Review

Targeting stress granules: A novel therapeutic strategy for human diseases

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ABSTRACT

Stress granules (SGs) are assemblies of mRNA and proteins that form from mRNAs stalled in translation initiation in response to stress. Chronic stress might even induce formation of cytotoxic pathological SGs. SGs participate in various biological functions including response to apoptosis, inflammation, immune modulation, and signalling pathways; moreover, SGs are involved in pathogenesis of neurodegenerative diseases, viral infection, aging, cancers and many other diseases. Emerging evidence has shown that small molecules can affect SG dynamics, including assembly, disassembly, maintenance and clearance. Thus, targeting SGs is a potential therapeutic strategy for the treatment of human diseases and the promotion of health. The established methods for detecting SGs provided ready tools for large-scale screening of agents that alter the dynamics of SGs. Here, we describe the effects of small molecules on SG assembly, disassembly, and their roles in the disease. Moreover, we provide perspective for the possible application of small molecules targeting SGs in the treatment of human diseases.

1. Stress granules

A large portion of mRNA in mammalian eukaryotic cells completes transcription in the nucleus and is then transported to the cytoplasm for translation and expression. When eukaryotic cells are stimulated or disturbed, the mature mRNA in cells cannot be translated into proteins immediately. These temporarily untranslated mRNA or translation-stalled mRNA then polymerize with RNA-binding proteins (RBPs) to form messenger ribonucleoprotein (mRNP) granules without a membrane structure, known as Cajal bodies, stress granules (SGs), processing bodies (P-bodies), RNA transport granules, or germ granules [1,2]. While mRNP granule types are complex and diverse, there are three commonalities between mRNP granules [3]: first, mRNP granules usually contain non-translated or poorly translated mRNA, and these mRNA can re-enter polysome for translation after cellular adaption or environmental recovery. Second, different mRNP granules may contain the

Fig. 1. SG dynamics. SG dynamics is observed by fluorescence microscopy with immunostaining against SG-nucleating proteins or cells expressing GFP-tagged SG-nucleating proteins including G3BP1, G3BP2 and TIA-1, which are commonly used as SG markers. (A). Immunostaining results show G3BP1 and TIA-1 are extensively overlapped in SA (sodium arsenite)-induced SGs. (B). HeLa cells stably expressing GFP-tagged G3BP2 protein show SGs are efficiently assembled after 30 min treatment of SA, partially disassembled after 1 h recovery (Rec) from SA treatment and completely disassembled after 3 h Rec from SA treatment.

same mRNA or RBP and these components can be relocated from one mRNP granule to another granule. Third, different mRNP granules can interact dynamically, involving docking, fusion, and becoming another mRNP granule after maturation. mRNP granules have a very important effect on mRNA function and cell signalling, and are also closely related to diseases [4]. One of the most studied mRNP granules is SGs. SGs are a type of dynamic granular substance formed of mRNA of stagnant translation and RBPs in the cytoplasm of eukaryotic cells, the formation of which is stimulated by various stresses including oxidative stress, heat shock, hypoxia, or viral infection (Fig. 1). It is an adaptive regulatory mechanism that protects cells from apoptosis under adverse conditions [5,6]. Besides, SG formation and dynamic can affect mRNA localization, translation, and degradation as well as signalling pathways and antiviral responses [7].

2. The biological functions of SGs

The assembly of SGs is closely related to the translation initiation of eukaryotes. The canonical translation initiation in eukaryotes begins with circularization of mRNA by eukaryotic initiation factor 4F (eIF4F) complexes and assembly of 43S preinitiation complexes (43S PIC) [8,9]. The 43S complexes start searching for the initiation codon along mRNA, and once the initiation codon is identified, eukaryotic initiation factor 2 (eIF2) hydrolyses GTP to GDP, which triggers dissociation of eIFs from ribosome and allow the join of 60S ribosomal subunit to form a complete 80S initiation complex. A key step in this process is the formation of ternary complex comprising eIF2, GTP and Met-tRNA$_i^{Met}$. eIF2 plays a central role in translation initiation and is tightly regulated. When eukaryotic cells encounter harmful external stimuli, such as heat shock, viral infection, oxidative stress, hypoxia, or other stresses, the α subunit of eIF2 (eIF2α) is phosphorylated at serine 51 by stress-induced kinases including PKR (protein kinase RNA-activated), PERK (PKR-like endoplasmic reticulum kinase), HRI (heme-regulated inhibitor) and GCN2 (general control non-derepressible 2). Phosphorylation of eIF2α blocks the formation of eIF2-GTP-Met-tRNA$_i^{Met}$ ternary complex, resulting in inhibition of global translation and activation of gene-specific translation such as yeast general control non-derepressible 4 (GCN4) and mammalian activating transcription factor 4 (ATF4). The translation-stalled mRNAs are then recruited by RBPs to promote the formation of SGs [10–15]. However, eIF2α phosphorylation cannot certainly cause SG formation. For example, doxorubicin (DOX) efficiently triggers phosphorylation of eIF2α while fails to promote SG formation [16]. The inactivation of eIF4F complexes through the disruption of eIF4E and eIF4G binding or inhibition of eIF4A activity by hippuristanol [17], H$_2$O$_2$ [18], pateamine A [19] can also lead to translation inhibition and SG formation [20].

In addition to mRNA, SG components include various RBPs, translation initiation factor and non-RBPs such as signalling proteins. SG composition is different in the same type of cells subjected to different types of stress or in different cells subjected to the same type of stress. SGs also share some ubiquitous RBPs to facilitate SG aggregation and nucleation, such as Ras GTPase-activating protein-binding protein 1 and 2 (G3BP1/2), cell cycle associated protein 1 (Caprin1) and T cell restricted intracellular antigen-1 (TIA-1) [21,22]. These proteins are often used as protein markers of SGs to facilitate SG research (Fig. 1). With the deepening of research, SG proteins and proteins that regulate SG assembly/disassembly have been discovered.

SGs are not of a uniform structure; rather, there are two distinct layers: the core and the shell [23]. These two layers of SGs may have different components, functions and dynamics. Two models for discrete phases of SGs assembly have been proposed, the cores first model and the liquid-liquid phase separation (LLPS) first model. In the cores first model, the core is surrounded by a less concentrated and more dynamic shell, and the core size and dynamics of SGs do not change over time [9, 24]. In the LLPS first model, the condensation of the liquid inside the cell can drive the assembly of SGs. In some special circumstances, liquid aggregates of SGs are transformed into solid-like states, which may causes fibrosis or devastating protein aggregation diseases [25].

As the stress disappears, the clearance of SGs from the cytoplasm of cells occurs rapidly, i.e., within minutes (Fig. 1). The SG depolymerisation process is opposite to the aggregation process. First, the less stable shell is dissolved and then the core is removed [26]. SGs are generally believed to be sites of temporary mRNA storage and triage. The mRNAs in SG will have at least two directions after depolymerisation. The first is that, after chaperone or autophagy-mediated clearance of SGs upon recovery from stress or stress adaptation, specific mRNAs reversely mobilise back to the polysome and translation is resumed. The second is that specific mRNAs are translocated from SGs to P-bodies for storage or degradation [26–30]. The disassembly of SGs depends on the activation of heat hock proteins and the dephosphorylation of eIF2α, as well as the regulation of the microtubule system [31, 32].

3. SGs and human diseases

Increasing evidence suggests that SGs are involved in many human diseases (Fig. 2), including cancers, neurodegenerative diseases, viral infections and autoimmune disease, well-documented by elegant reviews [33–37]. SGs affect multiple pathways by intercepting and sequestering signalling components, such as RACK1 (p38/JNK signalling), TRAF2 (NF-κB signalling), Raptor (mTOR signalling) and RhoA/ROCK1 (Wnt signalling) [33–37]. When exposed to stress environments, cells may arrest the cell cycle and repair stress-induced damage, or proceed to apoptosis. Acute stress-induced SGs selectively recruit and exclude mRNAs, thereby promoting the translation of stress-related response genes and enabling cells to quickly return to a normal state after stress. Conversely, if SG formation is blocked during stress, the cell survival rate is significantly reduced. However, SGs induced by pathological chronic stress (neurodegeneration, nutrient starvation) lack several classical SG components that contribute to the pro-survival functions of canonical SGs (RACK1, small ribosomal proteins), and conversely have a pro-death function [38,39].

3.1. Cancer

The tumour microenvironment is full of stresses, such as a high concentration of ROS, hypoxia and hypoglycaemia, which can strongly
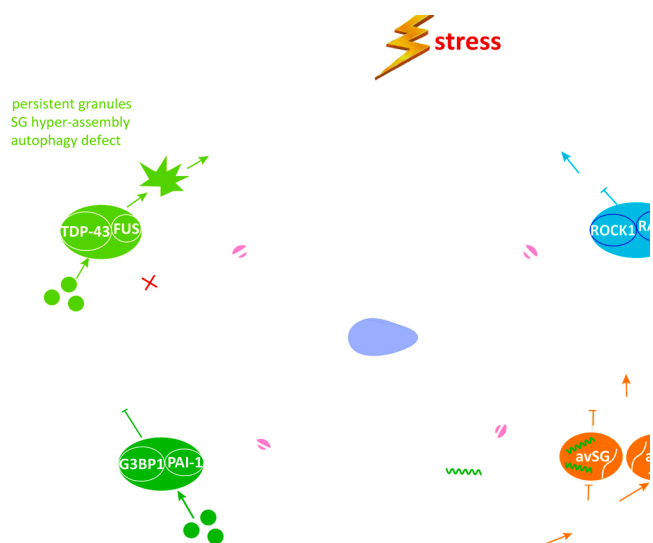
Fig. 2. The physiological and pathological roles of SGs. By recruitment of a variety of RBPs and signaling proteins into SGs to regulate cellular processes including stress response and adaption, SGs are important to maintain homeostasis and human health. Disruption of SG dynamics is involved in the progression of aging and many types of human diseases, including cancer, chemotherapy resistance, neurodegenerative diseases and viral infection. (A). Mutation in disease-associated RBPs (e.g., FUS, TDP-43, hnRNPA1) promotes the formation of abnormal and persistent SGs, which lead to neurotoxic protein aggregates and progress towards neurodegenerative diseases. (B). Tumor microenvironment and many chemotherapeutic drugs act as stressful condition and might promote SG formation. Tumor cells hijack SG property to escape from apoptosis, resulting in tumorigenesis and drug resistance. (C). Aging-associated changes in cellular environment might lead to disruption of SG dynamics. During aging, downregulation of G3BP1 expression causes defect in SG formation. Thus, aging-promoting protein PAI-1 fails to localize to SG, which further accelerates aging. (D). In response to viral infection, SGs are induced in host cells to inhibit viral replication, via repression on viral mRNA translation and stimulation on host innate immune response. However, viral proteins (e.g., protease and nucleocapsid protein) antagonize SG formation to favor viral mRNA translation. Alternatively, viral proteins could also induce aSG (atypical SG, viral mRNAs are excluded) to inhibit translation of cellular mRNA while allow translation of viral mRNA.

trigger SG formation. SGs are observed in many human cancers, such as pancreatic cancer, hepatocellular carcinoma, glioma/glioblastoma, sarcoma, mantle cell myeloma, colorectal cancer, head and neck cancers and prostate cancer [36]. SGs regulate the expression of oncogenes, tumour metabolism, and adapt tumour cells to the microenvironment, which are directly related to the development of tumours and the efficiency of anticancer drugs. Several anti-tumour drugs have been shown to induce the formation of SGs. On the one hand, SGs are partially involved in apoptosis regulation. For example, eIF2α phosphorylation-dependent SGs (Type I) induced by sodium arsenite (SA) and bortezomib [40] may protect cells in the stress response, inhibit apoptosis and promote cell survival by the sequestration of signalling molecules, such as RACK1 [41], ROCK1 [42] and Raptor [43]. Disturbing the formation of anti-apoptotic type I SGs not only influences tumour progression but also sensitises cancer cells to chemotherapeutic agents. On the other hand, eIF2α phosphorylation-independent SGs (Type II), induced by sodium selenite and silvestrol, may impair cell resistance to stress, reduce cell survival and promote apoptosis [39,44, 45]. Induction of pro-apoptotic type II SGs might be one mechanism underlying their anti-tumour effect.

3.2. Virus infection

Viruses are another type of adverse stress to host cells. Viruses interfere with the gene expression of infected cells, and antiviral responses drive the formation of SGs to control viral RNA translation and viral replication. Virus-induced SGs have a unique composition (such as Trim25) and function, and have been named anti-viral SGs (avSGs) to distinguish them from typical SGs [46]. Based on the ways of regulating SG formation, viruses are divided into different types. The first type of virus temporarily triggers SG formation early in the replication cycle, but later limits SG formation. These viruses include poliovirus, genovirus and orthovirus [47]. Mammalian orthoreoviruses (MRV) induce SG formation via eIF2α phosphorylation in a strain- and cell type-dependent manner. As the infection progresses, viral infections leads to the inhibition of SGs. In addition, several strains of MRV inhibit SG formation induced by arsenite to facilitate infection [48]. The second type of virus can inhibit SG formation during the entire infection process, such as influenza A virus, which inhibits SG formation by blocking eIF2α phosphorylation to avoid the repression of virus replication [49]. The third type of virus induces SG formation to help virus RNA replication. Respiratory syncytial virus (RSV), picornavirus and coronavirus may benefit from inducing SG formation as part of the mechanism by which they inhibit host cell protein synthesis and innate immunity [50–52]. The eIF2α kinase PKR is the primary sensor responsible for the rapid inhibition of translation initiation. PKR-mediated SGs can block the translation of viral RNA and interfere with virus replication in response to virus infection. Some viruses, however, encode for PKR inhibitors, thus avoiding PKR-mediated eIF2α phosphorylation and SG formation [53]. The drugs that induce SGs by bypassing PKR and/or eIF2α phosphorylation may have therapeutic potential to control viral infection.

Recently, SARS-CoV-2 nucleocapsid (N) protein has been identified to inhibit SG formation by LLPS of N protein from the SG essential protein G3BP1/2, which enhances viral RNA replication and translation [33,54, 55]. SARS-CoV-2 suppression of SG formation may be mediated by the interaction of N protein with G3BP1/2 and/or CSNK2B/CSNK2A2, the subunits of casein kinase 2 (CK2) [56]. CK2 activity is enhanced following SARS-CoV-2 infection in cells which increase SG disassembly through the promotion of G3BP1 phosphorylation. Several small molecules, including CK2 inhibitors, have been suggested to inhibit the process of viral RNA replication via SG disassembly [56,57].

3.3. Neurodegenerative diseases

SGs have been linked to several neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD) and

Alzheimer's disease (AD). A number of proteins in SG components, such as TDP-43, FUS, hnRNPA1, hnRNPA2, TIA1, MATR3, EWSR1, TAF15, ATX1 and ATX2 [58,59], have been shown to be associated with neurodegenerative diseases. Misfolded proteins or mutations in pathogenic RBPs cause abnormal protein aggregation and SG formation. When stress becomes chronic, persistent and more severe, original reversible SGs change into irreversible stable amyloid-like assemblies and insoluble fibre aggregates, leading to the loss of normal RBP function in the nucleus and the induction of neurotoxicity in the cytoplasm [58,59]. Moreover, partially mutated proteins (VCP, optineurin, p62, ubiquilin-2) may result in the dysfunction of autophagy and cause defects in the clearance of abnormal SGs, subsequently exacerbating the disease process [62]. At present, research on SGs and neurodegenerative lesions are focused on RBP biological models. RBPs guide the transport, translation and degradation of intracellular RNA, thereby regulating and controlling protein expression. Irreversible SGs impair the function of RBPs and interfere with mRNA transport and transcription, and worsen neurodegenerative diseases. Thus, reversing the irreversible solid-state of SGs may enables SGs to maintain reversible liquid properties, or promote SG disassembly and autophagy clearance, which is beneficial to the treatment of neurodegenerative diseases.

3.4. Aging

Aging is accompanied by many intracellular processes such as cellular metabolic change, mitochondrial dysfunction and abnormal protein quality control, which could result in disrupted protein homeostasis and the abnormal formation, maintenance, disassembly and clearance of SGs, consequently leading to chronic or pathological SGs [35]. These aberrant SGs may accelerate the aging process and aging-associated diseases. In aged *C. elegans*, the SG proteins PAB-1 and TIAR-2 can form aggregates, associated with shorter lifespan [63], suggesting a connection between SG protein aggregation and shorter lifespan. An abnormal protein-RNA interaction may lead these proteins to be aggregated and form irreversible aggregations [64], leading to persistent SGs, which would cause the pathogenesis of aging-related diseases [65–67]. In addition, the SG components can also disturb SG dynamics, i.e., TDP-43 can increase amyloid-β oligomeric aggregates and accelerate aging and the onset of neurodegenerative diseases [68,69]. However, studies have also shown that cellular senescence can cause eIF2α hyperphosphorylation and impair SG formation in the stress response in a G3BP1-dependent manner [70]. Omer and colleagues found that SG assembly is defective in aged cells and SG assembly has an anti-aging effect by recruitment of the pro-aging protein, plasminogen activator inhibitor-1 (PAI-1) to SGs [71]. Whether preventing chronic SG formation or inducing the formation of canonical SGs could retard aging process is worth investigating.

3.5. Inflammatory diseases

In inflammatory diseases, the inflammasome is an important component that activates proinflammatory cytokines and induces inflammatory cell death. However, SGs and the NLRP3 inflammasome compete for DDX3X molecules to coordinate the activation of innate responses and subsequent cell fate decisions under stress conditions. SG formation leads to the sequestration of DDX3X and thereby inhibits NLRP3 inflammasome activation [72]. In the immune response, the eIF2α of T cells undergoing antigen presentation for the first time is phosphorylated to form SGs that wrap the mRNA of cytokines inside. When antigen presentation occurs again, some mechanism is initiated to dissolve SGs and release the mRNA, thus translating and secreting cytokines [73]. SGs suppress the stress-induced inflammatory response by recruiting inflammatory factors TRAF2 and inhibiting TNF-α-mediated NF-κB proinflammatory signalling [74].

SGs are also associated with several other diseases, such as atrial fibrillation, organ fibrosis, autoimmune disease and brain ischemia [75]. It is expected that more diseases will be linked with SGs soon.

4. Small molecules targeting SGs

At present, many compounds have been identified to affect the process of SG assembly, maintenance and disassembly via different mechanisms. SGs transiently exist in specific stress states and are regulated by a large number of post-translational modifications, protein remodelling complexes and microtubule networks. Regulating SG assembly or disassembly may be a key method of controlling cell fate or treating disease. Hence, it is a promising field to develop potential therapeutic strategies by targeting SG proteins in related diseases. In this part, we will focus on the reported small molecules that affect SG aggregation or depolymerisation (Tables 1–4), summarise them according to their functions, and discuss the potential for drug development targeting SGs.

4.1. Targeting translation initiation

The accumulation of SGs and related pathogenesis is fundamentally regulated at three levels: formation, maintenance and clearance. The major SG formation-regulating signalling pathways include the eIF4F and eIF2α pathways, and the mammalian target of rapamycin (mTOR) also involved in signalling. The eIF2, GTP and Met-tRNA$_i^{Met}$ ternary complex and eIF4F complex are two major points for the regulation of translation initiation. Under stress conditions, eIF2α kinases are activated to induce the phosphorylation of eIF2α at position 51 serine. The phosphorylated eIF2α (p-eIF2α) reduces the actions of eIF2B and inhibits translation initiation, leading to the disassembly of polysomes; consequently, untranslated mRNAs and translationally arrested PICs initiate SG assembly. These eIF2α kinases are key components in the integration of intracellular and extracellular signals regulating cellular translation in the stress response. Four eIF2α kinases are responsible for this process with different stresses: GCN2, PERK, PKR and HRI, which provide effective drug targets for therapeutic intervention.
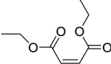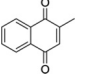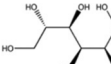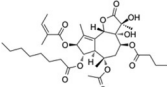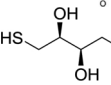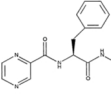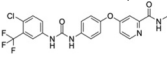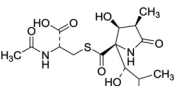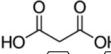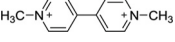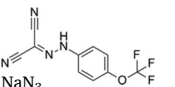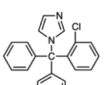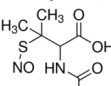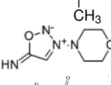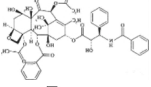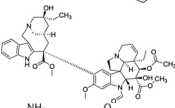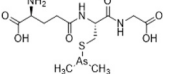
4.1.1. Phosphorylation of eIF2α

Several small molecules have been reported to induce eIF2α phosphorylation and SG assembly, although different eIF2α kinases are employed. The Raf1/Mek/Erk kinase inhibitor sorafenib is approved for advanced hepatocarcinoma [76], and has previously been reported to induce ER stress by the activation of PERK phosphorylation of eIF2α [77,78]. Recently, it has been found that sorafenib induces SG formation in various cancer cells via PERK-mediated eIF2α phosphorylation [78]. SG formation reduces the sensitivity of cells to sorafenib, reduces cell death and improves cell survival. Thus, the disruption of SG formation can restore cell sensitivity to sorafenib. The anti-tumour drug 5-fluorouracil (5-FU) [79] has been found to induce SG assembly, inhibit cell proliferation and promote apoptosis via PKR-mediated eIF2α phosphorylation. SG assembly is associated with the 5-FU metabolite FUrd that is further converted into FUTP and incorporated into RNA to promote SG assembly [80].

Bortezomib is a peptide boronate inhibitor of the 26S proteasome approved by the FDA for the treatment of myelomas and other haematological tumours [81,82]. Bortezomib efficiently induces SGs in many cancer cells, but fails to form SGs in Hs578 T breast cancer cells. The effects of bortezomib-induced eIF2α phosphorylation is mediated through the activation of HRI and GCN2, leading to SG formation, subsequently resulting in resistance to bortezomib in cancer cells [40].
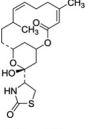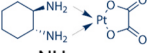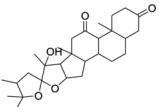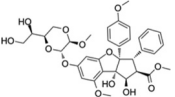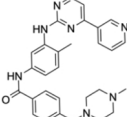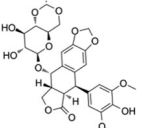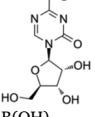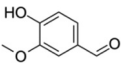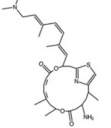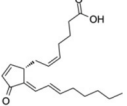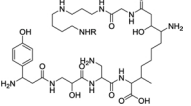
In contrast, several small molecules have also been reported to inhibit eIF2α phosphorylation and impair SG assembly. Psammaplysin F, a marine sponge-derived metabolite, has effective activity against the malaria parasite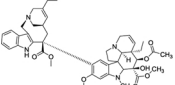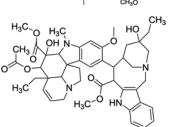 [83,84]. Psammaplysin F treatment may lead to resistance to SG inducers in many cell lines (e.g., the Vero, MCF7, T47D, HeLa and MCF7MDR cell lines). One possible mechanism is that psammaplysin F decreases the phosphorylation of eIF2α under stress. Trehalose is a natural disaccharide sugar, widely occurring in various organisms including bacteria, plants, insects, yeast, fungi and

Table 1
Compounds increasing SG assembly.

Category	Compound	Chemical formula	Mechanism	References
Oxidative stressor	Sodium arsenite	$NaAsO_2$	Induce eIF2α phosphorylation	[154]
	Sodium selenite	Na_2SeO_3	Induce 4EBP1 dephosphorylation	[39]
	Hydrogen peroxide*	H_2O_2	Induce 4EBP1 hypophosphorylation	[18]
	Diethyl maleate (DEM)		Induce eIF2α phosphorylation	[18]
	Menadione		Unknown	[156]
Osmotic and oxidative stressor	Sorbitol		Induce eIF2α phosphorylation	[124,157]
ER stressor	Thapsigargin		Induce eIF2α phosphorylation	[89]
	Dithiothreitol (DTT)		Induce eIF2α phosphorylation	[158]
Proteasome inhibitor	Bortezomib		Induce eIF2α phosphorylation	[40]
	Sorafenib		Induce eIF2α phosphorylation	[78]
	Lactacystin		Induce eIF2α phosphorylation	[151]
Mitochondrial inhibitor	Malonate		Induce 4EBP1 hypophosphorylation	[159]
	Paraquat		Induce eIF2α phosphorylation	[160]
	Carbonyl cyanide (trifluoromethoxy) phenylhydrazone (FCCP)		Induce enery deprivation	[161]
	Sodium azide	NaN_3	Decrease polysomes	[162]
	Clotrimazole		Unknown	[162]
Nitric oxide-generating compound	Nitroso-N-acetylpenicillamine (SNAP)		Induce eIF2α phosphorylation and 4EBP1 dephosphorylation	[90]
	3-Morpholinosydnonimine (SIN-1)		Induce eIF2α phosphorylation and 4EBP1 dephosphorylation	[90]
Microtubule stabilizer	Paclitaxel		Promote microtubule as-sembly and stabilization	[16,106]
Microtubule disruption drug	Vinorelbine		Induce eIF2α phosphorylation and 4EBP1 dephosphorylation	[16,109]
	Vinblastine*		Induce eIF2α phosphorylation and 4EBP1 dephosphorylation	[16,109]
	Vincristine		Induce eIF2α phosphorylation and 4EBP1 dephosphorylation	[16,109]
	Darinaparsin		Inhibits microtubule polymerization	[164]
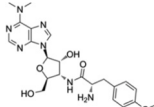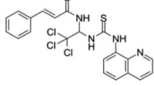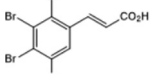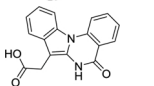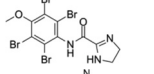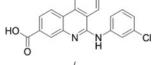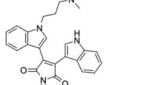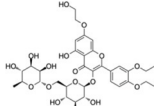
(continued on next page)

Table 1 (*continued*)

Category	Compound	Chemical formula	Mechanism	References
Actin polymerization inhibitor	Latrunculin B		Disrupt actin fiber and facilitate the motility of SG components	[109]
DNA damage drug	Oxaliplatin		Induce eIF2α phosphorylation	[165]
	Cisplatin		Induce eIF2α phosphorylation	[166]
eIF4A inhibitor	Hippuristanol		Inhibit eIF4A activity	[17,98]
	Silvestrol		Stimulate eIF4A RNA-binding activity	[45,167]
Tyrosine kinase inhibitor	Imatinib		Induce eIF2α phosphorylation	[45,167]
Topoisomerase II inhibitor	Etoposide		Induce eIF2α phosphorylation	[166]
RNA incorporating agents	Fluorouracil (5-FU)		Induce eIF2α phosphorylation	[44,80]
	6-Thioguanine		Incorporate into RNA	[80]
	5-Azacytidine		Incorporate into RNA	[80]
Others	Boric acid	B(OH)$_3$	Induce eIF2α phosphorylation	[116]
	Vanillin		Unknown	[168]
	Pateamine A and its analogs		Stimulate eIF4A RNA-binding activity	[19,97]
	Furfural		Unknown	[169]
	Deoxy-delta12,14-prostaglandin J2 (15d-PGJ2)		Promote eIF4A inactivation	[170]
	Edeine		Prevent 60S binding to the 48S complex	[132]

"*" represents that this drug has been reported that can both induce SGs assembly and inhibit SGs assembly, depending on cell type and dosage.

Table 2
Compounds enhancing SG assembly.

Category	Compound	Chemical formula	Mechanism	References
Protein synthesis inhibitor	Puromycin		Destabilize polysomes	[153]
eIF2α dephosphorylation inhibitor	Salubrinal		Inhibit eIF2α dephosphorylation	[171,172]
Casein Kinase 2 inhibitor	Tetrabromocinnamic acid		Prevent G3BP1 phosphorylation	[90]
	IQA		Prevent G3BP1 phosphorylation	[103]
	TMCB		Prevent G3BP1 phosphorylation	[56]
	Silmitasertib		Prevent G3BP1 phosphorylation	[56]
PKC inhibitor	GF109203X		Inhibit G3BP2 phosphorylation and delay eIF2α phosphorylation	[173]
Others	Troxerutin		Unknown	[117]

These compounds can not induce SGs assembly in large quantities when used alone. However, they enhance the role of other induced SGs production compounds, such as increase the size of sodium arsenite-induced SGs or block the disassembly of arsenite-induced SGs.

invertebrates. Trehalose protects cells from protein denaturation and exhibits neuro-protective effects in several neurodegenerative diseases under heat, freezing, oxidation, drying and dehydration conditions. This effect is accompanied by p-eIF2α dephosphorylation, which accelerates the restoration of translation. Trehalose pretreatment also decreases p-eIF2α induced by ER stress or heat shock, sensitizes cells to stress and impairs cell survival [85]. Inhibition of chemotherapy drug-induced SGs by this type of small molecule may be beneficial to relieving drug resistance in cancer patients.

The integrated stress response, mediated by eIF2α, regulates protein synthesis under stress. ISRIB targets eIF2B and is the first reported antagonist of the ISR that blocks signalling downstream of all eIF2α kinases [86]. In non-stress situations, 43S preinitiation complexes containing eIF2-GTP-Met-tRNA$_i^{Met}$ begin translation. To engage in a new round of initiation, eIF2-GDP released during this process needs to reload GTP to form new eIF2-GTP, in a reaction catalysed by the heteropentameric eIF2B [87]. ISRIB can reverse cognitive deficits following traumatic brain injury, protect against prion-induced neurodegeneration and prevent metastasis in a subset of cancers [86]. It has been reported that ISRIB reverses the effect of eIF2α phosphorylation and restores translation by targeting eIF2B. ISRIB prevents the formation of SGs induced by thapsigargin (a potent ER stressor that inhibits the ER calcium pump [88]) and arsenite through eIF2α phosphorylation, without reducing Pat-A or hippuristanol-induced SGs through eIF4A inhibition [89]. In another study, ISRIB also inhibited SIN-1-induced SG assembly in U2OS cells [90]. ISRIB also leads to the disassembly of pre-formed SGs by loading dissociating mRNAs with actively translating ribosomes [89]. ISRIB, with its ability to quickly disassemble SGs even in the presence of stress, is expected to develop into new therapies for SG-related diseases.
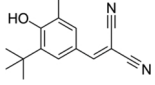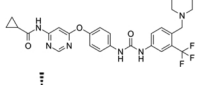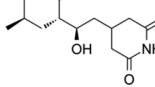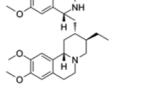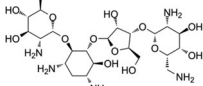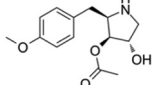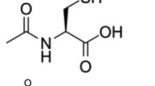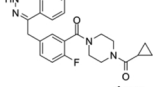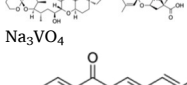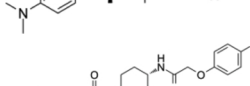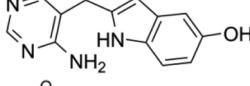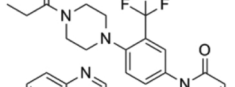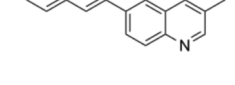
4.1.2. Disruption of the eIF4F complex

Assembly of the eIF4F complex requires eIF4A, eIF4E and eIF4G as a key step in mRNA circulation and translation initiation. It is tightly regulated by cell signalling, particularly through mTOR signalling. In normal conditions, mTOR phosphorylates eIF4E-binding protein 1 (4E-BP1), which blocks 4E-BP1 binding to eIF4E and forms the eIF4F complex. The eIF4F complex is a major target of environmental stress to induce eIF2α-independent SGs. In response to stresses or chemical compounds, mTOR is inactivated, which results in the accumulation of hypophosphorylated 4E-BP1 and enables its binding to eIF4E, causing displacement of the scaffolding protein eIF4G and the RNA helicase eIF4A from mRNA cap structures. Consequently, translation initiation and polysome formation are blocked, concomitantly with SG assembly. Disruption of the eIF4F complex may induce non-canonical SGs, which lack some of the core components of eIF2α phosphorylation-induced canonical SGs [91]. Thus, targeting the eIF4F complex might be a promising approach to regulating SG dynamics.

eIF4A inhibition and the disruption of eIF4E binding to eIF4G are the common mechanisms underlying the formation of eIF2α-independent SGs induced by several small molecules. Malonate, a classic competitive inhibitor of the respiratory electron transport chain, induces mitochondrial stress and inhibits mitochondrial respiration [92,93]. It has been reported that malonate induces SG aggregation through 4E-BP1-mediated inactivation of the eIF4E pathway, which may inhibit apoptosis in HeLa cells [94]. Like malonate, selenium, an essential micronutrient that promotes the production of reactive oxygen species (ROS) for targeting tumour cells [31], induces SG assembly by destroying the eIF4F complex via the dephosphorylation of 4E-BP1 [39]. Interestingly, selenite-induced SGs prompts U2OS human osteosarcoma cells to undergo apoptosis, probably because of the unique SG composition [39].
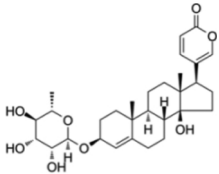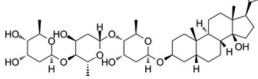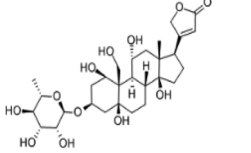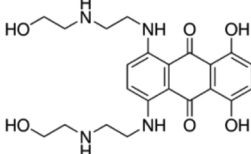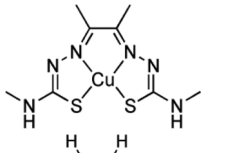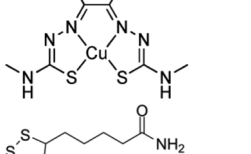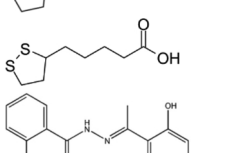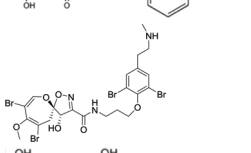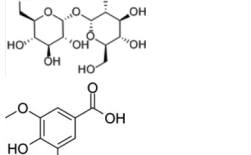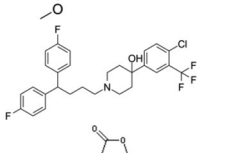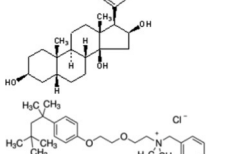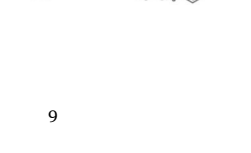
Table 3

Compounds inhibiting SG assembly.

Category	Compound	Chemical formula	Mechanism	References
Transcription inhibitor	8-Hydroxyquinoline		Unknown	[123]
Mitochondrial fission inducer	Tyrphostin A9		Unknown	[123]
β cell proliferation agonist	WS3		Unknown	[123]
Protein synthesis inhibitor	Cycloheximide		Stabilize polysomes	[153]
	Emetine		Stabilize polysomes	[153]
	Neomycin		Unknown	[123]
	Anisomycin		Unknown	[123]
ROS scavenger	N-Acetyl-L-cysteine		Reduce ROS production	[18]
PARP inhibitor	Olaparib		Inhibit PARP	[18]
Phosphatase inhibitor	Okadaic acid		Promote G3BP phosphorylation	[131]
HDAC inhibitor	Vanadate	Na_3VO_4	Promote G3BP phosphorylation	[131]
	Trichostatin A(TSA)		Inhibit HDAC6	[131]
PERK inhibitor	ISRIB		Target eIF2B and block signaling downstream of all eIF2α kinases	[89]
ATP-competitive mTOR inhibitor	Torkinib(PP242)		Inhibit mTORC1 activity and reduce accumulation of eIF4E-eIF4GI complexes	[91]
	Torin1		Inhibit mTORC1 activity and reduce accumulation of eIF4E-eIF4GI complexes	[91]
Na+/K+-ATPase inhibitor	Proscillaridin A		Unknown	[91]

Table 3 (*continued*)

Category	Compound	Chemical formula	Mechanism	References
	Digitoxin		Unknown	[123]
	Ouabain		Unknown	[123]
Topoisomerase II inhibitor	Mitoxantrone		Inhibit TDP-43 accumulation	[123]
Bis(thiosemicarbazonato)-Copper Complexes	Diacetylbis(methylthiosemicarbazonato)-copperII (CuII(atsm))		Inhibit TDP-43 accumulation	[174]
	Glyoxalbis(methylthiosemicarbazonato)-copperII (CuII(gtsm))		Inhibit TDP-43 accumulation	[174]
Others	Lipoamide		Inhibit FUS accumulation and disrupt SG phase separation	[124]
	Lipolic acid		Inhibit FUS accumulation and disrupt SG phase separation	[124]
	Hydroxy-N′-[1-(2-hydroxyphenyl)ethylidene]benzohydrazide		Target G3BP2 and influence G3BP2 function	[132]
	Psammaplysin F		Decrease eIF2α phosphorylation	[83]
	Trehalose		Promote eIF2α dephosphorylation	[85]
	Syringic acid		Unknown	[117]
	Penfluridol		Unknown	[123]
	Gitoxigenin		Unknown	[123]
	Benzethonium		Unknown	[123]
	Pyrvinium		Unknown	[123]

Table 3 (*continued*)
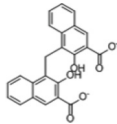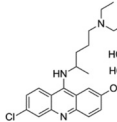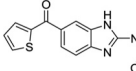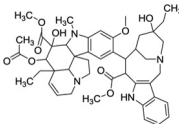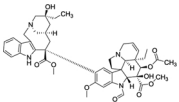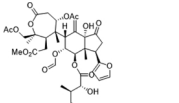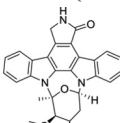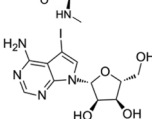
Category	Compound	Chemical formula	Mechanism	References
	Quinacrine		Unknown	[123]

Table 4
Compounds disturbing SG assembly.

Category	Compound	Chemical formula	Mechanism	References
Microtubule disruption durgs	Nocodazole		Promote microtubule depolymerization	[109]
	Vinblastine*		Promote microtubule depolymerization	[109]
	Vincristine*		Promote microtubule depolymerization	[16,123]
Actin filament stabilizer	Prieurianin		Unknown	[123]
ATP-competent kinase inhibitor	Staurosporine and its analog (RO-31−8220)		Disrupt the ATP-driven chemical reactions	[138]
	5′-Iodotubercidin		Disrupt the ATP-driven chemical reactions	[138]

These compounds interfere with the fusion and growth of SG, rather than the initial assembly of SG. When SA stimulates cells that pretreated with these compounds, the proportion of cells with SGs decreases, the number of SGs in each cell increases, and the proportion of large SGs decreases. "*" represents that this drug has been reported that can both induce SGs assembly and disturb SGs assembly, depending on cell type and dosage.

Of note, perturbing eIF4A activity induces SG assembly independent of eIF2α phosphorylation, which may exert anti-cancer activity [20,95]. 15-deoxy-delta12,14-prostaglandin J2 (15d-PGJ2) possesses multiple biological activities [96], which blocks translation by targeting cysteine 264 and inactivating eIF4A, without the involvement of eIF2α phosphorylation, suggesting that the translation inhibitory action of 15d-PGJ2 is likely to contribute to the antineoplastic activity of 15d-PGJ2 [96]. Pateamine A and its analogues, DMDA-PatA and B-PatA, induce SG formation through the prevention of eIF4A heterodimerisation with eIF4G and stabilising the eIF4A:mRNA complex, consequently limiting the availability of eIF4A incorporation into eIF4F complexes [19,97]. Hippuristanol and silvestrol have also been found to induce SG assembly by perturbing eIF4F ATPase, helicase and RNA-binding activities, explaining their anti-tumour and antiviral replication activity [17,98].

Some small molecules have been reported to have combined activities of eIF2α phosphorylation and eIF4F disruption. For example, the vinca alkaloids vinorelbine, vinblastine and vincristine promote polysome disassembly and SG assembly through simultaneous activation of eIF4E-BP1 and phosphorylation of eIF2α [16]. Blocking vinca

alkaloid-induced SG assembly by inactivating 4EBP1 or inhibiting eIF2α phosphorylation decreases cancer cell viability and promotes apoptosis [16]. Nitric oxide-generating compounds 3-morpholinosydnonimine (SIN-1) and S-nitroso-*N*-acetylpenicillamine (SNAP) are protein synthesis inhibitors that trigger SG formation through NO-induced eIF2α phosphorylation and 4E-BP1 dephosphorylation [90]. NO plays a major role in this process. Interestingly, SIN-1-induced pro-apoptotic SGs are less dynamic and less mobile compared to typical arsenite-induced anti-apoptotic SGs because of the loss of eIF3B over time [90].

4.1.3. Targeting mTOR signalling

While the dissociation of eIF4E-eIF4G induces eIF2α-independent SGs, association of eIF4E-eIF4G is required for the assembly of eIF2α-dependent SGs. Therefore, disruption of eIF4E-eIF4G could inhibit the assembly of eIF2α-dependent SGs, or promote the disassembly of pre-existing eIF2α-dependent SGs. This was proposed by Fournier and colleagues, who found that mTOR inhibitor-mediated disruption of eIF4E-eIF4GI impairs SG formation under mild stress [91]. When the cell is treated with a mild stressor such as arsenite or bortezomib (both induce eIF2α phosphorylation), eIF4E binds to eIF4G, but the eIF4E-4GI

complex is in an inactive state, accumulates gradually and eventually leads to the production of SGs. In this case, depletion or inactivation of mTOR leads to hypophosphorylation of 4E-BP1, which then blocks the interactions between eIF4E and eIF4GI to promote the disassembly of formed SG [91].

PP242 is an ATP-competitive inhibitor of mTOR that blocks the phosphorylation of Akt at S473 and suppresses mTOR activity. PP242 directly binds to the ATP-binding site of mTOR and inhibits the catalytic activity of both mTORC1 and mTORC2 [99]. It also decreases the phosphorylation of 4E-BP1 to prevent the binding of eIF4E to eIF4GI, thus inhibiting SG formation [91]. The depletion of mTOR significantly inhibits SG formation in cells treated with PP242 [91]. Similar to PP242, the kinase inhibitor torin1 is also a selective ATP-competitive inhibitor of mTOR [100], which inhibits mTORC1-dependent phosphorylation of 4E-BP1 and impairs SG formation [91]. These data suggest that the suppression of mTOR signalling may inhibit the assembly of mild stress-induced eIF2α-dependent SGs and sensitize tumour cells to apoptosis.

4.1.4. Inhibition of CK2

CK2 is a stress-activated protein kinase linked to the phosphorylation of several translation initiation factors, including eIF3 and eIF5 [101, 102]. CK2 accelerates SG disassembly through the promotion of G3BP1 phosphorylation to inhibit SG assembly [103]. Thus, CK2 inhibitors may block SG disassembly and recover from the stress. Tetrabromocinnamic acid (TBCA) is a specific CK2 inhibitor [104] and increases residual SGs in cells during recovery from stress [103]. The another CK2 inhibitor, 5-oxo-5,6-dihydroindolo-(1,2-a)quinazolin-7-yl acetic acid (IQA), also inhibits the disassembly of virus-induced SGs [103]. A recent study has suggested that the CK2 inhibitors TMCB and silmitasertib could interfere with SG disassembly by SARS-CoV-2 N protein, but this needs to be confirmed [56,57].

4.2. Targeting the cytoskeleton

The recruitment of mRNPs in the cytoplasm to fuse into mature SGs requires transportation along microtubules by motor proteins; thus, microtubule integrity is essential for SG assembly. Microtubules are intracellular structures assembled with heterodimers of α and β tubulin, and are responsible for various kinds of movements in cells. Microtubules are involved in cell division, the organisation of intracellular structure and intracellular transportation. Microtubule disruption delays SG formation. SGs are still formed, but they are smaller in size, greater in number and variable in distribution [105].

Tubulin inhibitors are used in the treatment of cancer because they induce cell cycle arrest. Paclitaxel, a first-line anti-cancer drug, is a β-tubulin inhibitor and promotes microtubule assembly and stabilisation [106,107]. Paclitaxel induces SG formation in many cells [16,105]. Similarly, latrunculin B, an actin filament inhibitor [108], has been reported to accelerate microtubule-based motility via actin disruption, thus facilitating the motility of SGs and promoting the formation of large SGs [109].

In contrast, tubulin depolymerising agents could trigger SG disappearance. As a reversible microtubule inhibitor, nocodazole binds to β-tubulin and disrupts microtubule assembly and disassembly kinetics, thereby preventing mitosis and inducing apoptosis in tumour cells [110]. Nocodazole inhibits SG formation but does not prevent SA-induced eIF2α phosphorylation, suggesting that nocodazole acts downstream of eIF2α phosphorylation [109].

Vinblastine is a therapeutic drug for Hodgkin's disease, lymphocytic lymphoma, histiocytic lymphoma, advanced testicular cancer, advanced breast cancer, Kaposi's sarcoma and Letterer-Siwe disease [111,112]. Vinblastine has higher affinity for tubulin and causes microtubule depolymerisation [113]. Similarly to nocodazole, vinblastine disrupts the formation of SGs and the disassembly of the formed SGs [109]. However, a higher concentration or prolonged treatment with vinblastine potently promotes translation repression and induces microscopically visible SGs [16,109]. Since the cytoskeleton also serves as scaffold and is closely associated with the polysome for the active translation of mRNA [114], vinblastine at a high dose may induce robust polysome disassembly through the collapse of microtubules and the secondary aggregation of SGs [16].

These studies have provided helpful strategies for targeting microtubules to regulate SG formation, as well as strategies for overcoming resistance to tubulin inhibitors in cancer patients.

4.3. Targeting SG components

The core components of SGs includes G3BP1/2, TIA-1, TIAR, Caprin1, TDP-43, a number of energy-driven protein/nucleic acid chaperones or remodelling complexes and untranslated mRNA. Targeting the components may influence SG dynamics.

4.3.1. TIA-1

TIA-1 has been reported to prevent cell proliferation, tumour growth and invasion [115]. Under physiological conditions, TIA-1 binds to an adenine/uridine-rich element in the 3'-UTR of TNFα mRNA and represses TNFα mRNA translation. In contrast, TIA-1 increases TNFα mRNA translation in response to stress. Boric acid exhibits anticarcinogenic and bone strengthening effects by balancing the anti-apoptotic eIF2α-SG pathway and pro-apoptotic eIF2α-ATF4 pathway via TIA-1 translocation from the nucleus to SGs [116]. Similarly, troxerutin, known as vitamin P4 with multiple nutritional and pharmacological properties (antioxidant, anti-inflammatory, radioprotective and hepatoprotective activities), has also been shown to promote SG formation in a TIA1-dependent manner. This effect is diminished in TIA-1 suppressed cells, suggesting that enhanced SG formation by troxerutin might recruit essential stress-fighting mRNAs/proteins into SGs and thereby impair the cellular machines required for survival and recovery [117].

4.3.2. TDP-43

TAR DNA binding protein 43 (TDP-43) is an RBP pathologically and genetically linked to ALS, FTD, Alzheimer's disease, Parkinson's disease and Huntington's disease [118,119]. Several studies have shown that the accumulation of TDP-43 in SGs is mediated through activation of the stress kinase c-Jun N-terminal kinase (JNK) [120]. Inhibiting extracellular signal-regulated kinase (ERK) could prevent the accumulation of TDP-43 and human antigen R-(HuR)-positive SG formation. Mutations in the TDP-43 coding gene TARDBP as well as changes in the expression level of TDP-43 proteins may disrupt the dynamics of SGs [120].

Mitoxantrone is a type II topoisomerase inhibitor approved for the treatment of acute myeloid leukaemia and multiple sclerosis [122]. Mitoxantrone reduces TDP-43 in SGs and decreases SG formation in disease-relevant cell models harbouring mislocalised TDP-43 protein [121,123]. Mitoxantrone prevents SG localisation of mutant TDP-43 and reduces the accumulation of mutant TDP-43 into persistent cytoplasmic puncta in induced pluripotent stem cell-derived spinal motor neurons (iPS-MNs). Mitoxantrone also significantly reduces the cumulative death rate in mouse primary neurons expressing TDP-43 (M337 V)-EGFP [123], suppresses the recruitment of FUS to SGs, and reduces the number and size of liquid FUS droplets formed in vitro [124].

Emerging evidence suggests that LLPS-driven SG formation is associated with the pathogenesis of neurodegenerative diseases, aging, cancer and virus infections [125] because of increased irreversible hydrogelation that interferes with normal cellular functions. Hyperphosphorylated TDP-43 undergoes LLPS to colocalise with other stress granule components and promote aberrant protein aggregation, forming chronic SGs. The small molecule inhibitors of tankyrase-1/2, XAV939, G007-LK and IWR-1-endo, suppress the formation of cytoplasmic TDP-43 and increase LLPS of TDP-43 from other SG components [126]; this prevents the pathogenesis of neurodegenerative diseases and may be used to

treat ALS and FTD. LLPS can be enhanced by poly(ADP-ribosyl)ation (PARylation), an important post-translational modification (PTM) involved in DNA damage repair and apoptosis. Additionally, a PARP inhibitor was found to attenuate TDP-43-mediated neurotoxicity in an ALS model [127]. It has been reported that the PARP inhibitor olaparib delays the recruitment of TDP-43 and hnRNP A1 to SGs and delays SG assembly, which is beneficial in ALS and FTD [127]. In pathologic fibrillation, SGs are unlinked from the chemical chaperone trimethylamine N-oxide (TMAO), which enhances RNP liquid condensation and inhibits protein fibrillation, suggesting a therapeutic strategy for TDP-43 aggregation-associated degenerative disorders [128].

4.3.3. G3BP

G3BP1 and G3BP2 contain RNA-binding domains, and are associated with RNA binding [129]. G3BP1/2 are core component of SGs and essential for SG formation. Arsenite induces SG formation, probably via the induction of the dephosphorylation of G3BP at Ser149 [22,130], and increases the interaction of G3BP with histone deacetylase 6 (HDAC6) [131]. Phosphatase inhibitors such as okadaic acid and vanadate inhibit the interaction of G3BP and HDAC6 and increase G3BP phosphorylation level and regulate SG formation [22,131].

Since G3BP1/2 are essential for the initiation of SG formation, small molecules targeting G3BP1/2 could be expected as a new approach for cancer therapy. Compound C108, 2-hydroxy-N-[1-(2-hydroxyphenyl) ethylidene]benzohydrazide, could alleviate the function of G3BP2 and inhibit the activity of tumour-initiating cells [132], showing anti-tumour activity. A recent study showed that SARS-CoV-2 N protein interacts with G3BP1/2 to inhibit SG formation; this inhibits virus RNA replication, suggesting a potential therapeutic target for COVID-19 [54]. However, the small molecules targeting this complex need to be tested.

4.3.4. ATPase

The assembly, fusion, depolymerisation and transportation of SG components require ATP. Consequently, intracellular ATP levels can affect SG dynamics [90]. The formation of SGs and maintenance of SG core proteins in the dynamic pool of SGs require ATP driven chemical reactions rather than specific kinase activity [133]. It seems that the different ATPase complexes have different effects on SG assembly and disassembly [133]. The movement of SGs along the microtubule also requires ATP energy [134,135]. Thus, ATPase inhibitors may affect both the assembly and disassembly of SGs.

Soil-dwelling microbes produce the chemical staurosporine and its analogue RO-31-8220, which are both ATP-competent kinase inhibitors [134,135] that may inhibit SA-induced SGs [138]. 5′-Iodotubercidin (5′-ITU), a kinase inhibitor [139], interferes with the fusion and growth of SGs by inhibiting ATPase activity, but does not affect the disassembly of SGs during recovery from SA stress [138].

4.3.5. FUS

The RNA/DNA binding protein fused in sarcoma/translocated in liposarcoma (FUS/TLS) is linked to ALS [140]. Like TDP-43, FUS/TLS is sequestered in the cytosol of ALS-affected motor neurons. Mutations in FUS cause severe loss of motor neurons in the spinal cord associated with juvenile ALS. The unique feature of FUS pathogenesis in ALS is their nuclear clearance and simultaneous cytoplasmic aggregation in affected motor neurons [118]. FUS accumulation in SGs is a reversible process, which may lead to pathological aggregation of FUS in SGs under chronic stress [60,61]. FUS droplets form by LLPS, and become gel-like forms containing amyloid fibrils and are thus less dynamic. Additionally, some disease-related FUS mutations can exacerbate this phenomenon and aggravate the condition [80]. Lipoamide and lipolic acid have been reported to act on FUS to reduce SG formation and promote the dissolution of pre-existing SGs. Lipoamide and lipoic acid have been shown to improve FUS condensation behaviour, prevent SG component aggregation in a *C. elegans* model, and recover neuron and organism FUS-associated defects in a *Drosophila* model of ALS [80].

4.4. Post-translational modifications

Post-translational modifications (PTMs) including methylation, acetylation, ubiquitination, sumoylation, glycosylation, phosphorylation and neddylation play crucial roles in regulating the functions of proteins by inducing their covalent linkage to new functional chemical groups. PTMs are involved in various signalling pathways that regulate distinct cellular processes. Dysregulated PTMs have been shown to influence RBPs, the key regulators in RNA dynamics, and cause various diseases including cancers, neurodegenerative disorders, virus infection and cardiovascular disease [141,142], suggesting that targeting PTMs is a therapeutic option for these diseases.

Ubiquitination and the ubiquitin-like proteins NEDD8 and SUMO, which mediate neddylation and sumoylation, regulate various cellular processes [143–145], and involved in the disassembly of SGs. Thus, the pharmacological inhibition of ubiquitination may improve the efficiency of cancer therapies. Neddylation induces SG assembly under a stress state via enhanced polysomic disassembly following translation arrest. However, so far, no pharmacological agent has been reported to act on neddylation.

Acetylation and deacetylation, another major form of protein modification, are also involved in SG formation and disassembly. Histone acetylases and histone deacetylases (HDACs) regulate the acetylation of proteins. It has been shown that HDAC6, but not other HDACs, may directly interact with the SG core component G3BP1 to enhance SG formation. Pharmacological inhibition or genetic ablation of HDAC6 blocks SG assembly and sensitises cells to apoptosis under stress [131]. This effect of HDAC6 might be linked to the deacetylation of tubulin [146,147], which is important for SG dynamics, since the pan-HDAC inhibitor TSA results in microtubule network hyperacetylation and decreases SG formation [131]. Thus, targeting HDAC6 deacetylase activity or its interaction with G3BP are worth considering for SG regulation and cancer therapy. Interestingly, the NAD$^+$-dependent deacetylase SIRT6 interacts with G3BP to induce SG assembly in response to stress [148], and inhibiting the activity of SIRT6 impairs SG formation. Thus, SIRT6 antagonists may be a strategy to inhibit the formation of anti-apoptotic SGs and induce apoptosis in tumour cells.

4.5. Targeting SG clearance

SGs are disassembled when the stress is relieved, which is accompanied by translation restoration. Similar to SG formation, the disassembly of SGs is also a multi-step process and is mediated by chaperones and autophagy [26]. The roles of the hsp70 family in chaperone-mediated SG dissolution have been studied by several groups. For example, hsp70, hsp40, hsp104 and HspB8 (the small heat shock protein hsp22) participate in SG removal and relieve the accumulation of untranslated ribosomal products in SGs. Therefore, it is worth investigating the effect of small molecules targeting these chaperone proteins on SG dynamics.

Autophagy removes SGs, in a process called granulophagy. Autophagic vesicles may engulf SGs and clear the particles. The process requires valosin-containing protein (VCP), an ATPase required for the autophagy disaggregation of SG components [29]. Trehalose, an autophagy enhancer [149], has been found to delay SG assembly and facilitate SG premature post-stress disassembly in neuroblastoma SH-SY5Y cells, implicating that trehalose is a possible therapeutic approach in neurodegenerative diseases. However, the action of trehalose is mediated via an increase in p-eIF2α rather than the autophagosome pathway. One study showed that only a minor fraction of aberrant SGs is cleared by autophagy, whereas the majority of SG disassembly requires the HSPB8-BAG3-HSP70 chaperone [149]. Whether autophagy enhancers could accelerate SG disassembly, and thereby be developed as therapeutic agent against cancers and neurodegenerative diseases, still needs further investigation.

The proteasome is also responsible for the degradation of mRNAs in SGs, which was confirmed by the induction of SG assembly by

pharmacological and clinically used proteasome inhibitors [150]. The proteasome inhibitor bortezomib induces SG assembly via the stimulation of eIF2α phosphorylation by the kinases HRI, PKR, GCN2 and PERK [40,151]. This disrupts multiple pathways in the cell and bone marrow microenvironment, leading to apoptosis and inhibiting cell cycle progression, angiogenesis and proliferation [82]; this is a common approach in the treatment of cancers [81,151]. In contrast, prolonged inhibition of the proteasome instead leads to partial recovery of translation and SG disassembly due to stress adaptation [40,151], suggesting that the assembly of SGs in response to proteasome inhibition is time-dependent and reversible [40,151]. Thus, the application of proteasome inhibitors has to consider the involvement of SGs.

4.6. Targeting polysome dissociation

The polysome is a complex formed by cytoplasmic RNAs and ribosomes undergoing active translation. During the stress process, the polysomes originally in the translationally active state begin to dissociate, and the excess free mRNA from ribosomes associate with aggregation-prone RBPs or misfolded proteins, thereby promoting SG protein recruitment and SG assembly [152].

Emetine and cycloheximide are protein synthesis inhibitors that stabilise polysomes by freezing ribosomes to keep ribosomes loaded with mRNA; this prevents the ribosome from reaching the stop codon. mRNA can shuttle between SGs and polysomes under stress. Emetine and cycloheximide prevent the assembly of SA-induced SGs by trapping mRNA in polysomes and maintaining polysomes, preventing free mRNA from accumulating in the cytoplasm [150,153]. On the contrary,

puromycin, an inhibitor of protein synthesis that destabilises polysomes by promoting translational premature termination, enhances SA-induced SGs rather than inhibiting SG aggregation, suggesting that the drugs promoting polysome stabilization or dissociation can affect SG formation.

5. Conclusion and perspective

Here, we have summarised recent advances in small molecules targeting SGs (Fig. 3). SGs have been identified in many biological processes and diseases. The assembly and disassembly of SGs determine further storage, translation remodelling or degradation of untranslated mRNA, which affect cell death or survival under specific conditions. In cancer treatment, on the one hand, the formation of SGs can lead to cell survival and increase cell resistance to chemotherapeutic drugs. The combined use of drugs that inhibit SG formation or promote SG disassembly with chemotherapeutic drugs may alleviate drug resistance. On the other hand, some drugs may enhance the effects of chemotherapy by inducing SG-mediated cell apoptosis. Furthermore, the persistence of stress particles leads to chronic SG formation and irreversible pathogenesis, for example in neurodegeneration and aging, It is therefore possible that targeting chronic SGs that inhibit the abnormal aggregation of related SGs or promote SG clearance may be a novel therapeutic strategy in neurodegenerative diseases and other chronic diseases. The formation and the biological functions of SGs are complex. Many questions need to be answered by research and the development of SG-targeting drugs. (1) Studies on SGs are largely confined to cell culture and *C. elegans* because of the absence of a suitable *in vivo* mammalian
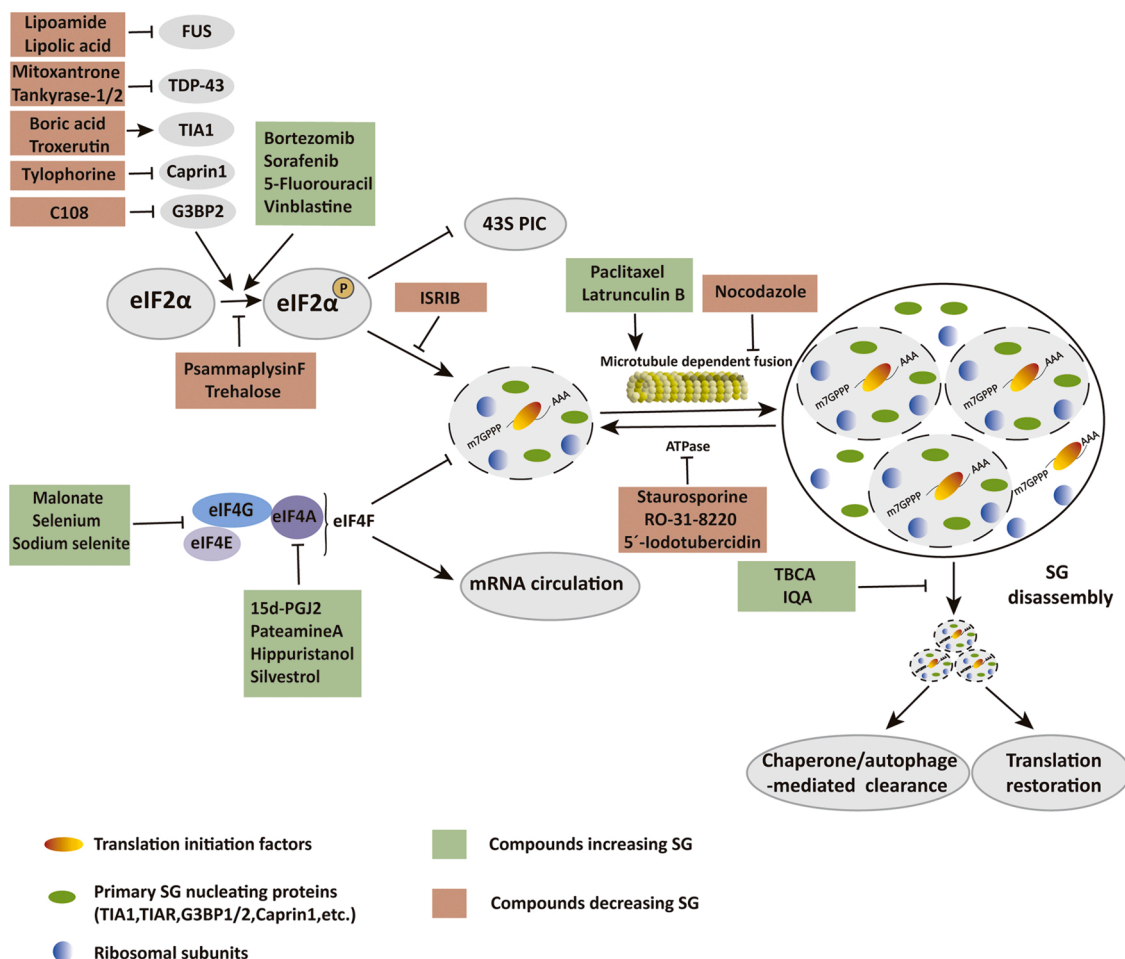

Fig. 3. Compounds targeting SG. Compounds targeting SGs at diverse steps of SG assembly and SG disassembly are summarized and schematically represented.

model. Ideally, a mouse model will be developed that can directly assess stress particles in living animals and more intuitively present direct associations between SGs, drugs and diseases. (2) The side effects of the long-term administration of SG-interfering agents remain unclear. (3) Most SG contents are not the direct target of small molecules. So, the identification of druggable targets in SGs will reveal new biological functions and mechanisms of SG biology. Thus, SGs deserve more in-depth research in order to find novel therapeutic strategies for human diseases.

Declaration of Copeting Interest

The authors have no competing interests to declare.

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