Biochimica et Biophysica Acta 1822 (2012) 509–521

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

δ-Catenin promotes E-cadherin processing and activates β-catenin-mediated signaling: Implications on human prostate cancer progression

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ARTICLE INFO

Article history:
Received 14 July 2011
Received in revised form 19 December 2011
Accepted 23 December 2011
Available online 11 January 2012

Keywords:
Prostate cancer
Catenin
LEF-1
E-cadherin
Androgen receptor
Wnt

ABSTRACT

δ-Catenin binds the juxtamembrane domain of E-cadherin and is known to be overexpressed in some human tumors. However, the functions of δ-catenin in epithelial cells and carcinomas remain elusive. We found that prostate cancer cells overexpressing δ-catenin show an increase in multi-layer growth in culture. In these cells, δ-catenin colocalizes with E-cadherin at the plasma membrane, and the E-cadherin processing is noticeably elevated. E-Cadherin processing induced by δ-catenin is serum-dependent and requires MMP- and PS-1/γ-secretase-mediated activities. A deletion mutant of δ-catenin that deprives the ability of δ-catenin to bind E-cadherin or to recruit PS-1 to E-cadherin totally abolishes the δ-catenin-induced E-cadherin processing and the multi-layer growth of the cells. In addition, prostate cancer cells overexpressing δ-catenin display an elevated total β-catenin level and increase its nuclear distribution, resulting in the activation of β-catenin/LEF-1-mediated transcription and their downstream target genes as well as androgen receptor-mediated transcription. Indeed, human prostate tumor xenograft in nude mice, which is derived from cells overexpressing δ-catenin, shows increased β-catenin nuclear localization and more rapid growth rates. Moreover, the metastatic xenograft tumor weights positively correlate with the level of 29 kD E-cadherin fragment, and primary human prostate tumor tissues also show elevated levels of δ-catenin expression and the E-cadherin processing. Taken together, these results suggest that δ-catenin plays an important role in prostate cancer progression through inducing E-cadherin processing and thereby activating β-catenin-mediated oncogenic signals.

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1. Introduction

δ-Catenin is a member of the p120-catenin (hereafter, p120) subfamily of armadillo proteins and is abundantly expressed in neuronal cells. In addition, δ-catenin is overexpressed in some cancers, including those of prostate, breast and lung [1–3]. Both p120 and δ-catenin are known to bind to the juxtamembrane domain regions of E-cadherin, and p120 has been shown to regulate E-cadherin stability, endocytosis and processing. Recently, δ-catenin accumulation in the urine of prostate cancer patients has been reported, and it promotes prostate cancer cell growth and tumorigenesis by altering the cell cycle and the profiles of survival genes [4,5]. However, siRNA-based genetic screening of human mammary epithelial cells identified δ-catenin as a potential suppressor of anchorage-independent growth [6]. Accordingly, the functions of δ-catenin in tumorigenesis require clarification.

E-cadherin, one of the major classical cadherins involved in epithelial cell–cell adhesion, is frequently absent or down-regulated in various human epithelial cancers. E-cadherin contains an extracellular domain, a transmembrane domain and a cytoplasmic domain. Particularly, its cytoplasmic domain associates with β-catenin, which links actin cytoskeletal networks via α-catenin [7,8]. The reduction in E-cadherin expression is correlated with malignant behavior in cancer, such as the loss of epithelial morphology and increased invasiveness and metastatic potential. E-cadherin down-regulation depends on either transcriptional or post-transcriptional modifications. As an example of transcriptional regulation, the presence of a somatic mutation or

Abbreviations: PS-1, Presenilin-1; MMP, Matrix Metalloproteinase; HGF, Hepatocyte Growth Factor; CTF, C-Terminal Fragment.

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doi:10.1016/j.bbadis.2011.12.015

hypermethylation in its promoter reduces E-cadherin expression, and the expressions of specific transcriptional repressors, such as Snail, Twist or ZEB, can also reduce E-cadherin expression [9–13]. In addition, the post-translational regulation of E-cadherin can be achieved by controlling its endocytosis, degradation or processing. The post-translational regulation of E-cadherin is particularly linked with p120. p120 promotes the cell surface trafficking of cadherins, regulates cadherin stability and turnover at the plasma membrane [14–17], and also facilitates the recycling of cadherin back to the cell surface [14,18].

E-cadherin processing occurs when cells are exposed to various stimuli that activate specific proteases. For example, E-cadherin cleavage results from the activation of presenilin-1 (PS-1)/γ-secretase, caspase, calpain, MMP-3, MMP-7, MT1-MMP, plasmin, kallikerin 7, ADAM10 or ADAM15, which can generate different N-terminal and C-terminal E-cadherin fragments [19–24]. Ectodomain shedding of E-cadherin releases soluble E-cadherin fragments, which affect its homophilic associations [25]. Recent reports demonstrate that ADAM15 activity causes ectodomain shedding of E-cadherin with subsequent ErbB receptor activation and signaling through the Erk pathway [26]. An E-cadherin C-terminal fragment that can enter the nucleus and regulate p120-Kaiso-mediated signaling has also been reported [27]. Accumulated evidence suggests that E-cadherin processing involves many proteases and affects many cellular functions, including adhesion, proliferation, migration and invasion/metastasis, due to E-cadherin loss- or gain-of-function. Although the presence of tumor-specific or bacteria-induced E-cadherin fragments suggests that the processing of E-cadherin should be tightly regulated [21,28,29], the exact mechanisms involved have not been elucidated.

Lu et al. reported that δ-catenin binds to E-cadherin [30]. Recently we found that δ-catenin preferentially binds to E-cadherin in epithelial cells and localizes at the plasma membrane when stable cell–cell junctions are formed [31]. However, the functions of δ-catenin in epithelial and epithelial-derived carcinoma cells remain undetermined. In the present study, we found that human prostate cancer cells overexpressing δ-catenin show an increase in multi-layer growth in culture and substantial processing of plasma membrane E-cadherin. Our results suggest that δ-catenin-induced E-cadherin processing was found to be dependent on the activities of MMP and PS-1/γ-secretase and on extracellular serum factors. Introducing deletion mutations on δ-catenin that deprives the ability to bind E-cadherin or to recruit PS-1 to E-cadherin totally abolishes the δ-catenin-induced E-cadherin processing and multi-layer growth of the cells. Interestingly, the levels of total and nuclear β-catenin, one of the major components of E-cadherin complex, are elevated in cells overexpressing δ-catenin, and the expressions of their downstream target gene for β-catenin/LEF-1 as well as androgen receptor-mediated transcription are increased in these cells. In accordance, human prostate tumor xenograft, which is derived from cells overexpressing δ-catenin, shows increased β-catenin nuclear localization and more rapid growth rates. Furthermore, metastatic xenograft tumor weights positively correlate with the level of 29 kD E-cadherin fragment, and primary human prostate tumor tissues also show elevated levels of δ-catenin expression and E-cadherin processing. Our results suggest that δ-catenin plays a role in prostate cancer progression through inducing E-cadherin processing and thereby activating β-catenin-mediated oncogenic signals.

2. Materials and methods

2.1. Plasmids and antibodies

The constructions of full length and ΔC787 δ-catenin in pEGFP-C1 have been previously described [32]. The WT and D257A mutants of PS-1 in pCS4-HA were generated by PCR amplification and cloned into pCS4-HA vector.

The antibodies used in the present study were as follows: anti-δ-catenin (C98320); and E-cadherin (C20820) (BD Biosciences), E-cadherin (#3195) (Cell signaling); anti-GFP (632376) and β-catenin (C2206) (Sigma); anti-α-tubulin (sc5286), β-tubulin (sc5274), cyclin D1 (sc20044), CDK4 (sc260), androgen receptor (sc7305) and E-cadherin (sc7870) (Santa Cruz Biotech.); α-Histone H3 (05–499) (upstate). For immunohistochemistry, anti-δ-catenin (ab54578) (abcam) and β-catenin(#9582) (Cell Signaling Technology) were used. HA epitope was detected using media from 12CA5 hybridoma.

2.2. Cell culture, transfection, and reagents

CWR22Rv-1 and PC3 human prostate cancer cell lines were maintained in RPMI and DMEM, respectively, supplemented with 10% FBS and 1% penicillin/streptomycin at 37 °C in a 5% CO_2 atmosphere. Rv/C or Rv/δ cells were grown in G418 (Sigma) 125 μg/ml supplemented media. Plasmid DNA was transfected using Plus/Lipofectamine reagent (Invitrogen), and siRNAs were transfected using Lipofectamin RNAiMAX (Invitrogen) according to the manufacturer's instructions. δ-Catenin siRNAs were purchased from Sigma-Aldrich : siRNA#1 (Oligo# 996742), siRNA#2 (Oligo#996743). For RT-PCR, the primer set for cyclin D1 was 5′-gaccatccccctgacggccgag-3′/5′-ccgcacgtcggt gggtctgc-3′, for c-myc was 5′-tctggatcaccttctgctgg-3′/5′-cctcttga catttctcctcg-3′, for c-jun was 5′-atgactgcaaagatggaaac-3′/5′-gtcac gttcttggggcaca-3′, and for GAPDH was 5′-atcaccatcttccaggagcga-3′/ 5′-agttgtcatggatgaccttggc-3′. Synthetic testosterone, R1881, was from NEN Life Science (Boston, MA, USA), and charcoal dextran-treated (CDT) FBS was from Invitrogen.

2.3. Immunostaining and image acquisition

Cells growing on glass coverslips were fixed with 4% PFA in PBS, blocked using PBS containing 1% goat serum for 1 h at RT, and incubated with primary antibody for 16 h at 4 °C. Cells were then washed in PBS, incubated for 1 h at RT with Rhodamine-conjugated or Alexa Fluor 633-conjugated secondary antibody, and washed in PBS. Cells were visualized under an inverted fluorescent microscope as previously described [31]. For confocal analyses, images were visualized and acquired using a TCS SP5 AOBS/Tandem microscope (Leica Microscope systems GmBh) at Korea Basic Science Institute, Gwangju center, Republic of Korea.

2.4. Immunoblotting and immunoprecipitation

Immunoprecipitation and immunoblotting were performed as previously described [33]. Immunoprecipitation was performed on lysates by incubation with primary antibody for 16 h at 4 °C and immunoprecipitates were pulled down using protein G-Sepharose beads (GE healthcare). All results are representative from at least three independent experiments.

2.5. Purification of cell surface proteins by biotinylation

Nearly confluent cells in 100 mm dishes were washed and collected in 1.5 mL microcentrifuge tubes using ice-cold reaction buffer (0.1 M phosphate, 0.15 M NaCl, pH 8.0). Cell surface proteins were labeled with biotin by incubating tubes with 0.5 mM Sulfo-NHS-SS-Biotin (Pierce) at RT for 30 min. Cells were then lysed in 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 PMSF, and protease inhibitor mixture) and cleared by centrifugation at 13,200 rpm for 15 min at 4°C. Equal amounts of protein lysates were then subjected to pull-down with streptoavidin-agarose beads (Pierce), and biotinylated plasma membrane E-cadherin was detected by immunoblotting with an E-cadherin antibody. For lysosomal-endocytic pathway inhibiting experiments, cells were treated with E-64 (10 μM) at 37°C for

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