Published in final edited form as:
Mol Cells. 2010 March ; 29(3): 233–237. doi:10.1007/s10059-010-0030-2.

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

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Abstract

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 the 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 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.

Keywords

p120 catenin; δ-catenin; E-cadherin; Adherens junction; protein stability

1. 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 (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 levels (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 in 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 studies have

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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 preselin-1(PS1) protein, which is prominently implicated in familial Alzheimer disease (Zhou *et al.*, 1997), and it was later suggesting that δ-catenin is a neuron-specific protein in brain (Lu, Paredes 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 cells (Burger *et al.*, 2002). Indeed, δ-catenin is known to interact and colocalize with E-cadherin, β-catenin and p120-catenin in epithelial cells (Lu, Paredes 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, Tebay 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.

2. Materials and Methods

Plasmids and antibodies

The constructions of wild–type (WT) in pEGFP-C1 have been 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% CO_2 atmosphere. In addition, CWR22Rv-1/GFP-δ-catenin and CWR22Rv-1/GFP cells were treated with G418 (Sigma) 125 μg/ml. 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 Na_3VO_4, 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).

3. Results

3.1. 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 expresses 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 +/+ and 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).

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

3.3. 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.

3.4. 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 E-cadherin (Lu, Paredes et al., 1999), and, as demonstrated in Figure 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, and there is no cytotoxicity using large amount of DNA and it was found that GFP-δ-catenin overexpression reduced amounts of E-cadherin/GFP-p120-catenin 1A(Fig. 4B). The same results were observed at the total cell lysates level (Fig. 4B). Likewise, GFP-p120-catenin 1A overexpression decreased amounts of E-cadherin-bound GFP-δ-catenin, and results were same at total cell lysates (Fig 4C). However, levels of bound E-cadherin and total β-catenin levels were not significantly affected by δ–catenin overexpression (Fig. 4A).

4. Discussion

The present study shows that cells overexpressing δ-catenin contain less p120-catenin than control cells at the cell-cell interface and that δ-catenin overexpression cause 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, Anastasiadis et al., 2000). Like p120-catenin, δ-catenin also interacts with E-cadherin containing amino acid residues 734–774 (Lu, Paredes et al., 1999). Lu et al. showed that increased δ-catenin immunoreactivity is related with the down-regulations of E-cadherin and p120-catenin (Lu, Dobbs et al., 2005). However, the underlying the 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-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 Fig. 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. Internalized 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 proteosome-dependent proteolysis inhibitor, it was observed that some cytosolic p120-catenin underwent proteosome-mediated degradation. However, the mechanism responsible for p120-catenin degradation in the cytosol is unclear. Alternatively, p120-catenin released by E-cadherin interacts 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 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, Tebay 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 MDCK (Madin-Darby canine kidney) epithelial cell line. But we used a prostate cancer cell line. A difference between normal and tumorous epithelial cell 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.

Acknowledgments

This study was supported by a grant to KK by the Korean Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (#A084462).

Abbreviations

CBD	catenin binding domain
JMD	juxtamembrane domain
PS1	preselin-1

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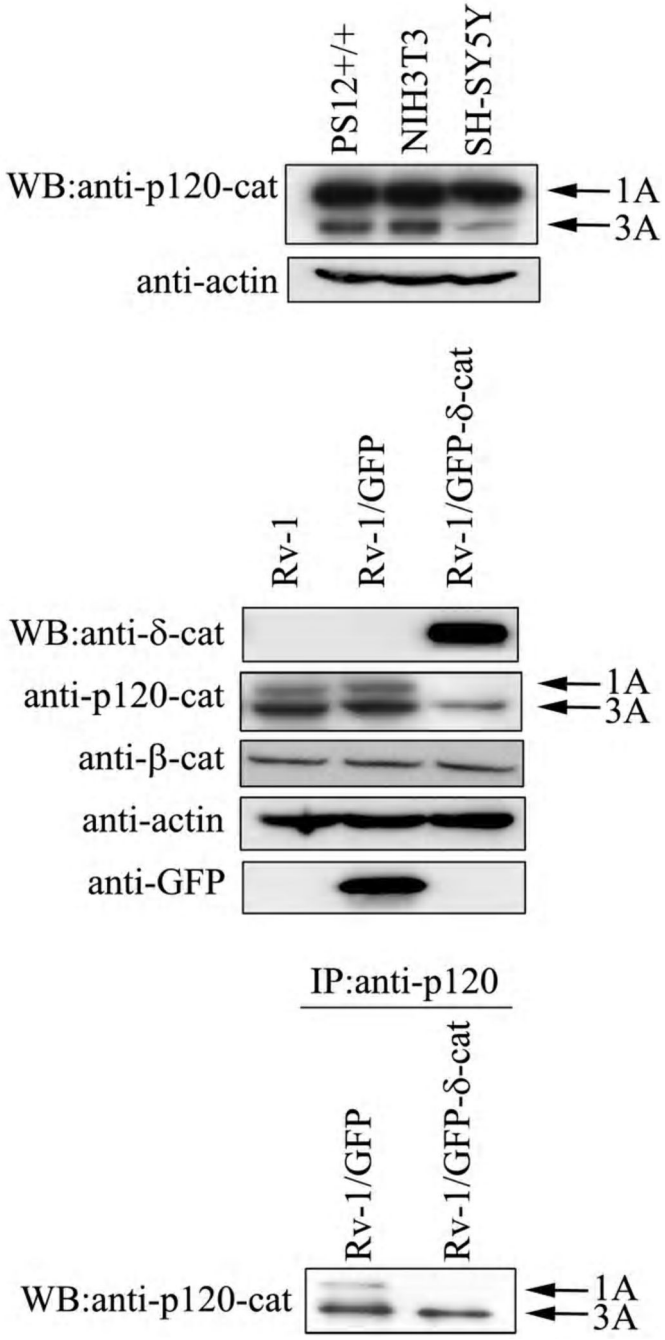

Fig. 1.

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 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.

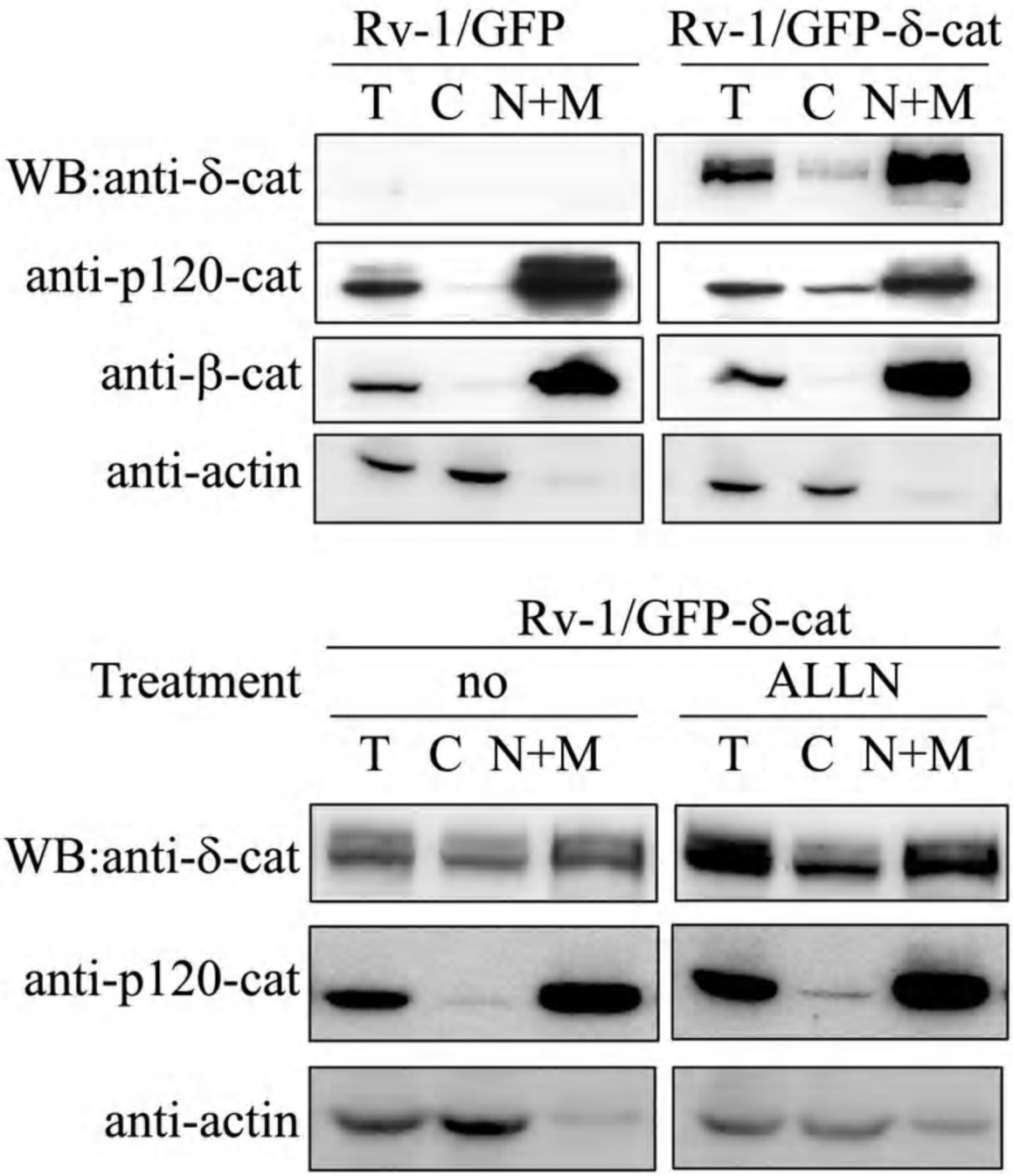

Fig. 2.

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 μM) for 4 hours prior to cell fractionation assays.

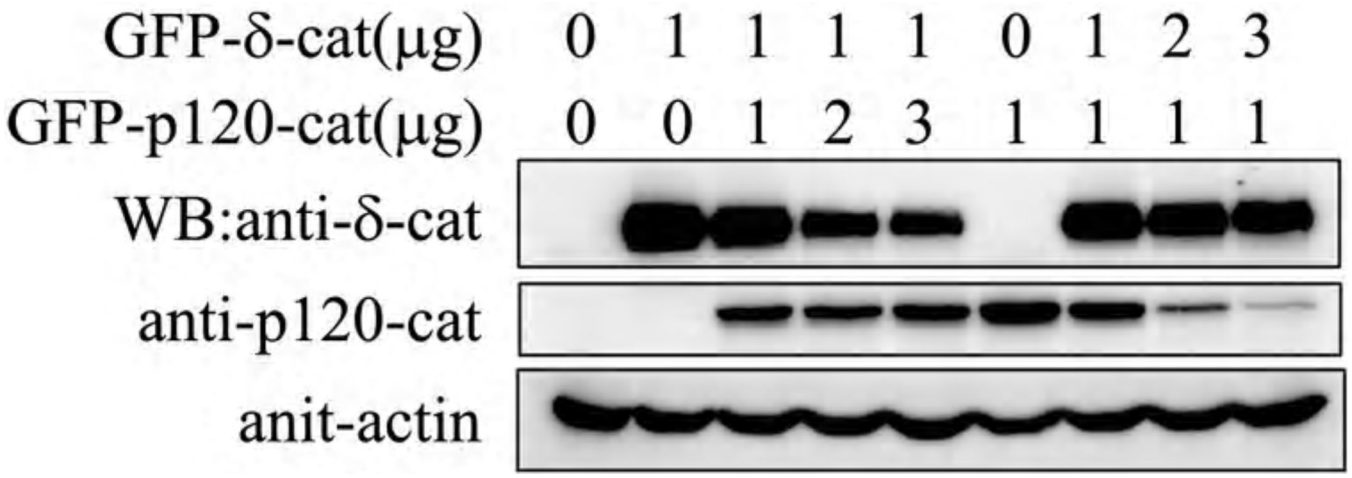

Fig. 3.

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.

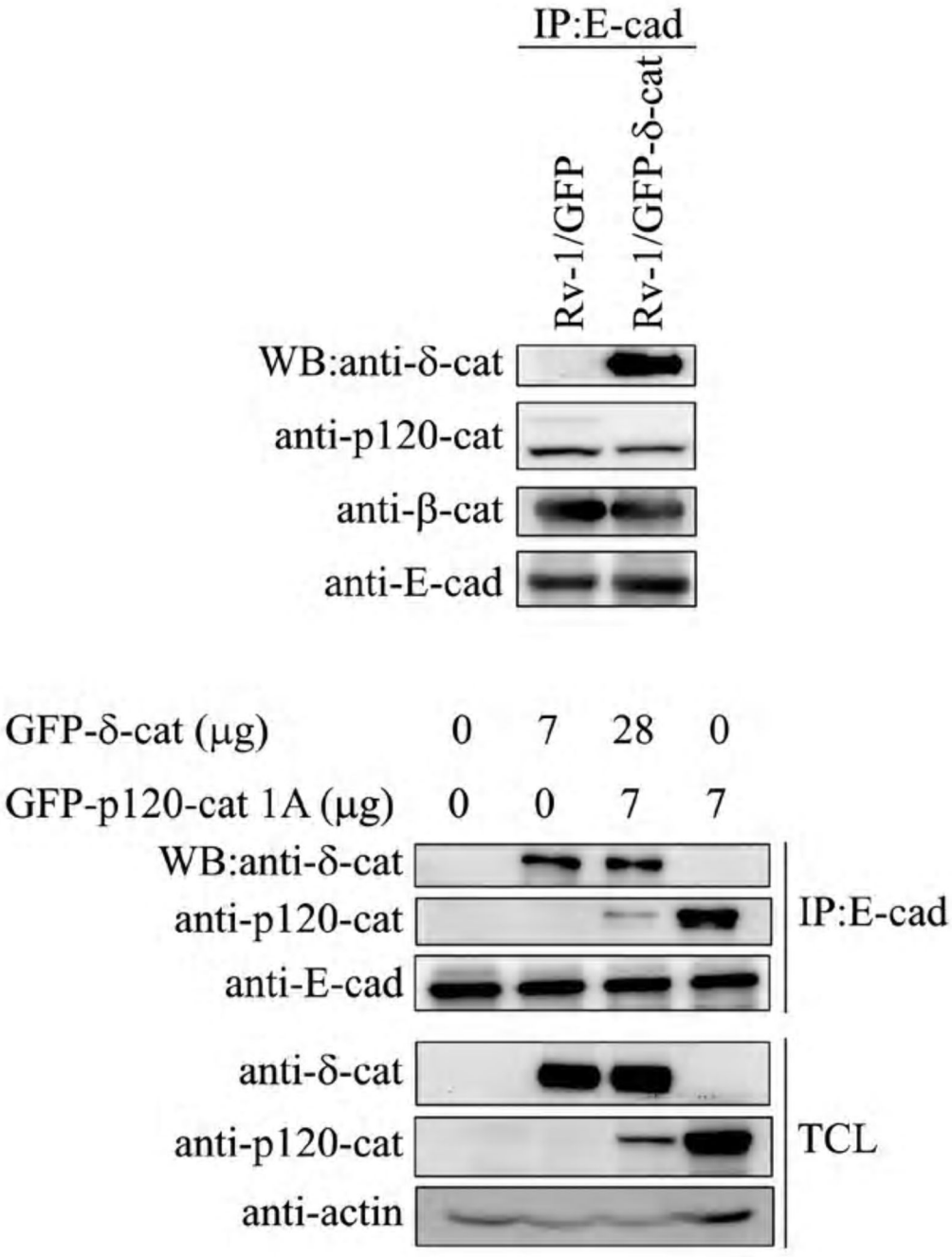

Fig. 4.

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.