科技部補助專題研究計畫成果報告
期末報告

PRMT1 催化之蛋白質精胺酸甲基化對壓力顆粒及旁斑點組成及肌萎縮側索硬化症病因關聯

計 畫 類 別 ： 個別型計畫
計 畫 編 號 ： MOST 106-2320-B-040-018-
執 行 期 間 ： 106年08月01日至107年10月31日
執 行 單 位 ： 中山醫學大學生物醫學科學學系（所）

計畫主持人： 李娟

計畫參與人員： 碩士級-專任助理：張建評

中 華 民 國 108 年 01 月 22 日

中文摘要：肌萎縮性側索硬化(ALS)的致病原因與細胞內沒有膜外包的次胞器如核醣蛋白顆粒(RNP)的形成調節有關。多數RNP 顆粒內的 RNA 結合蛋白同時也在 ALS 病人運動神經元的堆積中發現。這些蛋白除了帶有類普恩序列或低複雜區外，多含有可被精胺酸甲基轉移酶 PRMT1修飾的 RGG 或 RG 重複序列。RNA 結合蛋白低複雜區的交互作用不僅生理上影響RNP 顆粒在細胞內的組裝及動態變化，也是病理上ALS蛋白聚集形成的關鍵。研究指出ALS中細胞核旁斑點及其架構之長非編碼核酸（lncRNA）NEAT1的表現增加，在神經母細胞瘤細胞中降低PRMT1的表現或抑制其活性會誘發期衰老及增加p53及其下游p21和PAI-1的表現。資料庫分析顯示神經母細胞瘤細胞中PRMT1的表達與 NEAT1負相關。 我們觀察到抑制PRMT1引發NEAT1表現和旁斑點形成增加。預測催化旁斑點和其他 RNP 顆粒RNA 結合蛋白的甲基化作用，和引發沉澱並發展成神經退化性疾病有關。我們也將一來自的RG重複序列轉染至細胞中觀察表現和蛋白質精胺酸甲基化，並且將進一步以分析此修飾和低複雜區結合乃至糾結形成的影響。

中文關鍵詞：蛋白質精胺酸甲基化， 蛋白質精胺酸甲基轉移酶1， 旁斑點， 細胞核旁斑點組合轉錄物1

英文摘要：The etiology of amyotrophic lateral sclerosis (ALS) is related to the regulation of non-membrane bounded sub-organelles or RNP granules. Numerous RNA binding proteins in the granules overlap with the proteins accumulated in the motor neurons of the ALS patients. Besides prion-like sequences or low complexity domains, the majority of these proteins also contain repeated RGG or RG sequences that can be modified by the major protein arginine methyltransferase (PRMT) PRMT1. Stable PRMT1-knockdown (PRMT1-KD) cells showed reduced growth rates and cell cycle arrest at G2/M. They also exhibited senescent phenotypes and increased p53 expression. p21 and PAI-1, which are two p53 downstream targets critical for senescence, were significantly induced in SK-N-SH cells subjected to either PRMT1-KD or inhibitor treatment. Nuclear paraspeckle formation and its scaffold long non-coding RNA (lncRNA) NEAT1 have been shown to be up-regulated in ALS. We found that the expression of PRMT1 is negatively correlated with NEAT1 in neuroblastoma. When the PRMT1 gene is knocked down in a neuroblastoma cell line SK-N-SH, the expression level of NEAT1 and paraspeckle formation are highly elevated. PRMT1 catalyzed methylation of the RNA binding proteins should participate in the formation of paraspeckles and other RNP granules. Besides, we expressed an RG-rich sequence in cells to monitor the effects of the modification on the association of LCD and protein aggregation.

英文關鍵詞：protein arginine methylation, PRMT1, paraspeckle, NEAT1

Final report of MOST 106-2320-B-040-018

PRMT1 催化之蛋白質精胺酸甲基化對壓力顆粒及旁斑點組成及肌萎縮側索硬化症病因關聯

PRMT1 catalyzed protein arginine methylation on the physiological assembly of stress granules and paraspeckles with the implication in ALS etiology

Introduction and Specific aims

In this proposal, we proposed to study the involvement of the major protein arginine methyltransferase (PRMT) PRMT1 in neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). The etiology of ALS is related to the regulation of non-membrane bounded sub-organelles. We proposed to focus on the cytoplasmic stress granule that are comparable to the cytoplasmic inclusions found in ALS neurons and the nuclear body paraspeckle that has been related to ALS recently. Numerous RNA binding proteins in the RNP granules overlapped with the ALS accumulated proteins containing prion-like sequences or low complexity domain (LCD). A majority of the proteins contain the RGG or RG abundant sequences that can be modified by PRMT1. Interaction through the low complexity regions by liquid-liquid phase separation has been shown to be the key for physiological assembly and dynamics of the RNP granules and pathological aggregation in patient neurons. PRMT1 catalyzed modification on the proteins with LCD might be a critical modifier for the pathogenesis. TDP-43 and FUS that are mutated in familial ALS patients and present in cytoplasmic inclusions have been considered as the critical clues of ALS pathogenesis. Protein arginine methylation of FUS by PRMT1 has been shown to reduce cytoplasmic accumulation. In this study, we will investigate the putative methylation of the ALS core TDP-43 and the RG dipeptide translated from *C9ORF72*, another gene mutated in ALS. Recently, paraspeckle formation and its scaffold long non-coding RNA (lncRNA) NEAT1 have been shown to be up-regulated in ALS. From the results of online database analyses and knockdown experiments, we found that the expression of PRMT1 negatively correlated with NEAT1 in neuroblastoma. We thus would like to investigate why and how PRMT1 KD resulted in NEAT1 up-regulation and paraspeckle formation, especially in neuroblastoma cells. Paraspeckle proteins such as SFPQ and NonO have been shown to be arginine methylated. We had encountered these proteins in our previous studies (Chang et al., 2013; Hung et al., 2009) and would like to investigate the relation of PRMT1-catalyzed methylation of these proteins with their RNA binding activity and paraspeckle formation. Besides, we have analyzed PRMT1 methylation of an RNA binding protein SERBP1 and the assembly and disassembly of cytoplasmic stress granules (Lee et al., 2013).

Background

We are interested in the disease mechanism of ALS because we had been involved in the studies of poly-glutamine neurodegenerative diseases (Tsai et al., 2005; Tsai et al., 2003; Tsai et al., 2004; Wen et al., 2003) that also have protein inclusion problems. As we had studied

stress granule assembly/disassembly (Lee et al., 2013), we noticed their close relationship with ALS etiology. Besides, we always aware that the typical PRMT1 substrate FUS and its arginine methylation are involved in ALS. We studied PRMT1 and PRMT8 in the neural system using SK-N-SH neuroblastoma cells and showed that PRMT1 might be involved in paraspeckle formation and thus can affect ALS in a novel pathway. We propose the three-year project to investigate the effects of PRMT1 catalyzed protein arginine methylation on the physiological assembly of stress granules and paraspeckles with the implication in ALS etiology.

RNA and proteins can be specifically assembled into ribonucleoprotein particles (RNPs) or complexes. RNP bodies or RNA granules are non-membrane-bound cellular compartments such as processing bodies and stress granules in the cytoplasm or nucleoli and Cajal bodies in the nucleus. These physiological assemblies are highly dynamic with their components exchange with the surroundings constantly. Imbalanced RNA metabolism and protein homeostasis may impair the dynamics of RNP bodies and be the key factor for the pathogenesis of neurodegenerative RNP inclusion diseases. Typically, ALS and frontotemporal lobar degeneration (FTLD) may originate from altered assembly or clearance of SGs (Ramaswami et al., 2013).

ALS is an adult-onset neurodegenerative disease with degenerated motor neurons, progressive muscle weakening and paralysis. It is characterized by motor neuron loss with the degenerating neurons accumulated with protein aggregates. The major protein component of the inclusions is the 43-kD TAR-DNA–binding protein (TDP-43), a DNA- and RNA binding protein with two RRMs and a C-terminal a Gly-rich domain. Similarly, another RNA-binding protein fused in sarcoma/translocated in liposarcoma (FUS/TLS) has been shown to be deposited in the cytoplasmic inclusions of ALS and FTLD (Shelkovnikova et al., 2014). Mutations in *TDP-43* and *FUS* have been identified in ALS patients. Mutations in *TDP-43* are about 3% of patients with familial amyotrophic lateral sclerosis (fALS) and in 1.5% of patients with sporadic ALS. Mutations of FUS account for about 5% of familial ALS cases and also are present in FTLD (Lattante et al., 2013).

The liquid-liquid phase separation model proposed that the non-membrane-bound compartments behave like condensed liquid phases of the cytoplasm or nucleoplasm and the components within can dynamically exchange with the surroundings (Kato et al., 2012). These bodies are rich in specific RNA binding proteins and sometimes RNA may nucleate the assembly. Mutations or increased concentration of certain RNA binding proteins such as FUS may convert a liquid state to an aggregated (solid) state, which is reminiscent of the pathological state seen in ALS patients. Proteins with of low complexity domains (LCDs) have been considered "prion-like" as the structures are close to the cross –structures typical of the fibrils in neurodegenerative prion or Alzheimer's disease (Kato et al., 2012). Reversible aggregation of these regions may facilitate proteins to form higher-order assemblies and microscopically visible RNP granules (Toretsky and Wright, 2014). In fact, TDP-43, FUS and many functional RNP proteins as well as proteins that aggregates in neural degenerative

diseases contain LCD. Pathogenic mutations of RNA-binding proteins such as hnRNPA1 have been shown to strengthen fibril formation and may lead to multi-system proteinopathy (Kim et al., 2013).

Another hexanucleotide repeat expansion in the gene *C9ORF72* has been identified as the most frequent mutation of ALS and FTLD. Non-ATG translation of the hexanucleotide repeat expansion in *C9ORF72* can produce five dipeptide repeat (DPR) polypeptidess. The only toxic glycine-arginine (GR) or proline arginine (PR) polypeptides interact with the LCDs in the RNA binding proteins and can induce spontaneous assembly of poorly dynamic SGs (Lee et al., 2016).

Protein arginine methylation is a post-translational modification implicated in many important physiological functions such as epigenetic regulation, RNA processing, DNA repair, and cellular signaling (Bedford and Clarke, 2009). Protein arginine methyltransferases are widely distributed enzymes in eukaryotes responsible for the transfer of methyl groups from the donor S-adenosylmethionine (SAM) to guanidino nitrogens in specific arginines of the substrate proteins. The posttranslational modification (PTM) catalyzed by PRMTs occurs in a wide spectrum of proteins mostly involved in nucleic acid binding/processing with the glycine arginine rich (GAR) or typical arginine-glycine (RG) or arginine-glycine-glycine (RGG) and RXR sequences (Bedford and Clarke, 2009). Systematic proteomic analyses showed that a good portion of the modified arginines appear to be present in the arginine-proline (RP) context (Guo et al., 2014).

According to the addition of methyl groups to different nitrogen atoms of arginines, the PRMTs can be divided as type I methyltransferase that add the second methyl groups to the same guanidino nitrogen to form asymmetric dimethylarginines (ADMA), the type II methyltransferase that add the second methyl groups to the different guanidino nitrogen to form symmetric dimethylarginines (SDMA), and the type III enzyme that catalyze the formation of monomethylarginines (MMA) only. By now there are nine different PRMT genes identified in mammals by their sequence homology with the first identified PRMT1 and numbered according the identification order. The methyltransferase activities PRMT1, 2, 3, 4 (CARM1), 6 and 8 have been characterized as type I while PRMT5 and PRMT9 as the type II PRMT. PRMT7 is the only type III enzyme (Zurita-Lopez et al., 2012). The methylation will not change the positive charge on the arginine residue but can change the hydrogen bond donor to a hydrophobic methyl group, thus might affect further molecular interactions.

Of all PRMTs, PRMT1 is the first identified (Lin et al., 1996) and the most widely distributed protein arginine methyltransferase in mammals (Wang and Li, 2012). PRMT1 is the predominant protein arginine methyltransferase responsible for more than 85% of asymmetric dimethylarginine in mammals (Tang et al., 2000). It is well studied with broad substrate spectrum and plays roles in various cellular processes. For example, asymmetric dimethylation of histone H4 Arg-3 (H4R3me2a) by PRMT1 is part of the epigenetic histone code and thus

PRMT1 is a coactivator for some nuclear receptors as well as various transcription factors including p53 and YY1. Involvement of PRMT1 in signaling pathways was suggested by early identification of PRMT1 as an interacting partner of the cytoplasmic domain of INF receptor (Abramovich et al., 1997). Besides, PRMT1 can rapidly methylate estrogen receptor upon estrogen binding and facilitates further downstream signaling (Le Romancer et al., 2008). The mammalian forkhead transcription factor FOXO1 critical for oxidative stress resistance can be methylated by PRMT1. The methylation blocks Akt phosphorylation of FOXO1 and leads to the nuclear retention of FOXO1 and the stimulation of FOXO1 transcriptional activity (Yamagata et al., 2008). Similar cross talk was observed in arginine methylation and phophorylation of BAD (Sakamaki et al., 2011). Furthermore, PRMT1 is implicated in RNA processing and some nucleic acid binding proteins such as hnRNPA1, fibrillarin and Sam68 are among the earliest reported substrates of PRMT1 (Nicholson et al., 2009; Pahlich et al., 2006). FUS and other FET family members including EWS and TAF15 are also PRMT1 substrates. Our lab had shown that arginine methylation of SERBP1 (Lee et al., 2014) and CNBP (Wei et al., 2014) can affect the subcellular localization or RNA binding activity respectively.

Stress granule formation is generally considered to protect stalled mRNPs for their return to translation when the cells recover from stress. Many RNA binding proteins, various translation initiation factors, and mRNPs with mRNAs stalled at translation initiation are in SGs dynamically. SGs may be considered as a dynamic triage center, sorting mRNAs for storage, decay or re-initiation under stressful conditions. Inhibition of methylation appears to affect the assembly and disassembly dynamics of TDRD3-containing SGs (Goulet et al., 2008). We further demonstrate that adenosine periodate (AdOx) treatment delays the association/dissociation of SERBP1 with stress granules (Lee et al., 2014). Hypomethylation retains SERBP1 in the nucleus/nucleolus regardless of arsenite. Addition of AdOx increases the proportion of cells with granules, but decreases co-localization of FMRP granules with T-cell intracellular antigen-1 (TIA-1)-stained SGs. Nevertheless, recently Tsai et al showed that overexpression of G3BP1 induced SGs and the RGG domain is required for the induction. PRMT1 can also co-localize in the SGs. All these reports indicate that arginine methylation can participate in SG formation but the effects might be different on different proteins.

Paraspeckles are one type of the nuclear bodies identified close to the nuclear speckles (nuclear body for snRNP assembly) in most of the cultured cells (Nakagawa and Hirose, 2012). Paraspeckles are also apparent in ALS motor neurons, and NEAT1, a highly abundant lncRNA scaffold of paraspeckles, is up-regulated in the related condition frontotemporal lobar degeneration (Nishimoto et al., 2013). Paraspeckles are stress-responsive structures induced by viral infection, proteasome inhibition, and differentiation (Imamura et al., 2014). There are ~40 known paraspeckle proteins, mostly abundant nuclear RNA binding proteins enriched in

RNA recognition motifs (RRMs), zinc finger, and K homology domains (Naganuma et al., 2012). The core paraspeckle proteins that are essential for paraspeckle formation are two Drosophila behavior/human splicing (DBHS) proteins: non-POU domain-containing octamer-binding protein (NonO/p54nrb) and splicing factor proline- and glutamine-rich (SFPQ; also commonly known as polypyrimidine tract-binding protein-associated splicing factor PSF). NonO and SFPQ are homologues and binding partners and have been described as multifunctional nuclear proteins, implicated in subnuclear body formation; transcription initiation; coactivation and corepression; constitutive and alternative splicing; transcriptional termination; and DNA repair. Because SFPQ binds to the IL-8 promoter to suppress its expression, sequestering SFPQ to paraspeckles by high NEAT1 level can derepress the expression of IL-8 (Imamura et al., 2014). Other DBHS paraspeckle protein components include paraspeckle protein 1 (PSPC1) that can form intertwined dimer with NonO (Knott et al., 2016). Besides, FUS has been reported to be a core paraspeckle protein.

Like FUS, the core paraspeckle protein NonO and SFPQ can be arginine methylated. We had identified NonO to be in a spot with anti-methylarginine signals by mass spectrometry. Immunoprecipitated NonO was also detected by anti-dimethylarginine antibodies (Hung et al., 2009). We had also identified SFPQ as a putative candidate that due to protein methylation status, was differentially recognized by an anti-Sm positive antisera from an SLE patient (Chang et al., 2013).

Snijders et al. reported the methylation of SFPQ (Snijders et al., 2015). They showed that SFPQ can be methylated by PRMT1 and also identified SFPQ arginine methylation sites by mass spectrometry (MS) (R7,9,19,25 for DMA and 9,32 and 693 for MMA). Arginine methylation did not affect the interaction of SFPQ with NONO. Treatment with broad methyltransferase inhibitor AdOx or specific PRMT inhibitor AMI-1 reduced SFPQ binding to mRNA. Hu *et al* showed that CARM1 can modify NonO (p54nrb) to reduce its association with mRNAs containing IR*Alus* and can work as a transcriptional regulator to suppress the expression of *NEAT1*. CARM1 thus regulate the nuclear retention of mRNAs containing IR*Alus* at two different levels (Hu et al., 2015). In Hu's study, they showed that beside CARM1, NonO can also be methylated by PRMT1 and PRMT6 but to a less level. The methylation sites by MS analyses showed that R357, R365 and R378 are major methylation sites on p54nrb by CARM1 while R73, R184 and R207 maybe methylated by other PRMT as they were present in both scramble and CARM1 knockdown Flag-p54nrb IP samples.

As discussed before, RG or RGG rich sequences that are typical PRMT recognized arginine methylation sites are often present in proteins with low complexity sequences or are within the LCD. Many proteins involved in stress granule or paraspeckle formation overlap with the proteins identified in ALS aggregates. Most recent studies showed that hexanucleotide repeat expansion in the gene *C9ORF72* can results in non-ATG translation of five dipeptide repeat (DPR) polypeptidess. Among them only glycine-arginine (GR) or proline arginine (PR) peptides are toxic. These poly-GR and PR interact with the LCDs in the RNA binding proteins

and can induce spontaneous assembly of poorly dynamic stress granules. Intriguingly, two major type I and type II PRMT, PRMT1 and PRMT5 respectively, are among the genetic modifiers of GR/PR toxicity confirmed in fruit flies (Lee et al., 2016). It is thus tempting to propose that arginine methylation might affect the interaction of the protein components of cytoplasmic/nuclear RNP granules or non-membrane bounded sub-organelles and be related to the etiology of ALS.

We suspect that PRMT1 catalyzed arginine methylation can affect ALS at different levels:

1. Through paraspeckle: PRMT1 can repress NEAT1 expression and paraspeckle formation at transcriptional level or by affecting subcellular localization, RNA binding activity or stability of paraspeckle proteins at the post-translational level.
2. Through the mechanism of stress granule assembly and protein aggregation: PRMT1 can directly methylate a variety of proteins that are involved in stress granule formation that overlapped with ALS-causing proteins. How arginine methylation at the RG/RGG sequences in these proteins affects the association of LCD can be the core of protein aggregation in ALS.

In this study, we thus planned to explore the putative involvement of PRMT1 catalyzed methylation of the RNA binding proteins in paraspeckles as well as stress granules, and their putative participation in the development of neurodegenerative disease. The study should provide future directions and implications in the therapy of ALS and even be further extended to other neurodegenerative diseases.

RESULTS

PART 1. Published paper

Lee, YJ, Chang, WW, Chang, CP, Liu, TY, Chuang, CY, Qian, K, Zheng, YG and **Li, C**. (2018) Downregulation of PRMT1 promotes the senescence and migration of a non-*MYCN* amplified neuroblastoma SK-N-SH cells. **Scientific Reports** (accepted)

Abstract:

Protein arginine methyltransferase 1 (PRMT1) catalyzing the formation of asymmetric dimethylarginines has been implicated in cancer development, metastasis, and prognosis. In this study, we investigated the effects of low PRMT1 levels on a non-*MYCN* amplified neuroblastoma SK-N-SH cell line. Stable *PRMT1*-knockdown (*PRMT1*-KD) cells showed reduced growth rates and cell cycle arrest at G_2/M. They also exhibited senescent phenotypes and increased p53 expression. p21 and PAI-1, which are two p53 downstream targets critical for senescence, were significantly induced in SK-N-SH cells subjected to either *PRMT1*-KD or inhibitor treatment. The induction was suppressed by a p53 inhibitor and marginal in a p53-null SK-N-AS cell line, suggesting dependence on p53.In general, the DNA damage and ROS levels of the *PRMT1*-KD SK-N-SH cells were slightly increased. Their migration activity also increased with the induction of PAI-1. Thus, PRMT1 downregulation released the repression of cellular senescence and migration activity in SK-N-SH cells. These results might partially

explain the poor prognostic outcome of low PRMT1 in a non-*MYCN*-amplified cohort and indicate the multifaceted complexity of PRMT1 as a biological regulator of neuroblastoma.

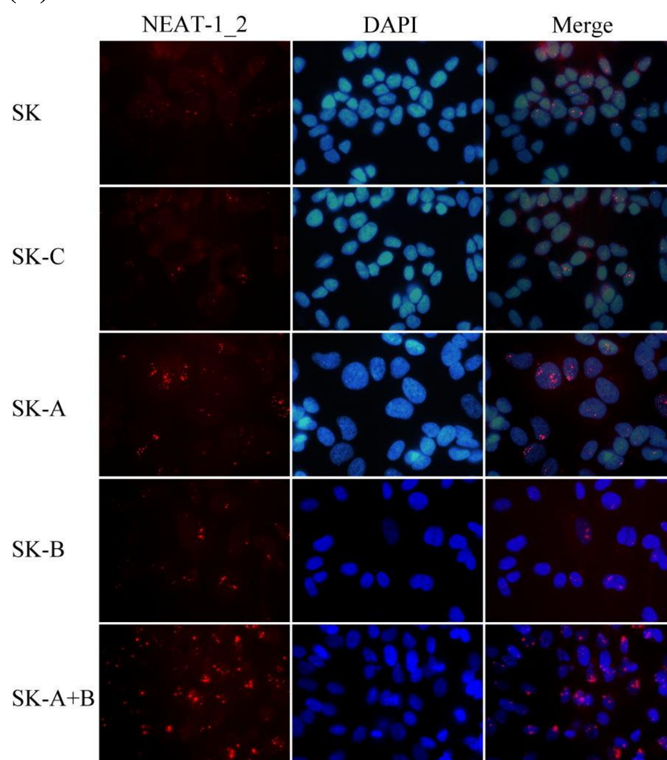
PART 2. Unpublished results

Part of the results of this studies that have not been published are shown below.

1. **Induced NEAT1 lncRNA expression and elevated paraspeckle formation in PRMT1 KD cells**

   Analyses of the expression correlation of the genes by the online database r2 (http://hgserver1.amc.nl/cgi-bin/r2/main.cgi) showed that expression of PRMT1 negatively correlated with NEAT1, similar to the relationship between CARM1 and NEAT1 in neuroblastoma. Interestingly, we found significant NEAT1 induction and paraspeckle formation in the neuroblastoma PRMT1 KD SK-N-SH cells. We then detected the NEAT1 RNA expression by RT-qPCR and in situ hybridization. The NEAT1 RNA was highly induced in PRMT1 knockdown cells and accumulated as small dots in the nucleus (Fig. 1A). The major paraspeckle protein NonO was also present as concentrated dots in the nucleus in PRMT1-KD cells (Fig. 1B). These dots are likely to be paraspeckles formed after PRMT1 KD with NEAT1 induction. Our results thus indicate a novel connection of PRMT1 and NEAT1 expression/paraspeckle formation.

(A)



(B)

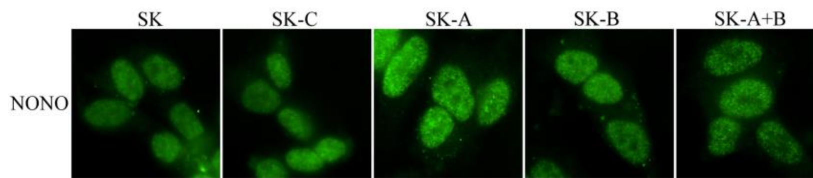


Fig. 1. Induced NEAT1 lncRNA expression and elevated paraspeckle formation in PRMT1-KD neuroblastoma SK-N-SH cells. (A) In situ hybridization of SK-N-SH cells using RNA probe for NEAT1_2. (B) Immunofluorescent analyses of SK-N-SH cells using anti-NonO antibodies.

2. Expression of exotic recombinant protein with the RGG rich sequences

RGG box with repeating RG/RGG sequences interrupted by few amino acids are present in many RNA binding proteins. Most of the proteins contain other RNA binding segments such as RRM domain and KH domain. The sequences usually last for around 10 amino acids like in hnRNP or 20-30 in some proteins, but in fibrillarin, the RGG box in the N-terminus can be extended to about 80 amino acid residues. In the residues from residue 8-72, there are 15 Rs and 47 Gs. Asymmetric dimethylarginines in fibrillarin has been identified in the early days by traditional protein sequencing (Lischwe et al., 1985). Our lab had used mouse fibrillarin as the substrate protein for in vitro methylation reactions. We had a construct expressing the N-terminal fibrillarin in *E. coli* as a RGG source in the pET vector. We also successfully placed PRMT1 in the same construct to express and purify arginine methylated RGG box protein in large amount (Wei HM, Master thesis of Department of Biomedical Sciences, Chung Shan Medical University, 2013).

We prepared GFP- tagged constructs to express full-length fibrillarin or the RGG sequence from fibrillarin. We transfected the constructs into HeLa and neuro2A cells and examined the subcellular localization of GFP-RG or GFP-fibrillarin by fluorescent microscopy. All constructs showed stronger nuclear than cytoplasmic distribution. Besides, the GFP-RG proteins form large granules in the nucleus while the GFP-fibrillarin proteins form smaller granules in the nucleus. These granules are likely to be the nucleoli. We then stained the transfected cells with an asymmetric dimethylarginine (ADMA)-specific antibody ASYM24 to examine if the protein arginine methylation pattern might be affected after the expression of exotic GFP-RG or GFP-fibrillarin proteins. ADMA-containing proteins detected by ASYM24 are clearly located in the nucleus but not the nucleoli. The distribution pattern are similar whether the cells express GFP fusion proteins or not. The expression of exotic GFP-RG or GFP-fibrillarin proteins in the nucleoli did not increase the detection by ASYM24 in these regions. The localization of the protein arginine methyltransferase PRMT1 was not affected with the exotic expression of GFP-RG or GFP-fibrillarin proteins. Similar results were obtained in neuro2a cells.

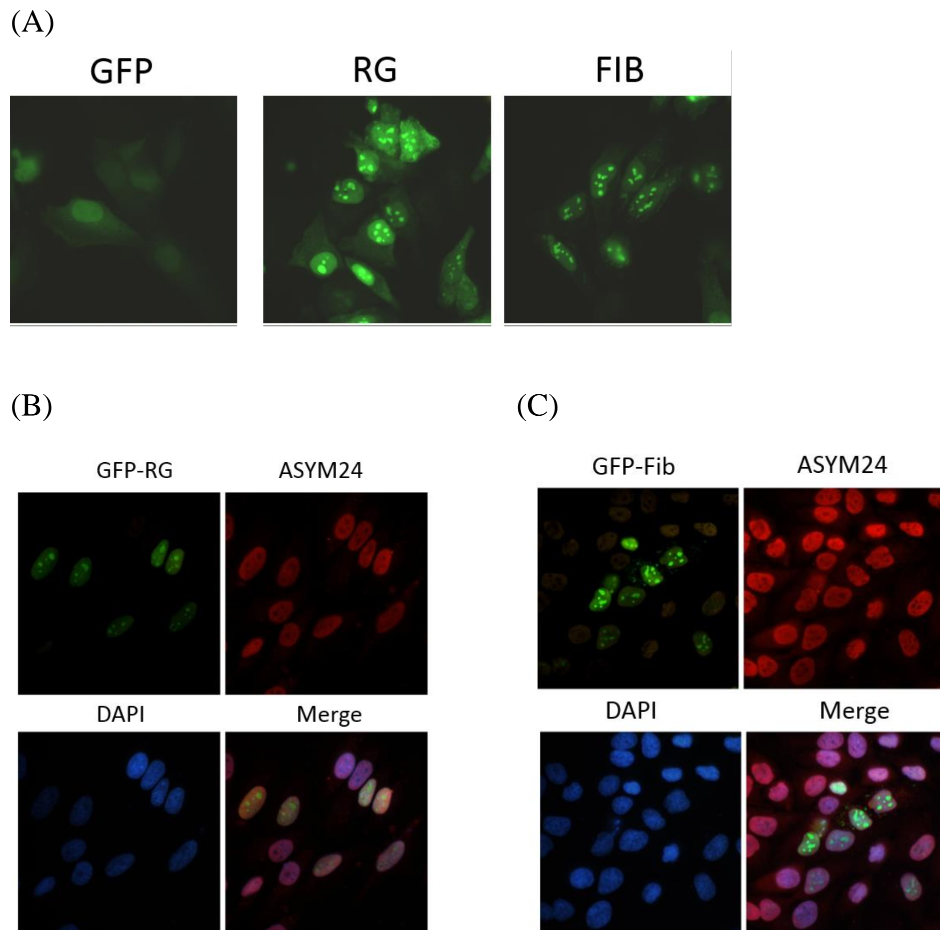


Fig. 2. Accumulation of GFP-tagged fibrillarin and glycine arginine rich (GAR or RG) sequence containing proteins in nucleolus in HeLa cells. (A) HeLa cells transfected with plasmids expressing GFP, GFP-RG of GFP-fibrillarin proteins observed by fluorescent microscopy. (B) HeLa cells transfected with plasmids expressing GFP, GFP-RG of GFP-fibrillarin proteins were observed by fluorescent microscopy, immunostained with ASYM24, stained with DAPI and merge of the images.

References

Abramovich, C., Yakobson, B., Chebath, J., and Revel, M. (1997). A protein-arginine methyltransferase binds to the intracytoplasmic domain of the IFNAR1 chain in the type I interferon receptor. EMBO J *16*, 260-266.

Bedford, M.T., and Clarke, S.G. (2009). Protein Arginine Methylation in Mammals: Who, What, and Why. Molecular Cell *33*, 1-13.

Chang, H.H., Hu, H.H., Lee, Y.J., Wei, H.M., Fan-June, M.C., Hsu, T.C., Tsay, G.J., and Li, C. (2013). Proteomic analyses and identification of arginine methylated proteins differentially recognized by autosera from anti-Sm positive SLE patients. J Biomed Sci *20*, 27.

Goulet, I., Boisvenue, S., Mokas, S., Mazroui, R., and Cote, J. (2008). TDRD3, a novel Tudor domain-containing protein, localizes to cytoplasmic stress granules. Human Molecular

Genetics *17*, 3055-3074.
Guo, A., Gu, H., Zhou, J., Mulhern, D., Wang, Y., Lee, K.A., Yang, V., Aguiar, M., Kornhauser, J., Jia, X., *et al.* (2014). Immunoaffinity enrichment and mass spectrometry analysis of protein methylation. Mol Cell Proteomics *13*, 372-387.
Hu, S.B., Xiang, J.F., Li, X., Xu, Y., Xue, W., Huang, M., Wong, C.C., Sagum, C.A., Bedford, M.T., Yang, L., *et al.* (2015). Protein arginine methyltransferase CARM1 attenuates the paraspeckle-mediated nuclear retention of mRNAs containing IRAlus. Genes Dev *29*, 630-645.
Hung, C.J., Lee, Y.J., Chen, D.H., and Li, C. (2009). Proteomic analysis of methylarginine-containing proteins in HeLa cells by two-dimensional gel electrophoresis and immunoblotting with a methylarginine-specific antibody. Protein J *28*, 139-147.
Imamura, K., Imamachi, N., Akizuki, G., Kumakura, M., Kawaguchi, A., Nagata, K., Kato, A., Kawaguchi, Y., Sato, H., Yoneda, M., *et al.* (2014). Long noncoding RNA NEAT1-dependent SFPQ relocation from promoter region to paraspeckle mediates IL8 expression upon immune stimuli. Mol Cell *53*, 393-406.
Kato, M., Han, T.W., Xie, S., Shi, K., Du, X., Wu, L.C., Mirzaei, H., Goldsmith, E.J., Longgood, J., Pei, J., *et al.* (2012). Cell-free formation of RNA granules: low complexity sequence domains form dynamic fibers within hydrogels. Cell *149*, 753-767.
Kim, H.J., Kim, N.C., Wang, Y.D., Scarborough, E.A., Moore, J., Diaz, Z., MacLea, K.S., Freibaum, B., Li, S., Molliex, A., *et al.* (2013). Mutations in prion-like domains in hnRNPA2B1 and hnRNPA1 cause multisystem proteinopathy and ALS. Nature *495*, 467-473.
Knott, G.J., Bond, C.S., and Fox, A.H. (2016). The DBHS proteins SFPQ, NONO and PSPC1: a multipurpose molecular scaffold. Nucleic Acids Res *44*, 3989-4004.
Lattante, S., Rouleau, G.A., and Kabashi, E. (2013). TARDBP and FUS mutations associated with amyotrophic lateral sclerosis: summary and update. Human mutation *34*, 812-826.
Le Romancer, M., Treilleux, I., Leconte, N., Robin-Lespinasse, Y., Sentis, S., Bouchekioua-Bouzaghou, K., Goddard, S., Gobert-Gosse, S., and Corbo, L. (2008). Regulation of Estrogen Rapid Signaling through Arginine Methylation by PRMT1. Molecular Cell *31*, 212-221.
Lee, K.H., Zhang, P., Kim, H.J., Mitrea, D.M., Sarkar, M., Freibaum, B.D., Cika, J., Coughlin, M., Messing, J., Molliex, A., *et al.* (2016). C9orf72 Dipeptide Repeats Impair the Assembly, Dynamics, and Function of Membrane-Less Organelles. Cell *167*, 774-788 e717.
Lee, Y.J., Wei, H.M., Chen, L.Y., and Li, C. (2013). Localization of SERBP1 in stress granules and nucleoli. FEBS J.
Lee, Y.J., Wei, H.M., Chen, L.Y., and Li, C. (2014). Localization of SERBP1 in stress granules and nucleoli. FEBS J *281*, 352-364.
Lin, W.J., Gary, J.D., Yang, M.C., Clarke, S., and Herschman, H.R. (1996). The mammalian immediate-early TIS21 protein and the leukemia-associated BTG1 protein interact with a protein-arginine N-methyltransferase. J Biol Chem *271*, 15034-15044.
Lischwe, M.A., Ochs, R.L., Reddy, R., Cook, R.G., Yeoman, L.C., Tan, E.M., Reichlin, M.,

and Busch, H. (1985). Purification and partial characterization of a nucleolar scleroderma antigen (Mr = 34,000; pI, 8.5) rich in NG,NG-dimethylarginine. J Biol Chem *260*, 14304-14310.
Nakagawa, S., and Hirose, T. (2012). Paraspeckle nuclear bodies--useful uselessness? Cell Mol Life Sci *69*, 3027-3036.
Nicholson, T.B., Chen, T., and Richard, S. (2009). The physiological and pathophysiological role of PRMT1-mediated protein arginine methylation. Pharmacological Research *60*, 466-474.
Nishimoto, Y., Nakagawa, S., Hirose, T., Okano, H.J., Takao, M., Shibata, S., Suyama, S., Kuwako, K., Imai, T., Murayama, S.*, et al.* (2013). The long non-coding RNA nuclear-enriched abundant transcript 1_2 induces paraspeckle formation in the motor neuron during the early phase of amyotrophic lateral sclerosis. Molecular brain *6*, 31.
Pahlich, S., Zakaryan, R.P., and Gehring, H. (2006). Protein arginine methylation: Cellular functions and methods of analysis. Biochimica et Biophysica Acta (BBA) - Proteins & Proteomics *1764*, 1890-1903.
Patel, A., Lee, H.O., Jawerth, L., Maharana, S., Jahnel, M., Hein, M.Y., Stoynov, S., Mahamid, J., Saha, S., Franzmann, T.M.*, et al.* (2015). A Liquid-to-Solid Phase Transition of the ALS Protein FUS Accelerated by Disease Mutation. Cell *162*, 1066-1077.
Ramaswami, M., Taylor, J.P., and Parker, R. (2013). Altered ribostasis: RNA-protein granules in degenerative disorders. Cell *154*, 727-736.
Sakamaki, J., Daitoku, H., Ueno, K., Hagiwara, A., Yamagata, K., and Fukamizu, A. (2011). Arginine methylation of BCL-2 antagonist of cell death (BAD) counteracts its phosphorylation and inactivation by Akt. Proc Natl Acad Sci U S A *108*, 6085-6090.
Shelkovnikova, T.A., Robinson, H.K., Troakes, C., Ninkina, N., and Buchman, V.L. (2014). Compromised paraspeckle formation as a pathogenic factor in FUSopathies. Hum Mol Genet *23*, 2298-2312.
Snijders, A.P., Hautbergue, G.M., Bloom, A., Williamson, J.C., Minshull, T.C., Phillips, H.L., Mihaylov, S.R., Gjerde, D.T., Hornby, D.P., Wilson, S.A.*, et al.* (2015). Arginine methylation and citrullination of splicing factor proline- and glutamine-rich (SFPQ/PSF) regulates its association with mRNA. Rna *21*, 347-359.
Tang, J., Frankel, A., Cook, R.J., Kim, S., Paik, W.K., Williams, K.R., Clarke, S., and Herschman, H.R. (2000). PRMT1 is the predominant type I protein arginine methyltransferase in mammalian cells. J Biol Chem *275*, 7723-7730.
Toretsky, J.A., and Wright, P.E. (2014). Assemblages: functional units formed by cellular phase separation. J Cell Biol *206*, 579-588.
Tsai, H.F., Lin, S.J., Li, C., and Hsieh, M. (2005). Decreased expression of Hsp27 and Hsp70 in transformed lymphoblastoid cells from patients with spinocerebellar ataxia type 7. Biochem Biophys Res Commun *334*, 1279-1286.
Tsai, H.F., Liu, C.S., Chen, G.D., Lin, M.L., Li, C., Chen, Y.Y., Wang, B.T., and Hsieh, M. (2003). Prenatal diagnosis of Machado-Joseph disease/Spinocerebellar Ataxia Type 3 in

Taiwan: early detection of expanded ataxin-3. Journal of clinical laboratory analysis *17*, 195-200.
Tsai, H.F., Liu, C.S., Leu, T.M., Wen, F.C., Lin, S.J., Liu, C.C., Yang, D.K., Li, C., and Hsieh, M. (2004). Analysis of trinucleotide repeats in different SCA loci in spinocerebellar ataxia patients and in normal population of Taiwan. Acta neurologica Scandinavica *109*, 355-360.
Wang, Y.-C., and Li, C. (2012). Evolutionarily conserved protein arginine methyltransferases in non-mammalian animal systems. FEBS Journal *279*, 932-945.
Wei, H.M., Hu, H.H., Chang, G.Y., Lee, Y.J., Li, Y.C., Chang, H.H., and Li, C. (2014). Arginine methylation of the cellular nucleic acid binding protein does not affect its subcellular localization but impedes RNA binding. FEBS Lett *588*, 1542-1548.
Wen, F.-C., Li, Y.-H., Tsai, H.-F., Lin, C.-H., Li, C., Liu, C.-S., Lii, C.-K., Nukina, N., and Hsieh, M. (2003). Down-regulation of heat shock protein 27 in neuronal cells and non-neuronal cells expressing mutant ataxin-3. FEBS Letters *546*, 307-314.
Yamagata, K., Daitoku, H., Takahashi, Y., Namiki, K., Hisatake, K., Kako, K., Mukai, H., Kasuya, Y., and Fukamizu, A. (2008). Arginine methylation of FOXO transcription factors inhibits their phosphorylation by Akt. Mol Cell *32*, 221-231.
Zurita-Lopez, C.I., Sandberg, T., Kelly, R., and Clarke, S.G. (2012). Human protein arginine methyltransferase 7 (PRMT7) is a type III enzyme forming omega-NG-monomethylated arginine residues. J Biol Chem *287*, 7859-7870.

106年度專題研究計畫成果彙整表

計畫主持人：李娟	**計畫編號：**106-2320-B-040-018-

計畫名稱：PRMT1 催化之蛋白質精胺酸甲基化對壓力顆粒及旁斑點組成及肌萎縮側索硬化症病因關聯

成果項目					量化	單位	質化（說明：各成果項目請附佐證資料或細項說明，如期刊名稱、年份、卷期、起訖頁數、證號...等）
國內	學術性論文	期刊論文			0	篇	
		研討會論文			0		
		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	
		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		
		積體電路電路布局權			0		
		著作權			0		
		品種權			0		
		其他			0		
	技術移轉	件數			0	件	
		收入			0	千元	
國外	學術性論文	期刊論文			1	篇	29. Lee, YJ, Chang, WW, Chang, CP, Liu, TY, Chuang, CY, Qian, K, Zheng, YG and Li, C. (2018) Downregulation of PRMT1 promotes the senescence and migration of a non-MYCN amplified neuroblastoma SK-N-SH cells. Scientific Reports (accepted)
		研討會論文			0		
		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	
		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		

		積體電路電路布局權	0		
		著作權	0		
		品種權	0		
		其他	0		
	技術移轉	件數	0	件	
		收入	0	千元	
參與計畫人力	本國籍	大專生	0	人次	
		碩士生	0		
		博士生	0		
		博士後研究員	0		
		專任助理	1		碩士級研究助理張建評
	非本國籍	大專生	0		
		碩士生	0		
		博士生	0		
		博士後研究員	0		
		專任助理	0		
其他成果 （無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。）			NA		

科技部補助專題研究計畫成果自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現（簡要敘述成果是否具有政策應用參考價值及具影響公共利益之重大發現）或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以100字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形（請於其他欄註明專利及技轉之證號、合約、申請及洽談等詳細資訊）

論文：■已發表　□未發表之文稿　□撰寫中　□無

專利：□已獲得　□申請中　■無

技轉：□已技轉　□洽談中　■無

其他：（以200字為限）

Lee, YJ, Chang, WW, Chang, CP, Liu, TY, Chuang, CY, Qian, K, Zheng, YG and Li, C. (2018) Downregulation of PRMT1 promotes the senescence and migration of a non-MYCN amplified neuroblastoma SK-N-S

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性，以500字為限）

本研究部分內容已發表，顯示在神經母細胞瘤細胞中降低PRMT1的表現或抑制其活性會誘發期衰老及增加p53及其下游p21和PAI-1的表現。 該研究對考量神經母細胞瘤細胞以PRMT1 為治療標的提供多方思考面向。

4. 主要發現

本研究具有政策應用參考價值：■否　□是，建議提供機關

（勾選「是」者，請列舉建議可提供施政參考之業務主管機關）

本研究具影響公共利益之重大發現：□否　□是

說明：（以150字為限）