Augmenting SOD2 Levels in Apoe−/− Mice with Peptide-mRNA Nanoparticles Preserves Atherosclerotic Plaque Stability in Advanced Atherosclerosis

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ABSTRACT
Erosion or rupture of the advanced atherosclerotic plaque is the leading cause of atherosclerotic complications such as acute coronary syndrome and stroke. Atherosclerotic plaques in aging hypercholesterolemic mice have decreased SOD2 levels and increased mitochondrial oxidative stress, which leads to cell apoptosis, necrotic core expansion, and fibrous cap thinning and rupture. We hypothesized that increasing SOD2 expression in advanced atherosclerotic plaque cells in mice using mRNA polyplexes would protect mitochondria from oxidative damage and preserve plaque stability. Macrophages isolated from Sod2−/− mice treated with amphilic cationic peptide pSRIIH:Sod2 mRNA polyplexes had markedly increased levels of Sod2 mRNA and mitochondrial SOD2 (p<0.01), higher SOD activity, which resulted in lower levels of mitochondrial and cellular reactive oxygen species (ROS) and improved mitochondrial function. RNA-Seq analysis showed that macrophages treated with Sod2 nanoparticles had broadly decreased inflammatory cytokines and immune activation pathways. In Apoe−/− mice, atherosclerotic lesions with vulnerable plaque features were induced in the carotid artery by placing a perivascular collar over the carotid artery and 4-months Western diet. Mice were treated for 4 weeks with saline or with 0.5 mg/kg pSRIIH:Sod2 mRNA polyplexes which selectively accumulated in atherosclerotic lesion macrophages. The carotid plaques of the mice treated with Sod2 mRNA differed significantly from the plaques of the control Apoe−/− mice by higher SOD2 expression, lower mitochondrial ROS and oxidative DNA damage, and reduced expression of CCL2, IL-1, IL-6, MMP-2 and 9 (p<0.01). Sod2 polyplexes-treated mouse plaques exhibited 25% fewer inflammatory macrophages (p<0.01) and 98% more smooth muscle cells (p<0.001) with a reduced lipid core, preserved fibromuscular cap, and decreased plaque vulnerability index (p<0.05). The results suggest that selective overexpression of Sod2 in plaque macrophages using nanoplexes reduces mitochondrial stress and preserves plaque stability without adverse effects. Using nanoparticle-based mRNA therapeutics to modulate plaque morphology has great potential in the prevention and treatment of atherosclerotic complications.

RESULTS


Figure 2. pSRIIH:Sod2 mRNA increases ROS levels and down-regulates inflammatory pathways in macrophages. (A) pSRIIH uptake by macrophages. (B) RT-PCR analysis of Sod2 expression in primary macrophages. (C) Western blot analysis of SOD2 expression in primary macrophages. (D) HPLC analysis of superoxide levels in primary macrophages. (E) SOD2 immunofluorescence in primary macrophages. (F) Seahorse analysis of mitochondrial function in primary macrophages. (G) Primary macrophages bulk RNA-seq analysis DEG volcano plot. (H) Gene ontology KEGG pathway enrichment analysis.

Figure 3. pSRIIH:Sod2 mRNA treatment in Apoe−/− mice with advanced atherosclerotic lesions. (A) Flow cytometry analysis of SOD2 expression. (B) Fluorescence images of carotid artery atherosclerotic lesions from pSRIIH:Sod2 mRNA-Cy3 treated mice. (C) Immunofluorescence microscopy analysis of SOD2 expression. (D) Cellular (DHE) and mitochondrial (MitoSOX) superoxide generation in carotid artery atherosclerotic lesions from control and pSRIIH:Sod2 mRNA treated mice.

Figure 4. Plaque vulnerability and inflammation are reduced after pSRIIH:Sod2 mRNA treatment in Apoe−/− mice with advanced atherosclerotic lesions. (A) Oil red O staining of carotid artery sections. (B) Immunofluorescence staining for macrophage and SMC markers. (C) Inflammatory and remodeling markers expression in carotid artery lesions.

Figure 5. Pro-inflammatory plaque macrophage populations were depleted after pSRIIH:Sod2 mRNA treatment in Apoe−/− mice with advanced atherosclerotic lesions. (A) Flow cytometry analysis of plaque cells phenotype. (B) t-SNE clustering of spectral flow cytometry data from carotid artery atherosclerotic lesions. (C) Heat-map representation of macrophage markers relative expression for each distinct cluster.

CONCLUSIONS

- Treatment with pSRIIH nanoplexes selectively targeted to plaque macrophages increases SOD2 levels.
- SOD2 augmentation modulates macrophage inflammatory phenotype through mitochondrial oxidative stress reduction and improved function.
- pSRIIH:Sod2 mRNA treatment in Apoe−/− mice with advanced atherosclerosis reduces inflammation and preserves plaque stability.