Next generation miRNA sequencing and changes in coagulation measured by thrombelastography (TEG) in patients with cardiovascular disease

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Background: Thrombelastography (TEG), an ex-vivo clotting assay can identify subjects at high risk of subsequent coronary thrombotic events. Synthesis of clotting factors is subject to post-translational regulation, which is modulated at least in part by miRNA.

Hypothesis: We hypothesized that miRNA sequencing may identify specific miRNA linked with measures of hypercoaguability by TEG.

Methods: Kaolin activated thrombelastography was performed in platelet poor citrate plasma from 61 subjects referred for cardiac catheterization. Time to clot formation (R), clot stabilization time (K), and maximal fibrin clot strength (MA) was measured. Next generation miRNA sequencing was done from RNA isolated from whole blood samples, which includes miRNA derived from leukocytes and platelets. Prediction of miRNA gene targets was performed with targetscan.

Results: Sequencing resulted in quantification of 371 distinct miRNA from whole blood samples. We found 13 miRNA correlating with alteration in TEG-R, 33 miRNA correlating with TEG-K, and 21 miRNA correlating with TEG-MA. Coagulation factors or genes associated with coagulation were found to be among predicted targets in 49 out of these 67 miRNA. Most common predicted targets included factors II, V, VII, X, XIII, fibrinogen, plasminogen-activator inhibitor, and tissue factor. Factor XIIIa1 was highly conserved gene target by miR-96-5p (one of only 3miRNA predicted for this gene). MiR-96-5p correlated with clot stabilization time (?=0.26, p=0.047) which has been shown to be dependent on FXIIIa activity. MiR-22-3p was significantly correlated with TEG-K (?=0.28, p=0.034) and was only miRNA with highly conserved target site for prothrombin (Factor II).

Conclusions: In patients with cardiovascular disease miRNA sequencing combined with coagulation phenotype in silico analysis may identify novel links to coagulation that are associated with increased thrombotic risk.