Bioinformatic and Structural Analyses of GCOM1 and its Interacting Genes Suggest New Functions

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INTRODUCTION

Since our discovery of the GCOM1 hub gene, we have focused on its roles in the central nervous system and deduced its likely functions. One method focuses on finding the genes with which GCOM1 proteins interact which we have termed Gints. The most significant interaction we have found thus far is between GCOM1 and GRIN1, which encodes the NR1 subunit of the NMDA receptor. Based on our observation that anti-GCOM1 antibodies protected neurons against NMDA toxicity, we hypothesized that GCOM1 and one or more Gints participate in a novel signal transduction pathway relevant to neuroprotection.

OBJECTIVES

- Demonstrate that GCOM1 and one or more Gints participate in a novel signal transduction pathway relevant to neuroprotection
  - –Focus on internexin-α (INA), which has been shown to interact with NMDAR,
  - –Provide evidence for a pathway of GRIN1> GCOM1> INA> downstream effectors.
- Prepare for our next series of biological experiments by performing advanced bioinformatic analyses of key GCOM1 and Gint proteins to determine the most likely pathways and structural correlates

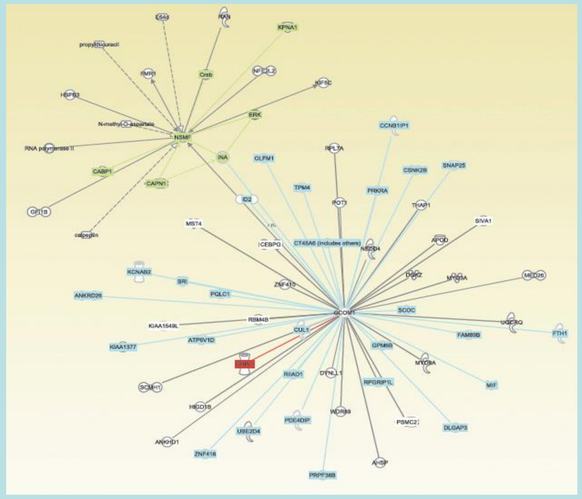

Figure 1. Path Designer analysis (IPA knowledgebase with additions from refs 2 and 7) showing network connections between GRIN1, GCOM1, INA, NSMF and other Gints.

METHODS

- Amino (codons 22-414) and carboxyl (332-550) cDNA "baits" of the human 550 aa Gcom1 mRNA/protein were cloned into the yeast two-hybrid (Y2H) vector pCWX200 and screened using TetR system against an adult human brain cDNA library. The Y2H screening yielded 27 distinct Gints.
- To derive Gcom15 cDNA, human and rat Gcom15 cDNAs were cloned from adult brain mRNA as overlapping 5' and 3' segments (Titan RT PCR system; Roche) which were combined by hybridization-extension PCR. The resulting large amplicons were ligated and transformed into competent *E. coli* using the TOPO-TA vector system (Invitrogen). Intact open reading frames (ORFs) coding for 765 and 761 amino acids for human and rat Gcom15, respectively, were identified and subcloned into the pCIneo expression vector (Promega). Each Gcom15 cDNA was transfected into HEK293 cells either with or without the mouse NR1-1a NMDAR subunit (a gift from R. S. Zukin) and incubated for 24 hours at 37 C. Membrane proteins were isolated for Western blots and immunoprecipitation (IP) with anti-GCOM1 S23-E38 or anti-NR1 Ab followed by Western blotting and staining with appropriate Ab (co-IP) (fig 2).
- Functional analyses were generated through the use of IPA (Ingenuity® Systems, www.ingenuity.com) Core Analysis, which assess over/under representation of signaling and metabolic pathways, molecular networks, and biological processes in the gene lists.
- Pathways were generated with IPA Path Designer, which builds pathways by connecting molecules and genes if any direct/indirect interaction or regulatory relationship exists between them either in the IPA knowledgebase, or provided by a user.
- Structural features and relationships were determined by programs from the internet, including Signal P, Motifs, Phyre, protein docking and I-TASSER.

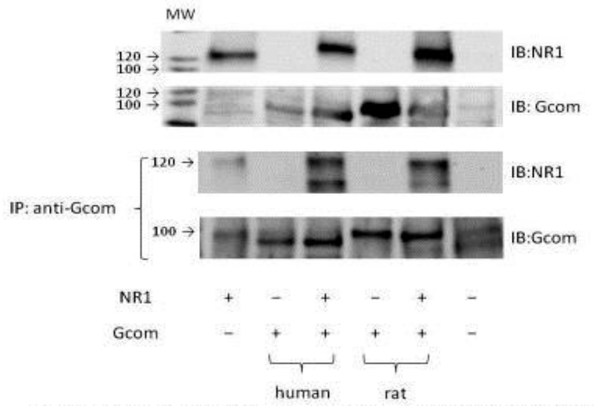

Figure 2. Expression, Immunoprecipitation and co-Immunoprecipitation of Gcom15 and NR1 cDNAs in HEK293 cells.

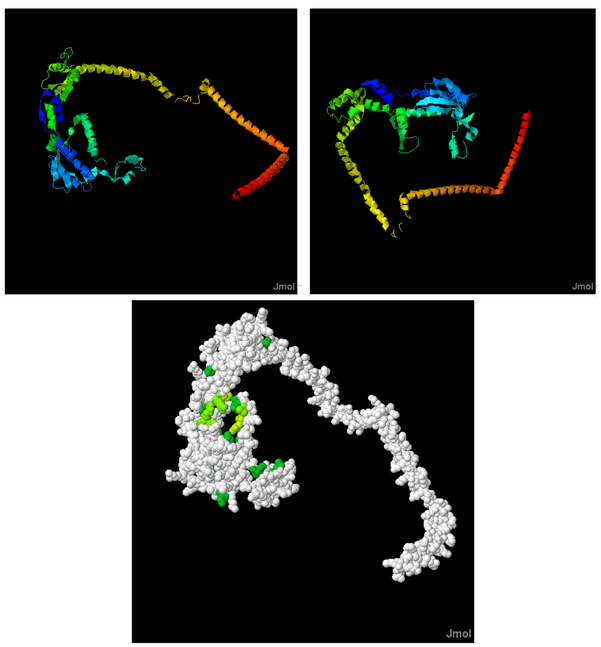

Figure 3. Predicted 3D structure of Gcom15 (Phyre Prediction Software: Protein structure prediction on the web: a case study using the Phyre server; Kelley LA & Sternberg MJE Nature Protocols. 4, 363 - 371 (2009)). Structure prediction software has significant consensus in Alpha Helix section between amino acids 41-489. Significant overlap between Gcom15 and smooth muscle myosin heavy chain was determined by software (overlap of 6%, e value 4.2 e-35).

RESULTS

Both Gcom1 and Gcom15 display structural features characteristic of a type I membrane protein with considerable coiled-coil and α-helix structure as in yotiao (AKAP9), which is also an interactor of the NR1 subunit (6), and to which the Gcom1 protein displays similarity(1). Path Designer analysis revealed network connections between GRIN1, GCOM1, INA, NSMF and other Gints (Fig 1). Human and rat Gcom15 proteins expressed in HEK293 cells migrated as ~105 kDa bands after staining with anti-GCOM1 Ab (Fig 2, top). HEK cells transfected with human or rat Gcom15 cDNA and NR1 cDNA revealed reciprocal co-IP of NR1 by anti-GCOM1 Ab (Fig 2, bottom) and vice versa (anti-NR1 IP not shown). The predicted 3D structure of Gcom15 further revealed significant overlap between Gcom15 and smooth muscle myosin heavy chain (Fig 3).

DISCUSSION

Bioinformatic in silico analyses strongly support our hypothesis that GCOM1 participates in a novel pathway that links synaptic activation of NMDARs to changes in neuronal gene expression. This idea is further supported by the fact that the Gcom1 and Gcom15 proteins share two large C-terminal exons with Gdown1, the 13th subunit of RNA polymerase II, whose key role in transcription is currently under intense investigation[8,9].

References:

1. Genomics 2004; 84: 265. 2
2. NeuroReport 2008; 19: 1721.
3. JNA 2009; 21: 399.
4. Nat Neurosci 2000; 3: 661.
5. ASA Ann Mtg 2010 Abstract A1521.
6. JNeurosci 1998; 18: 2017.
7. Cell 2013; 152: 1119.
8,9.Molec Cell 2012; 45: 38 and 51.

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Figure 2 was also shown in our 2010 ASA abstract A1521.

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