Revisiting the Stress Concept for A Better Recipe of Patient Prognostic: Implication of Stress Granule Markers

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ABSTRACT

Stress Granule formation is a pro-survival mechanism helping cells to cope with environmental challenges. Stress Granules have been studied for two decades in fundamental research, and are now being examined in the context of human pathogenesis. Here we review studies highlighting stress granules' involvement in cancer development through translational pattern modification.

Keywords: cancer; metastasis; stress granules; G3BP1; G3BP2; TIA-1; TIAR; CAPRIN-1; USP10; prognostic markers

1. Integrated Stress Response & Stress Granule Formation are linked to translation inhibition

Stress response is an ancestral evolutionary mechanism acquired by the first cellular organisms to protect them from sudden environmental changes [1]. This mechanism has been conserved throughout evolution [2].

At the level of the cells, any change in the environment that diverges from their optimal growth condition is considered as a stress and could induce a stress response. All the processes involved are part of what is now known as the *stress response* (ISR), and include the activation of stress response genes, such as those coding for heat shock proteins, known for decades, but also of another mechanism recently discovered: the formation of *Stress Granules* (SGs) [1]. Stress granules are membraneless cytoplasmic condensates of mRNA, RNA binding proteins and 40S ribosomes first discovered in 1999 by the laboratory of Dr. P. Anderson [3-6]. To date, the formation of SGs have been reported in plants, yeast, worms, insects and mammalian cells [3, 7-11]. They are visible by electron microscopy . The absence of membrane surrounding SGs and the extreme lability of the components has hindered purification of these structures and the identification of their components by global analysis. Currently, even if method are reported to purified SG marker {Youn, 2019 #3}, the candidate approaches is preferred for targeting specific components by immunofluorescence and FISH can robustly identify the proteins and mRNAs included in these structures. In 2015, a literature inventory mentioned more than a hundred of proteins known to be recruited to SGs, forming an eclectic mix from various signaling pathways. Even if there is yet no consensus to predict the recruitment of specific proteins to SGs, most of them interact with RNAs or are involved in RNA metabolism [12].

Under homeostatic conditions, active translation is facilitated by closed-loop mRNP formation (**Figure 1A**). This is a situation where the 5’ and 3’ ends of an mRNA are brought in close proximity. The 5’ mRNA cap is bound by eIF4E and the 3’ poly(A) tail is bound by PABP. These two proteins are bridged by the large scaffolding protein eIF4G. To initiate translation, the ternary complex, composed of eIF2:$tRNA_i^{Met}$:GTP facilitates decoding of the start codon which results in GTP to GDP hydrolysis. In response to stress, translation is rapidly inhibited, which, in some cases results in SG formation. Two translation inhibition pathways can induce the formation of SGs [4] (**Figure 1**). The phosphorylation of a subunit of eIF2, EIF2S1 (or

EIF2α), by one (or more) kinases, notably EIF2AK1 (or HRI), EIF2AK2 (or PKR), PERK (or EIF2AK3) and/or GCN2 (or EIF2AK4) [13], prevents the hydrolyzed GDP from leaving the ternary complex EIF2α-tRNA^{met}-GTP by block the formation of an active complex with ATP necessary for translation initiation (**Figure1 A-B**). Another stress response pathway centers on mTOR (**Figure 1C**). Under basal conditions, mTOR is active and constitutively phosphorylates eIF4E-BP protein (4EBP). Hyperphosphorylated 4EBP cannot interact with eIF4E, the mRNA cap binding protein. However, induction of a stress response inactivates mTOR leading to a rapid dephosphorylation of 4EBP thereby allowing it to interact with eIF4E. The eIF4E:4EBP interaction prevents blocks eIF4E:eIF4G complex formation. It is worth pointing out that these pathways are not mutually exclusive. Depending on the type of stress, either or both pathways could be activated [13, 14].

2. Stress Granules are pro-survival entities

Stressors triggering the formation of SGs can be as diverse as extreme temperatures (hot or cold), oxidative stress, osmotic stress, endoplasmic reticulum (ER) stress, mitochondrial stress, or UV irradiation (Reviewd in [12]). Several lines of evidence point toward a pro-survival benefits of SG formation, explaining the evolutionary conservation of this process. Upon mutations or knock-out of specific proteins involved in SG formation, or treatments decreasing the ability to form SG, cells die more easily and rapidly after stress exposition [15-19]. The pro-survival effect of SG formation could be explained by several independent mechanisms.

First, many pro-apoptotic signaling molecules, such as RACK1, TRAF2 and RSK2, are recruited to SGs [20-22]. Their sequestration in these bodies has been proposed to prevent their action in promoting a pro-apoptotic cascade.

Second, the level of G3BP1 expression inversely correlates with the generation of reactive oxygen species (ROS) after exposition to oxidative insult. Overexpression of G3BP1 reduces the level of ROS compared in reference to wild type cells. Cells expressing a truncated form of the protein that abrogates SG formation have an increased ROS production. Similar results were obtained using, USP10, another SG regulating protein. While not fully characterized, SGs seems to protect cells from oxidative insults by reducing the level of cellular ROS [18, 23].

Lastly, the translation repression upstream SGs formation reduces the cellular energetic needs during stress by restricting the process of translation, which is consuming much ATP [4], and by protecting mRNAs from stress-induced degradation, which will allow cells to restart translation as soon as the stress is resolved without having to re-synthetize fresh RNAs [24]. Also, SGs sequester the untranslated mRNAs consecutively to the global inhibition of translation [25]. Some mRNAs, such as chaperone mRNAs, are excluded from the SG structures so that they can be preferentially translated during the time of the stress and participate in proper protein folding to avoid functional defects [25, 26]. By those actions, SGs are described as a triage center for translation of mRNAs during stress exposure. One growing hypotheses is that SGs are able to reshape translation pattern under stress exposure [27].

More recently, further evidence of the pro-survival role of SGs in various human diseases has been made, particularly with regards to neurodegenerative diseases. In Amyotrophic Lateral Sclerosis (ALS), a plethora of proteins from many different pathways have been implicated in the disease [28]. For a long time, it was difficult to connect this diversity with the disease. A decade after demonstrating that ALS-relevant proteins are recruited to SGs [15, 29-34], the defect in stress response has become one of the leading hypothesis that explains neurons loss [12]. Antibiotic induced-ototoxicity results in cilia loss [27], the induction of SGs (using hydroxamate (-)-9) is able to rescue this defect [8].

However, in some cases, the SG-mediated survival of a cell might not benefit the host. For example, in a rapid growing tumor, cancer cells are also exposed to hypoxia and nutrient deprivation, which has been shown to induce the formation of SGs [35] and promote resistance to therapies [36]. Also, some chemotherapies induce the formation of SGs which are anti-correlated with patients' survival [37-42]. Blocking the induction of these chemotherapy-induced granules by interfering with the phosphorylation of EIF2S1 increases the efficiency of the treatment [42]. Also, after a screen of small molecules, β-estradiol, progesterone and stanolone prevent SG formation and hypoxia induced chemo-resistance in HeLa cells; however, the same molecules used in MCF7 did not have any of the mentioned effects [36, 42]. Blocking SG formation could be an option to counter cancer development but for this special use we are in need of a molecule with broad cellular specificity.

3. Investigating how regulation of mRNA and protein levels of SG components connects to cancer prognosis

During cancer development and dissemination, cells acquired driver mutations that are responsible for cell transformation, then aggressiveness of the disease. Aside from the acquisition of mutations, cancer development depends on modifications of the translation program of the cell. For example, Epithelial to Mesenchymal Transition (EMT), acquisition of stemness, acquisition of drug resistance involve specific modifications of translational programs. Recently, some studies have pointed out that this kind of translation pattern changes could occur after exposition to hypoxia stress [36, 43-45]. We know that a growing tumor is an extremely dynamic environment where stressors such as mechanical constriction, hypoxia, starvation play a role at multiple levels... SGs could be at the crossroad of all those stressors to initiate the translation pattern changes leading to cancer progression, [27, 36].

SG protein are multiple and too numerous to all be investigated. We focus our review analysis of key SGs regulators such as TIA-1 (T-cell-restricted intracellular antigen-1), TIAR (TIA-1-related protein), G3BP1 (Ras GTPase-activating protein-binding protein 1), G3BP2 (Ras GTPase-activating protein-binding protein 2), Caprin-1 and USP10 (Ubiquitin carboxyl-terminal hydrolase 10) (Figure 2).

- TIA-1 and TIAR, beside their role in immunity, have RNA Recognition Motifs (RRM) and play a role in RNA splicing. They are the historical SGs' markers [3] and have been reported to be recruited to SG in response to oxidative stress, heat shock, proteasome inhibition, ER stress as well as viral infection, in primary and all kinds of transformed cell lines [12]. Their overexpression induces spontaneous formation of SGs [3], but the knockout of each of them individually or simultaneously has never been reported to have an impact on SG formation.
- The G3BP1 protein also contains RRM, and has some helicase and RNAse activity under normal conditions [6, 46]. G3BP1 is closely related to another protein, G3BP2, with which it shares 98% identity. Nowadays, they are considered as the master regulators of SGs. Their overexpression also induces spontaneous formation of SGs [6, 47]. Individual knock out partially inhibits or delays the formation of SGs [24], but the double knock out completely abolishes the formation of SGs [48].

- Finally, USP10 and CAPRIN1 are two interactors of G3BP1, which compete with each other to interact with their target. Both are binding G3BP1 on the FGDF motif and have opposite effect on SG formation: CAPRIN1 favors SG formation by promoting G3BP1 aggregation with mRNAs to form SGs, whereas USP10 inhibits their formation by affecting G3BP1 conformation, inhibiting the condensation of SGs [48]. Those two proteins are not the sole regulators of G3BP1 aggregation, as the removal of the FGDF motif does not influence the formation of SGs.

Open access data on breast, colon and pancreatic cancer reveals that mRNA levels from *G3BP1, TIA-1, TIA-R* and *CAPRIN-1* are mostly upregulated in primary tumor compared to healthy tissues (Figure sup 1,2,3, Table 2) suggesting that an enhanced transcriptional regulation of proteins related to SGs might occur during primary tumor oncogenesis. Upregulation is not as homogenous between healthy tissues and metastases, but still significant in most tumor types. CAPRIN1 and G3PB1 show the most noticeable/prominent upregulation. On Kaplan-Meier curve we studied if the level of mRNA expression for SG components could correlate with patient overall survival and disease/recurrence/metastasis free survival. Most of the analyses did not show any correlation. When a correlation is observed, the difference does not go over 10%.

Those data are surprising because a correlation between upregulation of SG protein levels and poor prognosis exists. Indeed, G3BP proteins expression is of poor prognosis in various tumors, including sarcoma [49] colon [50], breast [50-53], thyroid [50], lung [54], head and neck [50], gastric [55, 56], hepatic [57] and prostate cancers [50, 58]. Higher TIA-1 protein levels correlate with poor prognostic in patients with colorectal cancer [59] and lymphoma [60]. High expression of CAPRIN1 protein correlates with poor prognostic for osteosarcoma [61], and hepatocellular carcinoma [62, 63]. Consistent with an inhibitory effect of USP10 on SG formation, high protein expression correlates with better prognostic in patients with gastric [64], ovarian [65], lung [66], small intestine [67], prostate [58] and gastric carcinoma [64]. The obvious conclusion would be that SGs related proteins are submitted to a double regulation during the disease development (figure 6):

- A transcriptional regulation early in the pathology which insures basal levels of the proteins involved in SGs function. This basal level is potentially the result of cancer cells subversion of the SGs mechanism to ensure their survival,
- A post-transcriptional regulation that impacts on the final level of proteins and has a prognostic role. This regulation occurs in the disease to become more aggressive and metastatic.

DISCUSSION & CONCLUSION

The early studies studies have established the pro-survival properties of SGs. The field is now evolving toward the implication of SGs in human pathogenesis. This was not an easy task because the SG pathway is composed of proteins that generally switch localization and function between basal and stress conditions. It is now well accepted that the mis-regulation of SGs is involved in the onset and development of at least one neurodegenerative disease.

In the cancer field, we are accumulating evidence for the SG role in the adaptation and survival of cancer cells. Not only SGs form in response to stressors involved in primary tumor development such as hypoxia and starvation, but also in response to chemotherapies to help cells to overcome treatment. When we look at the regulation of key SG modulators by microarrays we observe that the mRNA level of major SG components is upregulated in tumor tissues compared to unaffected ones, suggesting a role of those molecules in the tumorigenesis process. But, we do not find strong correlations between mRNA expression level and prognosis in patients. By combining those analyses and a deep literature review, we see that mRNA and proteins are elevated in tumors compared to the healthy tissues. Also even if mRNA expression levels do not correlate with patient prognosis, we can find several studies showing that protein expression levels are prognostic markers. For the proteins having a positive regulation of SGs, a higher expression correlates with poor prognosis (G3BP1, G3BP2, TIA-1, TIAR and Caprin-1). Whereas conversely, for USP10, that has an inhibitory role on SG formation, an increased level of expression correlates with better prognosis in patients (Figure 2). Those data point to an increased aggressiveness of cancer when SG formation capacity is increased. Altogether those data suggest a discrepancy between mRNA and protein expression implying a post-translational regulation. Increased

SG protein expression could imply an improved capacity of the malignant cells to form SGs in response to stressors.

Exposition to stressors such as hypoxia induces translation changes. We think that SG formation is one of the keys of this translation changes towards a more aggressive phenotype. The first step is probably the upregulation of SG components (mRNA and protein) to adapt intrinsic stress of tumor growth. Then, the most aggressive tumors are probably able to add a second layer of regulation by using SG formation to remodel the translation pattern and increase even more the level of proteins without further changes in transcription (Figure 6). The first proof of this pattern change is the upregulation of SG proteins without any change in mRNA level. Further studies are warranted to prove the role of SGs in this process. Inhibition of SG formation could be promising adjuvant therapy for refractory patients or in prevention of chemo-resistances.

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