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Structural Analysis of Nuclear Stress Bodies Formed in Response
to Thermal Stress

(熱ストレスに応答した核内ストレス体の構造解析)

2019 年 9 月
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Background and Purpose:

Nuclear stress bodies (nSBs) are primate-specific membrane-less subnuclear structures that are formed in response to thermal and chemical stresses. The assembly of nSBs is initiated alongside HSF1-dependent transcription of the primate-specific highly repetitive satellite III (HSATIII) long noncoding RNA (lncRNA) and heat shock-induced HSF1 aggregation. HSATIII lncRNAs are retained on chromosomes near their own transcription sites for several hours and recruit various RNA-binding proteins (RBPs). It is still poorly understood why nSBs are assembled in primates' stress response events. Decoding the protein composition and structural organization of nSBs were our first approach to elucidate the nSBs functions in primates' stress response events. Recently, our lab has identified 141 proteins as components of nSBs, most of which are likely RBPs involved in RNA splicing, processing, modification, and export.

Here, we elucidate the existence of two distinct nuclear stress bodies containing different sets of RNA-binding proteins that are formed with HSATIII upon thermal stress exposure, nSB-M and nSB-S. In addition, we report a previously unrecognized thermal stress-induced recruitment of the N⁶-methyladenosine (m⁶A) RNA modification machinery to nSBs. m⁶A is the most abundant RNA modification in eukaryotes and has been reported to have crucial regulatory roles in cancer proliferation, sex determination, cell differentiation, neuronal function, meiosis, circadian rhythm, and chromosomal silencing. Hence, m⁶A machinery recruitment to nSBs proposes a novel nSBs-dependent regulatory function of m⁶A in primates' stress response events.

Methods:

CRISPR/Cas9, ASO and siRNA were used for depletion of target of interest. RNA FISH and IF were used for detection of HSATIII, m⁶A RNAs, and proteins of interest. m⁶A Dot Blot was used for nSB-m⁶A RNAs enrichment detection. ChIRP was used for nSBs purification. Western blot was used for

detection of proteins of interest.

Results:

We observed adjacent localization of SAFB and HNRNPM, nSBs proteins, foci formed in response to thermal stress. First we confirmed that both entities utilize HSATIII lncRNAs as architectural scaffolds. Triple staining combined HSATIII RNA-FISH and immunofluorescence experiments have shown that both HNRNPM and SAFB foci recruit distinct RNA-binding proteins sets. We found that upon thermal stress exposure, HNRNPA1 and HNRNPH1 are strictly recruited to HNRNPM-foci, we named nSB-M, while SLTM, and NCOA5 are strictly recruited to SAFB-foci, we named nSB-S.

On the other hand, we detected a thermal stress-induced sequestration of the m⁶A writers, METTL3 and WTAP, and the m⁶A reader, YTHDC1, to nSBs. In addition, anti-m⁶A modification antibody confirmed the localization of m⁶A modified RNAs at nSBs. HSATIII knockdown was enough to abolish both stress-induced m⁶A machinery recruitment and m⁶A RNAs localization to nSBs. Moreover, we found that HSATIII transcripts are methylated and that at least HSATIII serves as target substrate for m⁶A machinery at nSBs. RNA-MS analysis has identified GGAAU, the highly repetitive sequence of HSATIII, as a novel m⁶A consensus motif. m⁶A machinery showed a unique recruitment/enrichment pattern at nSBs. During stress, the m⁶A machinery is recruited to nSBs and enriched upon stress removal 1h post-stress then releases from nSBs at 4h late recovery with exception of YTHDC1 that exhibit prolonged enrichment at nSBs. Finally, we found that TDP43 depletion dramatically inhibited nSBs-m⁶A enrichment highlighting a novel regulatory role of TDP43 in stress-induced m⁶A RNA modification.

Discussion:

We identified two distinct nSBs (nSB-S and nSB-M) containing different sets of RNA-binding

proteins that are formed with HSATIII arcRNAs upon thermal stress exposure. It is possible that nSB-S and nSB-M represent different stages of the synthesis and maturation of nSBs after thermal stress-induced HSATIII arcRNA synthesis. It is also possible that HNRNP proteins with high affinity to RNAs may rapidly bind to HSATIII to form the dense core of nSBs, and then SAFB and other components subsequently join the surrounding area to form the larger nSB-Ss. On the other hand, we report a previously unrecognized thermal stress-induced recruitment of m⁶A machinery to nSBs and m⁶A RNAs localization to most HSATIII thermal stress-induced foci. To investigate the target RNAs of the m⁶A machinery in nSBs, we re-purified HSATIII and found that HSATIII is enriched with m⁶A modification. In addition, RNA-MS has identified the third A of GGAAU in the highly repetitive HSATIII sequence as the major m⁶A sites. Moreover, RNA-MS has identified a small population of HSATIII m⁶A consensus that exhibit two adjacent m⁶A modification at AA of GGAAU. The known consensus motif of m⁶A sites is GGACU which contains one base mismatch with m⁶A sites in HSATIII, GGAAU lacking the C residue after A. The C residue in the m⁶A consensus has been reported to be essential for m⁶A deposition. Hence, shifting m⁶A consensus motif from GGACU to GGAAU might be thermal stress-dependent. Meanwhile, we cannot rule out the possibility that m⁶A machinery components and/or mechanism of action upon stress is distinct from normal condition. To elucidate the mechanism of action of the m⁶A machinery at nSBs, we screened the dynamics of m⁶A machinery components during stress/recovery phases. Remarkably, we found that m⁶A machinery has a unique recruitment pattern to nSBs, in which m⁶A machineries are recruited during stress and deposit m⁶A modification at HSATIII with relative enrichment in the early post-stress recovery stage then released from nSBs by 4h post-stress recovery stage. Only YTHDC1, the m⁶A reader, showed prolonged enrichment that might be due to additional non-m⁶A related function in nSBs during the late recovery stage. TDP43 was reported to specifically bind to GGAAU RNA foci formed in SCA31 disease. TDP43 depletion dramatically downregulated nSBs-m⁶A. Therefore, it raises the intriguing possibility

that TDP43 acts as the adaptor to link the m⁶A regulatory factors to HSATIII lncRNAs with GGAAU repeat sequences under stressed condition.

Conclusion:

- The nSBs components SAFB and HNRNPM exhibit adjacent localizations in response to thermal stress.
- Both SAFB-foci and HNRNPM-foci utilize HSATIII lncRNA as architectural scaffold.
- SAFB-foci (nSB-S) and HNRNPM-foci (nSB-M) sequester two distinct sets of RNA-binding proteins. SLTM and NCOA5 are strictly recruited to nSB-S while HNRNPA1 and HNRNPH1 are strictly recruited to nSB-M.
- m⁶A writers and readers' machineries are sequestered to nSBs upon thermal stress.
- m⁶A methylated RNAs are localized at HSATIII-based nSBs.
- HSATIII lncRNAs transcripts are the target substrates of m⁶A machineries at nSBs.
- The m⁶A consensus within HSATIII is the highly repetitive sequence GGAAU that is distinct from the global GGACU m⁶A consensus.
- With exception of YTHDC1, all m⁶A factors follow a consistent recruitment pattern at nSBs where they are gradually sequestered during thermal stress (2 hours HS) and enriched at early recovery stage (1 hour post-stress) then rapidly released from nSBs at late recovery stage (4 hours post-stress)
- TDP43 is a possible modulator of m⁶A methylation in nSBs.