Identification of Intracellular Proteases That Degrade Lipidated, Amide-Containing Chimeric Compounds

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Bifunctional chimeric compounds are essential molecular tools for chemical biology and drug development. In many cases, chimeric compounds are created by connecting two functional moieties, such as small-molecule ligands, lipids, and organic fluorescent dyes, via a linker with the amide bond. Chimeric compounds are then used in cell experiments on the assumption that they are stable in cells. However, as far as we know, the validity of the cellular stability of chimeric compounds has yet to be thoroughly evaluated.

In previous work, we developed a chimeric compound mgcTMP to control protein localization in mammalian cells[1] (Figure 1a, b). mgcTMP was created by connecting trimethoprim (TMP), a small-molecule ligand of *E. coli* dihydrofolate reductase (eDHFR), to a myristoyl-Gly-Cys lipopeptide via a linker. During the study, we unexpectedly found that mgcTMP was degraded in cells[2]. More specifically, we identified that the amide bond C-terminal to the Cys residue of mgcTMP was cleaved during the incubation with the cells, suggesting the involvement of proteases in mgcTMP degradation.

In this work, we first set out to identify proteases responsible for mgcTMP degradation. Using the diced electrophoresis gel (DEG) assay[3] and pharmacological validation (Figure 1c), we could identify two membrane-bound proteases involved in the cleavage of mgcTMP in cells. Given that mgcTMP contains two natural amino acids, Gly and Cys, the protease-mediated mgcTMP degradation might be reasonable. This prompted us to investigate whether a lipid-tethered chimeric compound containing no amino acids also undergoes intracellular protease-mediated degradation. We found that a simple lipid conjugate, in which trimethoprim and palmitic acid were attached to an ethylene glycol-based linker with amide bonds, was also cleaved by incubation with cells. On the other hand, we also found that chimeric compounds that contain amide bonds but do not have lipid moieties were stable in cells. This poster shows our results on identifying mgcTMP-cleaving proteases and characterizing chimeric compounds that do and do not undergo cellular degradation.

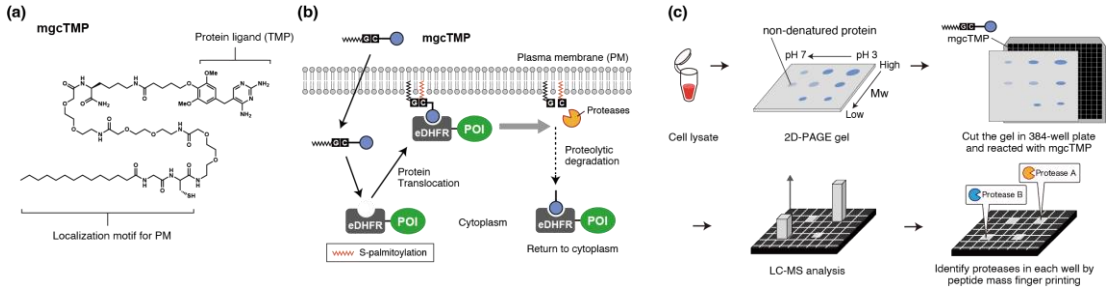

Figure 1. (a) Structure of mgcTMP. (b) Schematic illustration of protease-mediated mgcTMP degradation. (c) Strategy to identify mgcTMP-cleaving proteases by the DEG assay.

References

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[2] A. Nakamura et al. *ACS Chem. Biol.*, **2020**, *15*, 837–843.
[3] T. Komatsu et al. *J. Am. Chem. Soc.*, **2013**, *135*, 6002–6005.