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研究課題名（和文）Mechanistic elucidation of cellular responses to stress

研究課題名（英文）Mechanistic elucidation of cellular responses to stress

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研究成果の概要（和文）：DNA損傷や核小体ストレスなどのストレス要因に細胞を挑戦させることにより、細胞ストレス応答の性質を研究しました。 ING4やING5などの成長タンパク質阻害剤は、細胞ストレスの非常に早い段階で顆粒に移行することがわかりました。 レーザーマイクロ照射によって誘発されたDNA損傷に応答したING4の即時転座は、DNA損傷応答におけるINGタンパク質の新しい役割を示しました。 顆粒（細胞質および/または核）への変換-本質的に無秩序なドメインの含有量が高いタンパク質に見られる特徴は、INGタンパク質がストレス下の有害な影響から細胞を保護するという考えを支持しました。

研究成果の学術的意義や社会的意義
We expanded the knowledge of the nature of cellular stress responses. We agued novel functions of ING protein as guardians of cells from stress conditions beyond their given role in regulation of cell growth. The proposed mechanisms were involved in ING intrinsically disordered domains.

研究成果の概要（英文）：We studied the nature of the cellular stress response by challenging cells to stress factors such as DNA damage, and nucleolar stress. We have found that growth protein inhibitors, including ING4 and ING5, translocated to granules at a very early stage of cellular stress. The instant translocation of ING4 in response to laser micro-irradiation-induced DNA damage showed a new role for the ING protein in the DNA damage response. The transformation to granules (cytoplasm and / or nucleus)-a characteristic found in proteins with high content of intrinsically disordered domains supported the idea that ING proteins protect cells from harmful effects under stress.

研究分野：Molecular and Cellular Biology

キーワード：INGs　Cellular stress　ING4　Ing5

１．研究開始当初の背景

Nucleolus functions as a center of ribosome biogenesis that is tightly regulated because proliferating cells consume 60-80% of their energy for ribosome biogenesis. It is known that stress stimuli such as serum starvation and DNA damage induce structural abnormality in nucleoli and a halt in ribosome biogenesis, known as nucleolar stress (Boisvert FM et al. Nat Rev Mol Cell Biol. 2007). Recently, the nucleolar stress has been focused due to relevance to neurodegenerative diseases such as Alzheimer's disease and aging (Parlato R et al. J Mol Med. 2013). On the other hand, hyperactive ribosome biogenesis is often found in cancer cells (Tiku V. et al. Nat. Commun. 2017).

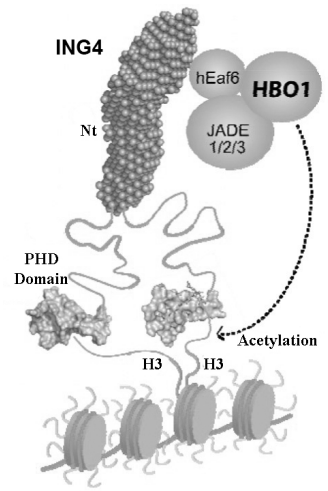


Fig. 1. ING4 with PHD domain forms a complex with HBO, a histone-acetyl transferase, JADE and hEaf6.

Inhibitor of growth 4 (ING4) belongs to a highly conserved 5-member ING protein family. They share a plant-homeodomain (PHD) at their C-termini. The PHD domain is known specifically to bind to tri-methylated histone H3 lysine 4 (H3K4me3) that is observed in active genes. ING4 has been demonstrated to exist in dimer and form complex with JADE (histone H4 binding protein) and a histone acetyltransferase, HBO1 *in vivo* (Doyon Y et al. Mol Cell. 2006) (**Fig. 1**). Like other ING proteins, ING4 has previously been considered as a tumor suppressor (Garkavtsev I. et al. Nature - 2004). However, I have recently reported that ING4 is a positive regulator of cell growth and ribosomal RNA synthesis by regulating histone acetylation of ribosomal DNA (Trinh et al. Sci Rep. 2019).

Recently, it has been found that when cells are stimulated with calcium ionophores or tumor necrosis factor, ING4 is citrullinated by peptidylarginine deiminases (PADs), which replace the positively charged aldimine group (=NH) of arginine with the neutrally charged ketone (=O) of citrulline. As the change in charges, this posttranslational modification can interfere with ING4 binding to p53 (Guo Q and Fast W J Biol Chem. 2011), thereby participating into regulation of ING4's functions.

２．研究の目的

The purpose of this project is to elucidate the role of ING4 in cellular stress responses, including nucleolar stress and DNA damage. This study will provide a novel and significant insight for its understandings.

３．研究の方法

The role of ING4 in DNA damage response: I have observed ING4 recruitment to the site of DNA damage, induced by laser micro-UV-irradiation. To evaluate the role of ING4 in DNA repair and cell viability, I will use the single cell gel electrophoresis assay (the comet assay) and the clonogenic assay in ING4 WT or KO cells. With the comet assay, I can compare the level of DNA break among samples, and with clonogenic assay, the repair scale will be judged through ability to form colonies of cells. Next, I will screen kinetic of common proteins that recruits to the damage site in DNA damage response such as 53BP1 and BRCA1 in ING4 WT, KO or rescued cells. It is well established that acetylation of ATM protein, a master kinase of DNA damage response pathway, activates its activity thereby activating the downstream pathway to respond to DNA damage (Sun Y et al. Mol Cell Biol. 2007). Because ING4 complex contains an acetyltransferase, HBO1, so I will examine if the rapid recruitment of ING4 contributes to acetylation of ATM using western blot with an anti - acetylated ATM antibody.

The effects of citrullination of ING4 on its function: I will induce various stresses on established cell lines that stably express Flag-ING4. Next, Flag-ING4 is purified and examined using the liquid chromatography-mass spectrometry (LC-MS) method that allows us to identify exactly the citrullinated arginine residues in ING4 sequence. Based on the method, I will investigate specific citrullination at arginine residues corresponding to types of stress, nucleolar stress or DNA damage stress. I will use mutagenesis method to replace the arginine identified above with lysine (similar positive charge, but unable for citrullination) and introduce to cells using CRISPR-Cas9 mediated knock-in. Then, I will challenge wild-type and the mutant cells with stress stimuli and evaluate rRNA synthesis and disruption of nucleolar. Thus, I would demonstrate the role of citrullination of ING4 in cellular.

４．研究成果

We have got following result so far.

1. ING4 translocation under cellular stress.

ING4 changed its localization coupled with inhibition of rRNA synthesis in the nucleolus at an early stage of nucleolar stress such as serum starvation while a nucleolar marker protein, nucleolin, remained to stay (**Fig. 2**).

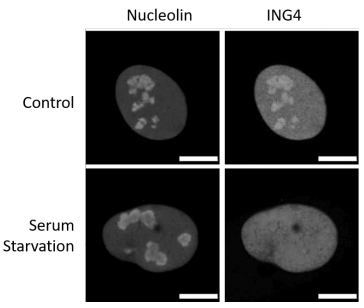
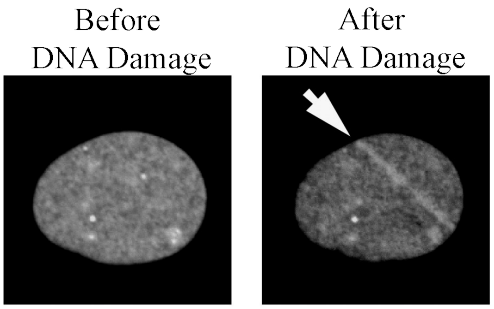


Fig. 2. Colocalization of GFP-ING4 and nucleolin in nucleoli. Serum starvation (lower row) caused disappearance of GFP-ING4 from nucleolus in the condition where nucleolin remained to stay there.

2. ING4 formed stress-related foci.

Examining genotoxic stress and ING4, we found that UV-laser scanning induced accumulation of ING4 at the region of DNA damage (**Fig. 3**). In addition, treatment of another cellular stress inducer – sodium arsenite also induced formation of ING4 granules. Accumulation at the DNA damage site is known as a common nature of DNA repair proteins.

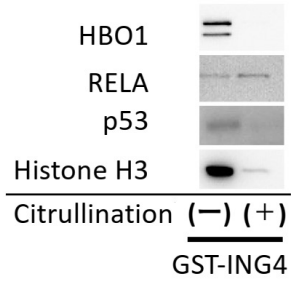
Fig. 3. UV-induced DNA damage (arrow) induced accumulation of GFP-ING4 at the site of DNA damage in the nucleus.



3. Citrullination altered ING4's pattern of interaction.

in vitro citrullination of ING4 decreased its affinity to HBO1, H3 and p53, but not to NF-kB subunit RELA **(Fig. 4)**. The selective effect of citrullination on ING4's ability to bind it's partner proteins opened the possibility that citrullination might be a mechanism to regulate ING4's functions.

Fig. 4. HBO1, p53 and histone H3 bound to GST-ING4, but not to citrullinated GST-ING4 in vitro (RelA bound to ING4 irrespective of its citrullination).



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５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関