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Puromycin induces SUMO and ubiquitin redistribution upon proteasome inhibition

Hotaru Matsumoto [a], Hisato Saitoh [a, b, *]

[a] Course for Biological Sciences, Faculty of Science, Kumamoto University, Kumamoto, Japan
[b] Department of Biological Sciences, Graduate School of Science and Technology, Kumamoto University, Kumamoto, Japan

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ABSTRACT

We have previously reported the co-localization of O-propargyl-puromycin (OP-Puro) with SUMO-2/3 and ubiquitin at promyelocytic leukemia-nuclear bodies (PML-NBs) in the presence of the proteasome inhibitor MG132, implying a role for the ubiquitin family in sequestering OP-puromycylated immature polypeptides to the nucleus during impaired proteasome activity. Here, we found that as expected puromycin induced SUMO-1/2/3 accumulation with ubiquitin at multiple nuclear foci in HeLa cells when co-exposed to MG132. Co-administration of puromycin and MG132 also facilitated redistribution of PML and the SUMO-targeted ubiquitin ligase RNF4 concurrently with SUMO-2/3. As removal of the drugs from the medium led to disappearance of the SUMO-2/3-ubiquitin nuclear foci, our findings indicated that nuclear assembly/disassembly of SUMO-2/3 and ubiquitin was pharmacologically manipulable, supporting our previous observation on OP-Puro, which predicted the ubiquitin family function in sequestrating aberrant proteins to the nucleus.

1. Introduction

Puromycin is an aminonucleoside antibiotic produced by *Streptomyces alboniger* [1]. When administrated to cells, it enters the ribosome A site, followed by incorporation into the nascent polypeptide chain, elongating either on the monoribosome or polyribosomes, which leads to covalent attachment of puromycin to the C-terminal end of the nascent polypeptides, referred to as 'puromycylation' [2–5]. Because puromycylation prematurely terminates elongation at any given site in the elongating polypeptide, the resulting puromycylated polypeptides are expected to be heterogeneous in size and improperly folded. Under standard conditions, such puromycylated polypeptides are subjected to an elimination reaction controlled by the ubiquitin-proteasome system (UPS) [6]. However, when the UPS is perturbed in cells, puromycylated immature polypeptides are expected to accumulate, causing cytotoxic effects, including stress response and growth defects, ultimately inducing cell death. To escape such a scenario, cells should contain an intrinsic protection system that would respond to accumulation of immature/aberrant proteins. However, the cellular defense strategy against immature/aberrant polypeptides remains largely unknown.

The small ubiquitin-related modifiers, SUMO-2/3 and SUMO-1, are highly conserved proteins, which can be covalently attached to multiple cellular proteins, referred to as SUMOylation [7,8]. Intriguingly, SUMOylation frequently, but not always, occurs with ubiquitin modification (ubiquitinylation) [9–12]. Many SUMOs and SUMOyalted proteins are detected in promyelocytic leukemia-nuclear bodies (PML-NBs), which are characterized by the presence of the tripartite motif family protein, PML/TRIM19, and their modification often occurs in response to environmental stresses, including oxidative stress, aging and virus infection [13–16]. Although the biological relevance of SUMOylation promiscuously with ubiquitinylation in PML-NBs remains elusive, the existence of RNF4/SNURF, an E3 ubiquitin ligase that promotes ubiquitinylation of SUMO-modified proteins, in PML-NBs, suggests a role for PML-NBs in controlling the stability of SUMOylated proteins with ubiquitinylation [17–20].

We have previously assessed the distribution of a derivative of puromycin, O-propargyl-puromycin (OP-Puro), in HeLa cells using

Abbreviations: SUMO, small ubiquitin-related modifier; PML-NBs, promyelocytic leukemia-nuclear bodies; RNF4, really interesting new gene (RING) finger protein 4; DAPI, 4′, 6-diamidino-2-phenylindole; OP-Puro, O-propargyl-puromycin.

* Corresponding author. Department of Biological Sciences, Graduate School of Science and Technology, Kumamoto University, 2-39-1 Kurokami, Chuo-ku, Kumamoto, 860-8555, Japan.
E-mail address: hisa@kumamoto-u.ac.jp (H. Saitoh).

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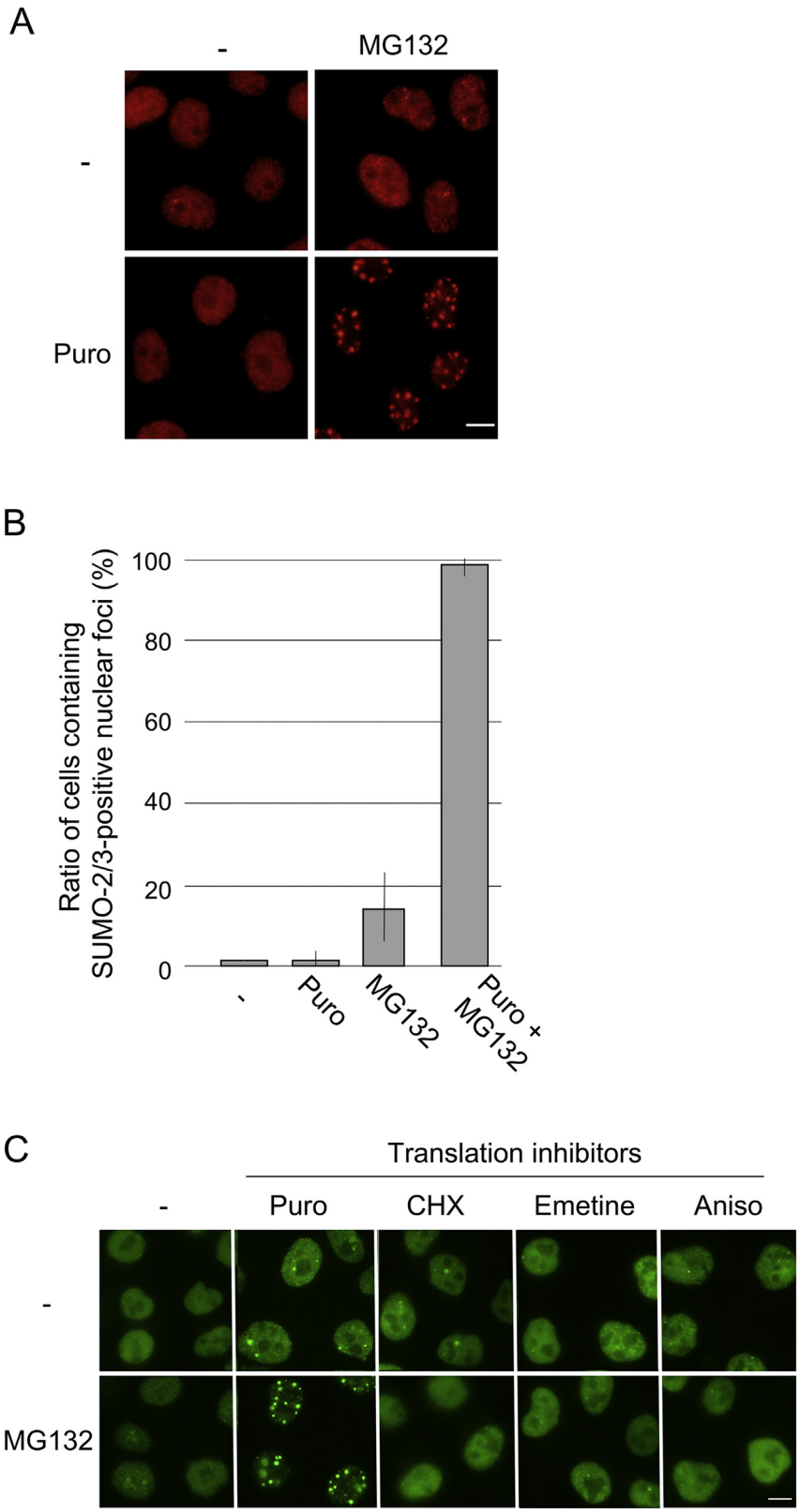

Fig. 1. Puromycin induces SUMO-2/3-positive nuclear foci formation upon proteasome inhibition. (A) Exponentially growing HeLa cells were exposed to vehicle alone (−, upper left), 10 μM puromycin (lower left), 10 μM MG132 (upper right) or 10 μM puromycin plus 10 μM MG132 (lower-right) for 4 h at 37 °C, and were then subjected to immunostaining using anti-SUMO-2/3 antibody. Scale bar indicates 20 μm. (B) HeLa cells were cultured and treated as indicated in (A). The proportion of cells forming SUMO-positive nuclear foci was evaluated for each treatment. Values shown represent the mean ± SD of three independent experiments. (C) Exponentially growing HeLa cells were exposed to vehicle alone (−, left column), 10 μM puromycin (Puro, middle left column), 10 μM cycloheximide (CHX, middle column), 10 μM emetine (middle right column) or 10 μM anisomycin (Aniso, right column) in the absence (upper panels) or presence of 10 μM MG132 (lower panels) for 4 h at 37 °C, and were then subjected to immunostaining using anti-SUMO-2/3 antibody. Scale bar indicates 20 μm.

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