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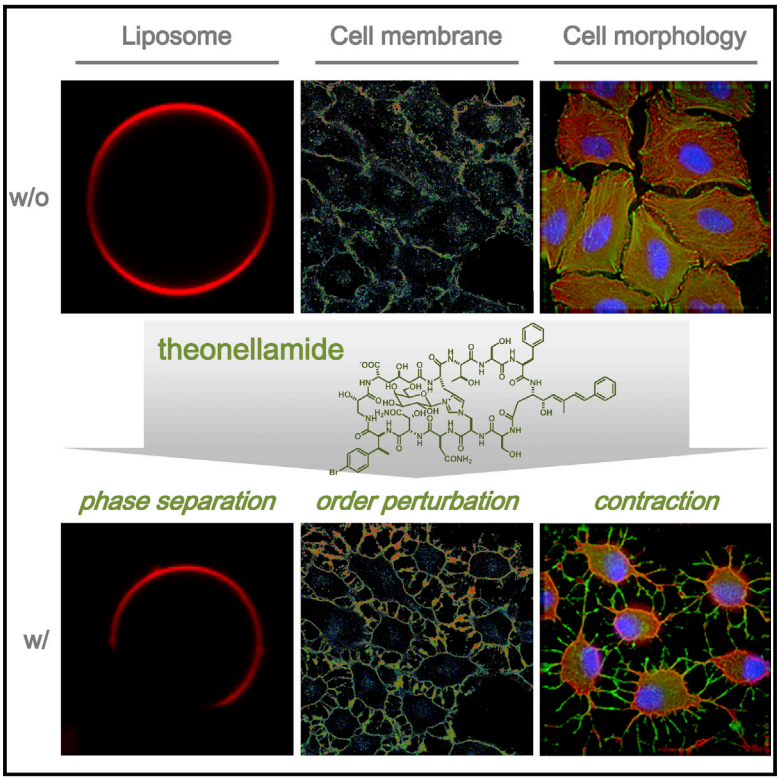
Targeting Cholesterol in a Liquid-Disordered Environment by Theonellamides Modulates Cell Membrane Order and Cell Shape

Graphical Abstract



Highlights

- TNMs, marine-derived peptides, recognize cholesterol in liquid-disordered domains
- TNMs modulate membrane order in model and cellular membranes
- Cells shrink in a cholesterol-dependent manner after TNM-A treatment
- The membrane order maintained by cholesterol is important for cell morphogenesis

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In Brief

Arita et al. report that theonellamides (TNMs), marine-derived peptides, exhibit a previously unrecognized mode of action of membrane-targeting natural products. Using TNMs as tools, the membrane order, which is maintained by cholesterol, was revealed to be important for proper cell morphogenesis.

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Targeting Cholesterol in a Liquid-Disordered Environment by Theonellamides Modulates Cell Membrane Order and Cell Shape

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SUMMARY

Roles of lipids in the cell membrane are poorly understood. This is partially due to the lack of methodologies, for example, tool chemicals that bind to specific membrane lipids and modulate membrane function. Theonellamides (TNMs), marine sponge-derived peptides, recognize 3β-hydroxysterols in lipid membranes and induce major morphological changes in cultured mammalian cells through as yet unknown mechanisms. Here, we show that TNMs recognize cholesterol-containing liquid-disordered domains and induce phase separation in model lipid membranes. Modulation of membrane order was also observed in living cells following treatment with TNM-A, in which cells shrank considerably in a cholesterol-, cytoskeleton-, and energy-dependent manner. These findings present a previously unrecognized mode of action of membrane-targeting natural products. Meanwhile, we demonstrated the importance of membrane order, which is maintained by cholesterol, for proper cell morphogenesis.

INTRODUCTION

Membrane lipids are not just solvents for membrane proteins but also regulate structural stability and proper functions of proteins (Coskun and Simons, 2011; Laganowsky et al., 2014; Lee, 2011; Singer and Nicolson, 1972). Furthermore, lipids cluster to form large membrane domains in artificial membranes and dead cells, which implies the potential existence of membrane domains in live cells (Lingwood and Simons, 2010; Simons and Ikonen, 1997). However, it is currently expected that the domain size is very small and short lived in cells under steady-state conditions (Kenworthy, 2008); for example, glycosylphosphatidylinositol (GPI)-anchored proteins form cholesterol-sensitive nanoclusters (less than 5 nm), which was deduced by fluorescence resonance energy transfer experiments combined with theoretical modeling (Sharma et al., 2004). Because of the limitation of biochemical analysis, the functions of such tiny domains largely remain to be investigated. One of the ways to unveil the role of membrane domains is chemical genetics; non-toxic, phenotypically drastic, lipid-targeting molecules would enable us to dissect the molecular events in the live cell membrane.

The cell membrane is one of the major targets of antibiotics and bacterial toxins. Several medically important molecules also target cell membranes, for example, amphotericin B (AmB) and daptomycin (Baltz, 2009; Murata et al., 2009; Volmer et al., 2010). On the other hand, some molecules have been used to detect the cellular localization of membrane lipids. Filipin, a polyene antibiotic, is a traditional sterol marker (Drabikow et al., 1973; Miller, 1984), while sterol-interacting proteins such as perfringolysin O (PFO, also known as θ-toxin), a bacterial cytolysin, have been successfully used to detect sterol molecules in fixed cells (Ohno-Iwashita et al., 2010). However, exogenous molecules targeting the cell membrane usually exhibit acute toxicity, hampering live cell analysis employing such molecules. Even filipin, the most commonly used sterol marker, has a critical limitation because of its cellular damage (Gimpl, 2010).

Theonellamides (TNMs, Figure 1) are marine sponge-derived bicyclic peptides that exhibit antifungal activity and moderate cytotoxicity (Bewley and Faulkner, 1994; Matsunaga and Fusetani, 1995; Matsunaga et al., 1989; Schmidt et al., 1998; Youssef et al., 2014). We previously reported that TNMs target 3β-hydroxysterols in liposomes and cell membranes (Espiritu et al., 2013; Ho et al., 2009; Nishimura et al., 2010, 2013, 2014). These peptides strictly recognize sterols with a 3β-hydroxy group, while recognition of these sterols appears to occur in shallow areas of membranes (Espiritu et al., 2013; Nishimura et al., 2010). It is noted

