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Original Article

Sub-cellular localization of Tudor-SN foci under different stress conditions

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Abstract: Tudor-SN (Tudor Staphylococcal Nuclease) protein is evolutionarily conserved in human, animals and plants, and participates in several biological processes, such as gene transcription, cellular stress and pre-mRNA splicing. Our previous data suggested that Tudor-SN binds G3BP protein and takes part in the SGs (Stress Granules) formation under oxidative stress or 45°C heat shock. Here, we further demonstrated that Tudor-SN containing SGs aggregated under the treatment of 10 mM DTT (Dithiothreitol) stress, 20 J/m^2 UVC exposure, but not 4°C 1 h, 32°C 20 h, and 1 μM thapsigargin 1 h. Tudor-SN foci fail to co-localize with organelles, including endoplasmic reticulum, mitochondria and Golgi apparatus. We also found that Tudor-SN foci disassembled gradually during the Prophase, Prometaphase, Metaphase and Anaphase, but reassembled in the Telophase of cell cycle.

Keywords: Tudor-SN, G3BP, stress granules, organelle, cell cycle

Introduction

When suffered from the various environmental stimuli (such as oxidative stress, heat shock, UV exposure, low permeability or virus infection), the protein translation situation in the cytoplasm can be altered to enable the eukaryotic cells to survive during stress [1]. The translation stalled mRNA molecules and the protein factors dynamically accumulate in the cytoplasm and form a kind of RNA foci structure named Stress Granules (SGs) [2, 3]. SGs contain the inhibited translation initiation complex, like eukaryotic translation initiation factor 2α (eIF2α) and Poly(A)-binding protein 1 (PABP-1), mRNAs released from polysomes, some miRNAs and various RNA binding proteins, such as Hu antigen R (HuR), Ras-GAP SH3 domain-binding protein (G3BP) and T-cell internal antigen-1 (TIA-1), which regulate mRNA translation, storage and stability during stress [4, 5]. The biological role of SGs components varies due to the stimuli type [2]. In addition, the efficient cell adaptation during stress depends on the coordination of a variety of cellular structures or processes, such as SGs, Processing Bodies (PBs), organelle, cell cycle, autophagy, and apoptosis [1, 6-9]. Thus, it is important to investigate the relationship between SGs and these structures or processes under different stress conditions.

Tudor staphylococcal nuclease (Tudor-SN) protein, also called p100 or staphylococcal nuclease domain containing 1 (SND1), was originally identified as transcriptional co-activator of EBNA2 (Epstein-Barr virus nuclear protein 2) [10]. Tudor-SN is composed by four N-terminal SN (staphylococcal nuclease-like) domains and a C-terminal TSN (Tudor-SN5) domain [11, 12]. SN domains mediate the binding of Tudor-SN with some transcription regulation proteins, like RNA polymerase II, CREB-binding protein (CBP), Signal transducer and activator of transcription 6 (STAT6) to form transcription complex, and

then enhance transcription activity [13, 14], while TSN domain is able to promote pre-mRNA splicing by promoting the formation of spliceosome [15, 16]. Several lines of evidence confirmed that Tudor-SN is a kind of SGs proteins in human, plant and animal [17-23]. However, the study on the Tudor-SN foci assembly in terms of cell cycle or organelle remains elusive.

Our previous data demonstrated that Tudor-SN efficiently binds and co-localizes with G3BP into SGs, when cells were treated with 45°C heat shock or arsenite sodium for 1 h [17]. Moreover, endogenous Tudor-SN knockdown can retard the aggregation of small G3BP-containing SGs into large SGs, and Tudor-SN protein is capable of influencing the assembly dynamics of G3BP granules [24]. In the present study, we further performed the sub-cellular localization analysis of Tudor-SN foci with different organelles, under different conditions, including cold stress, endoplasmic reticulum stress, UVC damage, or in different cell cycle phases.

Materials and methods

Cell Culture, plasmids and transfection

HeLa cells were cultured as described previously [14]. Plasmids were transfected by Lipofectamine 2000 (Invitrogen, Barcelona, Spain), according to manufacturer's protocol. pERFP-mito plasmid was kindly provided by Dr. Yongfeng Shang (Tianjin medical university, Tianjin, China). pEYFP-ER and pEYFP-Golgi plasmids were purchased from BD Biosciences Clontech (France).

Immunofluorescence (IF) assay

The IF assay was performed as described previously [16, 24, 25]. Cells were grown on glass cover slips and treated with drugs, including 0.5 mM sodium arsenite (Sigma Aldrich), Earle's balanced salt solution (EBSS) for 2 h (starvation), 10 mM DTT (Sigma Aldrich) for 1 h, 1 μM thapsigargin (ab120286, Abcam) for 1 h, 45°C for 1 h, 4°C for 1 h, 32°C for 20 h, 20 J/m^2 UVC, as indicated. Cells were fixed, premeabilized, and then incubated with specific primary antibodies at 4°C overnight. After washing, cells were incubated with fluorescent dye conjugated secondary antibodies (1:800 dilution) at 4°C overnight. The glass cover slips were adhered to the plates, using hard-set mounting medium, which containing 4',6-diamidino-2-phenylindole (DAPI) (sc-359850, Santa Cruz biotechnology). And the images were collected using Olympus FV1000 confocal microscope.

Primary antibodies were used: goat polyclonal anti-Tudor-SN (sc-34753, Santa Cruz Biotechnology), mouse monoclonal anti-G3BP (ab56-574, Abcam). Secondary antibodies were used: donkey anti-goat IgG (H+L) (TR) antibody (ab-6883, Abcam), donkey anti-goat IgG (H+L) (Dylight 488) antibody (ab96931, Abcam), Alexa Fluor 488-coupled donkey anti-mouse IgG (H+L) antibody (A21202, Molecular Probes).

Cell cycle synchronization

HeLa Cells were grown to 30%~40% of confluence and incubated in medium containing 2 mM thymidine (T1895, Sigma Aldrich) for 16 h. After washing with 1×PBS (phosphate-buffered saline), cells released in 10% FBS-DMEM growth media for 8 h. Then cells were treated again with 2 mM thymidine for 16 h to reach G1/S border and released at 4, 8 and 16 h in 10% FBS to reach S phase, G2/M phase, G0/G1 phase cells, respectively. The immunofluorescence assay with anti-Tudor-SN antibody was then performed to detect the formation of Tudor-SN foci during cell cycle. DAPI was used to stain the nucleus to further specify the cell cycle phase, including G1, S, G2, Prophase, Prometaphase (P1, P2, P3), Metaphase, Anaphase, Telophase (T1,T2) and G0.

Granule quantification and statistical analysis

The size and number of granules in cells was analyzed by the analyze particle tool in Image J software (version 1.46a, National Institutes of Health, USA), as described previously [24, 25]. All experiments were repeated at least three times, and more than 50 cells per experiment were scored. The data are presented as the box-plots (granule size analysis) or means ± se (Standard Error) and compared with one-way analysis of variance (ANOVA) using SPSS 16.0 software. *P* value less than 0.05 was considered statistically significant.

Results

The assembly of endogenous Tudor-SN foci varies in response to different stimuli

Previously, we found that Tudor-SN co-localizes with G3BP protein into SGs [17]. In this report,

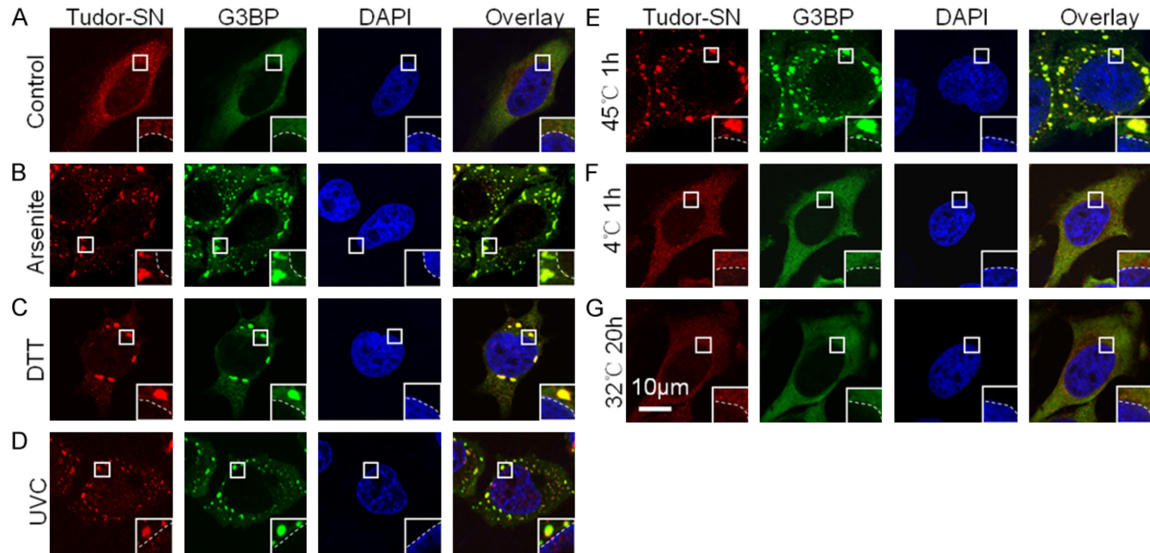

Figure 1. The formation of endogenous Tudor-SN foci under different stress conditions. HeLa cells were cultured and either untreated (Control, A) or treated with different stress stimuli, including 0.5 mM sodium arsenite for 1 h (B), 10 mM DTT for 1 h (C), 20 J/m^2 UVC (D), 45°C for 1 h (E), 4°C for 1 h (F), 32°C for 20 h (G), as indicated. Cells were fixed and stained with goat anti-Tudor-SN or mouse anti-G3BP (marker of SGs) primary antibodies, followed by donkey anti-goat IgG (H+L) (TR) or Alexa Fluor 488-coupled donkey anti-mouse IgG (H+L) secondary antibodies, respectively. After washing, the glass slides were adhered to the plates using hard-set mounting medium with DAPI. Confocal microscopy analysis was then performed. Bar, 10 μm.

we detected the formation of Tudor-SN foci in HeLa cells under different stress conditions, including endoplasmic reticulum stress, cold stress and UVC damage. The 10 mM DTT (Dithiothreitol) was used to mimic the endoplasmic reticulum stress. UVC damage stress was induced by the treatment of 20 J/m^2 UVC. The cold stress was also induced by 4°C for 1 h, 32°C for 20 h. As shown in **Figure 1**, both Tudor-SN and G3BP mainly located in the cytoplasm and failed to form the SGs under normal condition, whereas the stress conditions, including 0.5 mM arsenite sodium 1 h, 10 mM DTT 1 h, 45°C 1 h, 20 J/m^2 UVC, induced the assembly of Tudor-SN containing SGs. However, Tudor-SN foci were not aggregated in HeLa cells under 4°C for 1 h, 32°C for 20 h-mediated cold stress condition.

Tudor-SN foci fail to co-localize with endoplasmic reticulum, mitochondria, Golgi apparatus

The previous data of sucrose gradient fractionation indicated that Tudor-SN protein co-fractionated with GM130 (Golgi marker) and Calreticulin (endoplasmic reticulum marker) in lipid droplets of rat liver parenchyma and cultured hepatocytes [26]. Moreover, Tudor-SN was partially co-localized with the GM130 or Calreticulin in rat primary hepatocyte [26]. In addition, SG structure was reported to be associated with the endoplasmic reticulum stress (ERS) and mitochondrial stress [27-30]. For example, the treatment of 1 μM thapsigargin (a kind of intracellular endoplasmic reticulum calcium pump inhibitor) for 1 h was able to induce the ERS and the recruitment of Stau1 protein into SGs in mammalian cells [29]. In order to study the co-localization between Tudor-SN foci and mitochondria, endoplasmic reticulum or Golgi, HeLa cells were transfected with pERFP-mito, pEYFP-Golgi, pEYFP-ER plasmids and then treated 0.5 mM sodium arsenite, 1 μM thapsigargin for 1 h, or not. As shown in **Figure 2**, arsenite oxidative stress-induced Tudor-SN foci did not co-localize with the ectopically-expressed markers of mitochondria, Golgi and endoplasmic reticulum. Thapsigargin-mediated ERS also failed to efficiently induce the formation of Tudor-SN foci. The same results were also obtained in the IF assay of HeLa cells, using anti-GM130 (endogenous Golgi marker), anti-Calreticulin (endogenous ER marker) antibodies (data no shown).

Intriguingly, under normal condition, ~90% of HeLa cells, which was transfected with pERFP-

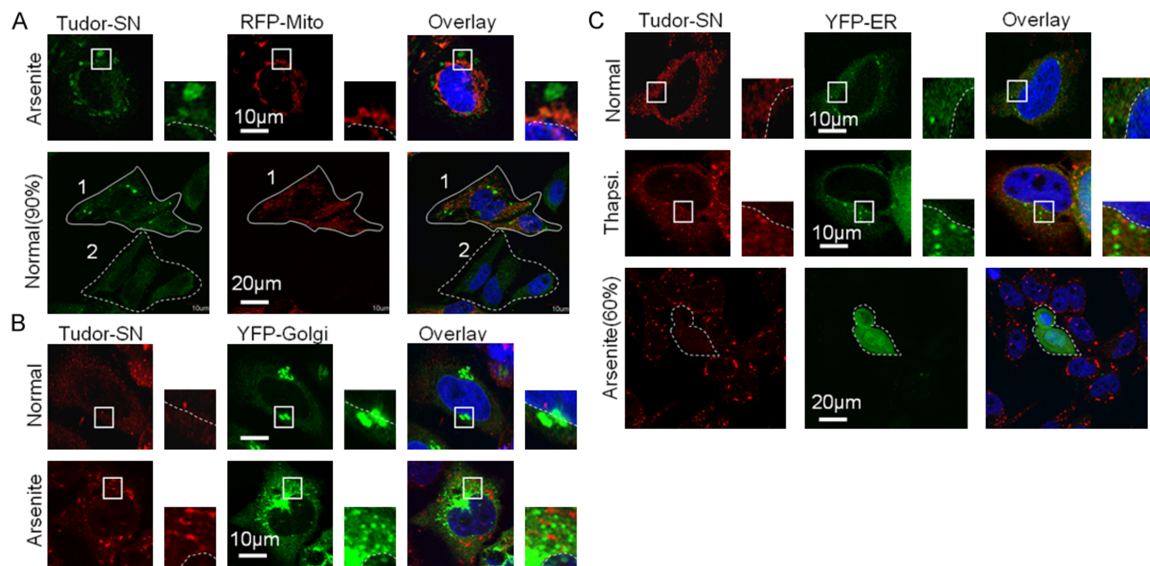

Figure 2. The co-localization between Tudor-SN and ectopically-expressed markers of mitochondria, endoplasmic reticulum and Golgi. HeLa cells were transfected with plasmids encoding RFP-tagged mito (A), YFP-tagged Golgi (B), YFP-tagged ER (C), and then treated with 0.5 mM sodium arsenite, 1 μM thapsigargin for 1 h, or not as indicated. Cells were fixed and stained with goat anti-Tudor-SN primary antibody and Dylight 488-coupled donkey anti-goat IgG (H+L) or donkey anti-goat IgG (H+L) (TR) secondary antibodies. Image data were collected using confocal microscopy. "1" in (A), RFP-tagged mito transfected HeLa cell. "2" in (A), RFP-tagged mito untransfected HeLa cell. Bar, 10/20 μm.

mito plasmid, were capable of accumulating the Tudor-SN foci (**Figure 2A**). pERFP-mito plasmid expresses the mitochondrial localization peptide of Thioredoxin 2 protein, which marks mitochondria and plays a role in the modulation of redox and antioxidant defense [31, 32]. The artificial plasmid transfection might interfere with normal mitochondrial redox equilibrium. In addition, when cells were transfected with pEYFP-ER plasmid, sodium arsenite failed to induce the formation of Tudor-SN containing granules in ~60% cells (**Figure 2C**). pEYFP-ER plasmid can express calreticulin fragments and the ER retention signal peptide (l-lysine-l-glutamic-aspartic acid-Leucine, Lys-Asp-Glu-Leu). Calreticulin, a kind of multifunctional calcium-binding protein, participates in the protein folding, ER calcium storage and release, and is commonly used as one marker protein of ER structure [33]. It is possible that the over-expression of calreticulin is associated with the inhibition of Tudor-SN containing SGs formation.

The above data indicated that Tudor-SN foci did not co-localize with mitochondria, endoplasmic reticulum or Golgi in morphology. However, we still could not rule out the possibility of functional link between Tudor-SN foci and mitochondria or endoplasmic reticulum stress.

Tudor-SN foci disassemble during cell cycle

Recently, we identified Tudor-SN as a new regulator of the G1/S transition, and Tudor-SN promotes cell cycle progression by facilitating E2F-1-mediated gene transcription under normal condition [34]. To analyze the Tudor-SN foci assembly in different phases of cell cycle during stress, HeLa cells were synchronized and collected at different cell cycle phases, including G1, S, G2, prophase, prometaphase, metaphase, anaphase, telophase and G0, by double-thymidine blocking and DAPI staining. After the cells were treated with 0.5 mM sodium arsenite for 0.5 h, IF assay was then performed to detect the formation of endogenous Tudor-SN foci using the anti-Tudor-SN antibody. As shown in **Figure 3A**, **3B**, Tudor-SN foci were accumulated in the G0, G1, S, G2 prophase phase in 75%~95% HeLa cells. And the foci number in most of cells is >20 (**Figure 4A**), the size of Tudor-SN foci was ~3 μm^2 (**Figure 4B**). However, in the phase of Prometaphase, Metaphase and Anaphase, the portion of cells containing Tudor-SN foci significantly reduced (**Figure 3**). And the foci number in cells reduced to 0~5; the size of Tudor-SN foci was less than 2 μm^2 (**Figure 4**, $P<0.05$). In Telophase phase, the rate of positive cells raised to 63% (**Figure**

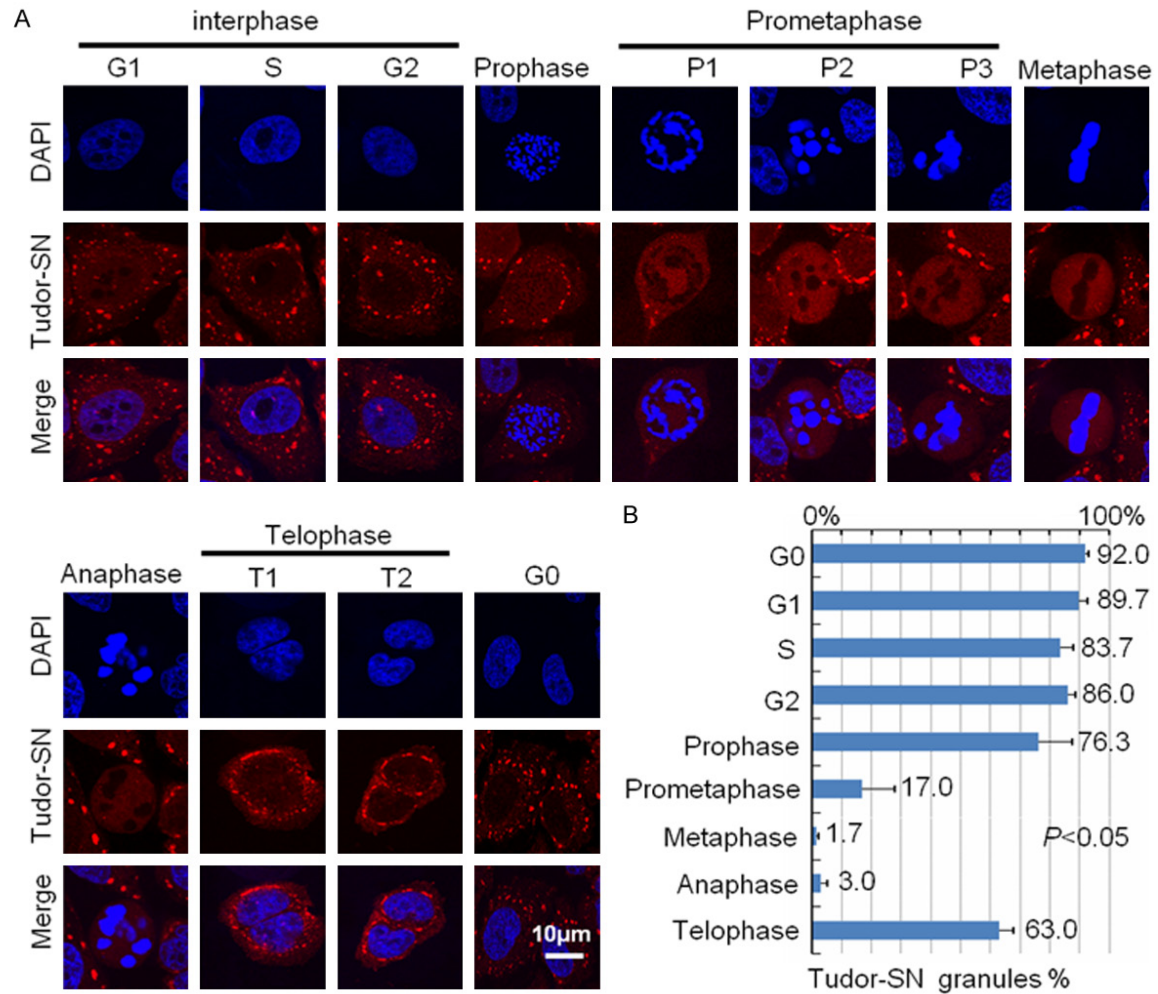

Figure 3. The formation of Tudor-SN foci during different cell cycle phase. A. HeLa cells were synchronized at different cell cycle phase (G1, S, G2, Prophase, Prometaphase, Metaphase, Anaphase, Telophase and G0), and then treated with 0.5 mM sodium arsenite for 0.5 h. Cells were fixed and stained with goat anti-Tudor-SN primary antibody and donkey anti-goat IgG (H+L) (TR) secondary antibody. After washing, the glass slides were adhered to the plates using hard-set mounting medium with DAPI. Confocal microscopy analysis was then performed. Bar, 10 μm. B. The numbers of Tudor-SN foci positive cells were counted and analyzed by One-way analysis of variance (ANOVA). Significant difference was indicated: *P*<0.05.

3), the foci number recovered to >20; and the foci size recovered to ~3 μm^2 (**Figure 4**, *P*<0.05). These indicated that Tudor-SN foci disassembled in the metaphase phase of cells and reassembled in telophase might for cellular adaptability.

Discussion

In the present study, we found that Tudor-SN foci accumulated in HeLa cells and showed the distinct assembly characteristics under the environmental stimuli of cold shock, mitochondrial stress, endoplasmic reticulum stress or UV irradiation. Accumulating evidence indicates that SGs are associated with cold stress [35-37]. For example, *Tudor-SN* mRNA in a New Zealand alpine stick insect was up-regulated in response to 0°C-induced cold shock for 1 h, suggesting that *Tudor-SN* is a kind of cold stress response gene [36]. The treatment of 0°C for 4 h was efficiently inducing the formation of SGs in S.cerevisiae. Yeast [37]. In our experiment, we failed to observe the aggregation of Tudor-SN/G3BP-containing SGs in HeLa cells under 4°C for 1 h, 32°C for 20 h-mediated cold stress conditions. Unfortunately, we did not detect the Tudor-SN foci formation under other cold stress conditions, such as 10°C for 10 h, which were unavailable in our Lab. The

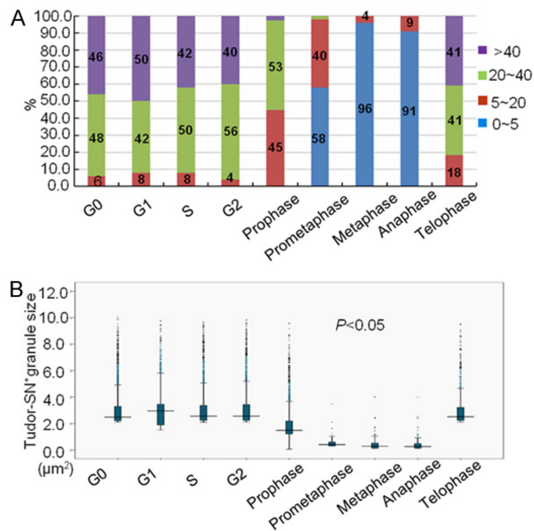

Figure 4. The analysis on the number/size of Tudor-SN foci during different cell cycle phase. A. The number of Tudor-SN foci per cell was measured by Image J. The cells were divided into four categories, 0~5, 5~20, 20~40 and >40, based on the number of granules per cell. B. Tudor-SN foci size was analyzed using the particle analysis tool in Image J and displayed as box-plots. One-way analysis of variance (ANOVA) was performed using SPSS 16.0 software. Significant differences are indicated as follows: $P<0.05$.

potential association of Tudor-SN foci and cold stress was thus not to be excluded, which needs further experiments.

The adaptive molecular defense mechanism of SGs assembly is complicated. Different SGs components exhibit different assembly kinetics. We found that exposure of 10 mM DTT for 1 h induced the formation of G3BP/Tudor-SN foci, suggesting the association of ER stress with SGs. However, the efficient aggregation of Tudor-SN foci was not observed in response to 1 μM thapsigargin for 1 h, even though Stau1 and TDP-43 (TAR DNA binding protein 43) were recruited into SGs after the treatment of thapsigargin [29, 30]. Tudor-SN foci did not co-localize with the ER structure as well, via pEYFP-ER plasmid transfection and the IF assay using anti-calreticulin antibody. And we were surprised that the expression of calreticulin fragment from the transfection of pEYFP-ER plasmid could hinder the formation of sodium arsenite-induced Tudor-SN foci. As reported, when COS7 or NIH3T3 cells were treated with heat shock, sodium arsenite for 30 min, calreticulin formed to dimers, and co-localized with TIA-1 protein in SGs [38, 39]. However, we observed that the foci of calreticulin were not co-localized with Tudor-SN foci in HeLa cells after the treatment of sodium arsenite. SGs assembly is reported to be associated with the inhibition of cellular apoptosis [1, 40]. Tudor-SN protein can be cleaved by caspase-3 and participates in the execution of apoptosis during stress [41]. Several researches had also shown that calreticulin can move to the cell membrane (Endo- to Ecto-) and induce apoptosis under oxidative stress situations [42-44]. Calreticulin protein might interrupt the aggregation of Tudor-SN foci via the modulation of cellular apoptosis. More work is needed to investigate this possibility.

Cell cycle refers to the whole process from a cell division to the next division. Here, the size and number of Tudor-SN foci in cells was found to decrease in the Metaphase phase and restore gradually in Telophase phase of cell cycle. This trend was similar to the behavior of eIF3B foci or decapping enzyme 1 a (DCP1a)-tracing PBs during cell cycle [9, 45, 46]. It is possible that the disassembly of SGs and PBs functions as an adaptive response to cell division, which facilitates normal mitosis and promotes cellular survival during stress [46]. Another possibility is that SGs or PBs could not assemble because of the characteristics of mitosis. During mitosis, the chromatin condenses into chromosome, translating ribosomes slow down and general proteins synthesis almost stops except for mitosis specific proteins [9, 47].

Here, we found that 20 J/m^2 UVC irradiation could induce the assembly of Tudor-SN foci in HeLa cells. Similarly, G3BP foci were also accumulated in 10 J/m^2 of UVC-irradiated mammalian NIH-3T3 cells, which is tightly related with the G1 phase arrest of cell cycle [48]. Our previous data indicated that Tudor-SN regulates the G1 to S phase transition [34]. It is likely that dynamic change of Tudor-SN foci contributes to G1/S border transition of cell after UV irradiation, implicating into the modulation of UV damage or cellular apoptosis. Moreover, Tudor-SN is phosphorylated in the G1/S border [34]. The phosphorylation modification is likely to be involved in the assembly dynamic of Tudor-SN foci during cell cycle. Further experiments can be performed to study the potential role of phosphorylated Tudor-SN foci in the G1/S border under stress conditions.

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Disclosure of conflict of interest

None.

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References

[1] Arimoto K, Fukuda H, Imajoh-Ohmi S, Saito H and Takekawa M. Formation of stress granules inhibits apoptosis by suppressing stress-responsive MAPK pathways. Nat Cell Biol 2008; 10: 1324-1332.

[2] Anderson P and Kedersha N. Stress granules: the Tao of RNA triage. Trends Biochem Sci 2008; 33: 141-150.

[3] Buchan JR and Parker R. Eukaryotic stress granules: the ins and outs of translation. Mol Cell 2009; 36: 932-941.

[4] Anderson P and Kedersha N. RNA granules: post-transcriptional and epigenetic modulators of gene expression. Nat Rev Mol Cell Biol 2009; 10: 430-436.

[5] Erickson SL and Lykke-Andersen J. Cytoplasmic mRNP granules at a glance. Journal of cell science 2011; 124: 293-297.

[6] Thomas MG, Loschi M, Desbats MA and Boccaccio GL. RNA granules: the good, the bad and the ugly. Cell Signal 2011; 23: 324-334.

[7] Buchan JR, Kolaitis RM, Taylor JP and Parker R. Eukaryotic stress granules are cleared by autophagy and Cdc48/VCP function. Cell 2013; 153: 1461-1474.

[8] Krisenko MO, Higgins RL, Ghosh S, Zhou Q, Trybula JS, Wang WH and Geahlen RL. Syk is Recruited to Stress Granules and Promotes their Clearance through Autophagy. J Biol Chem 2015; 290: 27803-15.

[9] Sivan G, Kedersha N and Elroy-Stein O. Ribosomal slowdown mediates translational arrest during cellular division. Mol Cell Biol 2007; 27: 6639-6646.

[10] Tong X, Drapkin R, Yalamanchili R, Mosialos G and Kieff E. The Epstein-Barr virus nuclear protein 2 acidic domain forms a complex with a novel cellular coactivator that can interact with TFIIE. Mol Cell Biol 1995; 15: 4735-4744.

[11] Shaw N, Zhao M, Cheng C, Xu H, Saarikettu J, Li Y, Da Y, Yao Z, Silvennoinen O and Yang J. The multifunctional human p100 protein "hooks" methylated ligands. Nat Struct Mol Biol 2007; 14: 779-784.

[12] Li CL, Yang WZ, Chen YP and Yuan HS. Structural and functional insights into human Tudor-SN, a key component linking RNA interference and editing. Nucleic Acids Res 2008; 36: 3579-3589.

[13] Valineva T, Yang J, Palovuori R and Silvennoinen O. The transcriptional co-activator protein p100 recruits histone acetyltransferase activity to STAT6 and mediates interaction between the CREB-binding protein and STAT6. J Biol Chem 2005; 280: 14989-14996.

[14] Yang J, Aittomaki S, Pesu M, Carter K, Saarinen J, Kalkkinen N, Kieff E and Silvennoinen O. Identification of p100 as a coactivator for STAT6 that bridges STAT6 with RNA polymerase II. EMBO J 2002; 21: 4950-4958.

[15] Yang J, Valineva T, Hong J, Bu T, Yao Z, Jensen ON, Frilander MJ and Silvennoinen O. Transcriptional co-activator protein p100 interacts with snRNP proteins and facilitates the assembly of the spliceosome. Nucleic Acids Res 2007; 35: 4485-4494.

[16] Gao X, Zhao X, Zhu Y, He J, Shao J, Su C, Zhang Y, Zhang W, Saarikettu J, Silvennoinen O, Yao Z and Yang J. Tudor staphylococcal nuclease (Tudor-SN) participates in small ribonucleoprotein (snRNP) assembly via interacting with symmetrically dimethylated Sm proteins. J Biol Chem 2012; 287: 18130-18141.

[17] Gao X, Ge L, Shao J, Su C, Zhao H, Saarikettu J, Yao X, Yao Z, Silvennoinen O and Yang J. Tudor-SN interacts with and co-localizes with G3BP in stress granules under stress conditions. FEBS Lett 2010; 584: 3525-3532.

[18] Zhu L, Tatsuke T, Mon H, Li Z, Xu J, Lee JM and Kusakabe T. Characterization of Tudor-sn-containing granules in the silkworm, Bombyx mori. Insect Biochem Mol Biol 2013; 43: 664-674.

[19] Weissbach R and Scadden AD. Tudor-SN and ADAR1 are components of cytoplasmic stress granules. RNA 2012; 18: 462-471.

[20] Gutierrez-Beltran E. Genome-wide analysis of uncapped mRNAs under heat stress in Arabidopsis. Genomics data 2015; 5: 7-8.

[21] Gutierrez-Beltran E, Bozhkov PV and Moschou PN. Tudor Staphylococcal Nuclease plays two antagonistic roles in RNA metabolism under stress. Plant Signal Behav 2015; 10: e1071005.

[22] Gutierrez-Beltran E, Moschou PN, Smertenko AP and Bozhkov PV. Tudor staphylococcal nuclease links formation of stress granules and processing bodies with mRNA catabolism in Arabidopsis. Plant Cell 2015; 27: 926-943.

[23] Yan C, Yan Z, Wang Y, Yan X and Han Y. Tudor-SN, a component of stress granules, regulates growth under salt stress by modulating GA20ox3 mRNA levels in Arabidopsis. J Exp Bot 2014; 65: 5933-5944.

[24] Gao X, Fu X, Song J, Zhang Y, Cui X, Su C, Ge L, Shao J, Xin L, Saarikettu J, Mei M, Yang X, Wei M, Silvennoinen O, Yao Z, He J and Yang J. Poly(A)(+) mRNA-binding protein Tudor-SN regulates stress granules aggregation dynamics. FEBS J 2015; 282: 874-890.

[25] Gao X, Shi X, Fu X, Ge L, Zhang Y, Su C, Yang X, Silvennoinen O, Yao Z, He J, Wei M and Yang J. Human Tudor staphylococcal nuclease (Tudor-SN) protein modulates the kinetics of AGTR1-3'UTR granule formation. FEBS Lett 2014; 588: 2154-2161.

[26] Garcia-Arcos I, Rueda Y, Gonzalez-Kother P, Palacios L, Ochoa B and Fresnedo O. Association of SND1 protein to low density lipid droplets in liver steatosis. J Physiol Biochem 2010; 66: 73-83.

[27] Stoecklin G, Stubbs T, Kedersha N, Wax S, Rigby WF, Blackwell TK and Anderson P. MK2-induced tristetraprolin:14-3-3 complexes prevent stress granule association and ARE-mRNA decay. Embo J 2004; 23: 1313-1324.

[28] Moujalled D, James JL, Parker SJ, Lidgerwood GE, Duncan C, Meyerowitz J, Nonaka T, Hasegawa M, Kanninen KM, GrubmanA, Liddell JR, Crouch PJ and White AR. Kinase inhibitor screening identifies cyclin-dependent kinases and glycogen synthase kinase 3 as potential modulators of TDP-43 cytosolic accumulation during cell stress. PLoS One 2013; 8: e67433.

[29] Thomas MG, Martinez Tosar LJ, Desbats MA, Leishman CC and Boccaccio GL. Mammalian Staufen 1 is recruited to stress granules and impairs their assembly. J Cell Sci 2009; 122: 563-573.

[30] Walker AK, Soo KY, Sundaramoorthy V, Parakh S, Ma Y, Farg MA, Wallace RH, Crouch PJ, Turner BJ, Horne MK and Atkin JD. ALS-associated TDP-43 induces endoplasmic reticulum stress, which drives cytoplasmic TDP-43 accumulation and stress granule formation. PLoS One 2013; 8: e81170.

[31] Lu J and Holmgren A. The thioredoxin antioxidant system. Free Radic Biol Med 2014; 66: 75-87.

[32] Yoshihara E, Masaki S, Matsuo Y, Chen Z, Tian H and Yodoi J. Thioredoxin/Txnip: redoxisome, as a redox switch for the pathogenesis of diseases. Front Immunol 2014; 4: 514.

[33] Gutierrez T and Simmen T. Endoplasmic reticulum chaperones and oxidoreductases: critical regulators of tumor cell survival and immunorecognition. Front Oncol 2014; 4: 291.

[34] Su C, Zhang C, Tecle A, Fu X, He J, Song J, Zhang W, Sun X, Ren Y, Silvennoinen O, Yao Z, Yang X, Wei M and Yang J. Tudor staphylococcal nuclease (Tudor-SN), a novel regulator facilitating G1/S phase transition, acting as a co-activator of E2F-1 in cell cycle regulation. J Biol Chem 2015; 290: 7208-7220.

[35] De Leeuw F, Zhang T, Wauquier C, Huez G, Kruys V and Gueydan C. The cold-inducible RNA-binding protein migrates from the nucleus to cytoplasmic stress granules by a methylation-dependent mechanism and acts as a translational repressor. Exp Cell Res 2007; 313: 4130-4144.

[36] Dunning LT, Dennis AB, Park D, Sinclair BJ, Newcomb RD and Buckley TR. Identification of cold-responsive genes in a New Zealand alpine stick insect using RNA-Seq. Comp Biochem Physiol Part D Genomics Proteomics 2013; 8: 24-31.

[37] Hofmann S, Cherkasova V, Bankhead P, Bukau B and Stoecklin G. Translation suppression promotes stress granule formation and cell survival in response to cold shock. Mol Biol Cell 2012; 23: 3786-3800.

[38] Carpio MA, Lopez Sambrooks C, Durand ES and Hallak ME. The arginylation-dependent association of calreticulin with stress granules is regulated by calcium. Biochem J 2010; 429: 63-72.

[39] Carpio MA, Decca MB, Lopez Sambrooks C, Durand ES, Montich GG and Hallak ME. Calreticulin-dimerization induced by post-translational arginylation is critical for stress granules scaffolding. Int J Biochem Cell Biol 2013; 45: 1223-1235.

[40] Thedieck K, Holzwarth B, Prentzell MT, Boehlke C, Klasener K, Ruf S, Sonntag AG, Maerz L, Grellscheid SN, Kremmer E, Nitschke R, Kuehn EW, Jonker JW, Groen AK, Reth M, Hall MN and Baumeister R. Inhibition of mTORC1 by astrin and stress granules prevents apoptosis in cancer cells. Cell 2013; 154: 859-874.

[41] Sundstrom JF, Vaculova A, Smertenko AP, Savenkov EI, Golovko A, Minina E, Tiwari BS, Rodriguez-Nieto S, Zamyatnin AA Jr, Valineva T,

Saarikettu J, Frilander MJ, Suarez MF, Zavialov A, Stahl U, Hussey PJ, Silvennoinen O, Sundberg E, Zhivotovsky B and Bozhkov PV. Tudor staphylococcal nuclease is an evolutionarily conserved component of the programmed cell death degradome. Nat Cell Biol 2009; 11: 1347-1354.
[42] Garg AD, Krysko DV, Vandenabeele P and Agostinis P. The emergence of phox-ER stress induced immunogenic apoptosis. Oncoimmunology 2012; 1: 786-788.
[43] Kageyama K, Ihara Y, Goto S, Urata Y, Toda G, Yano K and Kondo T. Overexpression of calreticulin modulates protein kinase B/Akt signaling to promote apoptosis during cardiac differentiation of cardiomyoblast H9c2 cells. J Biol Chem 2002; 277: 19255-19264.
[44] Prathyuman S, Sellappa S, Joseph S and Keyan KS. Enhanced calreticulin expression triggers apoptosis in the MCF-7 cell line. Asian Pac J Cancer Prev 2010; 11: 1133-1136.
[45] Yang Z, Jakymiw A, Wood MR, Eystathioy T, Rubin RL, Fritzler MJ and Chan EK. GW182 is critical for the stability of GW bodies expressed during the cell cycle and cell proliferation. J Cell Sci 2004; 117: 5567-5578.
[46] Aizer A, Kafri P, Kalo A and Shav-Tal Y. The P body protein Dcp1a is hyper-phosphorylated during mitosis. PLoS One 2013; 8: e49783.
[47] Alberts BJA, Lewis J, et al. Molecular biology of the cell. Biochem Mol Biol Education 2003; 13: 212-214.
[48] Moutaoufik MT, El Fatimy R, Nassour H, Gareau C, Lang J, Tanguay RM, Mazroui R and Khandjian EW. UVC-induced stress granules in mammalian cells. PLoS One 2014; 9: e112742.