**Effects of microRNAs on Endothelial Cell Dysfunction in Kawasaki Disease**

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**Introduction**

Kawasaki disease (KD) is the leading cause of acquired heart disease in children. KD is an acute vasculitis of unknown etiology that can lead to coronary artery lesions in 25% of untreated patients. The mechanisms behind the development of KD and consequent vasculitis remain unclear, and standard therapy with intravenous immune globulin does not work for 10-15% of patients. Therefore, identifying different factors that affect endothelial cells in KD may lead to better treatments.

Circulating microRNAs (miRNAs) are small, non-coding RNA molecules that control gene expression by inducing transcript degradation or by blocking translation. miRNA expression changes with KD and miRNAs may contribute to endothelial cell dysfunction. By treating endothelial cells in vitro with KD patient serum, we can determine the effects of changing miRNAs on endothelial cell gene expression.

**Methods**

Serum was isolated from patients with KD, healthy pediatric patients, or patients with non-KD febrile disease. For miRNA analysis, serum was freeze thawed 3X to release miRNAs, and miRNAs were reverse transcribed. 384 well TaqMan Low Density Arrays containing sequence-specific primers and Taqman probes in the ABI7900 were used to measure differences in miRNA levels in serum.1

Serum was also used to treat human umbilical vein endothelial cells (HUVECs), where half the serum was left untreated and half was subjected to freeze thaws, RNase treatment, and heat treatment to inactivate RNase.

**Results**

Circulating microRNAs (miRNAs) are small, non-coding RNA molecules that control gene expression by inducing transcript degradation or by blocking translation. miRNA expression changes with KD and miRNAs may contribute to endothelial cell dysfunction. By treating endothelial cells in vitro with KD patient serum, we can determine the effects of changing miRNAs on endothelial cell gene expression.

**Figure 1**: Heatmap of changes in miRNA levels between patients with KD (purple) and non-KD febrile controls (green). Published data.$^1$

**Figure 2**: Circulating miRNAs differentiate non-KD febrile controls from KD patients. (A) Random forest analysis of array data demonstrated that miR-210-3p, -184 and -19a-3p differentiated non-KD febrile controls (black squares) from all KD patients (red triangles). (B) Box plots showed that there are statistically significant differences (all p-values < 0.001) between the non-KD febrile controls and all KD patients. (C) Hierarchical clustering shows separation between the non-KD febrile controls and KD patients. (D) Receiver operating curve of the top 3 microRNAs showed an area under the curve of 0.769. Published data.$^1$

**Figure 3**: Treatment of human umbilical vein endothelial cells (HUVECs) with untreated or RNase-treated serum from children with KD, or non-failing, healthy controls (Ped NF), or non-KD febrile controls with either bacterial infections (bac) or viral infections (viral) induces changes in gene expression of pathological markers.

**Conclusions**

Patients with Kawasaki disease (KD) have increased expression of miRNA-210-3p, -184, and -19a-3p. miRNAs in KD serum induce increases in expression of cytokines, proteases, and markers of endothelial to mesenchymal transition. The effect of KD serum on endothelial cells is blunted by RNase treatment of the serum, indicating that the effect of serum on gene expression is primarily caused by RNAs in the serum. Since miRNAs decrease expression of their targets, it is likely that an intermediate mediator, such as a transcription factor, is responsible for inducing these changes in gene expression.

**Future Directions**

Transfer endothelial cells with specific miRNAs to determine their individual effect on expression of pathological markers.

Perform atomic force microscopy on serum-treated cells to determine whether KD serum induces a change in cell stiffness, which accompanies endothelial to mesenchymal transition, and may contribute to coronary artery lesions.

Determine RNA markers that distinguish KD patients with coronary artery lesions from patients that have no lesions, and distinguish between patients that respond to treatment from those who do not.

**References**


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