Macrophage autophagy is thought to be essential for protecting from atherosclerosis, and compromised autophagy in macrophages of the artery wall leads to a number of pathologic processes including activation of the inflammasome, defective efferocytosis, and impaired cholesterol metabolism. Autophagy of lipid droplets (LDs) or “lipophagy” catabolizes stored lipids to maintain cellular energy homeostasis and plays a key role in cholesterol efflux by regulating LD-cholesterol mobilization, a rate-limiting step in macrophage reverse cholesterol transport (RCT). MicroRNA-33 (miR-33) is a well-established post-transcriptional RCT regulator, yet the complete mechanisms by which anti-miR33 exerts its beneficial effects on cholesterol metabolism are not known. Notably, microRNA target prediction algorithms identify a number of essential autophagy-related proteins (ATG5, ATG7) and lysosomal effectors (lysosomal-associated membrane protein 1 [LAMP1], lysosomal acid lipase [LAL]) as putative miR-33 targets.

Quantitative PCR array profiling in mouse peritoneal macrophages revealed that a high proportion of autophagy genes are reciprocally regulated by miR-33 overexpression and inhibition. We validated a subset of genes in the autophagy pathway as bona fide miR-33 targets using 3’UTR luciferase assays and confirmed regulation of these targets by miR-33 using quantitative PCR and western blot analysis. Furthermore, we show that miR-33 indirectly regulates the expression of two master regulators of autophagy and lysosomal biogenesis gene programs: forkhead box O (FOXO) 3 and transcription factor EB (TFEB), via targeting of 5’ AMP-activated protein kinase (AMPK). Inhibition of miR-33 in peritoneal macrophage in vitro enhanced cellular autophagic flux, as observed by fluorescence microscopy and western blot analysis, and autophagy was required for anti-miR33 promotion of cholesterol efflux. Furthermore, anti-miR33 treatment of atherosclerotic Ldlr-/- mice enhanced autophagy in plaque macrophages and triggered atherosclerosis regression. These data describe a novel role for miR-33 in the regulation of autophagy and identify additional mechanisms by which anti-miR33 therapy protects against atherosclerosis.