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Original article

In silico identification of MicroRNAs targeting the key nucleator of stress granules, G3BP: Promising therapeutics for SARS-CoV-2 infection

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ABSTRACT

Stress granules (SGs) are non-membrane ribonucleoprotein condensates formed in response to environmental stress conditions via liquid–liquid phase separation (LLPS). SGs are involved in the pathogenesis of aging and aging-associated diseases, cancers, viral infection, and several other diseases. GTPase-activating protein (SH3 domain)-binding protein 1 and 2 (G3BP1/2) is a key component and commonly used marker of SGs. Recent studies have shown that SARS-CoV-2 nucleocapsid protein via sequestration of G3BPs inhibits SGs formation in the host cells. In this study, we have identified putative miRNAs targeting G3BP in search of modulators of the G3BP expression. These miRNAs could be considered as new therapeutic targets against COVID-19 infection via the regulation of SG assembly and dynamics.

1. Introduction

Cells often come across a variety of stresses and undergo a variety of responses to counterattack these stresses. To protect from various environmental stressors, cells undergo exceedingly organized cellular stress response pathways resulting in translation inhibition, polysomes dismantling, and mRNAs and proteins reshuffle into stress granules (SGs) (Ivanov et al., 2019; Kedersha and Anderson, 2002; Kiebler and Bassell, 2006). In mammalian cells, these membrane-less mRNA-protein condensates, SGs form in the cytoplasm in response to heat stress, arsenite exposure, UV irradiation, and viral infection (Kedersha et al., 2013; Protter and Parker, 2016; White and Lloyd, 2012). SGs are highly conserved and dynamic ribonucleoprotein (RNP) condensates that are formed through liquid–liquid phase separation (LLPS) results from protein and/or RNA interactions (Protter and Parker, 2016; Van Treeck and Parker, 2018). SGs are dynamic entities that are in equilibrium with actively translating polysomes, and the dynamics of SG tightly control the translation. Thus, dysregulation of SG assembly and disassembly is largely implicated in the pathogenesis of several diseases including cancer, inflammatory, neurodegenerative diseases, autoimmune diseases, and viral infections (Cao et al., 2020; Gao et al., 2019; Mahboubi and Stochaj, 2017; McCormick and Khaperskyy, 2017; Wolozin and Ivanov, 2019).

SGs formation is largely linked to exhibit both pro-survival and pro-death activities depending on the type and duration of stress. The protective SGs were formed in response to acute stress whereas chronic stress leads to the formation of harmful SGs (Arimoto et al., 2008; Reineke and Neilson, 2019; Zhang et al., 2019). The mechanism underlying SG assembly and disassembly has remained ill-defined, and their role during the stress response is still not clearly understood (Buchan and Parker, 2009; Marcelo et al., 2021; Panas et al., 2016). The mechanistic insights into SG dynamics and effects on cell signaling and cell survival events will enhance a better understanding of the disease pathology.

Several high-content studies have shown the antiviral role of SGs, however, several viruses utilize SGs to escape the host responses through several mechanisms which include, inhibition of post-translational modifications (Linero et al., 2011), elimination of critical SG proteins (Emara and Brinton, 2007; Nikolic et al., 2016), and through viral ribonucleoprotein (RNP) formation with critical SG proteins (Abrahamyan et al., 2010). Proteins that are

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crucial and sufficient to form SGs are known as scaffold proteins and includes certain translation repressors, such as caprin-1 and TIA-1, RNA-binding proteins, like GTPase-activating protein (SH3 domain)-binding protein 1 and 2 (collectively referred to as G3BP), and enzymes with ATPase activities (Buchan and Parker, 2009; Cao et al., 2020; Yang et al., 2020).

Recent studies showed that SARS-CoV-2 Nucleocapsid (N) protein undergoes RNA-induced LLPS (Cubuk et al., 2020; Perdikari et al., 2020; Savastano et al., 2020; Zheng et al., 2021) and this behavior is likely critical in viral genome packaging and virion assembly. SARS-CoV-2N interacts with several human RNPs involved in the formation of RNP granules including G3BP and sequesters these proteins which finally leads to attenuation of SG (Gordon et al., 2020a; Syed Nabeel-Shah et al., 2020; Zhou et al., 2021). These findings suggest that N protein sequesters G3BPs, reduces the SG formation, and is involved in the binding of several mRNAs of important genes which results in the modulation of metabolism and gene expression of the host cell by SARS-CoV-2N protein.

G3BP is the well-characterized SG nucleating proteins and are key nucleators for SG formation (Kedersha et al., 2016; Yang et al., 2020). Overexpression of G3BP results in SG formation even in the absence of stress (Tourriere et al., 2003) and deletion of G3BP removes SG in response to arsenite (Kedersha et al., 2016; Reineke et al., 2012; Zhang et al., 2019). The assembly and dynamics of SG can be regulated through extrinsic G3BP-binding factors that either strengthen or weaken the SG network. As G3BP expression level largely dictates SG dynamics and is a key factor for SG formation, we advocate that changes in gene expression of G3BP would lead to significant variations in SG formation and dynamics.

The microRNAs (miRNA) are short-length non-coding RNAs that control gene expression through binding with target messenger RNAs. Many miRNAs are important for cellular growth, differentiation, pathogenesis, development, disease progression, and cell death (Lou et al., 2019). The host miRNAs play important roles during viral infections in the modulation of host-virus interactions and thus control the viral infectivity and transmissibility (Bernier and Sagan, 2018).

In the background of the current COVID-19 pandemic, modulating SG formation can be crucial to inhibit viral replication and assembly and thus can prevent SARS-CoV-2 infection. In this study, we utilized different miRNA target prediction web server programs to identify potential miRNAs that can directly bind to G3BP and can regulate the expression of key SG nucleator protein and thus modulate the dynamics of SG assembly.

2. Methods

Several miRNA-gene interaction databases such as miRTarBase (Huang et al., 2020), miRbase 33333334(Kozomara et al., 2019), miRDB (Chen and Wang, 2020), and miRNet2 (Chang et al., 2020) were screened to identify the miRNAs interacting with the G3BP1 and G3BP2 genes. These databases also provide interaction validation information, if available, using the literature-based analysis. miRNA-gene interactions having at least one validation method were considered for further analysis. For identifying the common miRNAs interacting with both G3BP1 and G3BP2 genes an in-house Perl-script was prepared.

To predict the minimum free energy (MFE) secondary structure of the single sequence of miRNA, we have used the RNAfold web server that is one of the core programs of the Vienna RNA package (http://rna.tbi.univie.ac.at/) (Gruber et al., 2008). The ViennaRNA package has been used widely for RNA bioinformatics studies for almost two decades. Several characteristics of RNA secondary structures including Gibbs free energy ΔG, MFE, ensemble diversities, and the presence of MFE structures in the different ensembles can be determined, and these characteristics have been widely used in the detection of miRNA targets (Enright et al., 2003; Grün et al., 2005; Lorenz et al., 2011).

DIANA-TarBase v8.0 (Karagkouni et al., 2018) web tool was used to identify the effect of selected miRNAs on the target genes. The database is based on indexing of experimentally supported miRNAs and has more than 1 million indexed entries with ~670000 unique miRNA-target pairs. The database contains cell-type-specific gene-miRNA interaction information. Over the last 15 years, several in silico methods have been developed to identify the miRNA interactome (Shaker et al., 2020; Steinkraus et al., 2016). Many high throughput techniques also have allowed the identification of miRNA-gene interactions (Goodwin et al., 2016).

Furthermore, the enrichment analysis of common miRNAs targeting G3BP was performed using GeneTrail (V:3.0) (Gerstner et al., 2020) webserver. GeneTrail webserver uses gene ontology and miRPATHDB (Kehl et al., 2020) database for functional enrichment analysis at biological, molecular, and cellular levels. KEGG (Kanehisa and Goto, 2000) and Reactome (Jassal et al., 2020) databases were used for the identification of pathways related to the concerned miRNAs. Functions and pathways having a False Discovery Rate (FDR) < 0.05 were considered significant.

3. Results

3.1. Identification of potential miRNAs involved in the regulation of G3BP

In this study, we have utilized different miRNA target prediction tools to identify miRNA with the potential to target G3BP1 and G3BP2. This in silico analysis predicts 275 miRNAs having potential binding abilities to G3BP1 and 316 miRNAs binding to G3BP2 (Supporting Information Table 1). These identified miRNAs may increase or decrease the expression of G3BP by binding to them directly or indirectly and thus can be explored as a therapeutic strategy against SARS-CoV-2 infection by altering its replication and growth. Further, we found 34 common miRNAs targeting both G3BP1 and G3BP2 genes (Table 1). The interaction network of the identified 34 miRNAs and G3BP genes is represented in Fig. 1.

As miRNA binding to the target sequence is promiscuous (Brennecke et al., 2005), we further assessed the binding strength and significance of the identified miRNAs to G3BP1 and G3BP2 using the RNAfold web server. The stable binding of a miRNA to an mRNA is considered as the most likely target of that miRNA and is calculated by Gibb's free energy. The negative free energy, ΔG indicates the stability of the miRNA structure and the strength of the interaction between the miRNA and its target mRNA. The more negative ΔG (kcal/mol) indicates the more stable miRNA and strong association between miRNA and mRNA. The MFE analysis indicates that out of the 34 common miRNAs, a total of 15 miRNAs has a stable secondary structure and strongly bind with G3BP with $\Delta G < -1.5$ kcal/mol. Further, the miRNA: gene interactions were validated using the DIANA-TarBase webtool for these 15 miRNAs. The four miRNAs namely, hsa-miR-124-3p, hsa-miR-30a-5p, hsa-miR-101-3p, and hsa-miR-494-3p has been found to inhibit both the G3BP1 and G3BP2 target genes, whereas two miRNAs, hsa-miR-2110 and hsa-miR-23b-3p inhibit the G3BP1 gene only.

3.2. Functional and pathways enrichment analysis of common miRNAs

For the enrichment analysis of commonly identified miRNAs, we use the GeneTrail webserver for the gene ontology analysis along with pathways analysis. The GO analysis revealed that in the biological process the miRNAs were significantly enriched in

Table 1
List of potential miRNAs binding to both G3BP1 and G3BP2 genes along with their binding energies.

miRNA	Sequence (5′ – 3′)	Free energy, ΔG (kcal/mol)
hsa-miR-4696	ugcaagacggauacugucaucu	−4.2
hsa-miR-4418	cacugcaggacucagcag	−4.1
hsa-miR-124-3p	uaaggcacgcggugaaugccaa	−4.0
hsa-miR-519e-5p	uucuccaaaagggagcacuuuc	−4.0
hsa-miR-548u	caaagacugcaauuacuuuugcg	−3.7
hsa-miR-509-5p	uacugcagacaguggcaauca	−3.2
hsa-miR-103a-1-5p	ggcuucuuuacagugcugccuug	−3.1
hsa-miR-30a-5p	uguaaacauccucgacuggaag	−2.7
hsa-miR-651-3p	aaaggaaaguguaucuaaaag	−2.6
hsa-miR-7156-3p	cugcagccacuuggggaacuggu	−2.6
hsa-miR-2110	uuggggaaacggccgcugagug	−2.1
hsa-miR-515-5p	uucuccaaaagaaagcacuuucug	−1.8
hsa-miR-6868-3p	uuccuucuguugucugugcag	−1.7
hsa-miR-101-3p	uacaguacugugauaacugaa	−1.6
hsa-miR-23b-3p	aucacauugccagggauuaccac	−1.6
hsa-miR-494-3p	ugaaacauacacgggaaaccuc	−1.6
hsa-miR-509-3-5p	uacugcagacguggcaaucaug	−1.4
hsa-miR-103a-2-5p	agcuucuuuacagugcugccuug	−1.2
hsa-miR-506-3p	uaaggcacccuucugaguaga	−0.9
hsa-miR-2681-5p	guuuuaccacccuccaggagacu	−0.8
hsa-miR-484	ucaggcucagucccccucccgau	−0.7
hsa-miR-30b-5p	uguaaacauccuacacucagcu	−0.1
hsa-miR-4463	gagacuggggugggcc	−0.1
hsa-miR-7161-5p	uaaagacuguagaggcaacuggu	−0.1
hsa-miR-450a-2-3p	auuggggacauuuugcauucau	0
hsa-miR-3613-3p	acaaaaaaaaaagcccaacccuuc	0
hsa-miR-3925-5p	aagagaacugaaaguggagccu	0
hsa-miR-12123	uuauucauucacaaaagcuuua	0
hsa-miR-548c-3p	caaaaaucucaauuacuuuugc	0
hsa-miR-548n	caaaaguaauuguggauuuugu	0
hsa-miR-6890-3p	ccacugccuaugccccacag	0
hsa-miR-577	uagauaaaaauauugguaccug	0
hsa-miR-605-3p	agaaggcacuaugagauuuaga	0
hsa-miR-1-3p	uggaauguaaagaaguauguau	0

positive regulation of gene expression, positive regulation of the metabolic process, negative regulation of fibroblast proliferation, positive regulation of sprouting angiogenesis, negative regulation of programmed cell death, and positive regulation of cardiac muscle tissue development (Fig. 2). Whereas, the molecular functions were mainly enriched in RNA binding, mRNA binding, nucleic acid binding, and organic cyclic compound binding. According to KEGG and Reactome pathways analysis, the miRNAs participate in the Ionotropic activity of kainate receptors, PI3K/AKT activation, Epstein-Barr virus infection, ERK/MAPK targets, and peptide hormone biosynthesis pathways (Fig. 2).

4. Discussion

Accumulating evidence shows that SGs play important role in apoptosis, inflammation, immunomodulation, and various signaling pathways. SGs are also involved in aging, cancers, neurodegenerative diseases, viral infection, and several other debilitating diseases. Many studies suggest that troubles in SG dynamics cause many aging-associated neurodegenerative diseases and cancers (Mackenzie et al., 2017; Ramaswami et al., 2013; Wolozin and Ivanov, 2019). However, both the pro-survival and pro-apoptotic role of SG has also been described (Reineke and Neilson, 2019). Recently, SARS-CoV-2 nucleocapsid (N) protein has been reported to attenuate SG formation by LLPS of N protein and sequestration of G3BP, which increases the replication and translation of SARS-CoV-2 (Gordon et al., 2020a; Perdikari et al., 2020; Savastano et al., 2020; Syed Nabeel-Shah et al., 2020).

Recently studied SARS-CoV-2N protein interactome (Gordon et al., 2020b; Li et al., 2021) have identified the SG nucleating factor G3BP1 as a key component, suggesting that SARS-CoV-2 regulates SGs assembly largely through N protein. Moreover, N protein has been shown to form condensates with RNA in vitro(Carlson et al., 2020; Chen et al., 2020; Savastano et al., 2020), in the cytoplasm

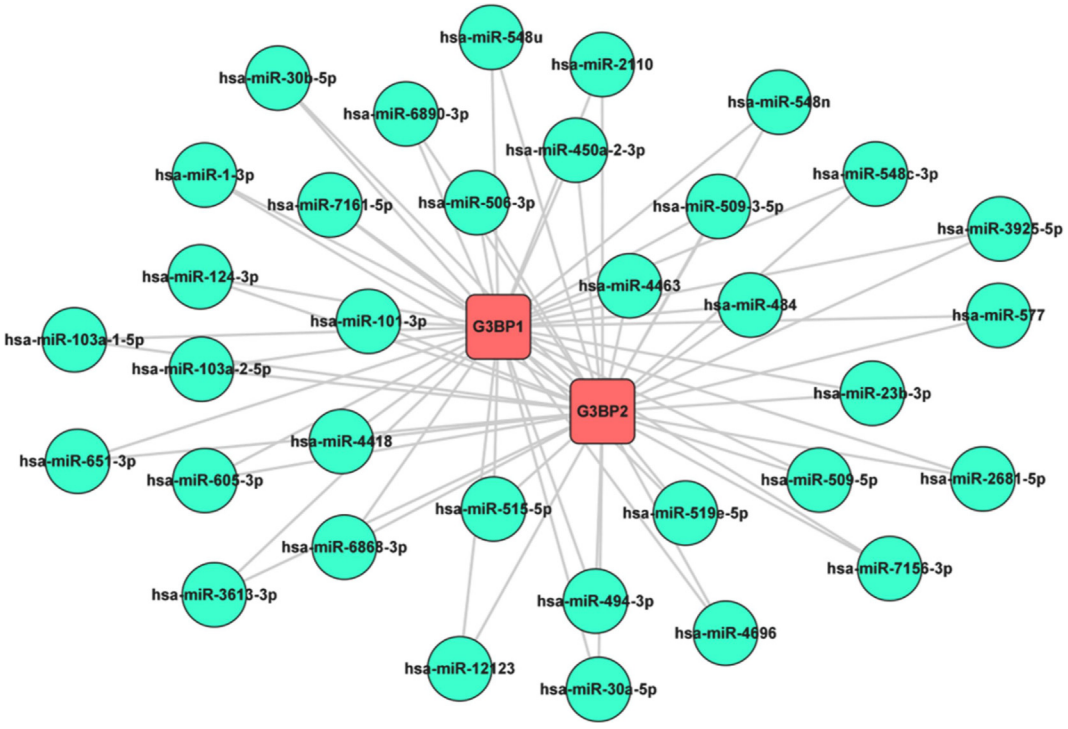

Fig. 1. G3BP-miRNA interactome. Common 34 miRNAs interacting with both G3BP1 and G3BP2 genes.

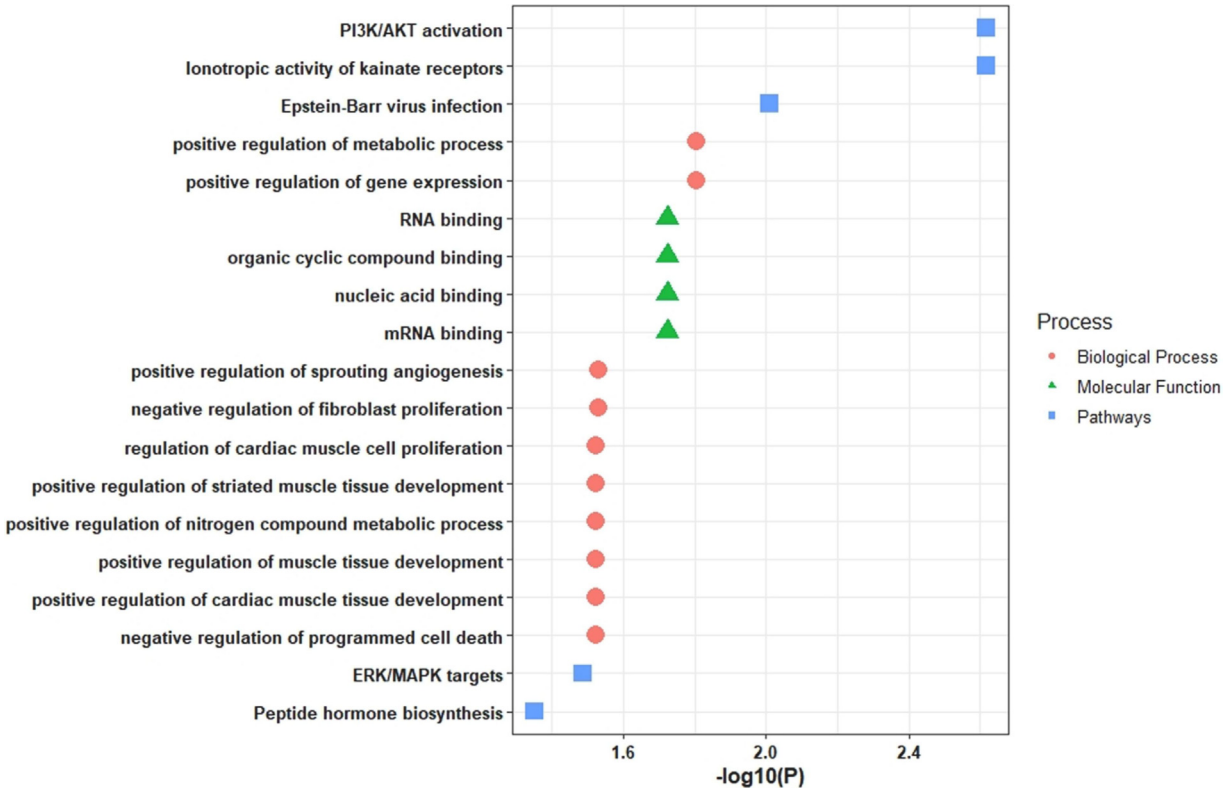

Fig. 2. Functional enrichment of the common miRNAs. Gene ontology analysis for biological and molecular functions along with KEGG pathways related to miRNAs interacting with G3BP.

of cells (Iserman et al., 2020; Lu et al., 2021), and also partially colocalizes within arsenite-induced SGs (Luo et al., 2021). N protein sequester G3BP1 and attenuate SGs formation which leads to the suppression of the host immune response and favors virus replication and growth (Luo et al., 2021; Savastano et al., 2020; Wang et al., 2021). Thus, targeting the function of G3BP1represents is a useful strategy to tackle COVID-19 infection.

Since miRNA plays important role in viral infection, we here utilized different bioinformatic approaches to identify miRNAs against G3BP and examine the interactions between them. The miRNA-G3BP interaction network suggests different signaling pathways in response to SARS-CoV-2 infection. The enrichment analysis indicates the two important pathways, PI3K/AKT and ERK/MAPK targeted by the identified miRNAs indicating their role in COVID-19.

The phosphoinositide 3 (PI3)-kinase/Akt pathway helps inflammation in many diseases (Hawkins and Stephens, 2015). The deletion of the Akt1 gene decreased inflammation and enhanced cardiac function in mice following myocardial ischemia (Kerr et al., 2013; Ma et al., 2014). Akt1 belongs to the serine/threonine-protein kinase family, Akt kinase (Akt1, Akt2, and Akt3) (Cohen, 2013). It has been shown that active Akt1 promotes viral protein synthesis (Wang et al., 2014). The replication of MERS coronavirus can be inhibited by administrating PI3K/Akt inhibitors (Kindrachuk et al., 2015). The PI3K/AKT pathway is involved in the SARS-CoV-2 entry into the host cell through endocytosis and in the development of immune responses, indicating that it could be considered as a therapeutic target (Khezri, 2021; Xia et al., 2020). Moreover, SARS-CoV spike and N proteins have been shown to increase ERK/MAPK phosphorylation with subsequent activation of pro-inflammatory pathways including increased cyclooxygenase-2 expression and IL-8 release (Mizutani et al., 2004; Wehbe et al., 2020). Lee et al. (2004) have shown that phosphorylated MAPK increased in CD14-positive monocytes in SARS-CoV infected patients. Increased MAPK activation in CD14 cells has been associated with high IL-8 levels and the MAPK signaling pathway is involved in the death of SARS-COV-infected cells (Mizutani, 2007). Recently, Zhang et al. (2020) suggested that SARS-CoV-2 infection leads to platelet activation, thrombus formation, and inflammatory responses in COVID-19 patients. This platelet activation is mediated by the MAPK pathway, located downstream of ACE2, and the platelet ACE2 expression decreased following the SARS-COV-2 infection (Zhang et al., 2020). Thus, both ERK/MAPK and PI3K/AKT signaling pathways are involved in cell proliferation and apoptosis, and have been demonstrated to be targeted by a broad range of viral pathogens, (Ehrhardt and Ludwig, 2009; Kindrachuk et al., 2015), and thus may represent novel drug targets for therapeutic intervention strategies.

5. Conclusions

Recent progress into identifying bona fide miRNA that can target viral genomes or host proteins may be useful in designing a drug to mitigate COVID-19 infection. In summary, we have identified and evaluated the potentials of miRNAs in controlling SARS-CoV-2 replication and assembly by regulating the expression of key SG nucleator, G3BP1, and G3BP2 genes. In view of the fact that G3BP could be an important drug target against COVID-19, the results of the study could be of therapeutic interest to tackle the ongoing COVID-19 pandemic.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2021.08.056.

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