

The role of stress granules in heavy metal-induced carcinogenesis

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Received date: December 13, 2024
Accepted date: January 06, 2025

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Abstract

In our recent published study entitled “Stress granules play a critical role in hexavalent chromium-induced malignancy in a G3BP1-dependent manner”, we explored how hexavalent chromium [Cr(VI)] exposure induces stress granule (SG) formation in human bronchial epithelial cells and how SGs contribute to the malignancy. We found that the loss of the SG core protein Ras-GAP SH3 domain-binding protein 1 (G3BP1) reduced SG formation and malignant properties. The results support that SGs play a critical role in mediating Cr(VI)-induced malignancy in a G3BP1-dependent manner, representing a novel mechanism and a potential therapeutic target. In this commentary, we introduce more in-depth background, summarize key findings of the published work, present additional data addressing a related question, and discuss emerging questions and future research directions.

Background on the Role of Stress Granules in Carcinogenesis

Stress granules (SGs) are dynamic, membraneless organelles that assemble in response to cellular stress, such as oxidative stress, hypoxia, or nutrient deprivation [1,2]. Protein translation typically is halted under stress conditions, leading to assembly of SGs containing mRNAs, RNA-binding proteins (RBPs), and other proteins. SGs help conserve cellular resources and promote cell survival under stress conditions. While this protective function is beneficial under acute stress, chronic or dysregulated SG dynamics is increasingly implicated in cancer development and progression. Aberrant SG formation and maturation have been observed in various types of cancer and are associated with tumor growth and resistance to therapy [3,4]. Other studies also suggested a potential connection between SGs and tumorigenesis. For instance, well-established tumorigenic molecules, such as the RAS oncogene, the mammalian target of rapamycin (mTOR), and histone deacetylase 6 (HDAC6), have been reported to upregulate SG formation [5-7]. A recent study showed that SG formation facilitated anchorage-independent survival of breast cancer cells [8].

Ras-GAP SH3 domain-binding protein 1 (G3BP1) and its interacting partners, cell cycle associated protein 1 (Caprin1) and ubiquitin specific peptidase 10 (USP10), play a critical role in regulating SG assembly and dynamics. The Caprin1-G3BP1 interaction promotes SG assembly, whereas the USP10-G3BP1 interaction facilitates SG disassembly [9]. We previously resolved the crystal structures of the Caprin1-G3BP1 and USP10-G3BP1 complexes. Both Caprin1 and USP10 interact with G3BP1 at the same binding pocket [10]. The SG formation is initiated upon the translational suppression via two different pathways. In the canonical pathway, the eukaryotic initiation factor-2 α (eIF2 α), which is a regulatory protein controlling the initiation of protein translation, is phosphorylated and the protein translation is stalled, leading to subsequent SG assembly [11]. In the non-canonical pathway, mTOR

kinase is inhibited by stresses, leading to the dephosphorylation of the eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1), suppression of the active translation initiation complex (eIF4F), and induction of SG assembly [12].

The core SG protein G3BP1 has been associated with increased tumor initiation, progression, and resistance to therapy [13,14]. Similarly, Caprin1 and USP10 contribute to cancer cell survival under stress conditions and facilitate the adaptation of cancer cells to hostile microenvironments [15,16]. Despite these findings, our understanding regarding the role of SGs in malignant transformation, cancer progression, and metastasis remain rather limited, especially as individual studies have focused on individual SG components. The broader impact of SG dynamics on mRNA metabolism, translation regulation, cellular signaling, and cancer biology is largely unclear.

The relationship between heavy metals and cellular stress responses has garnered significant attention, especially regarding carcinogenesis. Heavy metals, including arsenic, cadmium, chromium, and mercury, are well-known carcinogens that cause cancer [17]. They generate reactive oxygen species (ROS) on being up-taken into cells, leading to oxidative stress and SG formation [18]. SGs function as a protective mechanism by stabilizing mRNA transcripts that may be sensitive to oxidative damage [19]. Thus, SGs help prevent further ROS formation, acting as a cellular buffer during stress, further preventing apoptosis [18,20]. It is conceivable that SGs promote cell survival in stressful environments and allow for the continued growth of malignant cells. This mechanism is especially relevant to cells exposed to heavy metals, as SGs may help buffer against metal-induced oxidative stress and toxicity. Thus, chronic and persistent SGs can paradoxically contribute to malignant transformation.

Arsenic is a commonly used tool in the laboratory for inducing SG formation by treating cells with high doses (mM) acutely [21]. Arsenic is also a well-established carcinogen, as chronic exposure to low-dose (sub- μ M) arsenic causes cancer [17]. However, few studies have directly linked arsenic-induced SG formation to its carcinogenic effects. Similarly, the relationship between environmental Cr(VI) exposure and SGs is an intriguing yet insufficiently explored area. Thus, we were inspired to address the knowledge gap by examining how Cr(VI) exposure influences SG dynamics, and how Cr(VI)-induced SGs impact cellular processes leading to malignancy.

Findings of the Published Work

Our recently published work investigated how Cr(VI) exposure specifically induces SG formation and contributes to malignant transformation in bronchial epithelial cells. In the study, we used two methods to monitor SG formation: fixed-cell immunofluorescence microscopy and live-cell imaging. To evaluate malignant properties, malignant transformation markers, including p62, Nrf2, Bcl-2, and SOD2, were measured by western blot. Cell proliferation and sphere formation (a measure of cellular stemness) were also used to evaluate malignancy [22]. We found that acute exposure to high concentration Cr(VI) (10 mM) led to immediate SG formation within hours in the human bronchial epithelial cell line, BEAS-2B. In addition, chronic exposure at lower concentration Cr(VI) (1 μ M) induced SGs in BEAS-2B cells over several weeks, emulating prolonged environmental exposure scenarios. Cells chronically exposed to Cr(VI) demonstrated a heightened SG response, producing more SGs upon subsequent oxidative stress compared

to control cells. Chromium-induced transformed (CrT) cells were developed by subjecting BEAS-2B cells to 1 μ M Cr(VI) for 52 weeks. These malignantly transformed cells showed elevated levels of SG proteins, including G3BP2, Caprin1, and USP10, suggesting a cellular adaptation wherein SG components are upregulated as a part of the malignant transformation process. Moreover, SG response was up-regulated in CrT cells, supporting the notion that SGs may facilitate adaptation to the oxidative stress imposed by Cr(VI), aiding in cell survival and malignant progression.

Our published paper also suggested that among the SG proteins, G3BP1, a core protein for SG assembly, emerged as a critical player in Cr(VI)-induced malignancy [22]. Knockout of G3BP1 in CrT cells resulted in a significant reduction in SG formation and malignant characteristics. This included decreased cell proliferation, reduced sphere formation, a marker of cancer stemness, and lowered levels of cancer-associated proteins such as p62 (a marker for autophagy dysregulation) and SOD2 (a key antioxidant enzyme). These findings supported that disrupting SG assembly impairs the cancerous characteristics of Cr(VI)-exposed cells. Our work also suggested that G3BP1-dependent SG formation may be important for Cr(VI)-induced malignant transformation; however, this notion was not directly tested.

New Experiments Addressing a New Question

We carried out additional experiments to directly test the above notion, i.e., does the loss of G3BP1 in benign cells affect the Cr(VI)-induced transformation? In the new study, we first generated normal cells with G3BP1-knockout (BEAS-2B-G3BP1-KO cells) and chronically exposed them to low concentration (1 μ M) Cr(VI). The experiments were performed in a similar fashion as in the published study [22]. Briefly, relevant cell lines were seeded initially at 0.5×10^6 cells/well in triplicate in 6-well plates, cultured in DMEM complete medium (with 10% FBS and penicillin/streptomycin) and 1 μ M Cr(VI) for 24 days. As the most prevalent cell lines reached confluency, all lines were reseeded at the same number/well. The number of cells was counted for each line (three image fields for each treatment) at day 24 (7 days after the last seeding). On the final time point (day 24), an MTS assay (Promega) was performed in triplicate to determine cell viability. Data were quantified and presented as mean $\pm$ standard deviation (SD). Statistical analysis between two groups was conducted using an unpaired Student's t-test, with a P value ≤ 0.05 considered statistically significant. Data analysis and graph generation were performed using GraphPad Prism 10.

The study observed notable differences in survival between wild-type (WT) and G3BP1-KO cells under long-term Cr(VI) exposure (**Figure 1**). Upon exposure to low-concentration (1 μ M) Cr(VI), significantly more WT cells survived as compared to the G3BP1-KO cells (**Figure 1A**). As we recently published [22], WT cells gradually adapted to the stress and a portion of them eventually progressed and transformed to be malignant. Quantitation of cell numbers after 24 days of exposure showed consistently higher cell death in G3BP1-KO cells as compared to WT cells (**Figure 1B**). The cell viability MTS assay also confirmed less viable G3BP1-KO cells after 24 days exposure (**Figure 1C**). The experiment was aborted due to low viable cell numbers in the G3BP1-KO cells exposed to Cr(VI). The results suggest that loss of G3BP1 in benign cells leads to significant cell death, and that it is unlikely G3BP1-KO cells would survive the long-term Cr(VI) exposure.

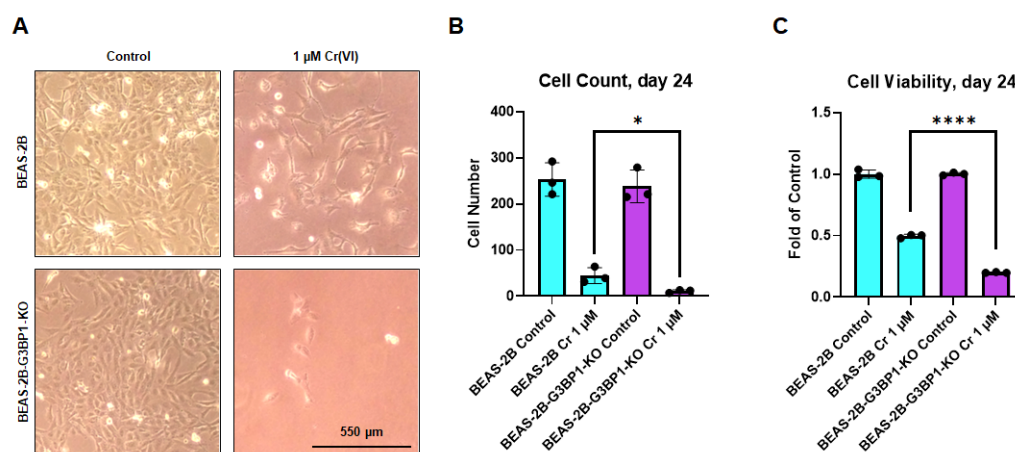


Figure 1. Loss of G3BP1 decreased viability of untransformed cells upon long-term Cr(VI) exposure. **A.** Human bronchial epithelial BEAS-2B cells and BEAS-2B cells with G3BP1 knockout (BEAS-2B-G3BP1-KO) were chronically exposed to 1 μ M Cr(VI) for up to 24 days. Cell morphology was imaged using an ECHO Rebel Microscope. Scale bar = 550 μ m. **B.** Quantitative analysis of cell numbers from panel A. Data were quantified from three different image fields. *: $p \leq 0.05$. **C.** MTS cell viability assay of BEAS-2B and BEAS-2B-G3BP1-KO cells after 24-day exposure. Equal cell numbers were seeded 7 days before the measurement. ****: $p \leq 0.0001$.

Future Directions

Our work supports that the membraneless organelle SGs function as an adaptive and protective mechanism to enable cells to survive and continue to grow under stress conditions such as chronic exposure to heavy metals, contributing to malignant transformation. Our work also opens several avenues for further exploration.

Firstly, are other SG proteins as critical as G3BP1 in Cr(VI)-induced or other malignant transformation? While G3BP1 is highlighted as essential for SG formation and Cr(VI)-induced malignancy, it remains unclear whether other SG proteins, such as Caprin1 and USP10, both upregulated in CrT cells, are equally critical in malignant transformation. Investigating their roles could provide a broader understanding of SG-mediated oncogenesis.

Secondly, can early G3BP1 knockout prevent malignancy? The timing of G3BP1 knockout seems to be crucial. In our published study [22], G3BP1 was knocked out in already transformed cells, reducing their malignant properties. A compelling question is whether knocking out G3BP1 prior to Cr(VI) exposure could entirely prevent transformation. Our new observations presented in this commentary demonstrate that BEAS-2B-G3BP1-KO cells exposed to low-dose Cr(VI) exhibit increased sensitivity to oxidative stress, dying within a month, as compared to wild-type cells that adapted and transformed (**Figure 1**). The new data underscores the critical role of G3BP1-mediated SGs in maintaining an ultimate balance between healthy cell survival versus cell transformation when cells are under stress conditions.

Thirdly, how do SG dynamics differ across different heavy metals? SGs appear to be a common response to various heavy metals according to the metal-induced ROS. Investigating whether different metals induce unique SG compositions or signaling pathways could enrich our understanding of SGs in cancers induced by these agents. It would be interesting to carry out a comparative analysis of SG composition, persistence, and cellular effects under exposure to Cr(VI), cadmium, arsenic, and other known carcinogenic metals.

Fourthly, can G3BP1 be a plausible therapeutic target in heavy metal-related cancers? Given the role of G3BP1 in SGs and SG-mediated stress adaptation, G3BP1 inhibitors may hold promise as therapeutic agents in cancers linked to environmental carcinogens. However, as SGs also support normal cellular stress responses, such inhibitors must be developed to selectively target SGs in cancer cells without disrupting homeostasis in healthy tissues. A better understanding of the mechanisms of SG-induced malignancy is therefore still needed. While SGs facilitate cell survival under stress, their interactions with other pathways that drive malignancy, such as apoptosis, autophagy, and DNA repair, remain poorly understood. Future research should explore these intersections using transcriptomic and proteomic approaches to uncover additional mechanisms through which SGs influence cancer progression in cells exposed to carcinogenic metals.

Lastly, can SG-associated biomarkers be utilized to predict susceptibility to metal-induced carcinogenesis or the effectiveness of G3BP1-targeting therapies? As discussed earlier, SGs are dynamic organelles and the conventional SG formation markers, such as phospho-eIF2 α [p-eIF2 α (Ser51)], are also dynamic. Thus, it is challenging to use these existing markers to monitor cells' susceptibility to metal-induced carcinogenesis or the effectiveness of G3BP1-targeting therapies. The development of novel and reliable biomarkers for these purposes is an area of ongoing research in SG studies.

Conclusions

In conclusion, our recent work highlights the critical role of SGs, particularly those mediated by G3BP1, in Cr(VI)-induced malignancy. The increased susceptibility to Cr(VI)-induced cell death of BEAS-2B-G3BP1-KO cells compared to that of WT cells underscores the protective function of SGs in cellular stress adaptation. Targeting G3BP1 could, therefore, weaken the ability of cancer cells to withstand environmental stress. New data presented in this commentary also suggests that loss of G3BP1 led to increased

vulnerability of untransformed cells under stress conditions. These findings align with research on other heavy metals or environmental risk factors, suggesting that SGs represent a general mechanism for cellular adaptation under stress induced by environmental risk factors. Our work opens exciting possibilities for therapeutic strategies aimed at disrupting SG pathways in cancer.

Acknowledgment

This research was partially supported by NIH/NINDS R01NS115507 (to H.Z.), VA I01 BX002149 and IK6 BX006316 (to H.Z.), University of Arizona College of Pharmacy R. Ken Coit endowment (to H.Z.), and University of Arizona Faculty Seed Grant (to L.W.).

Conflicts of Interest

The authors declare no conflicts of interest.

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