SUPPLEMENTAL MATERIAL

Detailed Methods

ICA collection and Patient Stratification

Carotid artery atherosclerotic plaque specimens and pertinent medical histories were obtained from 76 patients undergoing CEA in the Department of Surgery, Section of Vascular/Endovascular Surgery at the Ochsner Clinic. The Ochsner Internal Review Board approved the protocol and informed consent was obtained from all participants. Plaques were cross-sectioned into ~ 1cm pieces and the plaque shoulder was isolated using gross dissection, snap-frozen, and stored at -80 C until RNA isolation. The patients were stratified according to their neurologic presenting symptomatology; a stroke neurologist independently assessed all acutely symptomatic carotid patients. The mean NIH Stroke Score for the acutely symptomatic patients presenting with a stroke was 3.3 ±0.7. Patients without prior neurologic events but high-grade (>80%) carotid stenosis undergoing a prophylactic CEA were termed "asymptomatic" (n = 31). Patients with a prior neurologic event, including transient monocular vision loss, a transient cerebral ischemic event or stroke, indicated an acute plaque rupture event and were grouped according to time between the ischemic event and the CEA. Patients undergoing CEA within 5 days of rupture were termed "urgent" (n = 25) and those undergoing CEA greater than 5 days post rupture were termed "symptomatic" (n = 20). The mean times to treat in the urgent and symptomatic groups were 2.4 ± 0.4 and 38 ± 8 days, respectively.

RNA Isolation and Analysis

For each carotid plaque, the cross-section with the greatest plaque burden was identified and the circumferential boundary region of the plaque (plaque shoulder) was dissected away. Total RNA was isolated from these specimens using the miRNeasy mini kit (Qiagen Inc., Valencia, CA) with minor modifications. miRNA were measured using the miScript II RT Kit coupled with the miScript SYBR Green PCR Kit (Qiagen). STAT5A, c-Kit, and p27Kip1 were quantified using the One-step Quantitect SYBR Green PCR Kit (Qiagen). U6 snRNA and Hypoxanthine Phosphoribosyltransferase (Hprt) were used as loading controls for the miRNA assays and mRNA assays, respectively. The catalog numbers for the individual PCR assays are listed in the Supplementary Table I. Relative expression was calculated by the $2^{-\Delta\Delta Ct}$ method. The differences observed in miR-221/222 remained significant after adjusting for the individual performing the isolation.

Statistics

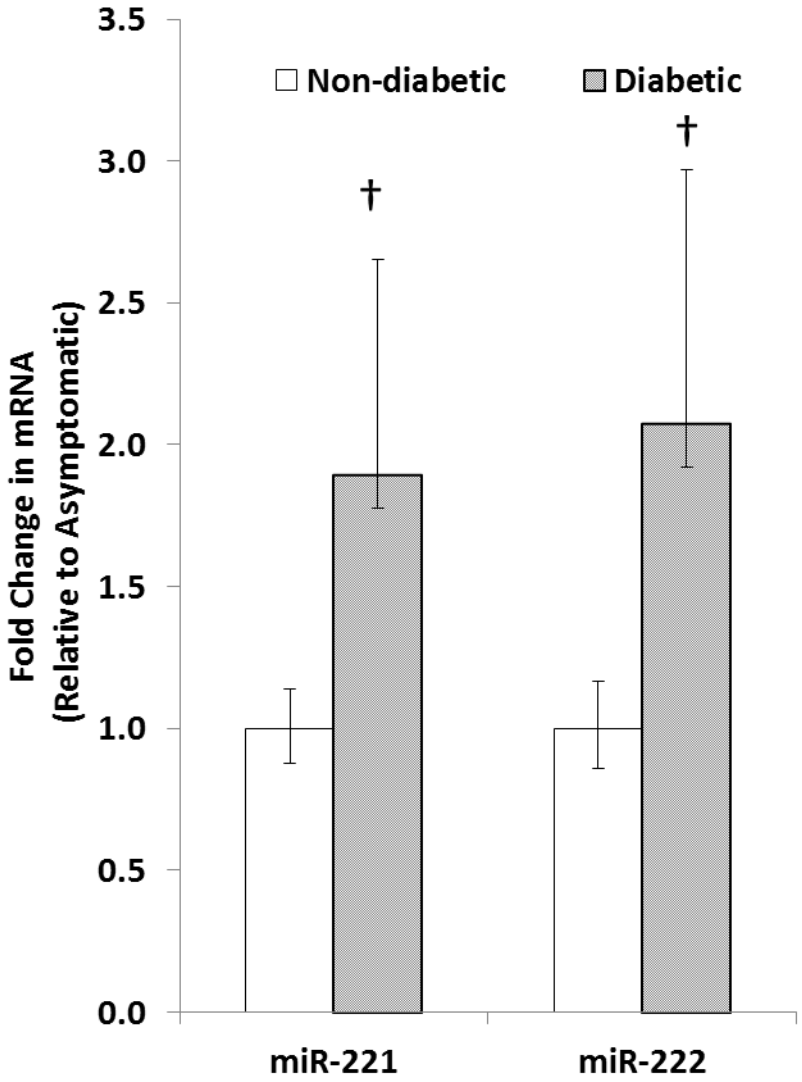
Data is expressed as the mean +/- standard error of the mean. Statistical analysis between three groups was performed using ANOVA coupled with Tukey's HSD test. For comparisons between two groups, Students t-test was used. X^2 analysis was used to compare categorical variables across groups. All analyses were performed using SPSS v19.0 (IBM).

Supplementary Table I

Supplemental Table: Primer Assays

Name	Qiagen Cat#
miR221	ms00003857
MiR222	ms00007609
MiR145	ms00003528
U6	ms00033740
P27	qt00596022
Stat5A	qt00066101
HPRT	qt00059066
c-kit	qt01679993

Supplementary Figure I



Supplementary Figure I. Expression of miRNA in the carotid plaque shoulder of diabetic and non-diabetic patients. † indicates $p < 0.05$.