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Thesis abstract

Substrate-induced activation of the rate-limiting cholesterol synthesis enzyme squalene monooxygenase

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Cholesterol is vital for membrane function, yet toxic in excess and associated with cardiovascular disease and cancer. Squalene monooxygenase (SM, also known as squalene epoxidase or SQLE) catalyses the rate-limiting and first oxygen-dependent step of the committed cholesterol synthesis pathway, and past research has shown that cholesterol modulates its protein levels to ensure that pathway flux is coupled with supply and demand. This is mediated by the N-terminal regulatory domain of SM (SM-N100), which senses high cholesterol levels and accelerates entry into the endoplasmic reticulum-associated degradation (ERAD) pathway. A mechanistic understanding of how SM is regulated by such stimuli is critical, as aberrant SM activity is oncogenic in a broad range of cancers. Thus, this thesis sought to identify additional metabolic factors controlling SM degradation. Using a chemical genetics screen and SM-N100 reporter constructs, we found SM is protected from degradation by the accumulation of its substrate, squalene. This feedforward regulation involves allosteric binding of squalene to the SM-N100 domain, which prevents its recognition by ERAD effectors. We next studied a lower molecular-weight form of SM routinely detected by immunoblotting, and through SM mutagenesis and targeting of the ERAD pathway found it arises through the rare phenomenon of partial proteasomal degradation. This disrupts the cholesterol-sensing SM-N100 domain but not the catalytic domain, rendering truncated SM constitutively active. Truncated SM, but not full-length SM, is also capable of localising to lipid droplets. Finally, we identified hypoxia as a physiological trigger for truncation through a combination of accelerated entry into ERAD and the accumulation of squalene, which prevents complete degradation of SM by the proteasome. Analysis of endometrial cancer tissues revealed a marked upregulation of SM truncation that was well-correlated with a hypoxic marker protein, suggesting hypoxia-induced truncation occurs in vivo and contributes to the oncogenic properties of SM. In summary, this thesis identifies dual mechanisms of substrate-induced SM regulation that impair its proteasomal degradation and preserve catalytic capacity. Beyond these fundamental insights into the control of cholesterol synthesis, our data highlight the significance of SM feedforward regulation under both homeostatic and pathophysiological conditions.

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