Stress granules, P-bodies and cancer

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Abstract

Cancer cells are exposed to adverse conditions in the tumor microenvironment, and utilize post-transcriptional control mechanisms to re-program gene expression in ways that enhance cell survival. Stress granules and processing bodies are RNA-containing granules that contribute to this process by modulating cellular signaling pathways, metabolic machinery, and stress response programs. This review examines evidence implicating RNA granules in the pathogenesis of cancer and discusses their potential as targets for anticancer therapies.

Keywords

Cancer; Stress Granules; P-bodies; post-transcriptional regulation; apoptosis; stress response

INTRODUCTION

Nascent mRNAs bind to an array of RNA-binding proteins (RBPs) and microRNAs that conspire to determine their fates. These transcripts are transported from the nucleus to the cytoplasm as a part of compositionally diverse complexes known as messenger ribonucleoprotein particles (mRNPs). The composition of these exported mRNPs determines whether transcripts are immediately translated or transported to specific subcellular regions for storage and/or localized translation. These mRNPs are also subject to quality control mechanisms that promote the degradation of transcripts that contain errors that could lead to the production of incomplete protein products (reviewed in [1, 2]).

There is an imperfect correlation between levels of mRNA and levels of the proteins they encode. Up to two-thirds of this variation can be attributed to post-transcriptional mechanisms that modulate mRNA stability and translation [3, 4]. Proteins that mediate post-transcriptional control bind to *cis* elements that are typically found in 5′- and 3′-untranslated

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regions of individual transcripts. These regulatory elements can be unique to a single RNA molecule (allowing targeted regulation of this mRNA) or be found in a subset of mRNAs encoding functionally related proteins (coordinated regulation). Individual RBPs can influence several aspects of mRNA metabolism, including mRNA decay/stabilization, subcellular localization or translation rate. The availability of these RBPs is under the control of cell-intrinsic and extracellular cues. The RBP components of RNP complexes assembled on mRNA transcripts coordinately determine mRNA stability, localization and translation to allow precise and dynamic control over protein synthesis. The reader is referred to several recent reviews on post-transcriptional regulation of gene expression by microRNAs and RBPs for more detailed information [5–7].

The intricate RBP network that regulates mRNA stability, localization and translation is spatially regulated by the assembly of stress granules and processing bodies, mRNP-containing cytoplasmic granules that influence multiple aspects of cell metabolism, especially during changing conditions. Perturbations in RNA granule functions lead to pathological phenotypes observed in multiple neurodegenerative, immunological and infectious diseases [8–11]. Involvement of RNA granules in cancer initiation and progression is an emerging concept in tumor biology, and is the subject of this review.

COMPLEX LIFE OF mRNA

Cytoplasmic mRNAs that pass quality control are modified with 5'-caps (m^7G) and 3'-poly(A) tails that are major determinants of mRNA stability. The eukaryotic decay machinery relies on ribonucleolytic activities that remove/hydrolyze the 5′-cap structure (decapping activity) and shorten or remove the poly(A) tail (deadenylation activity) to allow 5′-3′- or 3′-5′-exonucleolytic mRNA degradation, respectively [12].

Typically, bulk mRNA decay is initiated by deadenylation [13], which is performed by one of three deadenylases that reside in multi-subunit complexes containing regulatory proteins [14]. These deadenylases include the PAB-specific ribonuclease 2 and 3 (PAN2/3) complex, the CAF1/CCR4/NOT complex and a homodimer of poly(A) ribonuclease (PARN). The CAF1/CCR4/NOT complex often cooperates with PAN2/PAN3 or specific RBPs bound to selected mRNAs to remove poly(A) tails [15]. By removing the poly(A) tail from targeted transcripts, these deadenylase complexes also displace polyadenylate-binding protein (PABP), which is a major mRNA stabilization factor [12]. Following deadenylation, the decapping enzymes that remove m^7G from the 5′-ends of mRNA are activated (Figure 1). A decapping complex consisting of DCP1A and DCP2 decapping enzymes in association with the regulatory factors EDC3 (enhancer of decapping-3) and Hedls (also referred to as Ge-1 and Edc4) are responsible for hydrolysis of the cap structure [16]. Under steady-state conditions, the cap is protected from decapping by bound cap-binding eIF4F complex (consisting of translation initiation factors eIF4E, eIF4A, and eIF4G). The eIF4G scaffold protein communicates with the poly(A) tail through direct binding to PABP molecules, and deadenylation disrupts this communication (Figure 1). Following deadenylation/decapping, transcripts are degraded by the 5′-3′-exoribonuclease XRN1 or by exosome-associated 3′-5′-exoribonucleases RRP44 (hDIS3) or PM-SCL75 (RRP45, EXOSC10). In some cases, decay

of specific mRNAs is initiated by endoribonucleolytic cleavage within its body followed by degradation in both 5′-3′ and 3′-5′ directions [17].

Multiple other RBPs regulate recruitment of mRNA deadenylases/decapping enzymes in a transcript-specific manner. These RBPs bind to *cis*-elements found in the untranslated regions of selected mRNAs. A common example is the so-called ARE element (adenine and uridine-rich element) found in 3′-UTRs (Untranslated Regions) of short-lived RNA molecules [6]. AREs recruit numerous RBPs (ARE-BPs) that determine the stability of target transcripts. To date, more than 20 different ARE-RBPs have been identified. We will discuss ARE-BPs in the context of their association with RNA granules and possible roles in carcinogenesis.

Under steady-state conditions, recruitment of translation initiation complexes to the newly-exported mRNP promotes protein synthesis and the assembly of polysomes. The polysome-translated mRNA is circularized by interactions between eIF4G, a component of the cap-binding eIF4F complex, and 3′-poly(A) tail-bound PABP. This closed-loop configuration not only facilitates efficient translation by promoting the recycling of terminating ribosomes to the initiation complex, but also protects mRNA from degradation and ensures that only intact mRNAs are translated. Correspondingly, early views on the relationship between mRNA translation and mRNA decay were very simple: when mRNA is not translated, it is degraded. More recent data challenges this view and suggests a more sophisticated model in which RNA granules play a central role in regulating the assembly/disassembly of translating polysomes and the stability of specific mRNA transcripts (see Figure 1).

BASICS OF STRESS GRANULES AND PROCESSING BODIES

Although there are many types of RNA granules (e.g. Stress Granules (SGs), P-bodies (PBs), germ granules, neuronal granules, nuclear paraspeckles) [18], SGs and PBs are the most well understood and are closely associated with a variety of diseases including cancer. The classification of RNA granules is based on their composition (the presence of specific markers), subcellular localization (nuclear, cytoplasmic, axonal etc.), cell of origin (germ cells, neurons), response to stimuli (stress, viral infections), dynamic behavior and proposed functions (sites of mRNA storage/decay, stress response etc.) [9, 18].

PBs are cytoplasmic RNA granules that are enriched in the factors involved in mRNA degradation, mRNA surveillance, translational repression and RNA-mediated gene silencing. Initially they were discovered as "XRN1 foci" due to the presence of the exoribonuclease XRN1 in small granular structures within the cytoplasm of mammalian cells [19]. Later, other components of the RNA decay machinery such as decapping enzymes DCP1/DCP2 [20, 21] and decapping activators Hedls [22, 23], Edc3 [22], Pat1 [24, 25], LSm1–7 [20, 26, 27] and RCK (p54, DDX6) [28] were shown to co-localize with XRN1 foci. Further studies demonstrated that PBs are sites at which the key enzymes of cytoplasmic RNA degradation are concentrated (deadenylase complex CCR4/CAF1/NOT [29, 30] and its enhancer TOB2 [31], nonsense-mediated mRNA decay proteins UPF1, UPF2, UPF3, SMG5, SMG6 and SMG7 [32–34], ARE-mediated decay factors TTP, BRF1 and BRF2 [26, 35, 36]) and the RNAi machinery (GW182 [37], Argonautes [38, 39]). In

addition, PBs contain translation regulation-associated factors such as eIF4E [40], eIF4E-T [41], CPEB [28], PCBP2 [42] and FASTK [26]. This composition determines the type of mRNAs deposited in PBs: these mRNAs seem to be primed for degradation (e.g. they lack poly(A) tails) [30]. However, although initially proposed to be sites for mRNA translational repression, storage or decay, the physical integrity of PBs is not required for global or specialized decay pathways [24, 27]. Many PB proteins exchange rapidly with the cytoplasmic pool and are not stable PB components whereas others (e.g. Dcp2) are very stable PB components [26, 40, 43, 44]. Decay enzymes are also present on polysomes, and transcript degradation can take place while mRNAs are associated with actively translating ribosomes [45, 46]. PBs are not sites for storage of selected mRNA degradation factors (such as Argonautes) because less than 10% of the cytoplasmic pool of these factors is concentrated at PBs [43, 44]. However, this does not rule out the possibility that PBs are storage sites for selected mRNAs [47] or protein factors [43]. It is also possible that PBs are sites of recycling/modification of decay factors.

SGs are cytoplasmic foci wherein translation initiation factors, 40S ribosomal subunits and diverse RBPs translationally-stalled mRNAs coalesce into discrete granules which recruit various signaling molecules [48–50]. SGs are non-membranous structures formed in response to different stress stimuli, typically through the stress-induced phosphorylation of eukaryotic initiator factor (eIF) 2 (eIF2) [51] (Figure 1). This results from activation of one or more stress-sensing serine/threonine kinases that phosphorylate serine residue 51 of the alpha subunit of eIF2 (eIF2α) [52], resulting in impaired translational initiation and consequent polysome disassembly (Figure 1). eIF2 is a component of the translation-competent eIF2/tRNA$_i^{Met}$/GTP ternary complex, an early initiation factor that mediates binding of initiator tRNA$_i^{Met}$ to the 40S ribosomal subunit in a GTP-dependent manner [53]. To initiate mRNA translation, this ternary complex interacts not only with 40S ribosomal subunits but with a number of core translation initiation factors (such as multi-subunit eIF3, eIF4F complex, eIF5, eIF5B, eIF1, eIF1A) to form the 48S pre-initiation complex on the AUG start codon [54]. Productive assembly of the 48S complex on the start codon is followed by the joining of the large 60S ribosomal subunit to form a translation elongation-competent 80S ribosome. When eIF2α is phosphorylated, GDP-GTP exchange is inhibited, thus dramatically decreasing the levels of the active ternary complex, leading to the inhibition of translation initiation (reviewed in [53]). At the same time, protein synthesis is reprogrammed by the preferred translation of select mRNAs, including transcripts with upstream Open Reading Frames (uORFs) in the 5′ UTR. Such transcripts typically encode stress response proteins such as the transcription factor ATF4, to activate other pathways to activate long-term survival pathways [52].

Phosphorylation of eIF2α also leads to the assembly of SGs. Because translation elongation is not affected by eIF2α phosphorylation, ribosomes already engaged in translation "run-off" polysomes, converting them into non-canonical, translationally-stalled (so called 48S* complexes) pre-initiation complexes that lack selected initiation factors (such as eIF2 and eIF5) [55]. These 48S* complexes are the core constituents of SGs, but still require a subset of mRNA-associated RBPs to mediate SG assembly (Figure 1). The best characterized of these are TIA1 (T-cell internal antigen 1 [56]) and G3BP1 (Ras-GTPase-Activating Protein SH3-Domain-Binding Protein 1 [57]); these proteins contain aggregation-prone domains

that mediate the coalescence of 48S* complexes into SGs [58]. Over-expression of G3BP1 or TIA1 is sufficient to drive ("nucleate") SG formation even in the absence of stress, whereas siRNA-mediated knockdown of transcripts encoding these proteins significantly impairs with SG assembly despite phosphorylation of eIF2α and polysome disassembly. Moreover, multiple post-transcriptional modifications of various SG components contribute to SG assembly and disassembly (e.g. phosphorylation of G3BP1 at serine 149 impairs its ability to nucleate SGs [57]).

A defining feature of SGs is their dynamic nature: SGs are quickly assembled under stress and rapidly disperse after the stress is removed. The effects of pharmacological agents that either disassemble polysomes (puromycin) or freeze ribosomes on mRNA transcripts to stabilize polysomes (cycloheximide) reveal that SGs are in a dynamic equilibrium with polysomes [56]. Fluorescence recovery after photobleaching (FRAP) studies directly show that mRNPs within SGs rapidly shuttle in and out of SGs [26, 44, 56, 59]. SGs also recruit multiple enzymes and signaling molecules including kinases and phosphatases, scaffolding and adaptor proteins, ubiquitin-modifying enzymes, RNA helicases, ribonucleases, ribosyl-, glucosyl- and methyl-transferases. As signaling centers, SGs transiently alter multiple signaling pathways to ensure cell adaptation to stress (reviewed in [50]).

Despite their many differences, SGs and PBs share some protein and RNA components and are often observed in the close proximity to one another [26]. Like SGs, PBs can be induced by stress conditions and contain translationally-stalled mRNAs that can resume translation and be converted into polysomes [29, 56, 60]. Several proteins found in both PBs and SGs are known to regulate translation or stability of transcripts and include the cap-binding factor eIF4E [26, 40], the RNA helicase RCK/p54 (DDX6) [28], RNAi-associated Argonaute proteins [39, 44, 61, 62], the ARE-BPs TTP, BRF1 [26] and HuR [63], the translational silencer YB-1 (Y-box binding protein 1) [64], and CPEB [28]. Some proteins such as TTP and BRF1 promote docking between SGs and PBs which may allow mRNPs to move from one granule to the other [26, 35, 65] (Figure 1). Although SGs and PBs can fuse, each can also form independently of the other [26, 66, 67]. For more detailed overview of RNA granules, their components and functions in different organisms we refer the readers to recent reviews [9, 48, 50, 68–70].

SURVIVING THE HOSTILE CANCER MICROENVIRONMENT: ROLE OF RNA GRANULES

RNA granules contain hundreds of different molecules. Their direct roles in cancer metabolism, regulation of gene expression in cancer cells, and cancer cell adaptation to tumor microenvironments are yet to be uncovered. Here, we discuss some examples of PB- and SG-associated proteins that have been implicated in cancer cell physiology.

Stress responses and Cancer Microenvironment

The integrated stress response (ISR) is a program that receives information from different "stress sensors" and coordinates cellular adaptation to stress [71, 72]. Among these stress sensors are serine/threonine kinases that phosphorylate eIF2α [73]. They include GCN2

(which senses amino acid levels and is activated by amino acid deprivation [74]), HRI (which monitors oxidative stress/ROS levels and activated by heme deprivation in erythroid cells [75]), PERK (senses endoplasmic reticulum (ER) stress [76, 77]) and PKR (activated in response to UV exposure, viral infections and heat shock [78]). As eIF2α phosphorylation is the main trigger of SG formation [51], SG formation is part of the ISR that promotes adaptation and survival during changing local environmental conditions.

Responding and adapting to stress is important in both cancer development and the tumor response to anticancer therapies (Figure 2). During solid tumor development, cancers can quickly outgrow the existing vasculature, as high metabolic demands of rapidly proliferating cancer cells can out-strip the availability of oxygen and nutrients. This exposes the cancer cells to hypoxia, hyperosmolarity, and nutrient starvation [79]. Although tumors counter this by stimulating neovascularization (formation of new blood vessels), the new blood vessels are often leaky and disorganized. Moreover, the high proliferative rate and dysregulation of protein synthesis in cancer cells cause endoplasmic reticulum (ER) stress, a consequence of protein misfolding and overload in ER due to imbalanced protein synthesis and improper protein folding within secretory pathways [80]. All these condition create a hostile microenvironment that affects various aspects of tumor growth and metabolism (Figure 2).

Cancer cells adapt to this environment by uncoupling glycolysis from oxidative phosphorylation, a process facilitated by HIF1alpha (reviewed in [81]), which results in a high level of oxidative stress resulting from production of reactive oxygen species (ROS) and requires increased anti-oxidant capacity (reviewed in [82]). The altered glycolytic metabolism of cancer cells [81] is characterized by high mitochondrial membrane potential, elevated leakage of electrons, and production of O_2^- superoxide, which mitochondrial and cytoplasmic superoxide dismutases (SODs) metabolize into the longer-lived metabolite hydrogen peroxide, which acts as second messenger to activate ROS scavenging pathways (reviewed in [83]). Chronically elevated ROS depletes cellular anti-oxidants (reduced NADH/NADPH, peroxiredoxin, and glutathione), altering redox-dependent cell signaling [84] and activating several antioxidant defense pathways, including NRF2/KEAP1, HIF1, HSF and p53/myc (reviewed in [82]). The term "oxidative stress" is often equated with production of "reactive oxygen species" or ROS, but these are not precisely identical. ROS are small reactive molecules including superoxide, hydroxyl free radicals, and hydrogen peroxide (H2O2) that result from leaky or malfunctioning mitochondria or are produced enzymatically. ROS levels are balanced by antioxidants and ROS scavengers; when ROS production exceeds the antioxidant capacity, stress ensues.

The direct stress sensors of redox imbalance are largely cysteine residues in regulatory proteins such as KEAP1/NRF2 and HSF1. Arsenicals directly bind to reduced cysteines in proteins, and inactivate perhaps 200 enzymes, including key anti-oxidant enzymes (glutathione reductase, glutathione peroxidase, thioredoxin reductase and peroxidase), signaling kinases and phosphatases, key metabolic enzymes (pyruvate dehydrogenase) and signaling enzymes (reviewed in [85]). Increased ROS levels associated with arsenite treatment may be produced by cellular metabolism (mitochondrial electron transport; NAD redox reactions, etc) but accumulate because arsenite inactivates the protective antioxidant systems that would normally conteract the imbalance caused by ROS. Sodium arsenite was

first chemical found to potently induce SGs [51], and remains the most effective and consistent agent used to induce SG assembly. Induction of SGs by sodium arsenite [86] or hypoxia [87] can promote resistance to apoptosis. Although poorly understood, this may require both short-term suppression of apoptosis, and long-term adaption mediated by transcription factors such as NRF2 and HIF1alpha. SG-mediated, short-term responses may involve sequestration/inactivation of proapoptotic factors (such as RACK1 and TRAF2) in SGs [87, 88] or sequestration of mRNAs encoding pro-apoptotic proteins [89]. Treatment of diverse cancer cell lines with oxidative stress agents including sodium arsenite [56], H2O2 [90], or selenite [91] induces different types of SGs. Sodium arsenite activates the eIF2α kinase HRI which phosphorylates eIF2alpha, which is necessary and sufficient to induce SGs under normal culture conditions [56]. In contrast, although H2O2 also induces eIF2α phosphorylation, it triggers SGs through an eIF2a-independent pathway but requires eIF4E [90]. Selenite induces SG assembly through a combination of mechanisms, but the SGs it induces appear to lack several pro-survival molecules found in arsenite-induced SGs ([91] and see below).

The connection between SGs, ROS and redox imbalance is complex. A recent study [92] suggested that the balance between G3BP1 and one of its binding partners Ubiquitin-specific protease 10 (USP10) coordinately regulates ROS levels and inhibits apoptosis when both proteins are colocalized in SGs (induced by arsenite stress), but not in response to hydrogen peroxide, which did not induce SGs under the conditions they employed. This study did not determine what type of "arsenite-induced" ROS was produced [92], and did not consider differences between arsenite and H2O2 (reviewed in [85]), so this study is difficult to evaluate. An important earlier study clearly demonstrated that the balance between NAD(P)H and nonoxidized glutathione during stress conditions regulated both arsenite and G3BP1-induced SG formation [93], suggesting that redox stress rather than ROS is key to G3BP-mediated SG regulation. Another role for G3BP1 in redox regulation may involve its translational repression of the catalytic beta-subunit of mitochondrial H+-ATP synthase [94], suggesting that G3BP1-mediated translational repression is mediated by SGs, and that this repression may be relieved by release of the mRNA from the granule to sites proximal to the mitochondria [94]. G3BP1/USP10 are linked to autophagy [95], and other studies link each molecule independently to cancer: G3BP1 is important in cancer cell proliferation, migration and resistance chemotherapy [96], whereas USP10 stabilizes p53 [95] and SIRT6 [97] to suppress tumor formation.

SG formation and chemotherapy drugs

Several studies have linked the survival of cancer cells to the formation of SGs in response to chemotherapeutic treatments. Bortezomib (PS-341/Velcade), an FDA-approved drug that targets the 26S proteasome, displays significant anti-tumor activity against mantle cell myeloma and multiple myeloma. In contrast, many types of solid tumors are refractory to bortezomib treatment and resistant to bortezomib-induced apoptosis. Fournier et al. showed that bortezomib potently induces the formation of SGs in colon cancer (Caco), cervical cancer (HeLa) and lung cancer (Calu-I) cell lines [98]. Similarly to MG132 (another proteasome inhibitor used in chemotherapy) [99], bortezomib triggers phosphorylation of eIF2α promoting SG formation [98]. Interestingly, these drugs activate different eIF2α

kinases: MG132 activates GCN2 [100] while bortezomib activates HRI [98]. Upon HRI depletion, bortezomib fails to trigger SG assembly and cancer cells undergo efficient apoptosis correlating with the lack of pro-survival anti-apoptotic SGs. In addition, CUGBP1 (CUG triplet repeat RNA-binding protein 1) mediates recruitment of p21 (WAF1/CIP1) mRNA into SGs upon proteasome inhibition by MG132 or bortezomib [101]. p21 is a stress-responsive cyclin-dependent kinase inhibitor with tumor suppression functions that rely on cell cycle arrest or apoptosis induction. CUGBP1-mediated localization of p21 mRNA into SGs stabilizes this mRNA and promotes resistance to bortezomib-induced apoptosis. Conversely, knocking down CUGBP1 promotes bortezomib-induced apoptosis [101].

Interestingly, another chemotherapeutic agent, the antimetabolite 5-Fluorouracil (5-FU), also induces the assembly of SGs [102]. 5-FU is used for treatment of various types of cancers including breast, colorectal, head and neck cancers [103]. 5-FU cytotoxicity is caused by interference with enzymatic activities of thymidylate synthase and by its incorporation into DNA and RNA intermediates. 5-FU incorporation into RNA (5-FU-containing RNA metabolites) causes activation of PKR kinase [104], phosphorylation of eIF2α [104] and assembly of SGs that sequester RACK1, an important regulator of apoptosis [102]. RACK1-positive SGs are prosurvival and anti-apoptotic (see below). Other chemotherapeutic agents known to incorporate into RNA (such as 6-thioguanine and 5-azacytidine) also trigger assembly of SGs [102]. In contrast, DNA-incorporating chemotherapeutic metabolites (such as trifluorothymidine and gemcitabine) do not trigger SG formation but, surprisingly, potently increase the number of PBs in cancer cells [102]. The biological significance of these observations is not clear, although recently PBs have been implicated in the cellular response to hypoxia. Under hypoxic conditions, p54/Rck (a component of both PBs and SGs) regulates mRNA translation of the hypoxia inducible factor HIF-1α, a major regulator of cellular response to oxygen fluctuation [105]. SGs play a role in the resistance of cancer cells to radiation therapy and hypoxia by the regulation of HIF-1α and HIF-1α-regulated transcripts [89]; whether p54/Rck association with PBs/SGs is required for such regulation is to be determined.

Sodium selenite, a selenium-containing chemotherapeutic agent, promotes ROS production and selective apoptosis of cancer cells. It triggers ER stress resulting in phosphorylation of eIF2α and ROS-dependent formation of SGs in prostate cancer (DU145) and osteosarcoma (U2OS) cell lines [91]. Selenite-induced SGs lack translation initiation factor eIF3b, RACK1 and importin α1/β1, components of "canonical" sodium arsenite-induced SGs. The phosphorylation of eIF2α is dispensable for selenite-induced assembly of SGs; instead selenite relies on the action of eIF4E-binding protein 1 (4EBP1) for SG assembly and 4EBP1 depletion compromises its ability to assemble SGs. In response to selenite treatment, 4EBP1 disrupts the assembly of translationally-competent eIF4F complexes, resulting in the assembly of SGs. Importantly, selenite-induced SGs are functionally distinct from canonical SGs in that they promote cell death instead of cell survival in response to stress. This difference is at least partially attributed to the 4EBP1-mediated contribution to the cytotoxicity of selenite [91]. All these data suggest that SGs might be a part of the program by which cancer cells resist chemotherapy treatments and provide a basis for potential targeting of SGs in cancer.

Phosphorylation of eIF2α (p-eIF2α) is a key trigger of SG assembly and ISR, but p-eIF2α independent pathways of SG assembly also exist. Synthetic and natural compounds that induce SG assembly without eIF2α phosphorylation have been described, some of which are particularly relevant to cancer biology. One of them is 15-deoxy- (12, 14)-prostaglandin J2 (15d-PGJ2), a natural lipid inflammatory mediator belonging to a class of prostaglandins with diverse roles in cancer and inflammatory diseases. 15d-PGJ2 binds to the helicase eIF4A and inhibits eIF4A/eIF4G interactions, resulting in translation inhibition and assembly of SGs [106]. In leukemia and colorectal cancer cells, 15d-PGJ2 induces apoptosis and proliferative arrest [107]. The role of SGs in 15d-PGJ2-mediated apoptosis is proposed to be a consequence of global translational arrest, but the exact molecular details are lacking. Further studies of pro-apoptotic and anti-proliferative properties of 15d-PGJ2 are required to determine whether this compound is of a potential use for anti-cancer therapy.

At least three other drugs that target helicase eIF4A and induce SGs show promise as anti-cancer therapeutics: Pateamine A (PatA) [108, 109], hippuristanol [110] and silvestrol [111]. PatA is a metabolite isolated from the sea sponge *Mycale specious* that binds eIF4A [108, 109], stimulates its helicase and ATPase activities leading to the disruption of eIF4A/eIF4G interaction [108, 109, 112], reduction of translationally-competent eIF4F complex levels and assembly of SGs [100, 113]. PatA and its synthetic derivatives show potent anti-proliferative and proapoptotic activities in many tumor cell lines *in vitro* [109, 114, 115] and on melanoma xenograft models *in vivo* [115]. Silvestrol, a compound belonging to the class of rocaglates and isolated from plant *Aglaia foveolata*, stimulates the RNA binding and helicase activities of eIF4A leading to the sequestration of eIF4A from the eIF4F complex [111, 116] and thus inducing SG assembly [117] in a p-eIF2a-independent manner, similar to PatA [108, 109, 112]. Silvestrol is a potent inducer of apoptosis, effective inhibitor of cancer cell proliferation *in vitro*, and effective suppressor of tumor growth in xenograft models of breast, prostate and hepatocellular cancers [111, 117, 118]. Hippuristanol, a steroid isolated from coral *Isis hippuris*, is an allosteric inhibitor of eIF4A that reduces RNA binding, ATPase and helicase activities [110, 119] and thus promotes formation of SGs [100]. Hippuristanol induces cell cycle arrest and apoptosis in adult T-cell leukemia (ATL) cells and shows synergistic effects with other anti-tumor agents against diverse human leukemia and lymphoma cell lines and in the Eu-Myc lymphoma mouse model [120, 121]. Although all three eIF4A-targeting agents are promising anti-cancer drugs, the mechanisms underlying their anti-proliferative effects are still elusive. eIF4A is required for the translation of mRNAs with highly structure 5'-UTRs, which encode many proteins involved in carcinogenesis, therefore its inactivation and consequent SG formation may regulate the translation/ subcellular localization of such transcripts. Future studies relying on genome-wide approaches such as ribosome and/or polysome profiling correlated with subcellular localization of selected mRNA targets could dissect mechanisms of SG-inducing chemotherapy agents in cancer.

mTOR, SGs and cancer

SGs may regulate the activity and localization of the mammalian target of rapamycin (mTOR) kinase, a key regulator of cell metabolism and growth, which is widely implicated in cancer [122]. mTOR regulates cell growth and mRNA translation in response to insulin

signaling, growth factors and amino acid availability. mTOR is the catalytic subunit of two structurally distinct complexes associated with different subcellular compartments: mTORC1 (composed of mTOR, regulatory-associated protein of mTOR (Raptor), mammalian lethal with SEC13 protein 8 (MLST8) and associated non-core components PRAS40 and DEPTOR) and mTORC2 (composed of mTOR, rapamycin-insensitive companion of mTOR (Rictor), MLST8 and mammalian stress-activated protein kinase interacting protein 1 (mSIN1)). mTORC2 is an important regulator of the cytoskeleton, while mTORC1 constitutes an energy/nutrient/redox sensor coupling metabolism to protein synthesis through the regulation of phosphorylation of eIF4E-binding proteins (4EBPs), the key regulators of eIF4E functions (reviewed in [123, 124]).

In human cells, osmotic or oxidative stress promotes the recruitment of mTOR and its mTORC1-specific partner Raptor to SGs; their targeting to SGs is controlled by DYRK3 (dual specificity tyrosine-phosphorylation regulated kinase 3) [125]. Oxidative stress affects mTORC1 activity via both inhibitory and stimulatory inputs. While redox-induced hyperactivation of mTORC1 is mediated by the inhibition of the upstream tuberous sclerosis complex 1/2 (TSC1-TSC2) [126], redox-induced inhibition of mTORC1 is mediated by astrin, a binding partner of Raptor [127]. In response to redox and metabolic stresses, astrin inhibits Raptor:mTOR association by binding and sequestering Raptor in SGs, thus inhibiting mTORC1 assembly and activation [127]. Consequently, SG-mediated regulation of mTORC1 activity leads to changes in cellular translation by regulating eIF4F complex assembly. In addition, mTOR-regulated recruitment of TIA1/TIAR proteins into SGs selectively modulates translation of mRNAs with 5'-terminal oligopyrimidine tracts (5'TOP) [49, 128]. Specifically, mTORC1 cooperates with GCN2 kinase to regulate translation of 5'TOP mRNAs under conditions of nutrient starvation and stress [128]. This subclass of mRNAs predominantly encodes protein biosynthesis factors such as translation factors and ribosomal proteins [129]. As cancer cells have elevated needs for protein synthesis, the interplay between SGs, mTOR activity/localization and translational control of 5′ TOP mRNAs in the stressful tumor microenvironment is of vital importance for cancer cell metabolism.

SG-associated RBPs and regulation of their target cancer-relevant mRNAs

The cap-binding protein eIF4E is a component of the eIF4F complex that integrates information from major receptor signaling pathways and orchestrates cell proliferation and cell death/survival. eIF4E expression and activity are elevated in ~30% of all cancers, and its overexpression is correlated with poor prognosis, especially in hematological malignancies. In fact, eIF4E is an oncogene itself, and even modest over-expression of eIF4E enhances cancer cell proliferation and resistance to cell death (reviewed in [130]). While eIF4E is a general regulator of cap-dependent translation, a specific subset of mRNAs is especially sensitive to the levels of this factor [52, 131]. These eIF4E–sensitive transcripts encode cancer-related protein kinases, transcription factors, cell cycle and apoptosis regulators, growth factors and cytokines. Some proteins that activate eIF4E function (e.g., Akt, Myc and Ras) also increase the transcription of eIF4E (e.g., c-Myc). Importantly, eIF4E cooperates with c-Myc to promote cancer progression by inhibiting apoptosis [132, 133]. While localization of eIF4E into SGs and PBs is well documented, it remains to be

determined whether eIF4E-sensitive mRNA targets are preferentially recruited to these granules in response to extra- and intra-cellular stimuli.

RACK1, another SG component, is a highly conserved intracellular adaptor protein belonging to the Trp-Asp (WD) repeat protein family. It is a scaffolding protein that interacts with a vast array of signaling molecules including kinases and phosphatases, membrane receptors, G proteins, ion channels and apoptosis-related molecules [134]. RACK1 is a component of the 40S ribosomal subunit, binds eIF3, and regulates mRNA translation and quality control [135]. Up-regulation of RACK1 mRNA and protein expression is observed in many cancers (lung, gastric, breast cancers, gliomas) [136]. It is proposed to both suppress and promote cancer cell proliferation via its interactions with different partners that influence various signaling pathways. In one case, RACK1 interacts with the pro-apoptotic proteins Fem1b and BIM to inhibit cancer cell apoptosis. Sequestration of RACK1 into SGs has a negative impact on the stress-activated p38 and JNK (c-Jun N-terminal kinase)/MAPK pathways causing inhibition of apoptosis. However, RACK1 also can exert pro-apoptotic effects by interacting with apoptosis-related proteins such as BAX, a member of BCl-2 family [136]. The extent to which RACK1 interactions with pro- and anti-apoptotic molecules is regulated by stress-induced RACK1 localization into SGs, and whether RACK1 binding to 40S subunits and/or eIF3 is required for its targeting to SGs is presently unknown.

HuR (ELAV1) and TTP (Tristetraprolin) are among the best-characterized ARE-BPs with multiple functions in cancer. Both proteins are components of SGs, but exhibit opposite effects on their target mRNAs: while HuR generally stabilizes transcripts and regulates their translation, TTP actively promotes degradation of mRNA targets. The shared targets TTP and HuR include mRNAs encoding inflammatory cytokines, cell growth factors, angiogenesis-, apoptosis- and differentiation-related factors. Surveys of TTP expression in cancer cell lines and human tumors suggest that TTP functions as tumor suppressor [137], and its levels negatively correlate with tumor progression in breast and prostate cancer [138]. In a v-H-*ras*-dependent mast cell tumor model, where tumor development *in vivo* is dependent on the induction of autocrine IL-3 secretion, over-expression of TTP reduces levels of IL-3 mRNA and suppresses tumor growth [139]. In epithelial EpRas cells, knock down of TTP promotes epithelial-to-mesenchymal transition (EMT) and metastasis [140]. A microRNA frequently deregulated in hematological, cholangiocytic and lung cancers (miR-29a) dampens expression of TTP. Consequently, over-expression of miR-29a in EpRas cells leads to suppression of TTP levels followed by disruption of epithelial polarity, EMT and metastasis [140]. Similarly, over-expressed TTP impairs Myc-induced lymphomagenesis and abolishes the transformed state by disabling Myc-mediated proliferative responses [141]. While down-regulation of TTP is observed in tumors of different origin (e.g., cancers of the breast, colon, esophagus, kidney, lung, pancreas, prostate, skin, stomach, and thyroid) up-regulation of HuR is often observed in cancer [142]. In pancreatic and colon cancers, high HuR levels correlate with high levels of VEGF, potent angiogenic factor, and poor prognosis. In mouse xenograft models, over-expression of HuR in carcinoma cells lead to the development of larger tumors, while HuR depletion reduces

the tumor size [142–144]. While SGs regulate TTP/HuR localization and activities, the importance of these relationships to cancer development is still unclear.

YB-1 and LIN28 (LIN28A) are multifunctional RBPs belonging to the family of cold shock domain (CSD) proteins [145]. Both factors shuttle between the nucleus and cytoplasm and localize to SGs and/or PBs under stress. Mutations in the CSD of these proteins affect RNA binding and localization into SGs/PBs [64, 146]. While YB-1 is highly expressed in both adult and developing tissues, expression of LIN28 is highest in embryonic tissues and stem cells. Both YB-1 and LIN28 are overexpressed in a wide range of solid tumors and hematological malignancies (reviewed in [147, 148]). Although these proteins are closely related, their functions seem to be non-overlapping. LIN28 regulates gene expression by two mechanisms: 1) blocking the processing of the *let-7* family of microRNAs, and 2) enhanced translation of mRNAs encoding metabolic enzymes, splicing factors and cyclins (reviewed in [149]). YB-1 efficiently stabilizes its mRNA targets and can either inhibit or stimulate their translation [147]. In cancer, LIN28-mediated down-regulation of *let-7* microRNAs, which have tumor suppressor activity, leads to up-regulation of *let-7* mRNA targets that encode pro-proliferative, oncogenic and anti-apoptotic factors. Lin28 overexpression is sufficient to transform NIH/3T3 cells while depletion of Lin28 in human leukemia cells increases *let-7* levels and reduces cancer cell proliferation [150]. The role of YB-1 in cancer is even more complex. YB-1 inhibits translation of mRNAs associated with control of cellular proliferation and the epithelial phenotype but simultaneously facilitates activation of a mesenchymal gene expression program. YB-1-mediated EMT is associated with preferential translation of mRNAs encoding EMT-inducing proteins such as *Snail1, Twist* and others [151]. Overall this results in the paradoxical down-regulation of cancer cell proliferation and simultaneous increased mobility and invasiveness as well as survival in anchorage-independent conditions and metastasis.

Both angiogenin (ANG) and its inhibitor RNH1 (also known as ribonuclease inhibitor, RI) are components of SGs [152] and have well-characterized roles in cancer and angiogenesis (reviewed in [153]). ANG is a secreted ribonuclease (RNase) with angiogenic properties that is over-expressed in many cancers. It stimulates cancer cell proliferation by enhancing ribosomal RNA (rRNA) transcription and promotes cancer cell survival during stress [154]. Several stressors induce ANG translocation from the nucleus into the cytoplasm, where it cleaves transfer RNAs (tRNAs) in the anticodon loop to produce tRNA-derived stress-induced RNA (tiRNAs) [155, 156]. Selected tiRNAs inhibit mRNA translation by interfering with eIF4F complex functions, and promoting the assembly of pro-survival SGs in an eIF2α phosphorylation-independent manner that requires YB-1 [157–159]. The RNase activity of ANG is absolutely required for ANG-mediated angiogenesis, enhanced survival and proliferation [153]. RNH1 binds ANG and inhibits tRNA cleavage, rRNA transcription, and cell migration, but promotes apoptosis. Down-regulation of RNH1 promotes tumor growth and cancer cell proliferation, migration and metastasis (bladder, prostate and hematological cancers, melanoma) while up-regulation suppresses melanoma growth and metastasis [160–162]. While molecular details of RNH1-dependent suppression are still unclear, available data suggest that RNH1 regulates transcription and translation of EMT-associated transcription factors Twist, Snail, Slug and ZEB1 [161].

Several other SG- and/or PB-associated proteins can contribute to carcinogenesis. eIF5A, a hypusine-modified factor involved in various aspects of mRNA metabolism, promotes polysome disassembly and assembly of SGs [163]. The hypusine modification affects eIF5A-associated effects on polysomes and SGs [163] and is required for eIF5A-mediated promotion of tumor growth in pancreatic cancer [164]. The conserved RNA-dependent helicase Ded1/DDX3, which works both as an activator and repressor of translation, is a SG-associated protein [165, 166] with anti-apoptotic properties that promotes transformation by modulating hypoxic responses in breast cancer [167] or by regulation of *Rac1* mRNA translation and Rac1-mediated signaling in medulloblastomas [168]. Finally, Argonautes, key effectors of the RNAi pathway (reviewed in [169]), are components of both SGs and PBs that possess multiple cellular functions that are de-regulated in cancer cells. Their contribution to cancer initiation and progression via small RNA pathways is well documented and is a matter of several recent reviews [170–172].

We have described here only some RNA granule-associated proteins and their proposed roles in cancer. Note, however, that a number of studies trying to determine SG function(s) rely on deletion or mutation of one or more SG-associated proteins. Distinguishing between the functional effects of deletion/mutation of one or more SG-associated proteins on cell metabolism from the ancillary effects that these proteins exert on SGs (preventing/inducing) is a consistent problem in the field. Nonetheless, direct or indirect interactions between these and other proteins and apoptosis/signaling-related molecules suggest that the crosstalk between RNA granules and apoptotic machinery regulates the balance between cell survival and cell death. While the precise mechanistic details of crosstalk between RNA granules and apoptotic machineries are still elusive, the current evidence supports the notion that RNA granules could act as important signaling/regulatory centers exploited by cancer cells to allow their survival in hostile tumor microenvironments, as well as response to chemotherapy and radiotherapy.

CONCLUSIONS AND PERSPECTIVES

Over the past several years, substantial research has shown that RNA granules are not merely sites of mRNA storage or degradation. Instead, they are flexible and versatile regulators of gene expression, and act as signaling hubs to influence multiple aspects of cell metabolism central to carcinogenesis and metastasis. While the exact mechanisms by which RNA granules impact the development and progression of cancer are largely unknown, it is clear that the post-transcriptional mechanisms mediated by RNA granule-associated proteins and RNAs play major roles in adaptation to stress, cancer cell proliferation and tumor growth.

An intriguing aspect of RNA granule assembly, in particular SG assembly, is their responsiveness to chemotherapeutic drugs. As discussed above, some drugs promote the assembly of non-canonical SGs whose composition is different than that of classical SGs. Inclusion or exclusion of signaling molecules such as RACK1 or TRAF2 has profound effects on cancer cell viability, e.g. selenite-induced SGs promote cell death whereas 5-FU-induced SGs promote cell survival. Their assembly often relies on different signaling, e.g. selenite-induced SG assembly requires eIF4EBP1-mediated remodeling of the eIF4F

complex and is independent of eIF2α phosphorylation. A precise understanding of the mechanisms by which chemotherapeutic drugs induce SG assembly could identify new targets for cancer therapy.

Many questions remain, and a number of areas require further studies. Cancer cells within tumors are very heterogeneous. Do these tumor cell subpopulations assemble SGs in response to stress and/or chemotherapeutic drugs with different efficiency? Do cancer-associated mutations in key signaling molecules influence assembly of prosurvival or pro-apoptotic SGs? What are the key mRNA targets and proteins that are assembled into RNA granules in response to chemotherapy treatment? Developing a detailed spatiotemporal knowledge of the specific functions of SG subtypes, their composition and their regulation by different signaling molecules represent a major challenge, and a thorough understanding of the roles of RNA granules in cancer cell metabolism may identify the next important targets for cancer therapy.

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HIGHLIGHTS

Stress Granules and PBs house cancer-associated RNA-binding proteins

Many Stress Granule-associated proteins are aberrantly expressed in cancer

SG and PB-associated proteins alter gene expression and cancer initiation/progression

Chemotherapy drugs modulate assembly of Stress Granules

SGs and PBs are potential targets and biomarkers for cancer therapy
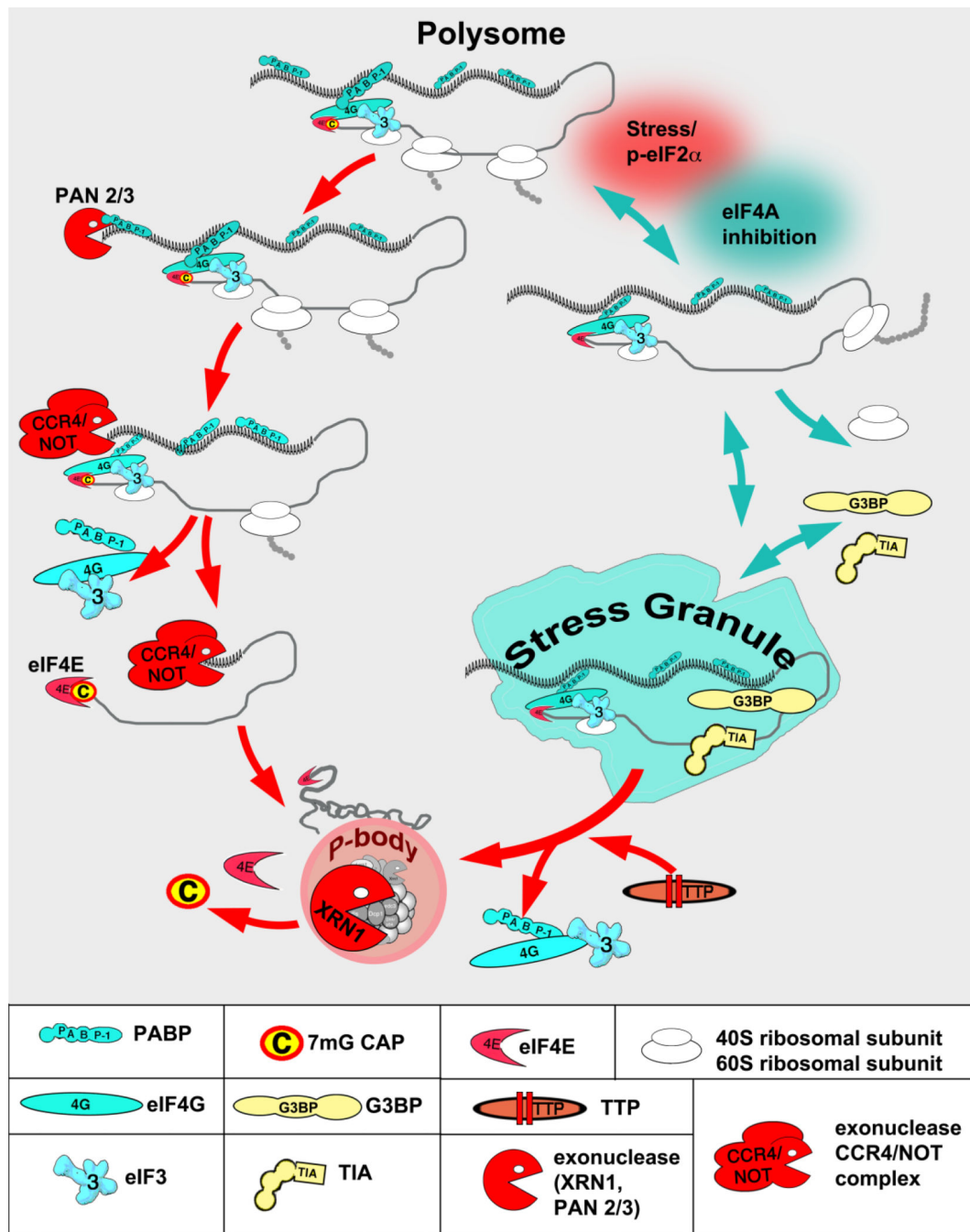
Figure 1. RBP-RNA granules interplay on the target mRNA

Under steady-state conditions, mRNA is actively engaged into translation as a part of polysome and in the form of translationally-competent mRNP consisting of several translation initiation factors (such as cap-binding complex eIF4F and multi-subunit eIF3) and mRNA-bound RBPs (such as poly(A)-bound PABP). In response to cell-intrinsic and extracellular changes mRNA translation is arrested and transcript can be routed from the polysomes to SGs (blue arrows) or PBs (red arrows). Transcripts destined for assembly into PBs are first deadenylated by one or more mRNA deadenylases (PAN2/3 and CCR4/NOT

are shown as examples). This causes the release of PABP molecules from the mRNA poly(A) tail, and the remodeling/decircularization of the mRNP which primes it for decapping. PB-associated decay enzymes subsequently may mediate mRNA decapping and decay within PBs. In contrast, transcripts assembled into SGs retain their poly(A) tails and associated PABP. Stresses, which may trigger phosphorylation of eIF2α (red shading) or not (such as inhibition of eIF4A, aqua shading), disrupt translational initiation of the mRNA, resulting in the runoff of ribosomes and the accumulation of stalled mRNPs. Binding of mRNAs by aggregation-prone RNA binding proteins (such as TIA-1, G3BP1) subsequently promotes its coalesce into SGs. Certain decay-promoting proteins (TTP, BRF1) can promote deadenylation and decay of SG-associated transcripts in tandem with promoting SG-PB fusion/interactions. Multiple additional proteins and signaling molecules associate with SGs or PBs once they are formed, depending on conditions.
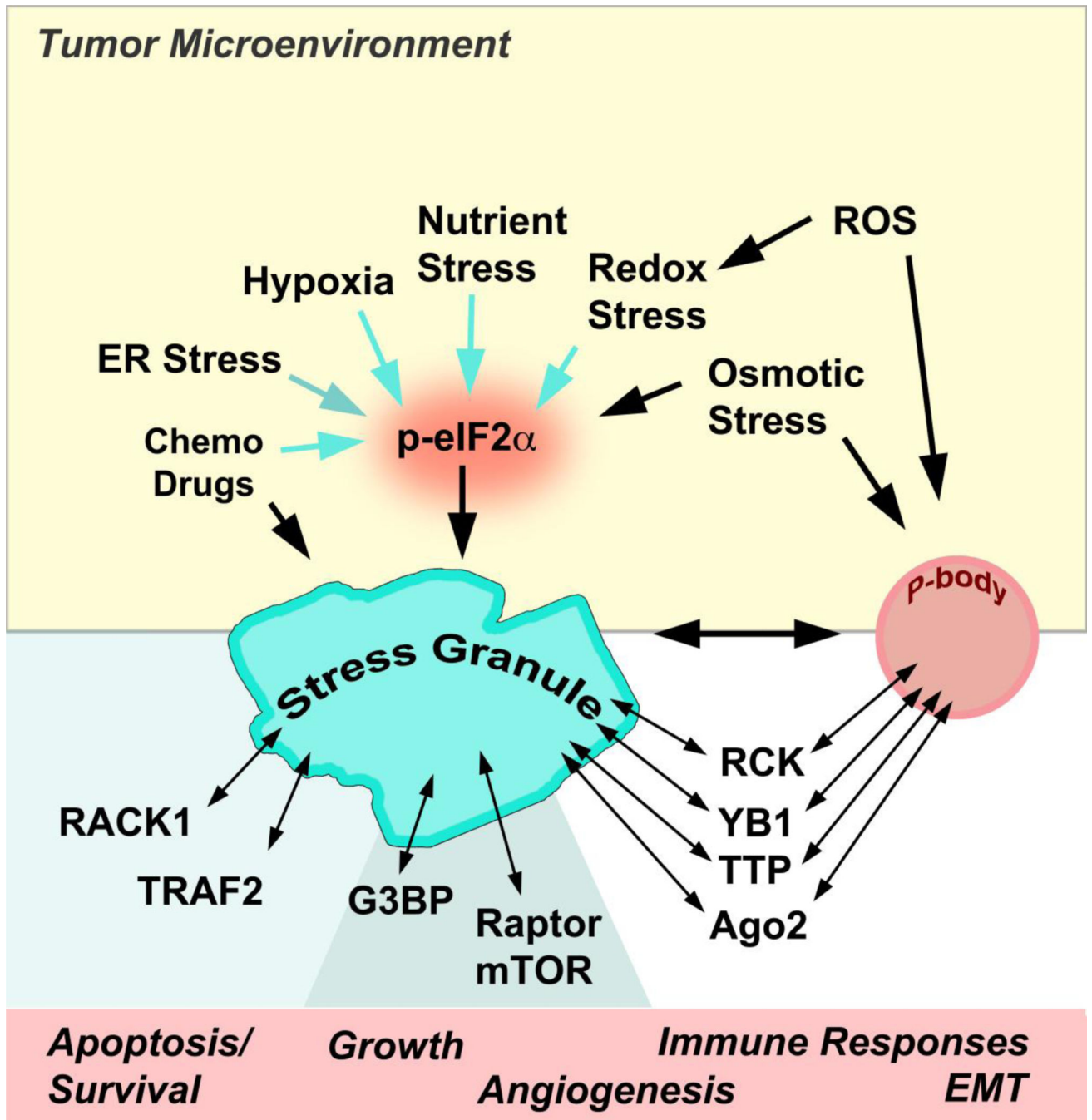
Figure 2. RNA granules and tumor microenvironment
Collective contributions from the different stress-associated conditions and surrounding
cellular environment result in the unique niche where cancer cells exist, known as the tumor
microenvironment (yellow). The tumor microenvironment changes dynamically during
tumor progression, which requires constant adaptation of cancer cells to the changing
intracellular and extracellular conditions. Hypoxia and nutrient starvation force cells to alter
their metabolism/cellular energetics ultimately causing chronic ROS production that has
multiple effects on cancer cell (patho)physiology. Chronic ER stress results from the

imbalance between increased protein synthesis due to the high demands on cancer cell proliferation and ER capacity often. These stresses, often augmented by chemotherapy, trigger formation of PBs/SGs, spatial manifestations of ISR (Integrative Stress Response) that orchestrates cellular adaptation to changing conditions. The integration and balanced management of these cancer-associated stresses through SGs and PBS modulate multiple physiological responses (bottom red panel) including activation of anti-apoptotic, pro-survival mechanisms, enhanced tumor growth, modulation of immune responses, promotion of angiogenesis and/or activation of a novel developmental regulatory program/ transformation referred to as the "epithelial-mesenchymal transition" (EMT). Multiple RNA-binding proteins with signaling properties (Ago2, RCK, TTP, YB-1, G3BP) and signaling molecules (RACK1, TRAF2, Raptor, mTOR) dynamically associate with SGs and PBs (double-headed arrows). They may transduce signals incoming from tumor microenvironment (yellow) to the physiological responses (bottom red panel), thus mediating both short- and long-term adaptations critical to tumor growth and metastasis. For other details, see text.