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Drs Myers and Semigran are employees of Renovacor, Inc and have either equity or options in the company. Mr Landsberg is a consultant with Renovacor, Inc. Mrs Bologna is a consultant to the company and has equity in Renovacor, Inc., Dr Feldman is the founder of Renovacor, Inc; has equity in the company; is a consultant to the company; and has pending U.S. patents that have been optioned by Temple University to Renovacor, Inc. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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RESEARCH LETTER

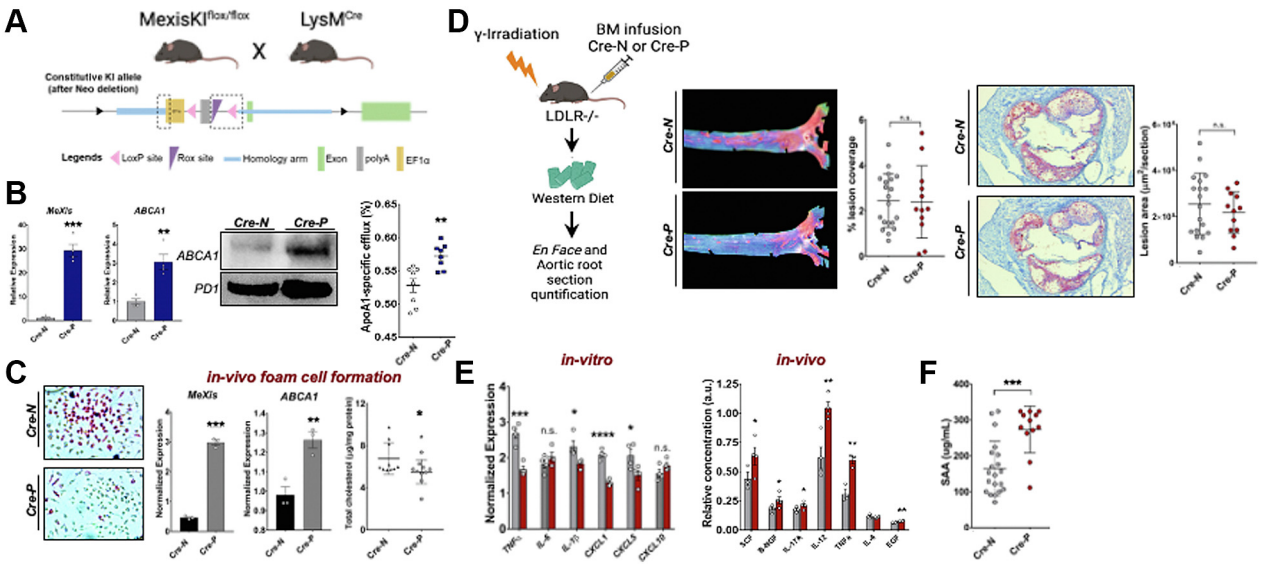
# LncRNAs in Inflammation

## Lessons From a Preclinical Investigation of *Mexis* Therapy in Atherosclerosis

Work in the last decade established long noncoding RNAs (lncRNAs) as critical regulators of many biologic processes required for life including X-inactivation.[1] However, the significance of lncRNAs in cardiovascular disease is still in question. Hundreds of lncRNA genes have been proposed to be critical for cardiovascular health but in vivo genetic perturbations have either been missing or showing subtle effects.[2] In addition, development of lncRNA-based therapies remains an aspirational goal. Our group discovered that conserved lncRNA *Mexis* acts as a key modulator of cholesterol efflux and atherosclerosis development in mice and humans.[3] *Mexis* orchestrates macrophage responses to sterol overload by boosting Abca1 expression and in part explains spatial variations in Abca1 across tissues. *Mexis*-based therapies may be highly attractive because they would enhance cholesterol efflux. Conversely, recent evidence suggests that sustained activation of DDX17 (the binding partner of Mexis) by RNAs triggers noncanonical inflammasome signaling.[4] Thus, we aimed to decipher how enhancing *Mexis* in macrophages would impact multiple nonredundant pathways causality linked with risk of atherothrombotic disease.

Our previous work showed that deletion of *Mexis* reduces Abca1, enhances inflammation and foam cell formation, as well as promotes atherosclerosis development.[3] To test the therapeutic effects of lncRNAs within lesions, we used a novel genetic model that allows spatial control of *Mexis* expression from the endogenous locus (**Figure 1A**). Our study was approved by the UCLA Institutional Animal Care and Research Advisory Committee. We crossed conditional *Mexis* knockin mice with $Cre^{LysM}$ to generate macrophage-specific enhanced *Mexis* expression (**Figure 1A**). Isolation of peritoneal macrophages from Cre+ mice confirmed a marked increase in *Mexis* compared with Cre- controls (**Figure 1B**). Consistent with the notion that *Mexis* acts in *trans* to boost *Abca1* levels, we observed an increase in *Abca1* mRNA and protein (**Figure 1B**). *Mexis* overexpression was associated with enhanced cholesterol efflux to an ApoA1 acceptor (**Figure 1B**). In addition, enhancing *Mexis* reduced foam cell formation in vitro and in vivo (**Figure 1C**). To test the efficacy of *Mexis*-based therapy on atherosclerosis we performed a bone marrow transplant in $LDLR^{-/-}$ mice with Cre+ and Cre- marrow (**Figure 1D**). We confirmed bone marrow reconstitution and found that *Cre+* bone marrow transplantation did not impact serum cholesterol, triglycerides, or fasting glucose (not shown). Analysis of atherosclerosis lesions by en face and aortic root section did not show differences in plaque burden between groups (**Figure 1D**). Surprisingly, and in contrast to the in vitro results, enhanced *Mexis* expression in the atherosclerosis model led to significant elevation in systemic inflammatory markers including serum amyloid A (**Figures 1E to 1F**).

**FIGURE 1 Sustained Overexpression of *MeXis* in Atherosclerosis**




**(A)** Schematic of *MeXis* knock-in strategy. **(B)** expression of *MeXis* and *Abca1* in *MeXisKI*$^{f/f}$*/LysM_Cre*-positive and *MeXisKI*$^{f/f}$*/LysM_Cre*-negative donor mice as measured by qPCR (n = 4), Western blot denoting levels of ABCA1 in peritoneal macrophages harvested. Equal amounts of protein were pooled from five animals per group and run intriplicate. The experiment was repeated three times with similar results. Cholesterol efflux in the presence of *ApoA1* (15 μg/mL) from donor *MeXisKI*$^{f/f}$*/LysM_Cre*-positive and *MeXisKI*$^{f/f}$*/LysM_Cre*-negative mice (n = 8). **(C)** Oil-red-O staining of BMDMs collected from *MeXisKI*$^{f/f}$*/LysM_Cre*-positive and *MeXisKI*$^{f/f}$*/LysM_Cre*-negative mice treated with oxLDL (100 ug/mL) for 72 hr. Images are representative of three independent biological replicates. qPCR analysis of *MeXis, Abca1* expression (n = 3) and quantification of total cholesterol content (n = 10) in peritoneal macrophages collected from *MeXisKI*$^{f/f}$*/LysM_Cre*-positive and *MeXisKI*$^{f/f}$*/LysM1_Cre*-negative mice injected with *AAV_PCSK9* and fed WD for 3 weeks. **(D)** Schematic of BMT experiment, representative photographs and percentage of aorta surface area with atherosclerotic plaque determined through *enface* analysis and Oil-Red-O stain of frozen sections from the aortic root and quantification of lesion area from Oil-red O -stained aortic root sections (n = 12 *Cre*-P mice, n = 20 *Cre*-N mice). **(E)** qPCR analysis of inflammatory gene expression in BMDMs collected from *MeXisKI*$^{f/f}$*/LysM_Cre*-positive and *MeXisKI*$^{f/f}$*/LysM_Cre*-negative mice treated with LPS (100 ng/mL) for 4 hr (left) and in-*vivo* quantification of serum cytokine levels by Luminex (n = 4). **(F)** in-*vivo* quantification of SAA levels by ELISA (n = 12 *Cre*-P mice, n = 20 *Cre*-N mice). Values are mean ± SEM of 3 (**C** [middle]), 4 (**B** [left], **E**), 8 (**B** [right]), 10 (**C** [right]), 12 (**D, F** [*Cre-P*]), or 20 (**D, F** [*Cre*-N]) independent biological replicates. *P* values were calculated using unpaired two-tailed *t*-test (**B-F**). **P<0.05; ** P<0.01; *** P<0.001.* BMDM = bone marrow-derived macrophage; BMT = bone marrow transplantation; ELISA = enzyme-linked immunosorbent assay; oxLDL = oxidized low-density lipoprotein; SAA = serum amyloid A; WD = Western diet.

Our results indicate that enhanced *Mexis* expression in macrophages may not be atheroprotective as hypothesized, but the mechanisms at play here are highly intriguing. Despite evidence of enhanced Abca1 in vivo, chronic *Mexis* expression was associated with unrestrained inflammation. *Mexis*, similar to many lncRNAs, contains multiple short interspersed nuclear elements (SINEs) which are repeats thought to have arisen from integration of retroviruses into the host genome. These RNAs are normally expressed at low levels but enhanced SINE RNA expression has been shown to activate the NLRC4 inflammasome through the activity of DDX17, an established binding partner of *Mexis*.[4] Thus, it is conceivable that "supra-physiologic" RNA expression was associated with inflammation. Although enhancing *Mexis* in macrophages reduced foam cell formation in vitro and in vivo, we did not see differences in Oil-Red-O staining. These results may be explained by compensatory effects in lipid handling properties of cells involved in the atherosclerosis study. Indeed, compensation of lncRNA effects has been reported in chronic perturbations in the context of lipid metabolism.[5] In addition, we cannot exclude contributions of other cell types within lesions that show Oil-Red-O stain enhancement or late changes in lipid accumulation due to enhanced inflammation in macrophages. Our study expands the interweaving of lipid handling properties with inflammation, suggesting that meaningful changes in atherosclerosis burden mediated by enhanced cholesterol efflux capacity may be negated by enhanced inflammatory

activation. In addition, our findings have important implications for proposed therapeutic strategies that aim to enhance lncRNA expression for chronic disease treatment and different modes by which lncRNAs impact inflammation.

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