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Comparative Interaction of Cadherin Cell Adhesion Proteins with Guanine Nucleotide-Binding Proteins Gα12 and Gα13

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Abstract

Cell migration, proliferation, and attachment are critical processes for both normal tissue growth, and the development of malignant disease. The G12/13 class of heterotrimeric guanine nucleotide-binding (G) proteins and their interaction with cadherin cell adhesion proteins has been implicated in the regulation of these processes. The two members of the G12/13 class, Gα12 and Gα13 are structurally similar, sharing 67% amino acid homology, but show only partial overlap in effector signaling.[1] Previous work demonstrated that Gα12 binds an acidic 11 amino acid region of the cytoplasmic C-terminal domain of epithelial (E)-cadherin, a membrane spanning cell adhesion protein responsible for adherens junction formation. This interaction was shown to cause cell disassociation and cell migration,[2] a hallmark of metastasis. While this region of E-cadherin was implicated in this binding event, the interacting residues and affinities of Gα12 and Gα13 with E-cadherin and other cadherin family members have yet to be elucidated. Here, epitope tagged mutants of Gα12 and chimeric constructs of Gα12 and Gα13 were used to examine binding affinities of these proteins with several members of the cadherin family. Interestingly, the activated form of Gα12 had a higher affinity for all cadherins tested when compared to Gα13, suggesting Gα12 may play a larger role in the regulation of these cell adhesion proteins. Pursuant to previous investigations, six sextet cassette mutants of eGFP tagged activated $G\alpha12^{QL}$ spanning the switch region I were utilized to examine binding to E-cadherin and VE-cadherin, which surprisingly failed to uncouple interaction. The E-cadherin deletion mutant that lacks the 11 amino acid region and had been demonstrated to disrupt binding of Gα12[2] was tested alongside Gα13 and strikingly showed strong binding to $G\alpha12^{QL}$ but abrogated binding to $G\alpha13^{QL}$. These data show that a potentially different activational conformation exists between $G\alpha12^{QL}$ and $G\alpha13^{QL}$, but that both G proteins bind a common region of the cytoplasmic domain of E-cadherin.

Keywords: Gα12/13, Cadherins, Cancer

1. Introduction

Heterotrimeric guanine nucleotide-binding proteins (G proteins) mediate numerous cellular physiological pathways, including cell proliferation, cytoskeletal changes, cell growth, and oncogenic transformation. These proteins are composed of a tightly associated βγ dimer and a α subunit, whose activation is dependent on GDP-GTP exchange. Upon stimulation of the G protein's G protein coupled receptor (GPCR), the α subunit binds GTP and disassociates from the βγ dimer, allowing it to interact with its downstream effectors.[1] Recent studies have shown that Gα12 and Gα13 are unique among the G protein subfamilies in that overexpression, and not mutational activation, of these

proteins drives neoplastic transformation in cells, making these molecules excellent targets of research for cancer therapies.[1,3,4]

Classical cadherins are a large family of single-pass transmembrane proteins that mediate cell adhesion through the formation of adherens junctions.[5] Cadherins consist of an extracellular N-terminal domain and a cytoplasmic C-terminal domain. The extracellular domain binds homotypically with cadherins of the same isoform in a Ca^{2+} dependent manner, and it is this homotypic binding of cadherin types that is largely responsible for the demarcation seen between discrete tissues.[5] The cytoplasmic domain of cadherins have several binding partners necessary for the structural integrity of the protein and maintenance of the cytoskeleton, including α-catenin, β-catenin, and p120-catenin, with α-catenin serving to bind the actin cytoskeleton, stabilizing the cytoskeleton at the adherens junction. In addition to facilitating cellular adhesion, there is evidence that E-cadherin acts as a tumor suppressor.[6] Many cancers of epithelial tissue show a marked decrease in E-cadherin expression, the principle cadherin of epithelial cells. This decreased expression is a hallmark of highly invasive cancers, while a higher expression of E-cadherin correlates to less invasive neoplastic tissue.[6] As mentioned, the cytoplasmic domain also serves to bind the transcriptional co-activator β-catenin, which has been demonstrated to activate several oncogenes once inside the nucleus.[7]

Previous investigation has demonstrated that Gα12 bound to an acidic 11 amino acid sequence of the E-cadherin cytoplasmic tail and caused cell migration and the dislocation of β-catenin from the cytoplasmic tail complex to the cytoplasm.[2] While this revealed a region of E-cadherin implicated in binding Gα12, the surfaces of Gα12 responsible for this interaction were not studied. Recently, it was found that Gα13 interacts with the cytoplasmic tail of vascular endothelial (VE)-cadherin at a cytoplasmic tail sequence of high similarity to that of E-cadherin by Gα12, and that it is the switch region I of Gα13 that binds to this distinct region on VE-cadherin.[8] The similarity of Gα12 and Gα13 in structure and the structural homology between E-cadherin and VE-cadherin, served as the impetus to determine if the switch region I of Gα12 is responsible for E-cadherin interaction. A comparative interaction study of Gα12 and Gα13 with E-cadherin, VE-cadherin, N-cadherin, and Cadherin-14 was also undertaken to better understand the binding affinities between these G proteins and several members of the cadherin family, using eGFP tagged forms of the G proteins. These interactions were also tested with eGFP tagged chimeras of Gα12 and Gα13 to more accurately define the regions of each G protein responsible for interaction. Finally, because Gα13 was not examined in relation to the acidic 11 amino acid sequence of E-cadherin, eGFP tagged $G\alpha13^{QL}$ was employed in co-precipitation assays with this E-cadherin deletion mutant.

2. Materials and Methods

2.1 Epitope Tagging of Gα12 Constructs

2.1.1 GFP Tagging of Gα12 NAAIRS Mutants

All NAAIRS mutants had previously been tagged with a myc epitope (amino acid sequence: EQKLISEEDL) with SGGGGS linkers by a silent restriction site (AgeI) at residues P^{139} V^{140} and were housed in pcDNA 3.1 (-). NAAIRS mutants designated FF, GG, HH, II, JJ, KK were digested with AgeI HF (New England Biolabs) at 37°C for 1 hour alongside a pcDNA 3.1 (-) plasmid containing eGFP. After 1 hour, 1.5μL of calf intestinal phosphatase (New England Biolabs) was added to the NAAIRS mutant plasmids and allowed to continue at 37°C for 1 hour. Gel electrophoresis was used to separate the resultant DNA on 1% (w/v) agarose and the NAAIRS mutant plasmids and the GFP insert were excised and isolated from the gel by mini column (Promega Wizard Kit). 500ng of plasmid was then ligated with 9μL of eGFP insert DNA using T4 DNA ligase (New England Biolabs) for 1 hour at room temperature then overnight at 4°C. JM109 competent cells were transformed with 1μL of the respective ligations and were grown on 100μg/mL ampicillin agar plates for 12-16 hours. Resulting singular colonies were picked and grown in 75μg/mL ampicillin Luria Broth (LB) for 12 hours. DNA was isolated from cells by mini column (Qiagen) and quantified by spectroscopy at 260nm. DNA was then transfected into HEK293 cells and examined under fluorescent microscopy for stable GFP expression.

2.1.2 GFP and Myc tagging of Gα12/Gα13 Chimeras

A silent mutation producing an AgeI cut site was introduced into each of the four chimeric constructs by polymerase chain reaction (PCR) using the following oligonucleotides:

FW – GAACAAGGCAGGGCTACCGGTGGAGCCTGCCACC
RV – GGTGGCAGGCTCCACCGGTAGCCCTGCCTTGTTC

Two separate reactions and cycles of forward alone oligonucleotide and reverse alone oligonucleotide were performed at 95°C for 35 seconds, 54°C for 1 minute, and 68°C for 12 minutes, before being combined and continued for an additional 15 cycles at the same conditions. Reactions were then digested with DpnI for 1 hour to eliminate parent DNA present in the reaction and these samples transformed into JM109 cells for DNA propagation. Presence of successful AgeI site insertion was determined by diagnostic restriction enzyme digest and subsequent gel electrophoresis. Splicing of eGFP epitope tag was then performed as described above.
Because the DNA sequence for the myc epitope tag was too small to be visualized and gel excised, phosphorylated oligonucleotides
(FW – [PHOS]CCGGTGAGCGGAGGAGGAGGAAGCGAGCAGAAGTTAATCAGCGAAGAAGATTTAAGCGGAGG AGGAGGATCA / RV – [PHOS]CCGGTGATCCTCCTCCTCCGCTTAAATCTTCTTCGCTGATTAACTTCTGCTCGCTTCCTCCTCCTC CGCTCA) were used to produce the double stranded DNA encoding the myc tag sequence. Both oligonucleotides were combined at 1μM in a microfuge tube using elution buffer (Qiagen) as the diluent and heated for 5 minutes at 95°C. Approximately 1.5L of water was brought to 85°C and the annealing reaction was placed in the water bath and the beaker removed from heat. The reaction continued for 2 hours, until the water bath reached 40°C. This myc DNA sequence was then ligated into the chimeras using the methods previously described.

2.2 Cloning of Gα12/Gα13 Chimeras into pcDNA 3.1(-)

Chimeras of Gα12 and Gα13 were generously donated by Dr. Barry Kreutz of the University of Illinois at Chicago. To facilitate the eGFP epitope tagging of these constructs, it was necessary to move them to pcDNA 3.1(-) to avoid extra AgeI cut sites in pCMV that originally housed the chimera DNA and would have interfered with the ligation process. Two different sets of forward and reverse primers were required for PCR to amplify the chimeras due to their ends being either Gα12 or Gα13 sequence. Also, BamHI sites and KpnI sites were inserted in the forward and reverse oligonucleotides, respectively, to enable cloning of these chimeras into pcDNA 3.1 (-). Cut sites are underlined.

Chimeras #1 & # 2: FW 5'-GTTGGGATCCACCACCATGTCCGGGGTGGTGCGGACC- 3'
RV 5'-GTTGGGTACCTCACTGCAGCATGAGCTGCTTCAG- 3'

Chimeras #3 & #4: FW 5'- GTTGGGATCCACCACCATGGCGGACTTCCTGCCGTCG- 3'
RV 5'- GTTGGGTACCTCACTGCAGCATGATGTCTTTC- 3'

The PCR conditions for the amplification of the chimeras were as follows: 95°C for 2 min, 94°C for 45 sec, 53°C for 1 min, 72°C for 2.5 min at 27 cycles, followed by a final 72°C 5 min elongation step. PCR products were then ligated into pcDNA3.1 (-) as previously described and successful ligation was confirmed by diagnostic restriction digest.

2.3 Creation of GST-VE-cadherin C-terminal tail

Full length VE-cadherin gene sequence was purchased from GE healthcare in bacterial glycerol stock. Oligonucleotides were designed to amplify the DNA sequence representing the 98 C-terminal amino acids of the VE-cadherin gene. A forward primer BamHI site and a reverse primer EcoRI site were inserted to facilitate the ligation of the PCR product into the GST harboring pGEX-KG plasmid (GE healthcare). Below are the amplifying primers:

FW: 5'- CGATGGATCCCCGAGGCACGCGCCTGGGGCAC- 3'
RV: 5'- TAGCGAATTCCTAATACAGCAGCTCTCCCG- 3'

PCR conditions used were as described in section 2.2.

2.4 Expression and Purification of GST fused proteins

Vectors containing the GST-cadherin cytoplasmic tail sequences were transformed into BL21(Gold)DE3 *E. coli*. Individual respective colonies for each GST construct were then inoculated in 12mL of Luria Broth (LB) with ampicillin (amp) added to a concentration of 75μg/mL. These cultures were then grown for 12-16 hours at 37°C under 220rpm agitation. After this growth phase, 5mL of this turbid culture was then inoculated in 500mL of LB under the same growth and amp concentration conditions. After 90 min, this 500mL culture was sampled and its concentration measured by spectrophotometry at 600nm. When the A_{600} value was between 0.5 and 0.8, induction of protein expression was stimulated by isopropyl-β-D-thiogalactopyranoside addition and the culture incubated for 3 hours. Bacteria were collected by centrifugation at 6000 x *g* at 4°C for 15 min. These cells were then homogenized in 5.5mL of cold GST Buffer A (2.3M sucrose, 50mM Tris pH 7.7, 1mM EDTA) containing a 1:500 dilution of protease inhibitors. 10mL of cold GST buffer B (50mM Tris pH 7.7, 10mM KCl, 1mM EDTA, 1mM DTT, 1:500 protease inhibitors) was then added to the homogenized bacterial sample and mixed. Following a 4-5mg lysozyme addition, the homogenized bacterial sample was incubated on ice for approximately 1 hour and mixed every 10 min. Following this incubation, 175μL of 10% sodium deoxycholate, 260μL of 1M $MgCl_2$, and 25μL of 5mg/mL DNAse I was added to the crude lysate and further incubated on ice for approximately 10 min. This mixture was rocked by hand every 2 min until a noticeable loss of viscosity was observed. This mixture was then refined by centrifugation at 15,000 x *g*, 4°C for 40 min and the resulting supernatant was added to 0.35mL of glutathione-sepharose beads (GE healthcare) that had been previously washed in T_{50}ED buffer (50mM Tris pH7.7, 1mM EDTA, 1mM DTT). This supernatant and glutathione-sepharose was machine rocked for 45 min at 4°C to allow interaction. The GST immobilized proteins were then washed further with T_{50}ED buffer supplemented with 150mM NaCl four times and aliquots of these purified proteins were snap frozen in liquid N_2.

2.5 Preparation of Gα12 and Gα13 constructs from whole cell lysates

Human embryonic kidney (HEK293) cells were cultured in Dulbecco Eagle medium with 10% fetal bovine serum and antibiotics, incubated in a 5% CO_2 atmosphere at 37°C. Approximately 10μg of construct DNA and 2mg/mL of polyethylenimine (PEI) were used to transfect HEK293 cells in a 10cm dish, with cells at 90% confluence. DNA and PEI were mixed with 500μL of DMEM and incubated for 15 min at room temperature, then introduced drop-wise to the cell plates. At 36-48 hours post transfection, the plates were rinsed with 4°C sterile phosphate buffered saline (PBS), scraped and collected in 3mL of PBS by centrifugation at 500 x *g* at 4°C. Each cell pellet then received 500μL of ice-cold lysis buffer (50mM HEPES pH 7.5, 1mM EDTA, 3mM DTT, 10mM $MgSO_4$, 1% polyoxyethylene-10-lauryl ether (LPX), 50% of volume MP 2X protease inhibitor mix, 1:500 protease inhibitor mix) and cells were lysed on ice by trituration. Samples were mixed by inversion at 4°C for 30 min, and then centrifuged at approximately 64,000 x *g* 3°C for 1 hour. Supernatants were then collected and 60μL aliquots of each construct were snap frozen in liquid N_2.

2.6 Co-precipitation of GST-cadherins and Gα12/13 constructs

Each 60μL lysate containing the Gα12/13 construct to be assayed was diluted with HEDM buffer (50mM HEPES pH 7.5, 1mM EDTA, 3mM DTT, 10mM $MgSO_4$) to lower the LPX concentration to approximately 0.075%. From this dilution, 40μL was removed to serve as the load control for the lysate being assayed, and the remainder equally distributed to be incubated with the GST-cadherin construct. These samples were allowed to interact for approximately 3 hours at 4°C by inversion. Interaction tubes were then centrifuged at 1300 x *g* at 2°C and the supernatants removed. Pelleted GST-sepharose beads were then washed 3 times with 1mL of HEDM buffer containing LPX at a concentration of 0.05%. Denaturation was achieved by addition of SDS sample buffer supplemented with 10% DTT at 72°C for approximately 10 min. Denatured proteins were then separated by SDS-PAGE, with 3μL of each sample used as the loading control for the Coomasie Blue stain gel, to determine equal amounts of GST-fused cadherin available in each *in vitro* interaction condition. Remaining protein was separated by SDS-PAGE and transferred to nitrocellulose paper for Western blotting. eGFP primary antibody (Thermo Scientific) was diluted to 1:1000 in 5% milk and TBST (Tris buffered saline, Tween-20) solution and incubated with immunoblot

for 3 hours at room temperature with agitation. Following three 10-minute washes of the immunoblot with plain TBST, a 1:7500 dilution of alkaline phosphatase conjugated secondary mouse antibody (Promega) in 5% milk and TBST was introduced and incubated for 1 hour under constant agitation. Three additional TBST washes were followed by detection of protein bands colorimetrically using NBT/BCIP in alkaline phosphatase buffer solution. Resulting blots were quenched with ddH_2O and photographed using Kodak GelLogic 100 imaging system and Gaussian fit densitometric data was obtained by Carestream 5X software.

3. Results

The highly similar amino acid homology between Gα12 and Gα13 led to the question of whether binding affinities with the cadherin types differed between Gα12 and Gα13. In order to facilitate the detection of both G proteins with immunoblot analysis, without the need for using two specific antibodies, a common epitope tag was needed. The eGFP (*e*nhanced *g*reen *f*luorescent *p*rotein) tag was chosen because it adds approximately 26kDa of mass to the G proteins, allowing the epitope tagged protein to be easily distinguishable from endogenous Gα12 and Gα13 proteins from whole cell lysates (Fig.1A). eGFP is also beneficial because it can be inserted into the alpha helical domain of both G proteins and does not disrupt the functional conformations of either protein, as evidenced by standard SRE luciferase response[9], of which Gα12 and Gα13 are potent stimulators (Fig. 1B). In addition, eGFP tagged Gα12 and Gα13 can be visualized by fluorescent microscopy (Fig. 1C).

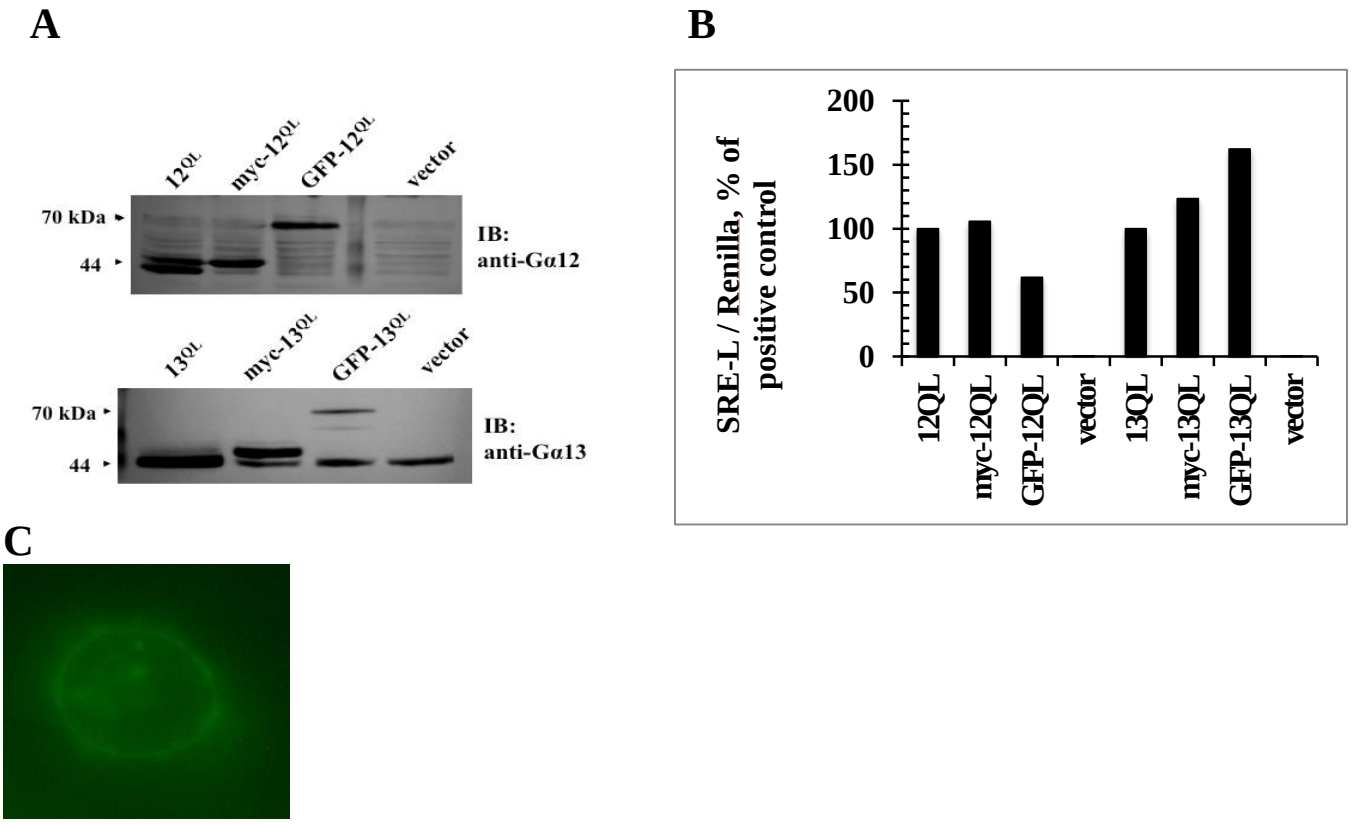


Figure 1. eGFP tagged Gα12 and Gα13 retain functionality. (1A) Immunoblot demonstrating the increased molecular weight of eGFP tagged Gα12 and Gα13 using anitbodies for both Gα12 and Gα13. The myc tag is another epitope tag that is commonly used but was chosen against due to poor immunoblot detection. (1B) SRE/Renilla response assay of epitope tagged Gα12 and Gα13 along with untagged protein. This assay is highly

robust and regularly evidences proper folding conformation of both proteins. (1C) HEK293 cell showing eGFP tagged Gα12 within the cell.

To further interrogate the findings that the switch region I of Gα13 is necessary for the interaction with VE-cadherin[8], constitutively activated NAAIRS cassette substitution mutants of Gα12 spanning the switch region I were implemented in co-precipitation assays with E-cadherin and VE-cadherin. These cassette substitution mutants were utilized due to their highly tolerated sequence of asn-ala-ala-ile-arg-ser (NAAIRS) within the conformational structure of Gα12 (Fig.2). Surprisingly, no switch region I spanning NAAIRS mutant failed to uncouple binding to the 98 C-terminal amino acids of either E-cadherin (Fig. 3A,B) or VE-cadherin (data not shown) in co-precipitation assays, with precipitate to load ratios all greater than 1, demonstrating strong interaction. These results were particularly surprising due to the high sequence homology seen between the switch region I of Gα12 and Gα13.

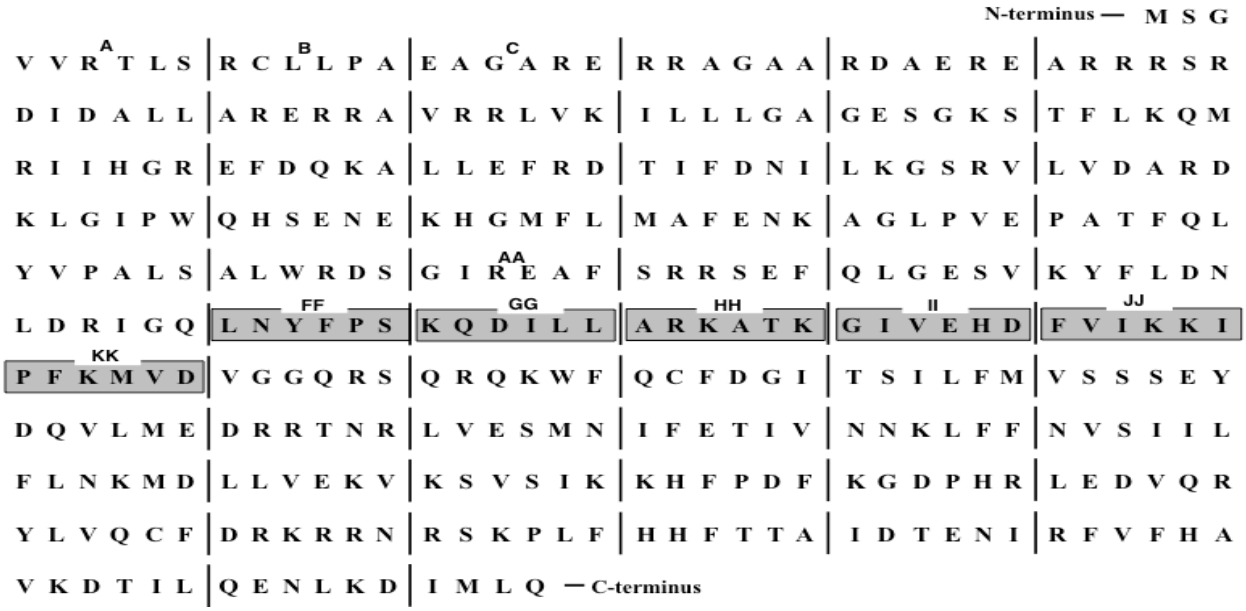


Figure 2. Highlighted boxes show the regions of primary Gα12 sequence that are substituted with the NAAIRS mutant sequence. The actual switch region I is composed of the ARKATKGIVEH sequence, within the HH and II NAAIRS mutants. The double lettered alphabetical nomenclature system designates the specific NAAIRS mutant.

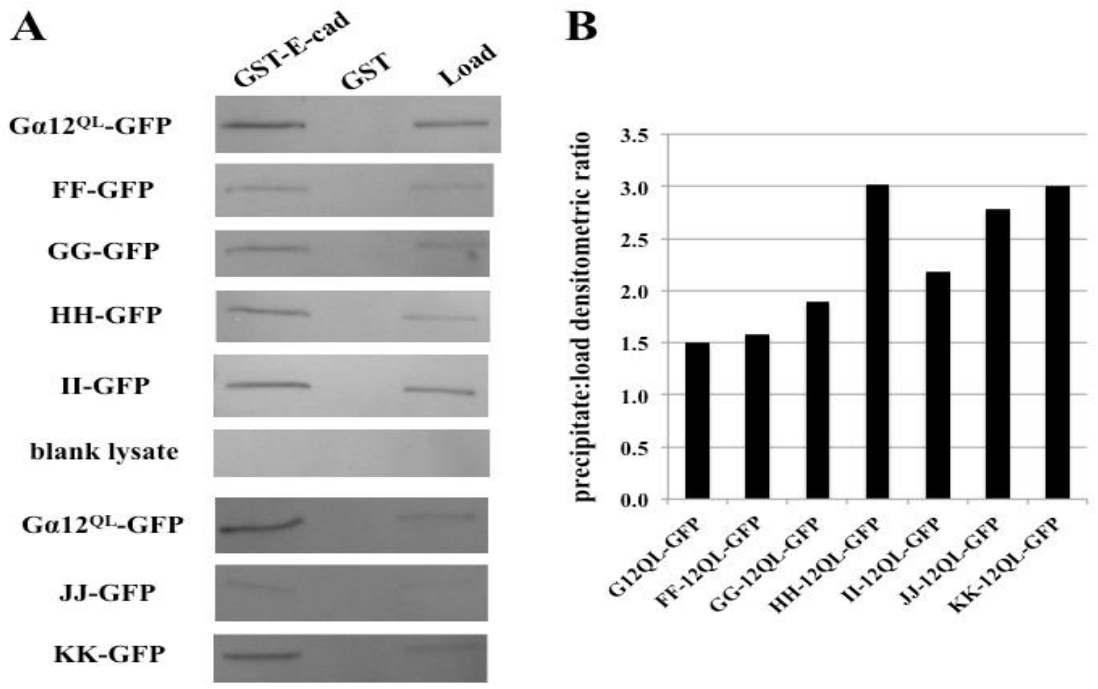
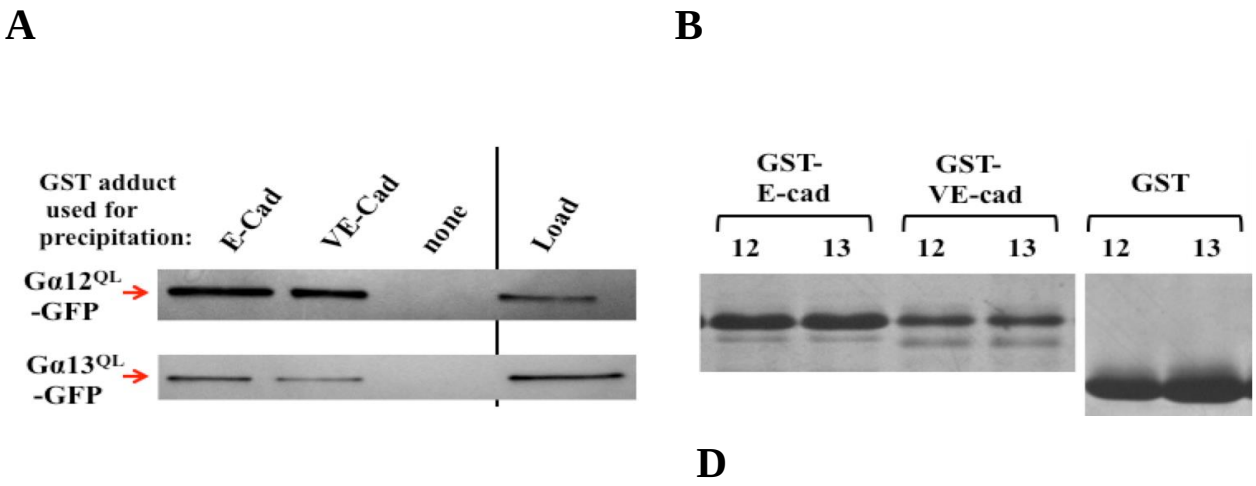


Figure 3. No Gα12^{QL}-GFP NAAIRS mutant shows inhibited binding to E-cadherin or VE-cadherin. (3A) Immunoblots of co-precipitation experiments examining FF-NAAIRS through KK-NAAIRS Gα12^{QL} mutants with the GST-fused 98 C-terminal amino acids of E-cadherin, showing no impaired binding. Gα12^{QL} NAAIRS mutants were detected by eGFP antibody. (1B) Densitometric data for the displayed co-precipitation experiments, showing the precipitate to load ratio for each immunoblot.

Previous investigations into vascular endothelial tissue disruption have found that the interaction of Gα13 with VE-cadherin leads to vascular barrier leakiness and inflammation in human lung tissue.[8] However, this study did not examine Gα12's ability to bind VE-cadherin. Also, because Gα12 and Gα13 display differing effector interaction, it was sought to determine if preferential binding occurs between the two G proteins and the cadherins tested. Interestingly, it was discovered that the Gα12^{QL} bound more strongly to E-cadherin, VE-cadherin, N-cadherin and cadherin-14 compared to Gα13^{QL} (Fig. 4A-D).



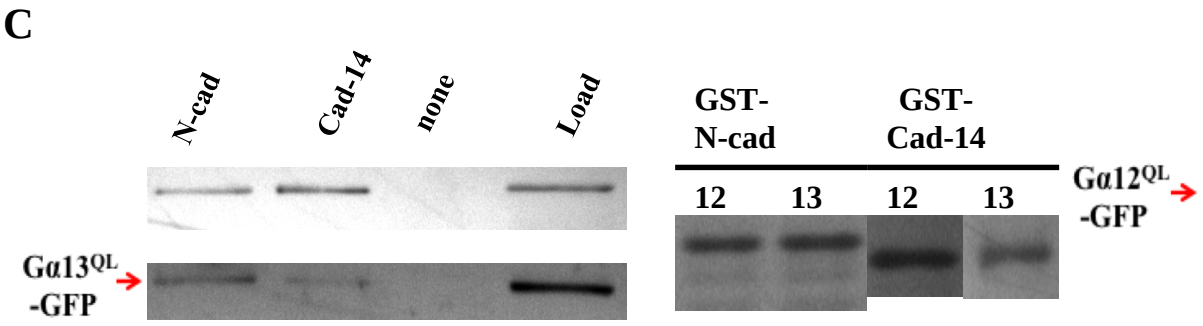


Figure 4. Co-precipitation of activated Gα12 shows preferential binding to all cadherins tested. (1A) Western blot band intensity and precipitate to load ratio shows clear higher affinity binding of $G\alpha12^{QL}$ to E-cadherin and VE-cadherin compared to $G\alpha13^{QL}$. (1B) The Coomasie Blue protein stain is used as a loading control to determine that each experimental co-precipitation received the same amount of available GST-fused cadherin that would be available to bind. This stain reveals that each condition had equal amounts of E-cadherin and VE-cadherin available for interaction. (1C) shows the co-precipitation data for $G\alpha12^{QL}$ and $G\alpha13^{QL}$ binding to N-cadherin and Cadherin-14, with $G\alpha12^{QL}$ exhibiting higher affinity binding. 1D) Cooblue stain again showing levels of available GST-fused protein for G protein binding.

To determine which regions of $G\alpha12^{QL}$ and $G\alpha13^{QL}$ were responsible for binding to the cadherins, four chimeric constructs previously described [10] were used in co-precipitation experiments (Fig. 5). Cell lysates expressing the chimeric constructs showed remarkably similar precipitate to load ratios of binding for all the C-terminal tails of the cadherins examined, with cadherin-14, the only type II cadherin tested, showing the most decreased signal. E-cadherin, VE-cadherin and N-cadherin each showed similarly strong binding affinity to all four chimeras, which, in combination with the uniformly weak cadherin-14 signal, made interpretation of these results particularly difficult (Fig.6).

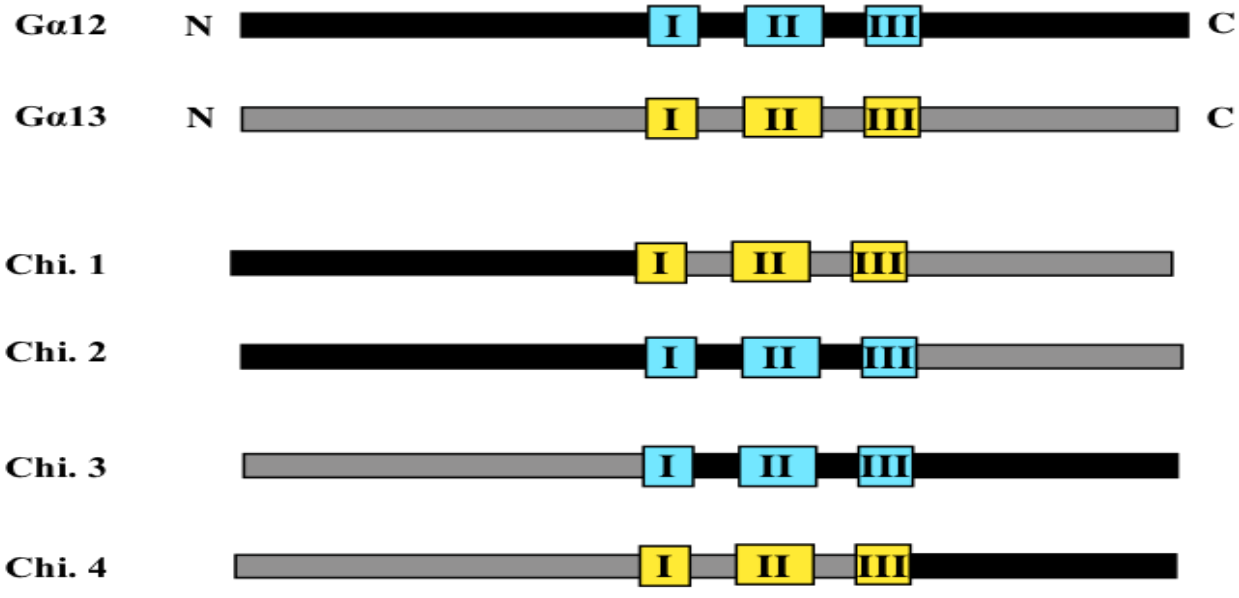


Figure 5. Schematic maps of Gα12, Gα13 and the four chimeras used for co-precipitation with cadherins. Gα12 amino acid sequence is in black with cyan switch regions labeled I, II, and III, while Gα13 sequence is gray, with

switch regions highlighted in yellow. Each chimera harbored the constitutively activating $Q^{229}L$ mutation and was epitope tagged with eGFP.

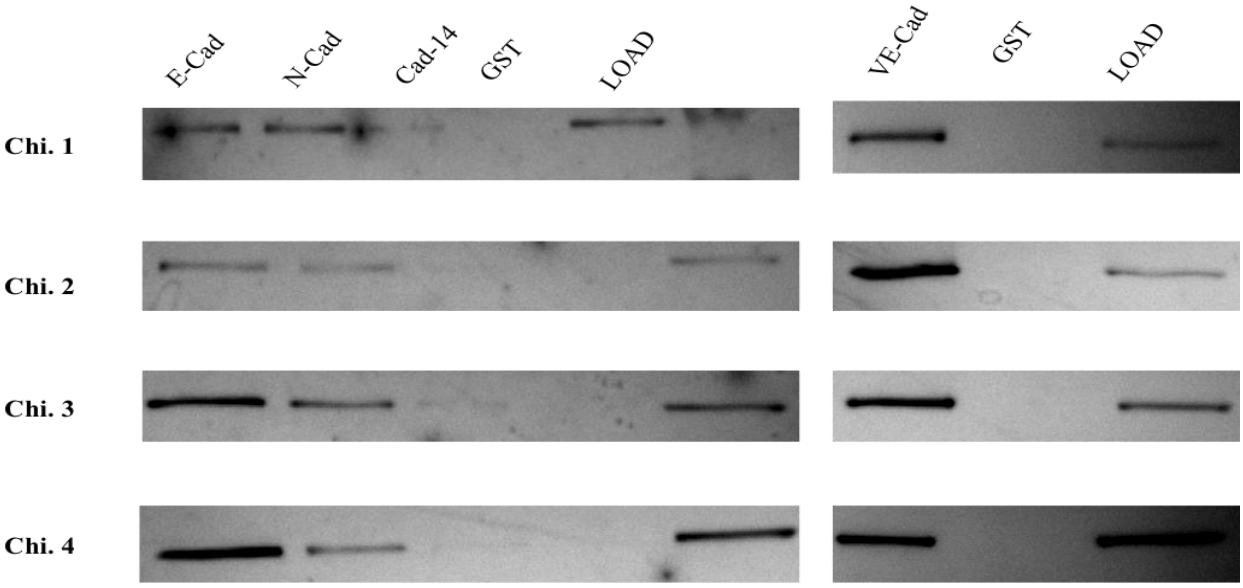


Figure 6. Chimeras of Gα12 and Gα13 fail to delineate specific determinants of binding to cadherins. Representative Western blots of co-precipitation with GST-fused cadherin tail experiments. The cadherins aligned at the top were GST fused and served as the co-precipitation, or "pulldown" agent. The "loads" are at the far right after chimera 1. Each chimera produced similar precipitate to load ratios with every cadherin, except chimera 2 with VE-cadherin, which showed a strong pulldown signal when compared to the other cadherins precipitated with chimera 2. Protein bands were detected by eGFP antibody as described in section 2.5.

To further investigate previous findings that an acidic 11 amino acid region of the E-cadherin tail is necessary for its interaction of $G\alpha12^{2}$ (Fig.7A), constitutively active $G\alpha13^{QL}$ was co-precipitated with this E-cadherin deletion mutant. Strikingly, it was found that $G\alpha12^{QL}$ still bound strongly to this deletion mutant (E-cadΔG12) while $G\alpha13^{QL}$ binding was drastically reduced (Fig.7B). While surprising, this finding suggests that this region of E-cadherin is also necessary for Gα13 interaction, while also introducing the possibility that these mutationally active constructs may differ in their three dimensional active conformations.

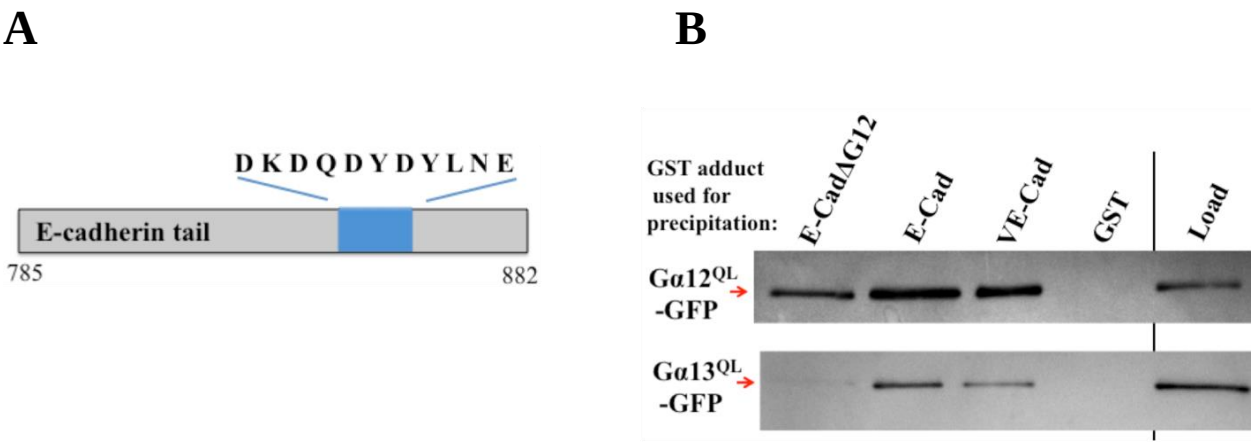


Figure 7. The Gα12 interacting domain of E-cadherin is bound by Gα13. (1A) A schematic map of the 98 C-terminal amino acids of the cytoplasmic tail of E-cadherin. The blue box represents the 11 amino acid region that is

deleted in E-cadΔG12 with its sequence shown in single letter amino acid code. (1B) Western blots showing the abrogated binding of Gα13^{QL} –GFP to the E-cadΔG12 mutant in parallel with E-cadherin and VE-cadherin, while again demonstrating the preferential binding of Gα12 to E-cadherin and VE-cadherin. Immunoblots shown are representative of four separate trials.

4. Discussion

Present results have failed to reveal which residues of Gα12 or Gα13 are responsible for their interaction with cadherin cell adhesion molecules. The failure of the Gα12^{QL} NAAIRS mutants of the switch region I to uncouple interaction with both E-cadherin and VE-cadherin was surprising, especially when compared to previous investigations into the interaction of Gα13 with VE-cadherin, which found this region necessary for the interaction. Still, the possibility remains that although these G proteins share a high level of homology, this region of Gα12 may not be involved with its interaction with cadherin proteins. Interestingly though, two switch region I NAAIRS mutants, HH and II have proved successful in uncoupling interaction with another class of cell adhesion protein, β3 integrin.[11] A potentially useful tool for future experimentation could involve the production of these NAAIRS mutants in Gα13,which could be utilized in similar co-precipitation assays to replicate previous research into its interaction with VE-cadherin, and to further test the other cadherins studied here. Constitutively active Gα12 consistently showed higher affinity binding to all the cadherin molecules tested. This finding could suggest that Gα12 may play a larger physiological and pathological role in the regulation of these adhesion proteins than Gα13, though both Gα subunits can bind these molecules, and have been shown to disrupt cell adhesion.[12] The nebulous results from the chimeric analysis could be explained by the highly similar amino acid structures of both Gα12 and Gα13. Both Gα12 and Gα13 effectively bind to the cadherins examined, and it could be that the residues necessary for the interaction exist in both proteins and are still functional in the four chimeras. More specific chimeric constructs that swap switch region I of both Gα subunits could potentially illuminate the determinants of binding to these adhesion proteins. Though the residues of Gα12 and Gα13 responsible for binding cadherins were not determined, it was found that Gα13 binds the same 11 amino acid region of the E-cadherin cytoplasmic tail. More specific mutagenesis of this 11 amino acid region of E-cadherin could reveal the individual residues that mediate this interaction with both Gα subunits. The seemingly contradictory result that activated Gα12 still binds this E-cadherin deletion mutant could be explained by the activation strategy used. This study was performed using mutationally activated Gα12^{QL}, in which glutamine^{229} is mutated to a lysine residue. This mutation creates a GTPase deficient protein, simulating the active conformation. The previous finding that Gα12 cannot bind this E-cadherin deletion mutant used purified Gα12 that was activated by GTPγS loading.[2] It is possible that these two different activation methods could create different activational conformations of the protein. Furthermore, these QL mutationally active constructs are derived from whole cell lysates of HEK293 cells; this could also contribute to differing results due to the cellular context in which these constructs are produced. Another activational mutant of Gα12, arginine^{203} to cysteine has been engineered by the author and is currently being tested for binding to this E-cadΔG12 mutant and for stimulation of SRE response to illuminate possible discrepancies between this mutant and the canonical QL activating form.

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