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EDITORIAL

DRP1: a novel regulator of PCSK9 secretion and degradation

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This editorial refers to 'Dynamin-related protein 1 inhibition reduces hepatic PCSK9 secretion' by M.A. Rogers *et al.*, doi: 10.1093/cvr/cvab034.

Proprotein convertase subtilisin/kexin type 9 (PCSK9) regulates the circulating levels of low-density lipoprotein (LDL) cholesterol (LDL-C) through its ability to bind the LDL receptor (LDLR) and mediate its degradation within the lysosomal compartment. Using genetics in families with lipid disorders, Dr Catherine Boileau and colleagues first revealed that a mutation within the PCSK9 gene causally led to the development of familial hypercholesterolaemia, spurring further efforts to discover sequence variants within the PCSK9 gene that associate with LDL-C levels and coronary atherosclerosis.[1] The confirmation of the association between genetic variants of PCSK9, LDL-C, and cardiovascular disease in large genome-wide association as well as Mendelian randomization studies implicated PCSK9 as a novel therapeutic target. Monoclonal antibodies to disrupt the interaction between PCSK9 and the LDLR are already in clinical use.[2] The highly anticipated ORION-4 clinical trial is ongoing, determining the long-term efficacy and safety of Inclisiran, an RNA interference method of PCSK9 targeting.[2] Although targeting PCSK9 efficiently reduces LDL-C even on top of high-intensity statin therapy, the costs of PCSK9 inhibitors has hampered their widespread use. Small molecule-based inhibition of PCSK9 has proven difficult primarily due to the relatively large binding interface between PCSK9 and the epidermal growth factor-like repeat A domain of the LDLR.[3] Thus, it has been postulated that deciphering the intracellular mechanisms controlling PCSK9 production and secretion could uncover novel therapeutic targets that could result in more cost-effective treatments.

Rogers *et al.*[4] develop previous work upon dynamin-related protein 1 (DRP1)[5] to uncover a novel regulator of PCSK9 secretion that was targetable with the small molecule inhibitor mitochondrial division inhibitor-1 (mdivi-1). The authors used a wide range of elegant approaches to reveal a role of DRP1, a member of the dynamin family of large GTPases and the key controller of mitochondrial fission, in the regulation of the hepatic secretion of PCSK9, while also highlighting a PCSK9-independent effect of DRP1 upon atherosclerotic development in a diabetic mouse model (*Figure 1*).

The initial observation that DRP1 may play a broad role in the cellular trafficking and subsequent protein secretion was made by Rogers *et al.* in smooth muscle cells in which the inhibition of DRP1 by mdivi-1 reduced collagen secretion.[5] Secondly, it has been previously shown that DRP1 may also regulate the hepatic secretion of very low-density lipoproteins.[6] Utilizing a quantitative stable isotope labelling (SILAC) mass spectrometry approach upon the secretomes of HepG2 cells treated with mdivi-1, Rogers *et al.* now report that DRP1 inhibition is able to reduce the secretion of many proteins, with PCSK9 being the most significant. Interestingly, this non-specificity in the reduction of protein secretion upon mdivi-1 treatment that also includes apolipoproteins-A1 (ApoA1) and B (ApoB), is indicative of the importance of mitochondrial and endoplasmic reticulum (ER) cross-talk in the secretory pathway. Further evidenced through the use of electron microscopy, the authors reveal the ability of DRP1 to mediate ER-microdomain remodelling, through the tethering of trafficking vesicles at the ER, highlighting a novel role of DRP1 beyond its regulation of mitochondrial fission.

The ubiquitous ER-resident chaperone, glucose-regulated protein 94 (GRP94) participates in the regulation of protein folding and is a target of X-box binding protein 1 directed transcription under instances of ER stress and the subsequent unfolded protein response. GRP94 binding to PCSK9 prevents PCSK9-mediated LDLR degradation within the ER upon the co-production of both proteins and their translocation through the secretory pathway.[7] Rogers *et al.* expand upon the relationship between GRP94 and PCSK9 to reveal that, upon mdivi-1 inhibition of DRP1, GRP94 cytosolic levels increase, acting to chaperone PCSK9 towards proteasomal degradation.[4] A proposed therapeutic avenue of PCSK9 targeting is through the promotion of PCSK9 ER-retention.[2] PCSK9 autocatalytic cleavage is a prerequisite for ER exit. The uncleavable PCSK9Q152H mutant, results in low circulating levels of PCSK9 and LDL-C, while unexpectedly also having a protective role in reducing ER stress through the stabilization of key ER-stress response chaperones including GRP94.[8] The GRP94-axis, however, may prove difficult for therapeutic targeting specifically due to membrane barriers. Instead, mdivi-1, a DRP1 inhibitor, is commonly used to inhibit mitochondrial fission. However, in a recent study mdivi-1 was shown to reversibly inhibit complex 1 and mitochondrial reactive oxygen species (ROS) production rather than the DRP1 GTPase activity as expected.[9] The study by Rogers *et al.* benefits from dissecting the mechanism of DRP1 and PCSK9 not only through the use of mdivi-1 but also through CRISPR/Cas9 deletion of DRP1 in cells and through the use of *Drp1*-deficient

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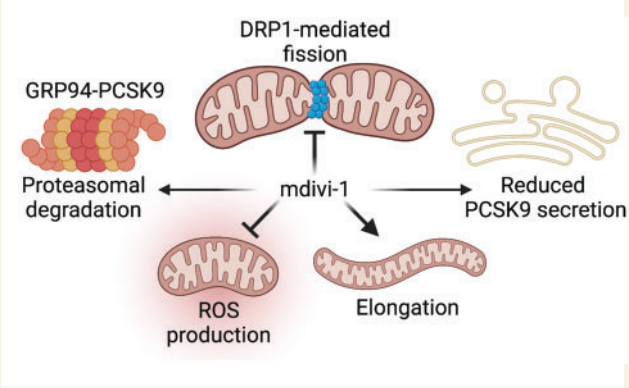

Figure 1 mdivi-1 inhibition of DRP1 induces PCSK9 degradation. DRP1 is central to mitochondrial fission and is targetable with the small molecule inhibitor mdivi-1. The inhibition of DRP1 promotes mitochondrial elongation while also reducing mitochondrial ROS generation. Rogers *et al.*[4] reveal that inhibition of DRP1 also reduces PCSK9 secretion and promotes the PCSK9 chaperone GRP94 to translocate from the ER to the cytosol, leading to increased PCSK9 proteasomal degradation.

mice. DRP1 inhibition in multiple biological settings has been posited to be beneficial, in settings of ischaemia–reperfusion in the kidney and heart through dampened ROS production, while also promoting cancer regression and reductions in pulmonary arterial hypertension.[10]

Rogers *et al.* propose mdivi-1 as a novel small molecule inhibitor of PCSK9. However, a lack of specificity of mdivi-1 and the wide-ranging biological pathways influenced by mdivi-1 might prevent its clinical use. PCSK9 therapeutics are rapidly advancing with recent studies highlighting the ability to deliver an oral antisense oligonucleotide for PCSK9 inhibition,[11] as well as *in vivo* CRISPR base editing of PCSK9 in primates.[12] To conclude, the study by Rogers *et al.*[4] uncovers a critical mechanism regulating PCSK9 secretion and cellular degradation, while also determining a beneficial role of mdivi-1 treatment in the context of calcific atherosclerosis and macrophage-driven inflammation. It awaits to be seen whether intracellular PCSK9 processing can provide novel drug targets with clinical utility.

Conflict of interest: S.A.B. and M.M. are named inventors on a patent application filed by King's College London for PCSK9 measurements.

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