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Journal of Cell Science & Therapy

ISSN: 2157-7013

Short Communication

Activators of G-Protein Signaling 3 (AGS3) Puncta: Potential Novel Biomolecular Condensates in Signal Transduction

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DESCRIPTION

AGS3 background and subcellular positioning

AGS3, also known as G-Protein Signaling Modulator 1 (Gpsm1), is composed of two distinct modules: Seven N-terminal Tetratricopeptide Repeats (TPR) and four C-terminal G-Protein Regulatory Motifs (GPR), linked by a linker region. The TPR motifs are crucial for determining the subcellular localization of AGS3 through interactions with various binding partners. The GPR motifs function as Guanine Nucleotide Dissociation Inhibitors (GDI), engaging with the Gαi/o/t family of G proteins and maintaining the Gα subunit in its GDP-bound state. AGS3 plays a versatile role in numerous cellular and systemic functions, including neuronal asymmetric cell divisions, autophagy, regulation of lysosomes, phagocytosis, trafficking of membrane proteins, leukocyte migration, metabolic processes, cardiovascular activities, responses to addiction and craving behaviors, renal responses to injuries, as well as in polycystic kidney disease and inflammatory responses. The mechanism behind AGS3's ability to contribute to such diverse functions remains unclear, but it likely involves its influence on fundamental cellular processes that vary between cell types and biological systems (Figure 1).

Figure 1: Schematic of AGS3 domain organization: Illustrates the Tetratricopeptide Repeats (TPR) and the G-Protein Regulatory Region (GPR) of AGS3, along with the G alpha inhibitory subunit (Gαi). **Note:** (▬) TPR; (▬) GPR.

To deepen our understanding of how the biochemical properties of AGS3 contribute to its varied functional roles, our research has concentrated on examining the subcellular distribution and controlled activity of AGS3 within cells. Our findings indicate that AGS3 regularly transitions between cytosolic and membrane-bound states, traversing various subcellular compartments such as the cytosol, cell membrane, distinct punctate structures within the cytosol, centrosome, the Golgi apparatus, and the aggresomal pathway, in a dynamic and regulated fashion [1,2].

Key discovery: AGS3's propensity to form distinct punctate structures

A major advancement in our research is the discovery of the inherent ability of AGS3 to form distinctive, non-membranous punctate structures. These puncta are unique because they do not correlate with any known vesicle or organelle markers and do not possess an outer membrane. AGS3 generates puncta of varying sizes, from 200 nm to 2.5 µm, and these can form either with tagged GFP or untagged in different cell lines. Alterations in AGS3's structure-such as truncations of the TPR motifs or amino acid substitutions at critical conserved positions within the TPR and GPR motifs-uniformly trigger puncta formation across all tested cell types [1,3] (Figure 2).

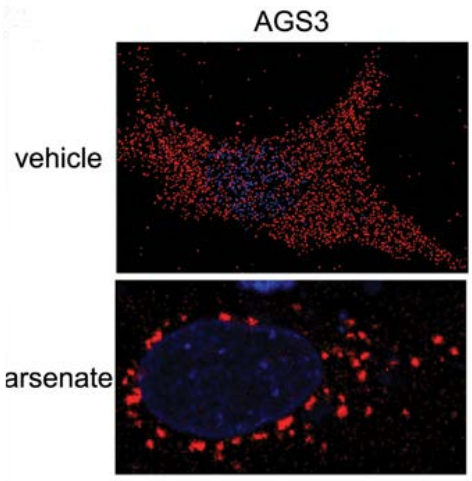

Figure 2: Subcellular distribution of endogenous AGS3 in COS-7 Cells: The upper panel shows a non-homogeneous distribution, while the lower panel, after treatment with arsenate, shows punctate distribution.

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Received: 15-May-2024, Manuscript No. jcest-24-31456; **Editor assigned:** 17-May-2024, PreQC No. jcest-24-31456 (PQ); **Reviewed:** 27-May-2024, QC No. jcest-24-31456; **Published:** 14-Jun-2024, DOI: 10.35248/2157-7013.24.15.462

Citation: Vural A (2024) Activators of G-Protein Signaling 3 (AGS3) Puncta: Potential Novel Biomolecular Condensates in Signal Transduction . J Cell Sci Therapy. 15:462.

Biomolecular condensates and AGS3 puncta

Our latest study explores the subcellular behavior of AGS3 and its regulated assembly into BMCs [4]. BMCs are novel, non-membranous structures newly identified in cell biology, comparable in size to organelles but formed through phase transitions. These condensates aggregate specific molecules, isolating them from the broader cytoplasm, thus supporting biological functions such as enhancing biochemical reactions, isolating harmful substances, storing biomolecules, and augmenting signal transmission.

The major factors driving phase transitions include multivalency of the protein, presence of intrinsically disordered sequences, and sensitivity to environmental stressors. Characterized by its multivalency and significant intrinsic disorder in the linker and GPR regions, AGS3 was examined using fluorescence microscopy to determine its localization in HeLa and COS7 cells under various stress conditions, including oxidative, pH, and thermal stresses. Remarkably, these stressors robustly induce the formation of AGS3 puncta, subsequently identified as AGS3 BMCs [4].

AGS3 engagement with defined biomolecular condensates

To elucidate the functional mechanisms of AGS3 Biomolecular Condensates (BMCs), our research explored AGS3's interactions with other proteins that show similarly regulated movements within cytosolic BMCs. These include signaling puncta (DVL2 signalosomes), stress granules, P-bodies, purinosomes, and elements of the misfolded protein pathway. Previously, we found that AGS3 is directed to DVL2 signaling puncta under normal conditions, where it engages in regulated interactions. The distribution of AGS3 to these DVL2 puncta is modulated by cell-surface G-protein-coupled receptors and the heterotrimeric Gαi subunit, indicating a collaborative role of AGS3 and DVL2 at the nexus of cell signaling networks [5].

Our current findings reveal that this AGS3-DVL2 engagement, in the context of BMCs, is disrupted by oxidative stress. Subsequent biochemical and biophysical assays have verified that AGS3 BMCs are distinct entities. Initial biochemical assessments using cell fractionation assays showed a pronounced segregation of AGS3 to the membrane pellet fraction under oxidative stress. To further characterize the material properties and biophysical nature of BMCs, we utilized Fluorescence Recovery After Photobleaching (FRAP). The analysis demonstrated that while AGS3-DVL2 BMCs are typically mobile and dynamic, the BMCs induced by stress exhibit constrained diffusion, reflecting a more rigid internal structure akin to phospho-deficient AGS3 BMCs [4], (Figures 3 and 4).

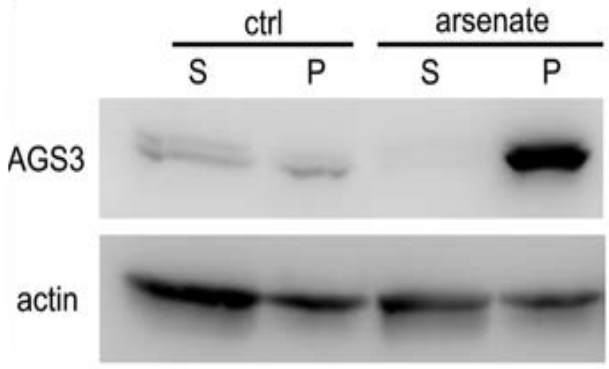

Figure 3: Subcellular fractionation of AGS3 under oxidative stress: Displays the results of AGS3 fractionation upon exposure to 0.5 mM arsenate for 30 minutes, highlighting changes in subcellular localization due to oxidative stress **Note:** S - Supernatant, P - Pellet.

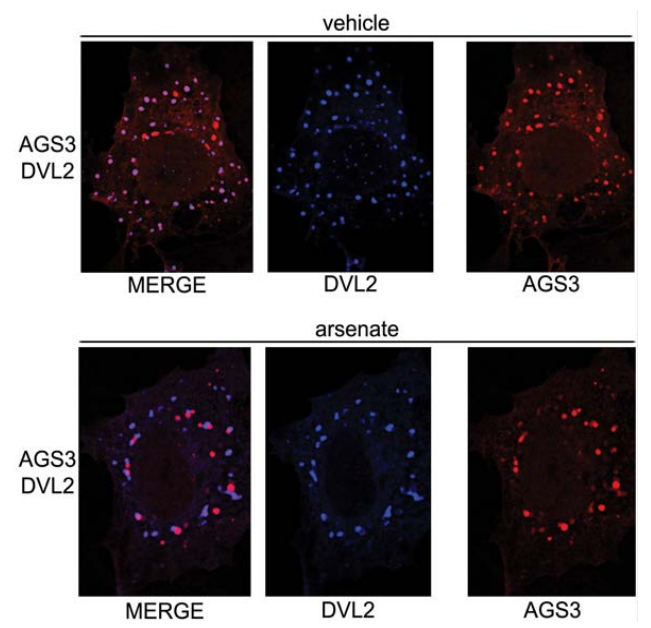

Figure 4: Redistribution of AGS3 in response to conditions: Under control conditions, AGS3 is found in DVL2 puncta (upper panel). In contrast, under arsenate-induced oxidative stress, AGS3 forms distinct BMCs that differ from DVL2 puncta (lower panel).

In terms of interactions with other BMCs, AGS3 engages with P-bodies under normal conditions but not with stress granules. Upon oxidative stress, the emergent AGS3 BMCs, immobile and rigid, are notably distinct from canonical stress granules, which are marked by G3BP1, or P-bodies indicated by Dcp1a. The formation of these stress-induced AGS3 BMCs is also impeded by its binding partner, the G-protein Gαi3 [5].

CONCLUSION

AGS3 BMCs or puncta-like structures are also observed endogenously in multiple cell types across different species. Our findings highlight two fundamental insights: Firstly, AGS3 consistently engages with specific BMCs in a controlled manner. Secondly, due to its regulated interactions with key cell signaling proteins, AGS3 may be a core component of a novel class of BMCs, acting as under recognized nodes in signal and protein processing. Based on these observations, we suggest that AGS3-BMCs may function as key signaling and protein processing hubs, which could elucidate the broad multifunctionality of AGS3.

FUNDING

These studies were supported by National Institutes of Health grants NS24821 and DA025896 to Stephen M. Lanier.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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