Squalene and cholesterol in the balance at the ER membrane.

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Squalene and cholesterol sensing at the ER membrane.

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Targeting the cholesterol biosynthetic pathway has become a mainstay for the treatment of ischaemic heart disease (1). However, the importance of cholesterol metabolism is not just confined to atherosclerosis, as cholesterol is an essential component of membranes, a precursor for other metabolic pathways, and can fuel tumour growth (2). Understanding how the cholesterol synthetic pathway is regulated is therefore of broad biological interest. Cellular cholesterol abundance is tightly regulated through a combination of uptake through LDL receptors and synthesis, with cholesterol sensing occurring at the ER membrane (3, 4). Cholesterol is detected by ER-resident proteins with sterol sensing domains, which both govern the stability of cholesterol synthetic enzymes and control the release of the SREBP2 transcription factor, regulating the transcription of genes required for cholesterol synthesis and uptake (4). This process is classically exemplified by the sterol sensitive degradation of HMG-CoA reductase (HMGCR), a rate limiting step in cholesterol synthesis, and the target of statins (1) (**Figure 1**). In PNAS, Yoshioka et al. present findings relating to squalene monooxygenase (SM, also known as squalene epoxidase, SQLE), a further rate limiting step in the cholesterol synthetic pathway, identifying a previously unappreciated role for squalene in regulating SM stability (5) (**Figure 1**).

SM catalyses the first oxidation step for cholesterol formation, oxidising squalene to 2,3-oxidosqualene. Its biological relevance has been recently highlighted by SM perturbations in cancers. High SM expression decreases the growth of non-alcoholic fatty liver disease (NAFLD) induced hepatocellular carcinoma (HCC)(6), whereas SM loss drives the cholesterol dependence of a subset of lymphomas (7) and small cell lung cancer lines

(8). Targeting SM may be therefore a tractable therapeutic option for some solid organ tumours dependent on SM for cholesterol synthesis.

SM inhibitors are already in clinical use for treating fungal infections. SM is required for ergosterol synthesis in fungi, and is inhibited by terbinafine, which is commonly used to treat cutaneous fungal infections (9). Several human SM inhibitors have also been developed, including NB-598, a potent small molecule inhibitor, with an IC50 of approximately 60 nM (10, 11). Recent structural studies identify how NB-598 binds to the SM catalytic domain, providing a basis for understanding the relative resistance of human SM to terbinafine (11). While these structural approaches are important for the development of active-site inhibitors, other regulatory domains within SM exist, particularly within the hydrophobic N-terminus that has not been well resolved in structural studies.

The N-terminal 100 amino acids of SM (SM-N100) contain a regulatory domain for SM stability and sterol sensing. Cholesterol accelerates the degradation of SM at the ER membrane by facilitating ubiquitination by the ER-resident MARCH6 E3 ligase and subsequent proteasomal degradation (12, 13). An amphipathic helix within SM-N100 is required for this process (14), which is recognised by MARCH6 and requires two E2 enzymes, UBE2G2 and UBE2J2 (15). This degradation pathway is distinct from the sterol sensing mechanism of HMGCR, where sterol binding domains are integral to the ERAD pathway, as it relies on the relative abundance of cholesterol within the membrane, and presumably conformational changes that promote recognition of the amphipathic degron (16) (**Figure 1**).

In PNAS, Yoshioka et al. devised a chemical screening approach to explore the dynamics of SM stability using a luciferase reporter (5). The top hits included fungal SM inhibitors, such as terbinafine, which stabilised their reporter. Using the more specific human SM inhibitor, NB-598, they confirmed that inhibition stabilised SM, and showed that stabilisation was independent of the catalytic domain, and could be observed with just the SM-N100 region. Perhaps these findings would be expected, as NB-598 treatment decreases cholesterol synthesis and intracellular cholesterol content (17), potentially decreasing MARCH6 mediated degradation of SM. However, Yoshioka et al. find that blocking squalene synthase (SQS), which is upstream of SM in the synthetic pathway, did not stabilise SM, arguing against a general role for changes in cholesterol flux altering SM degradation.

Instead, they showed that squalene abundance itself mediates SM stability, and that the addition of squalene to cells where SQS is inhibited is sufficient to increase SM N-100 levels.

How does squalene alter SM stability? Squalene accumulation following SM inhibition has been shown to occur in several compartments, and particularly within lipid droplets (8). However, the regulatory SM N-100 region localises to the ER membrane, suggesting that squalene may alter the recognition of the amphipathic MARCH6 degron. Yoshioka et al. used subcellular fractionation and immunohistochemistry to confirm the ER localisation of the SM N-100 region, and demonstrated little accumulation of squalene in cellular fractions likely to contain lipid droplets. They also used photoaffinity squalene probes to show a direct association of squalene to SM N-100, and demonstrated a reduction in MARCH6 mediated degradation of SM. These findings are all consistent with squalene influencing SM stability in the ER membrane. The discrepancy between squalene accumulation and SM localisation may relate to a protective role of lipid droplets, which potentially allow squalene sequestration away from other membranes (8). Further studies are required to delineate how squalene localisation is regulated, its relative abundance in the ER membrane, and how it may impact on SM function.

Yoshioka et al. showed that siRNA mediated depletion of MARCH6 increases SM N-100 and endogenous SM, which is partially altered with NB-598 treatment. The interaction between overexpressed MARCH6 and SM N-100 was also affected, suggesting that squalene may alter the recognition of the SM amphipathic helix by MARCH6. However, the interpretation of these experiments is complex, as MARCH6 depletion has a dominant effect on SM stabilisation, irrespective of the contribution to cholesterol mediated degradation of the N100 region. The authors' identification of a further endogenous truncated SM species that accumulated following NB-598 treatment points to additional mechanisms involved in SM stability. HMGCR, the first rate limiting step for cholesterol synthesis, is highly regulated at the ER membrane by three ubiquitin ligases (18), and it will be interesting to explore whether squalene levels have further regulatory roles on SM function.

An intriguing outcome from the studies presented is that the relative abundance of cholesterol and squalene alter membrane conformation, dictating turnover of the enzyme. This is distinct from the detection of changes in the labile cholesterol pool by sterol sensing domains (19), as Yoshioka et al. propose that the relative abundance of cholesterol and squalene within the membrane alters recognition of the SM amphipathic degron (5)(**Figure**

1). How this occurs is unclear. It is possible that lipid composition alters membrane curvature (20) or protein conformation, as have been observed in other membrane associated proteins (21). Alternatively, lipid composition may influence the interaction or conformation of ERAD E3 ligases, which can form channels, allowing retro-translocation of their substrates (22). Therefore, how the SM amphipathic degron engages with MARCH6 is an area that requires further study, and is likely to be relevant to the ubiquitination and degradation of other components of the cholesterol synthetic pathway.

Therapeutic targeting of SM continues to gain interest as a strategy to modulate cholesterol formation. The involvement of squalene as an allosteric regulator of SM stability by Yoshioka et al. reveals a fascinating mechanism of a lipid intermediate fine-tuning this key pathway. It is possible that SM stability responds to further alterations in the lipid membrane, which will be important to explore. However, the allosteric regulation of SM stability by squalene provides a potential new approach to alter SM activity and target cholesterol synthesis.

Acknowledgements

The author's research is supported by the Wellcome Trust (215477/Z/19/Z) and a Lister Institute Research Fellowship. This work was also supported by the NIHR Cambridge Biomedical Research Centre.

Declaration of Interests

The author declares no competing interests.

Figure Legend

Figure 1: Sensing of sterols and precursor lipids at the ER membrane. HMGCR and SREBP2 are regulated at the ER membrane dependent on sterol sensing domains present in INSIG, SCAP and HMGCR (left). INSIGs are more sensitive to oxysterols, whereas SCAP binds cholesterol. Sterol binding promotes HMGCR degradation by three E3 ligases (GP78, RNF145 and Hrd1). Sterol binding retains SREBP2 in the ER, preventing trafficking to golgi and subsequent cleavage by Site-1 and Site-2 protease. SM stability is regulated by the abundance of sterols and squalene within the ER membrane (right). Increased cholesterol promotes ubiquitination of the N-terminal region, and subsequent degradation. Squalene stabilises the N-terminal region, blunting MARCH6 mediated ubiquitination.

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