenin levels (Fig. 3). We also confirmed that δ -catenin overexpression reduced p120-catenin levels in a concentration-dependent manner in human prostate carcinoma cells.

The association between p120-catenin and E-cadherin is diminished by δ -catenin

As stated above, δ -catenin and p120-catenin bind to the JMD of

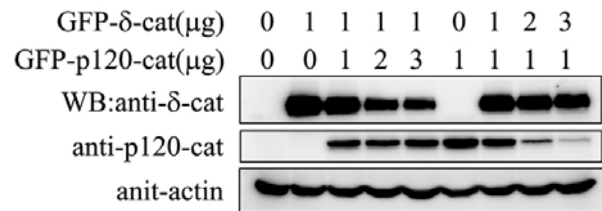


Fig. 3. δ -Catenin and p120-catenin reciprocally affected their protein stabilities. CWR22Rv-1/GFP cells were transfected with GFP- δ -catenin or GFP-p120-catenin 1A at different gene dosage ratios as indicated above panels. Total cell lysates were immunoblotted using for δ -catenin, p120-catenin, and actin.

E-cadherin (Lu et al., 1999), and, as demonstrated in Fig. 3, δ -catenin and p120-catenin reciprocally affect their stabilities. To determine whether overexpressed δ -catenin competes with p120-catenin for binding to E-cadherin, we performed immunoprecipitation assays using anti-E-cadherin in CWR22Rv-1/GFP- δ -catenin and control cells. The results obtained showed that CWR22Rv-1/GFP- δ -catenin cells contained less E-cadherin-bound p120-catenin than control cells (Fig. 4A). To investigate whether the overexpressions of p120-catenin or δ -catenin affect E-cadherin binding, we transiently transfected GFP-p120-catenin 1A or GFP- δ -catenin at different gene-dosage ratios into CWR22Rv-1/GFP cells. While there was no cytotoxicity using large amount of DNA, it was found that GFP- δ -catenin overexpression reduced the amounts of E-cadherin/GFP-p120-catenin 1A (Fig. 4B). The same results were observed at the total cell lysate level (Fig. 4B). Likewise, GFP-p120-catenin 1A overexpression decreased the amounts of E-cadherin-bound GFP- δ -catenin, and results were the same at the total cell lysate level (Fig. 4C). In addition, levels of bound E-cadherin and total β -catenin were not significantly affected by δ -catenin overexpression (Fig. 4A).

DISCUSSION

The present study shows that prostate cancer cells overexpressing δ -catenin contain less p120-catenin than control cells at the cell-cell interface and that δ -catenin overexpression causes the re-localization of p120-catenin from the plasma membrane to the cytosol. Furthermore, we found that p120-catenin and δ -catenin compete with each other for E-cadherin and that their stabilities are reciprocally affected by this competition. The core region of p120-catenin binding to E-cadherin is composed of amino acid residues 758-773 (Thoreson et al., 2000). Like p120-catenin, δ -catenin also interacts with E-cadherin containing amino acid residues 734-774 (Lu et al., 1999). Lu et al. showed that increased δ -catenin immunoreactivity is associated with the down-regulations of E-cadherin and p120-catenin (Lu et al., 2005). However, the underlying mechanism involved remains unclear. Thus, we decided to use biochemical methods to test the hypothesis that δ -catenin and p120-catenin compete with each other for E-cadherin.

Two pieces of evidence show that δ -catenin and p120-catenin do compete for E-cadherin. First, stable cell lines overexpressing δ -catenin showed dramatic reductions in total p120-catenin 1A and 3A levels and in E-cadherin/p120-catenin levels. Although p120-catenin 1A and 3A are expressed differentially in a cell type-dependent manner, δ -catenin overexpression affected both p120-catenin isoforms regardless of isoform type. Second, we also observed that transiently transfected δ -catenin and p120-catenin 1A compete for E-cadherin. Interestingly, E-cadherin/ β -

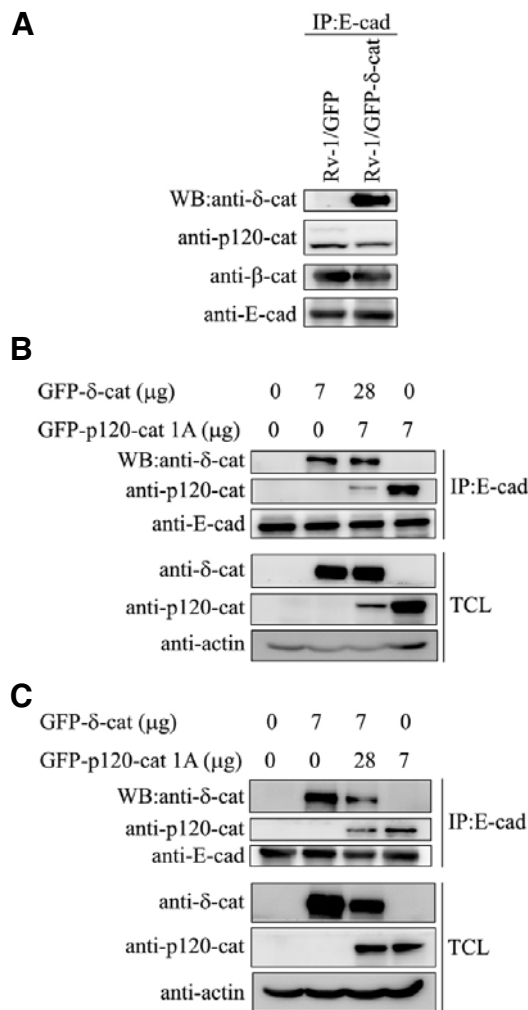


Fig. 4. δ -Catenin and p120-catenin competed with each other for E-cadherin binding. (A) Proteins from CWR22Rv-1/GFP- δ -catenin or CWR22Rv-1/GFP cells were immunoprecipitated with anti-E-cadherin antibody, and then immunoblotting assays were performed using different antibodies as indicated on the left of the panels. (B, C) CWR22Rv-1/GFP cells were transfected with GFP- δ -catenin and GFP-p120-catenin 1A at different gene dosage ratios as indicated above panels. Immunoprecipitation assays were performed on total lysates using anti-E-cadherin Ab, and immunoblotting assays were performed using different antibodies as indicated on the left of the panels. TCL indicates total cell lysate.

catenin (formed by an interaction at the cytoplasmic tail region of E-cadherin) was not affected by increases in p120-catenin or δ -catenin expression. Differences of the level of p120-catenin among Figs. 1B, 2A, and 4A results from cell lysis buffers. Normal cell lysis buffer was used in Fig. 1, and fractionation buffer was used in Fig. 2, and low stringency buffer was used in Fig. 4. Stable cell lines overexpressing δ -catenin displayed lower p120-catenin 1A and 3A isoforms levels (both total and E-cadherin-bound) and less translocation of p120-catenin from the plasma membrane to the cytoplasm than control cells. Re-distributed p120-catenin may undergo protein degradation in the cytosol, or alternatively, interact with other nuclear factors in the nucleus. When CWR22Rv-1/GFP- δ -catenin cells were treated with ALLN, a specific proteasome-dependent proteolysis inhibitor, it

was observed that some cytosolic p120-catenin underwent proteasome-mediated degradation. However, the mechanism responsible for p120-catenin degradation in the cytosol is unclear. Alternatively, p120-catenin released from E-cadherin may interact with transcription factors, including Kaiso and Glis2, in the nucleus (Daniel and Reynolds, 1999). Interestingly, modification of the E-cadherin to p120-catenin balance is known to lead to the translocation of p120-catenin from membrane to cytoplasm and finally to polyploidy (Chartier et al., 2007). E-cadherin downregulation leads to the accumulation of cytoplasmic and/or nuclear catenins and induces signaling by catenins released from the membrane. Indeed, β -catenin released from the membrane is either degraded in the cytoplasm or contributes to oncogenic transcription via the Wnt signaling pathway (Waltzer and Bienz, 1999). The present study demonstrates that cells overexpressing δ -catenin contains less E-cadherin-bound p120-catenin than control cells. It is worth noting that some tumor cells, such as, prostate, breast, and esophageal cancer cells, have been shown to overexpress δ -catenin (Burger et al., 2002). Therefore, in addition to E-cadherin down regulation, δ -catenin overexpression may modulate the E-cadherin/p120-catenin balance, and thereby, participate in tumorigenesis. The results on the modulation of p120-catenin levels by δ -catenin are in contrast to an earlier report in the literature (Lu et al., 1999) which showed that p120-catenin was not affected by δ -catenin and also that the association with E-cadherin was not altered. This might be due to the different cell lines used. The earlier report used a non-transformed MDCK (Madin-Darby canine kidney) epithelial cell line. But we used a prostate cancer cell line. A difference between normal and tumorous epithelial cells might affect this result.

p120-Catenin is known to affect the stability of E-cadherin by controlling its endocytosis (Davis et al., 2003). Our unpublished data show that δ -catenin overexpression causes E-cadherin processing (unpublished data). Therefore, our results imply that δ -catenin not only affects E-cadherin assembly but also causes p120-catenin re-localization from the plasma membrane to the cytosol or nucleus, and that this might play an important role in tumorigenesis.

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