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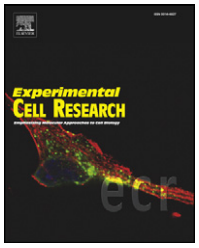

Research Article

Gα$_{12}$ binds to the N-terminal regulatory domain of p120^{ctn}, and downregulates p120^{ctn} tyrosine phosphorylation induced by Src family kinases via a RhoA independent mechanism

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ABSTRACT

p120 catenin (p120^{ctn}) regulates cadherin stability, and thus facilitates strong cell-cell adhesion. Previously, we demonstrated that Gα$_{12}$ interacts with p120^{ctn}. In the present study, we have delineated a region of p120^{ctn} that binds to Gα$_{12}$. We report that the N-terminal region of p120^{ctn} (amino acids 1-346) is necessary and sufficient for the interaction. While the coiled-coiled domain and a charged region, comprising a.a 102-120, were found to be dispensable, amino acids 121-323 were required for p120^{ctn} binding to Gα$_{12}$. This region harbors the phosphorylation domain of p120^{ctn} and has been postulated as important for RhoA regulation. Downregulation of Src family kinase-induced tyrosine phosphorylation of p120^{ctn} was observed in the presence of activated Gα$_{12}$. This down-regulation was triggered by three different Gα$_{12}$ mutants uncoupled from RhoA signalling. Furthermore, a dominant active form of RhoA did not reduce Src-induced phosphoryaltion of p120^{ctn}. In summary, our results suggest that Gα$_{12}$ binds to p120^{ctn} and modulates its phosphorylation status through a Rho-independent mechanism. Gα$_{12}$ emerges as an important regulator of p120^{ctn} function, and possibly of cadherin-mediated adhesion and/or cell motility.

Introduction

Cadherins are members of a family of functionally related transmembrane glycoproteins that mediate calcium-dependent cell-cell adhesion by homophilic interaction with cadherins on adjacent cells [1]. Cadherins play critical roles in development, morphogenesis and cancer [1,2]. Regulation of cadherin function occurs through the interaction of the intracellular, C-terminal domain of cadherins with a group of cytoplasmic proteins called catenins. All catenins belong to the subfamily of armadillo repeat domain proteins. β-catenin or γ-catenin bind tightly in a mutually exclusive fashion to the distal region of the cadherin C-terminus and link the cadherin complex to the actin cytoskeleton via interaction with α-catenin [1,3,4]. The catenin p120 (p120^{ctn}) is a

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distant relative of β-catenin that binds to the juxtamembrane domain of cadherins and controls their stability and retention at the cell surface (reviewed in [5]).

Four different ATGs located in the p120^{ctn} N-terminus are used as transcriptional start codons, giving rise to four different isoforms of p120^{ctn} [5]. The shortest isoform, p120 4A, lacks the N-terminal domain and is much more efficient in the positive regulation of cadherin function than the longest isoform, p120 1A [5]. p120^{ctn} is also an important regulator of RhoGTPases (reviewed in [6]). Specifically, p120^{ctn} has been shown to inhibit RhoA and activate Rac1 and Cdc42 [6]. In addition, p120^{ctn} is a substrate for non-receptor tyrosine kinases (Src, Fyn, Yes), receptor tyrosine kinases, and serine-threonine kinases [5]. Many of the phosphorylation sites are clustered in an N-terminal region of p120^{ctn} termed the phosphorylation domain [7]. Although the roles for individual phosphorylation sites have not been completely dissected, it is clear that this region is important for p120^{ctn} activities, including regulation of RhoA and cell adhesion. Recently, it has been shown that the differential phosphorylation of tyrosines 112, 217 and 228 by Fyn and Src kinases modulates the ability of p120^{ctn} to bind and inhibit RhoA [8].

The G12 subfamily of G proteins is comprised of the ubiquitously expressed members Gα$_{12}$ and Gα$_{13}$, which share 67% amino acid identity [9]. These proteins regulate a variety of cellular responses including transformation of fibroblasts [10], activation of JNK and serum response element [11,12], neurite retraction in PC12 cells [13], apoptosis [14], cell migration [15,16] and RhoA-dependent stress fiber formation [17]. The activation of RhoA-dependent mechanisms induced by the G12 family members occurs through binding and activation of Rho guanine nucleotide exchange factors containing an RGS-like domain (RGS-RhoGEFs) (reviewed in [18]). In addition, Gα$_{12/13}$ have been found to interact with the cytoplasmic tail of E-cadherin in a region different from β-catenin and p120^{ctn} [19,20], and trigger the release of β-catenin from cadherin complexes, thereby disrupting cadherin-mediated cell-cell adhesion [21].

Previously, we have demonstrated that the activation of RhoA by G12 subfamily proteins can counteract the p120^{ctn}-mediated inhibition of RhoA, and that both members of the G12 subfamily can interact with p120^{ctn} in the presence or absence of E-cadherin [22]. In this report, we have further investigated this interaction and show that Gα$_{12}$ binds to a region overlapping with the phosphorylation domain in the N-terminus of p120^{ctn}. Furthermore, Gα$_{12}$ downregulates the overall tyrosine phosphorylation on p120^{ctn}, including phosphorylation of residue Y228 induced by Src and EGF. We also show that the inhibitory activity of Gα$_{12}$ on p120^{ctn}-mediated RhoA regulation does not require Gα$_{12}$ binding to p120^{ctn}. Furthermore, we demonstrate Gα$_{12}$-mediated down-regulation of p120^{ctn} tyrosine phosphorylation to be independent of its ability to stimulate Rho activation.

Experimental procedures

Cell culture and Transfection

Human embryonic kidney cells (HEK293) cells were obtained from Invitrogen (Carlsbad, CA, USA), while HCT116 were obtained from American Type Culture Collection (ATCC) (Manassas, VA, USA). All cell types were grown in Dulbecco's modified Eagle's medium (DMEM) (Sigma, St. Louis, MO, USA), supplemented with 10% fetal bovine serum (EuroClone, Italy). NIH3T3 cells (ATCC) were grown in DMEM supplemented with 10% calf serum (Invitrogen). Cells were transiently transfected either with Lipofectamine (Invitrogen) or Fugene 6 (Roche, Switzerland) according to the manufacturer's instructions.

cDNA constructs

The pRcCMVmp120 1A construct was a generous gift from A. Reynolds (Vanderbilt University School of Medicine, Nashville, Tennessee, USA). The pRcCMVp120 1A Δ622-628 construct was kindly provided by P. Z. Anastasiadis (Mayo Clinic, Jacksonville, Florida, USA). FLAG-tagged full-length p120^{ctn} and deletion constructs were obtained from S. Aho (Thomas Jefferson University, Philadelphia, Pennsylvania, USA). The p120^{ctn} phosphorylation mutants phr-p120Y112E-GFP and pcDNA3-p120Y217E were a kind gift from M. Duñach (Universitat Autònoma de Barcelona, Bellaterra, Spain) and cDNA for active Fyn kinase, pEF-BOS-active Fyn was obtained from A. Carrera (Centro Nacional de Biotechnología, Madrid, Spain). The activated mutants of human Gα$_{12}$ and RhoA, pcDNA3-Gα$_{12}$Q231L and pcDNA3RhoAG14V respectively, were obtained from Guthrie Research Institute (Sayre, PA, USA), while the constitutively inactivated mutant of Gα$_{12}$, pCis-Gα$_{12}$G228A, was provided by S. Offermanns (University of Heidelberg, Germany). The activated mutant of mouse Gα$_q$, pCis-Gα$_q$R183C was kindly provided by M. Simon (Caltech, USA). The Gα$_q$R183C was subcloned into a pcDNA3 vector and used for further studies. The Gα$_{12}$ NAAIRS substitution variants with replacement of native residues 106-111, 232-237, 340-345 were described previously [23], with 106-111 replaced by the sequence Asn-Ala-Ala-Ile-Arg-Ser in mutant R, 232-237 replaced in mutant MM, and 340-345 replaced in mutant EEE. The cDNA for constitutively activated Src, pSM-CAM-Src, was from J.S. Gutkind (NIH, Bethesda, Maryland, USA). Expression plasmids for different p120^{ctn} constructs in pcDNA3 were engineered as follows: p120 1A was amplified by PCR using pRcCMVmp120 1A as a template, with the following forward primer containing a KpnI site (underlined): 5'- CTT GGT ACC GAA TGG ACG ACT CAG AGG TG- 3'and the reverse primer containing an EcoRI site (underlined): 5'-GCG AAT TCC TAA ATC TTC TGC ATC AAG GGT G- 3'. p120 NT was generated using the forward primer with the KpnI site (as above) and the following reverse primer containing an EcoRI site (underlined): 5'-ACG AAT TCC TAG TCT TCA TAG CTC CTG AG-3'. The isoforms 2, 3, 4 and the other N-terminal truncated mutants of p120^{ctn} were generated using forward primers with the KpnI site (as above) joined to the isoform-specific 5'sequence at either Met 55 (p120 2A), Met 102 (p120 3A), Met 121 (p120ΔN120), Met 158 (p120ΔN157), Pro 222 (p120ΔN221; here, an additional ATG was included in the primer in front of the codon for Pro), Met 245 (p120ΔN244), Met 298 (p120ΔN297) or Met 324 (p120 4A). Expression constructs for the deletion mutants p120Δ102-120, p120Δ121-221, p120Δ123-255, p120Δ173-310 and p120Δ222-323 were made by a two-step PCR strategy. In the first reaction, the N-terminal and C-terminal regions were amplified using the KpnI forward primer and EcoRI reverse primer for p120 1A as described above. These primers were used along with internal primers flanking the region to be deleted. In the second PCR, the products from the first PCR were mixed and used as templates with the same primer set used for amplification of p120 1A above. This reaction sewed the N- and C-terminal domains together with the desired regions deleted. The PCR products were digested with