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Invertebrate Gα12/13 chimeras show selective binding to RhoGEFs while retaining growth signaling

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Abstract

The G12/13 subfamily of heterotrimeric guanine nucleotide binding proteins (G proteins) has been shown to regulate the RhoA-mediated serum response element (SRE) signaling pathway. The α-subunit Gα12/13 does this by binding Rho-specific guanine nucleotide exchange factors (RhoGEFs) at their regulator of G protein signaling (RGS) homology (RH) domain and activating them, and these activated RhoGEFs subsequently bind and activate RhoA. The SRE growth signaling pathway has been implicated in gene transcription, cell migration, and proliferation, processes critical to carcinogenesis and metastasis. Chimeric Gα12/13 proteins containing the N-terminal region of the homologous *Drosophila* protein Concertina were found to exhibit selective binding to different RhoGEFs, interacting with leukemia-associated RhoGEF and PDZ-RhoGEF but not p115RhoGEF. Because these chimeric G proteins show normal growth signaling to SRE, an evolutionary and structural comparison was performed to identify N-terminal amino acids in Gα12/13 that are different or absent in Concertina. Based on these findings, we constructed a mutant Gα13 lacking Thr127 and Arg128, two residues previously shown to provide rgRGS contact points that were found to be absent in Concertina. Future work will analyze binding between this mutant Gα13 and known downstream effectors. p115 has also been found to promote the proliferation of gastric cancer cells through interacting with and stimulating the secretion of macrophage migration inhibitory factor (MIF), a cytokine that has been demonstrated to promote tumor progression. Identification of specific G12/13 residues critical for binding p115 could provide a novel target for cancer therapeutic strategies.

1. Introduction

Cells employ a plethora of pathways to perceive and respond to their environment. Many are initiated through the reception of extracellular signals by integral membrane proteins termed G protein-coupled receptors (GPCRs). GPCRs are numerous and diverse, collectively capable of binding ligands including neurotransmitters, hormones, and odorants, in addition to sensing light.[18] Recognition of an appropriate stimulus induces a conformational change of the GPCR, enabling activation of heterotrimeric guanine nucleotide-binding proteins (G proteins).[18,21] Heterotrimeric G proteins are intracellular signaling molecules comprising an α, β, and γ subunit. These proteins are inactive when their α-subunit is bound to GDP; GPCR stimulation induces the exchange of GDP for GTP, dissociating Gα from the Gβγ heterodimer. Gα subunits exhibit intrinsic GTPase activity; they eventually hydrolyze their bound GTP to GDP and reassociate with Gβγ.[8] Hydrolysis can be expedited by interaction with GTPase activating proteins (GAPs), which bind the Switch regions of activated Gα subunits and stabilize the transition state for GTP hydrolysis, thereby accelerating it.[1,24] Once dissociated, Gα and Gβγ subsequently activate downstream effector proteins.[18,19]

Gα proteins have been classified into four subfamilies based on amino acid sequence: Gs, Gi, Gq, and G12/13. Their distinctive effector interactions are imparted by their unique binding surfaces.[2] The G12/13 subfamily in vertebrates contains proteins Gα12 and Gα13, which govern diverse signaling networks that regulate cell migration and

proliferation.[10,23] Gα12 and Gα13 evolved from an ancestral G12/13 homolog encoded in invertebrates and share 67% sequence identity, enabling them to interact with many of the same downstream effector proteins. Both Gα12 and Gα13 bind RhoA-specific guanine nucleotide exchange factors (RhoGEFs) with regulator of G protein signaling homology domains (RH-RhoGEFs), inducing conformational changes that enable RH-RhoGEFs to subsequently exchange GDP for GTP on RhoA.[7,15,22] The RH-RhoGEFs include p115RhoGEF (p115), PDZ-RhoGEF, and leukemia-associated RhoGEF (LARG).[10] Activated RhoA stimulates the polymerization of cytoplasmic G-actin to form F-actin filaments, unbinding myocardin-related transcription factor A (MRTF-A).[17,22,27] Freed MRTF-A is translocated to the nucleus and transcriptionally coactivates serum response factor (SRF).[17,22,27] Activated SRF binds to serum response element (SRE), inducing transcription of immediate early response growth oncogene c-Fos.[17,22,27] Overexpressed wild type and constitutively activated Gα12 and Gα13 have both been demonstrated to induce oncogenesis and metastasis.[10] Additionally, Gα12 has been observed to be significantly upregulated in prostate cancer and in the earliest stages of breast cancer.[12,13]

Gα12 and Gα13 are capable of binding proteins other than RH-RhoGEFs. Gα12, but not Gα13, has been observed to bind the non-RhoGEF ARAF, a member of the Raf family of protein kinases.[4] The Raf protein kinases have been demonstrated to play important roles in signal transduction through regulating the MEK and ERK cascades, which have been implicated in cell migration.[4] Another protein reported to bind both Gα12 and Gα13 is protein phosphatase 5 (PP5), whose catalytic activity is purportedly activated when complexed with other proteins such as heat shock protein 90 (Hsp90), Gα12, and Gα13.[6,26] The TPR domain of PP5 acts as its binding surface.[6,26] An additional protein reported to interact with Gα13 is radixin, which is part of the ezrin/radixin/moesin (ERM) family of actin-binding proteins.[9,25] The ERM proteins are involved in cytoskeletal remodeling and cell migration, sharing overlapping roles with the Gα12 and Gα13 signaling networks.[9,25] A further protein that is hypothesized to bind to RhoA and possibly to Gα12 and Gα13 is p114RhoGEF (p114), but more experimentation is needed to know for certain.[16]

Recent research in our laboratory demonstrated that *Drosophila* G12/13 homolog Concertina was unable to drive SRE signaling in mammalian cells, then subsequently utilized this protein as a platform to identify key determinants of growth signaling in the Switch regions and C-terminal region of Gα12.[18] These data indicated that "chimera 4," a N-terminally myc-tagged and constitutively activated construct comprising the N-terminus of Concertina (residues 1-275) and the Switch regions and C-terminus of Gα12 (residues 202-379), signaled to SRE slightly stronger than constitutively activated Gα12.[18] A constitutive activation mutation of Q226L renders Gα12/13 incapable of hydrolyzing its bound GTP, thus remaining permanently activated.[18] Protein interaction assays were performed to assess the affinity of Gα12 chimera 4 for various potential binding partners including radixin, ARAF, the RhoGEF p114, and the RH-RhoGEFs p115 and PDZ-RhoGEF, but these trials were limited to using recycled anti-Gα12 antibody because it was no longer available. These assays were of poor quality due to mistakes such as overdevelopment of immunoblots and degraded primary antibody. Taking an alternative approach, a Gα13 version of chimera 4 was engineered and analyzed in protein interaction assays with radixin, PP5, and the RH-RhoGEFs p115, LARG, and PDZ-RhoGEF. These assays revealed an apparent abolishment of binding to p115, prompting further investigation.

A previous study by Chen and colleagues was able to crystallize the chimera "Gα13/i-5," a construct containing the helical domain and all three Switch regions of Gα13 on the backbone of Gαi, in complex with the rgRGS domain of p115.[3] Crystallographic analysis indicated that residues T127 and R128 of the chimera were in contact with the rgRGS domain of p115.[3] An evolutionary and structural comparison between Concertina and Gα13 using LALIGN highlighted that these two residues were unaligned with Concertina, spurring my hypothesis that these two residues were critical for Gα13 to bind p115.[20] To investigate the role of these two residues in binding with p115, a mutant Gα13 lacking them was engineered via PCR mutagenesis. p115 has also been found to promote the proliferation of gastric cancer cells through interacting with and stimulating the secretion of macrophage migration inhibitory factor (MIF), a cytokine that has been demonstrated to promote tumor progression.[14] Identification of specific G protein residues critical for binding p115 could provide a novel target for cancer therapeutic strategies.

2. Materials and Methods

2.1 DNA Constructs and Materials

Gα12 chimera 4 and Gα13 chimera 4 DNA constructs were obtained from Dr. Ted Meigs (University of North Carolina, Asheville) and were made as described previously.[18] N-radixin (residues 1-318) GST fusion beads, ARAF GST fusion beads, p114 (long) fusion beads, PP5-TPR GST fusion beads, GST beads, and BL21(Gold)-DE3 *E. coli*

cells (Agilent Technologies) transformed with DNA plasmids encoding GST fusion constructs of p115, LARG, and PDZ-RhoGEF stored in frozen glycerol stocks at -80 ºC were also obtained from Dr. Ted Meigs (University of North Carolina, Asheville).

2.2 Preparation of Glutathione *S*-Transferase (GST) Fusion Proteins

BL21(Gold)-DE3 *E. coli* cells (Agilent Technologies, Santa Clara, CA) transformed with DNA plasmids encoding GST fusion constructs of p115, LARG, and PDZ-RhoGEF were streaked onto Luria Broth (LB)-agar plates containing 100 µg/mL ampicillin using sterile technique and allowed to grow overnight. Sterile 12 mL LB cultures containing 75 µg/mL ampicillin were inoculated with single bacterial colonies and shaken at 37 ºC, 220 rpm for 12-16 hours. 6 mL from each 12 mL culture were transferred into sterile 500 mL LB cultures containing 75 µg/mL ampicillin and shaken at 37 ºC, 220 rpm. After 90 minutes, and every 20 minutes thereafter, absorbance readings at 600 nm were taken until an optical density of 0.5-0.8 was reached, then 0.5 mM isopropyl-β-D-thiogalactopyranoside (Fisher Scientific, Pittsburgh, PA) was added. All 500 mL cultures were shaken at 37 ºC, 220 rpm for 3 hours. Each 500 mL culture was divided into 3 large centrifuge bottles and centrifuged for 15 minutes at 4 ºC, 6,000 x g, and all pellets for each culture were resuspended and recombined in one allotment of 2.5 mL cold GST buffer A [2.3 M sucrose, 50 mM Tris pH 7.7, 1 mM EDTA, 1:500 dilution protease inhibitor mix). Each sample received 10 mL cold GST buffer B [50 mM Tris pH 7.7, 10 mM KCl, 1 mM EDTA, 1 mM dithiothreitol (DTT), 1:500 dilution protease inhibitor mix), was swirled, received 4-5 mg lysozyme powder, was swirled rapidly, and was incubated on ice for 1 hour, being swirled every 10 minutes. Each sample was transferred to an Oak Ridge centrifuge tube (Nalgene, Rochester, NY) on ice, received 175 µL 10% w/v sodium deoxycholate, 260 µL 1 M $MgCl_2$, and 25 µL 5 mg/mL DNAse I, and was rocked by hand every 2 minutes for 10 minutes. Samples were spun for 40 minutes at 4 ºC, 15,000 rpm in a Fiberlite F13-14x50 cy Fixed Angle Rotor (Thermo Scientific, Waltham, MA). While spinning, three 15 mL sterile conical tubes received 0.35 mL resuspended glutathione-sepharose beads and were washed three times with 14 mL ice-cold T_{50}ED buffer [50 mM Tris pH 7.7, 1 mM EDTA, 1 mM DTT]. The supernatant of each sample was decanted into one of the 15 mL sterile conical tubes of washed glutathione-sepharose beads and they were rocked on the Orbitron for 45 minutes at 4 ºC. The samples were spun for 3 minutes at 4 ºC, 1,300 x g, their supernatants were discarded, and they were washed four times with 14 mL modified T_{50}ED buffer [50 mM Tris pH 7.7, 1 mM EDTA, 1 mM DTT, 150 mM NaCl]. On the last wash, liquid was removed until the meniscus was at twice the height of the settled sepharose beads, 30-40 50 µL aliquots were prepared from each sample, snap-frozen in liquid N_2, and stored at -80 ºC.

2.3 Preparation of Detergent-Soluble Extracts of Gα Chimeras

Human embryonic kidney (HEK293) cells were grown in Dulbecco's modified Eagle's medium (Corning, Corning, NY) supplemented with 10% fetal bovine serum (Gibco, Billings, MT). Polyethylenimine was used to transfect a 10 cm dish of 90% confluent HEK293 cells with 10 µg plasmid DNA encoding G protein chimeras. Cells were washed with phosphate-buffered saline and scraped from the dish ~36 hours post-transfection, then centrifuged at 500 x g for 3 minutes. Pellets were resuspended and solubilized in lysis buffer [50 mM HEPES pH 7.5, 1 mM EDTA, 3 mM DTT, 10 mM $MgSO_4$, 1% (w/v) polyoxyethylene-10-lauryl ether (LPX)] containing protease inhibitors 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride (1.67 mM), leupeptin (2.1 µM), pepstatin (1.45 µM), Nα-tosyl-L-lysine chloromethyl ketone (58 µM), tosyl-L-phenylalanylchloromethane (61 µM), and phenylmethylsulfonyl fluoride (267 µM). Lysates were continuously inverted at 4 ºC for 30 minutes and centrifuged at 80,000 x g for 1 hour. Supernatants were aliquoted and snap-frozen in liquid N_2 and stored at -80 ºC.

2.4 Protein Interaction Assays

Cell lysate extracts were diluted in HEDM buffer [50 mM HEPES pH 7.5, 1 mM EDTA, 3 mM DTT, 10 mM $MgSO_4$]. 3% of each diluted lysate sample was reserved prior to the interaction experiment for the positive control (load). Sepharose-bound GST-fusion proteins and GST were diluted ~10-fold with HEDM buffer and combined with the lysate samples. Samples were inverted continuously for 90 minutes at 4 ºC, centrifuged for 3 minutes at 2 ºC, 1,300 x g, and washed twice with HEDLM buffer (HEDM buffer with 0.05% (w/v) LPX). Pellets were often too small to visualize, so the remaining ~25 µL of sample was mixed with a 1:10 ratio of 1 M DTT to 4X Laemmli sample buffer. Samples were incubated for 10 minutes at 72 ºC and stored at -20 ºC. Samples were subjected to SDS-PAGE and immunoblot analysis using a primary antibody specific to either the Gα12 N-terminus (Santa Cruz Biotechnology, Santa Cruz, CA), Gα13 (MilliporeSigma, Burlington, MA), or the myc epitope tag (MilliporeSigma, Burlington, MA),

followed by alkaline phosphatase-conjugated secondary antibodies (Promega, Madison, WI). Immunoblots were developed using alkaline phosphatase 1% Tween20 (AP1) buffer, 5-Bromo-4-chloro-3-indolyl phosphate (BCIP), and nitro blue tetrazolium (NBT). Additional polyacrylamide gels were run with 3 µL of each sample and stained with Coomassie Brilliant Blue to ensure interactions or lack of interactions were not due to quantities of GST-fusion proteins.

2.5 Sequence Analysis

The sequences of Gα12, Gα13, and Concertina were compared using LALIGN (University of Virginia, Charlottesville, VA) with the "global without end-gap penalty" alignment method.

2.6 PCR-Based Mutagenesis

The mutant Gα13 was engineered using PCR-based mutagenesis. It was constructed from two initial PCR amplimers derived from Gα13 and designed to have a 23 bp overlap. The sequence of the reverse oligonucleotide used to construct the first amplimer was 5′-AGCCATGGGGGCATCAAATGCCATCAACTTGTC-3′, and the sequence of the forward oligonucleotide used to construct the second amplimer was 5′-TGGCATTTGATGCCCCCATGGCTGCCCAGG-3′. The template Gα13 encoded a myc-tagged, constitutively activated variant. Primary PCR products were gel-extracted and subjected to a second round of PCR using end primers containing 5′-end restriction sites NheI and KpnI for cloning into the mammalian expression plasmid pcDNA3.1(-) (Invitrogen, Carlsbad, CA). The DNA construct was digested with NheI and KpnI (New England Biolabs, Ipswich, MA) and ligated into the plasmid, but it has not yet been purified or verified by sequencing.

2.7 Imaging Analysis

All SDS-polyacrylamide gels, immunoblots, and agarose gels were imaged using a Gel Logic 100 Digital Imaging System (Kodak, Rochester, NY) equipped with Molecular Imaging 5.X software (Carestream Health, New Haven, CT).

3. Results

3.1 Protein Interaction Assays with Gα12 Chimera 4

The prior finding by our laboratory that $G\alpha12^{QL}$ N-myc chimera 4 signaled to SRE slightly stronger than wild type $G\alpha12^{QL}$ prompted the experimental exploration of its affinities for downstream effector proteins.[18] Five protein interaction assays were performed with various characterized and potential binding partners of Gα12: ARAF, p114 long, N-radixin, LARG, p115, and PDZ-RhoGEF.[4,9,10,16,25] However, four of the trials were inconclusive due to complications such as under- or over-developed blots, and protein degradation due to unrefined technique, but also degraded antibody because only recycled anti-Gα12 antibody was available. In the one successful trial, it appeared that the weak binding of wild type $G\alpha12^{QL}$-myc to p115 was absent in $G\alpha12^{QL}$ N-myc chimera 4 (Figure 1).

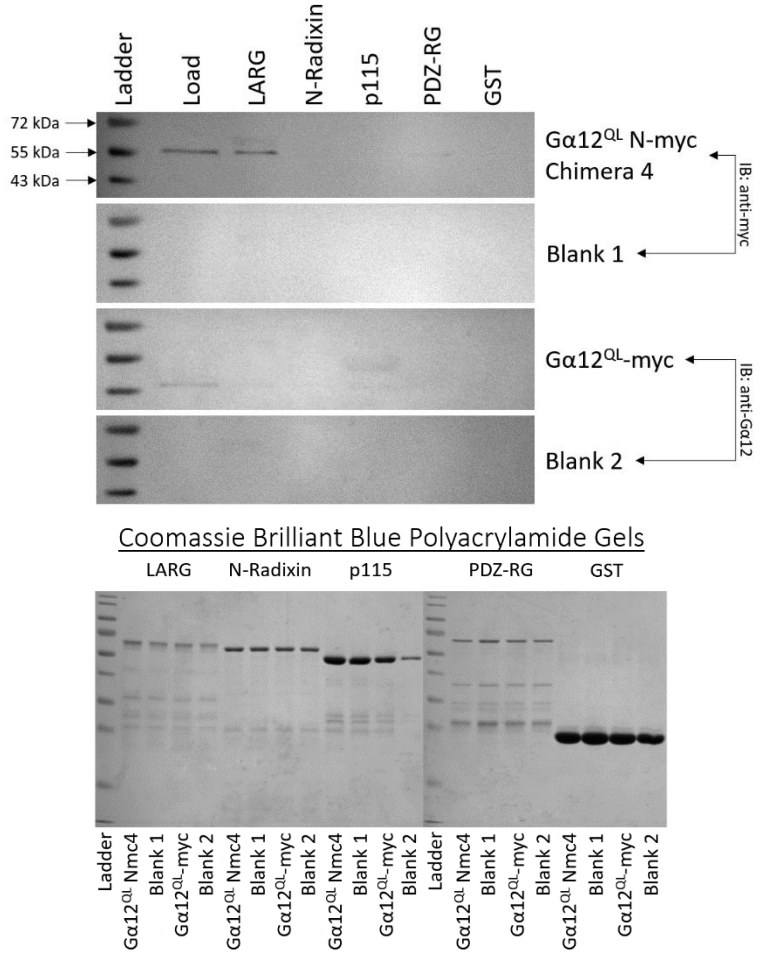

Figure 1. A N-terminal *Drosophila* Concertina chimera of Gα12^{QL} exhibits a possible loss of binding to p115.

Figure 1. Protein interaction assays were performed using lysates of Gα12^{QL}-myc and Gα12^{QL} N-myc chimera 4,[18] and GST-fusions of the N-terminal 318 residues of radixin and RH-RhoGEFs LARG, p115, and PDZ-RhoGEF. Data was representative of one trial. The N-terminus of Gα12 appears to possess a structure capable of binding p115 that is absent in the N-terminus of Concertina. Uniformity of GST-fusion protein levels across samples were analyzed using Coomassie Brilliant Blue staining.

3.2 Protein Interaction Assays with Gα13 Chimera 4

We then decided to take the alternative approach of examining the binding interactions of a Gα13^{QL} version of N-myc chimera 4. The 67% sequence identity between Gα12 and Gα13 and their overlap in several downstream binding targets made this a promising pursuit. Three protein interaction assays were performed with a more restricted set of characterized binding partners of Gα13: N-radixin, PP5-TPR, LARG, p115, PDZ-RhoGEF.[6,9,10,25,26] Regrettably, the first trial was ruined from overdeveloping the blots because the anti-mouse secondary antibody developed much faster than anticipated. In the following two successful trials, it was demonstrated that the strong binding of wild type Gα13^{QL}-myc to p115 was completely abolished in Gα13^{QL} N-myc chimera 4 (Figure 2).

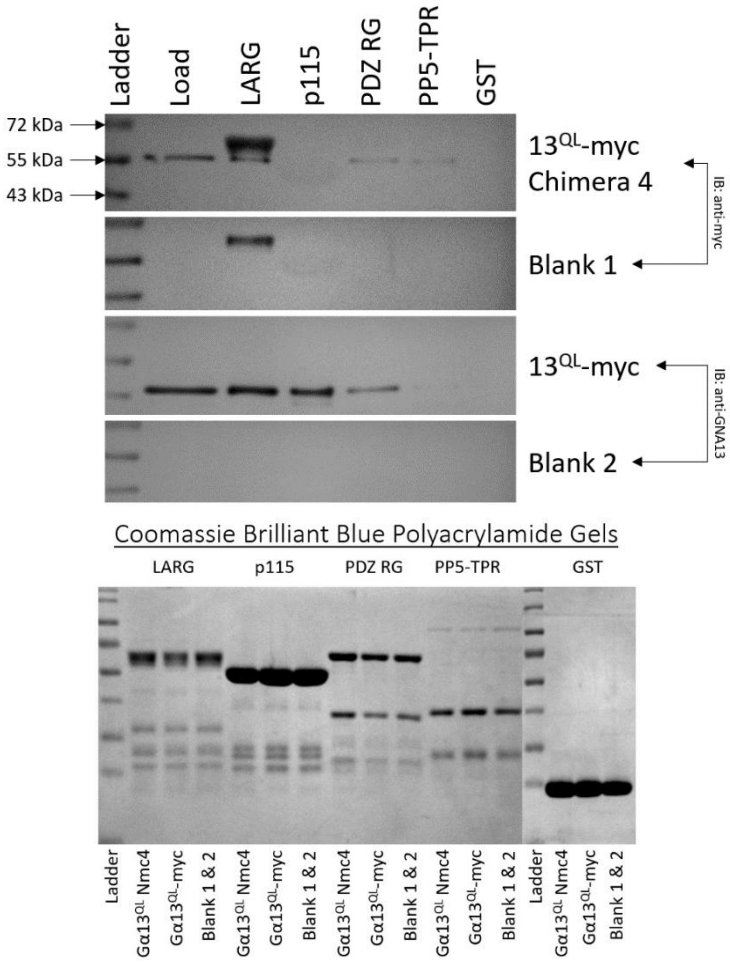

Figure 2. A N-terminal *Drosophila* Concertina chimera of Gα13^{QL} exhibits a complete loss of binding to p115.

Figure 2. Protein interaction assays were performed using lysates of Gα13^{QL}-myc and Gα13^{QL} N-myc chimera 4,[18] and GST-fusions of the TPR domain of PP5 and RH-RhoGEFs LARG, p115, and PDZ-RhoGEF. Data was representative of two trials, except for PP5-TPR, which was only assayed in one trial. The N-terminus of Gα13 appears to possess a critical structure for binding p115 that is absent in the N-terminus of Concertina. Uniformity of GST-fusion protein levels across samples were analyzed using Coomassie Brilliant Blue staining.

3.3 PCR Mutagenesis of Gα13 Lacking T127 and R128

Chen and colleagues performed a crystallographic analysis of chimera "Gα13/i-5," a construct containing the helical domain and all three Switch regions of Gα13 on the backbone of Gαi, in complex with the rgRGS domain of p115. These results indicated that residues T127 and R128 of the chimera were in contact with p115 rgRGS.[3] An evolutionary and structural comparison between Concertina and Gα13 using LALIGN highlighted that these two residues were unaligned with Concertina, leading me to hypothesize that these two residues were critical for Gα13 to bind p115.[20] To investigate the role of these two residues in binding with p115, a mutant Gα13 lacking them was engineered via PCR mutagenesis. The DNA construct was digested with NheI and KpnI (New England Biolabs, Ipswich, MA) and ligated into the plasmid, but it has not yet been purified or verified by sequencing (Figure 3).

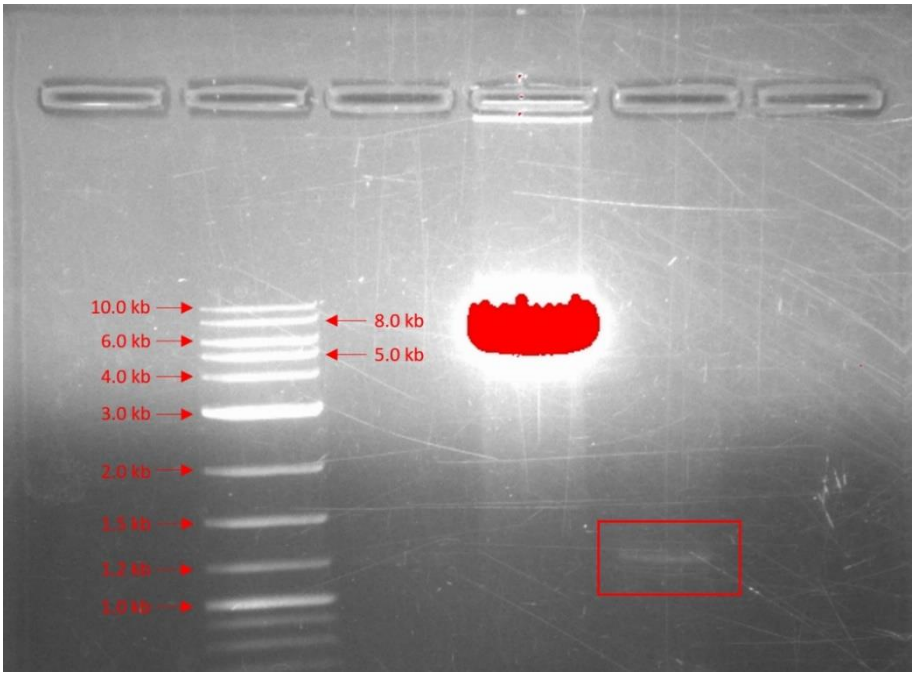

Figure 3. The 1.25 kb DNA construct encoding the Gα13^{QL} T127 and R128 double-deletion mutant is in the red box.

Figure 3. Agarose gel containing Quick-Load Purple 1 kb Plus DNA Ladder (New England Biolabs, Ipswich, MA) in lane 2, pcDNA3.1(-) mammalian expression plasmid (Invitrogen, Carlsbad, CA) digested with NheI and KpnI (New England Biolabs, Ipswich, MA) in lane 4, and the digested 1.25 kb DNA construct encoding the Gα13^{QL} T127 and R128 double-deletion mutant is in the red box in lane 5. The DNA construct and plasmid were subsequently purified with Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI), and the former was ligated into the latter.

4. Discussion

This study illuminated the loss of binding to p115 that was caused by the replacement of the N-terminus preceding the Switch regions of Gα13^{QL}, and possibly Gα12^{QL}, with that of *Drosophila* G12/13 homolog Concertina. It also partially produced a double-deletion mutant Gα13^{QL} lacking two residues known to contact p115 rgRGS in chimera Gα13/i-5.[3] While this mutation alone may not result in the abolishment of binding to p115, future work should aim to purify and sequence this mutant, then perform several protein interaction assays beginning with RH-RhoGEFs. Figure 2 shows that all other RH-RhoGEFs and PP5-TPR retain binding, with a slight possible increase in affinity for PP5-TPR and minor possible decrease in affinity for PDZ-RhoGEF (Figure 2). This suggests a potential regulatory target for knocking out the p115 pathway, which may prove to be useful for gastric cancer therapeutic strategies.[14] Residues T127 and R128 in Gα13 align with the functionally equivalent residues N134 and K135 in Gα12, indicating that this potential regulatory target may also exist in Gα12.[20] Therefore, a double-deletion mutant Gα12 lacking these two residues should also be investigated when fresh anti-Gα12 antibody is available.

The trial not shown that analyzed interaction with N-radixin displayed no bands for the chimera, Gα13^{QL}-myc, or the blanks, which is suspicious because N-radixin was reported to bind Gα13^{QL}.[9,25] Future work will involve the further characterization of the binding affinities of both Gα12^{QL} and Gα13^{QL} N-myc chimera 4 proteins to expand our understanding of the signaling pathways created by the divergent evolution of these proteins from their ancestral invertebrate homolog. Another useful approach would be to recreate these chimera 4's using the N-terminus of the α-subunit of *Caenorhabditis elegans* G12/13 homolog Gpa-12, establish each one's capability of signaling to SRE, and examine their interactions with Gα12/13 RhoGEFs.

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6. References

1. Berman DM, Kozasa T, Gilman AG. 1996. The GTPase-activating protein RGS4 stabilizes the transition state for nucleotide hydrolysis. J Biol Chem. 271(44):27209-27212.
2. Cabrera-Vera TM, Vanhauwe J, Thomas TO, Medkova M, Preininger A, Mazzoni MR, Hamm HE. 2003. Insights into G protein structure, function, and regulation. Endocr Rev. 24:765-781.
3. Chen Z, Singer WD, Sternweis PC, Sprang SR. 2005. Structure of the p115RhoGEF rgRGS domain–Gα13/i1 chimera complex suggests convergent evolution of a GTPase activator. Nat Struct Mol Biol. 12(2):191-197.
4. Gan X, Wang C, Patel M, Kreutz B, Zhou M, Kozasa T, Wu D. 2013. Different Raf protein kinases mediate different signaling pathways to stimulate E3 ligase RFFL gene expression in cell migration regulation. J Biol Chem. 288(47):33978-33984.
5. Gilman AG. 1987. G proteins: transducers of receptor-generated signals. Annu Rev Biochem. 56:615-649.
6. Golden T, Swingle M, Honkanen RE. 2009. Role of serine threonine protein phosphatase type 5 (PP5) in the regulation of stress induced signaling networks and cancer. Cancer Metast Rev. 27(2):169-178.
7. Hajicek N, Kukimoto-Niino M, Mishima-Tsumagari C, Chow CR, Shirouzu M, Terada T, Patel M, Yokoyama S, Kozasa T. 2011. Identification of critical residues in Gα13 for stimulation of p115RhoGEF activity and the structure of the Gα13-p115RhoGEF regulator of G protein signaling homology (RH) domain complex. J Biol Chem. 286(23):20625-20636.
8. Hollinger S, Hepler JR. 2002. Cellular regulation of RGS proteins: modulators and integrators of G protein signaling. Pharmacol Rev. 54(3):527-559.
9. Ivetic A, Ridley AJ. 2004. Ezrin/radixin/moesin proteins and Rho GTPase signalling [*sic*] in leucocytes. Immunology. 112(2):165-176.
10. Juneja J, Casey PJ. 2009. Role of G12 proteins in oncogenesis and metastasis. Brit J Pharmacol. 158(1):32-40.
11. Kelly P, Casey PJ, Meigs TE. 2007. Biologic functions of the G12 subfamily of heterotrimeric G proteins: growth, migration, and metastasis. Biochemistry-US. 46(23):6677-6687.
12. Kelly P, Moeller BJ, Juneja J, Booden MA, Der CJ, Daaka Y, Dewhirst MW, Fields TA, Casey PJ. 2006. The G12 family of heterotrimeric G proteins promotes breast cancer invasion and metastasis. P Natl Acad Sci USA. 103(21):8173-8178.
13. Kelly P, Stemmle LN, Madden JF, Fields TA, Daaka Y, Casey PJ. 2006. A role for the G12 family of heterotrimeric G proteins in prostate cancer invasion. J Biol Chem. 281:26483-26490.
14. Li XJ, Luo Y, Yi YF. 2013. P115 promotes growth of gastric cancer through interaction with macrophage migration inhibitory factor. World J Gastroenterol. 19(46):8619-8629.
15. Mao J, Yuan H, Xie W, Wu D. 1998. Guanine nucleotide exchange factor GEF115 specifically mediates activation of Rho and serum response factor by the G protein α subunit Gα13. P Natl Acad Sci USA. 95(22):12973-12976.
16. Medina F, Carter AM, Dada O, Gutowski S, Hadas J, Chen Z, Sternweis PC. 2013. Activated RhoA is a positive feedback regulator of the Lbc family of Rho guanine nucleotide exchange factor proteins. J Biol Chem. 288(16):11325-11333.
17. Miralles F, Posern G, Zaromytidou AI, Treisman R. 2003. Actin dynamics control SRF activity by regulation of its coactivator MAL. Cell. 113:329-342.
18. Montgomery ER, Temple BRS, Peters KA, Tolbert CE, Booker BK, Martin JW, Hamilton TP, Tagliatela AC, Smolski WC, Rogers SL, Jones AM, Meigs TE. 2014. Gα12 structural determinants of Hsp90 interaction are necessary for serum response element-mediated transcriptional activation. Mol Pharmacol. 85(4):586-597.
19. Oldham WM, Hamm HE. 2008. Heterotrimeric G protein activation by G-protein-coupled receptors. Nat Rev Mol Cell Bio. 9:60-71.

20. Pearson, W. 1996. LALIGN [Internet]. Charlottesville (VA): University of Virginia; [cited 2020 Dec 6]. Available from https://fasta.bioch.virginia.edu/fasta_www2/fasta_www.cgi?rm=lalign
21. Rosenbaum DM, Rasmussen SG, Kobilka BK. 2009. The structure and function of G-protein-coupled receptors. Nature. 459:356-363.
22. Siehler S. 2009. Regulation of RhoGEF proteins by G12/13-coupled receptors. Brit J Pharmacol. 158(1):41-49.
23. Suzuki N, Hajicek N, Kozasa T. 2009. Regulation and physiological functions of G12/13-mediated signaling pathways. Neurosignals. 17:55-70.
24. Tesmer JJ, Berman DM, Gilman AG, Sprang SR. 1997. Structure of RGS4 bound to AlF4--activated G(i alpha1): stabilization of the transition state for GTP hydrolysis. Cell. 89(2):251-261.
25. Vaiskunaite R, Adarichev V, Furthmayr H, Kozasa T, Gudkov A, Voyno-Yasenetskaya TA. 2000. Conformational activation of radixin by G13 protein alpha subunit. J Biol Chem. 275(34):26206-26212.
26. Yamaguchi Y, Katoh H, Mori K, Negishi M. 2002. Galpha(12) and Galpha(13) interact with ser/thr protein phosphatase type 5 and stimulate its phosphatase activity. Curr Biol. 12(15):1353-1358.
27. Yu OM, Brown JH. 2015. G protein-coupled receptor and RhoA-stimulated transcriptional responses: links to inflammation, differentiation, and cell proliferation. Mol Pharmacol. 88(1):171-180.