

Upregulation of miR-18a-5p in colony forming unit Hill's as a biomarker of subclinical cardiovascular disease; study on type 1 diabetes and the effect of metformin.

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Introduction

- MicroRNA (miR), a class of small noncoding RNA, has been shown to participate in the pathogenesis of diseases including cardiovascular disease (CVD), the predominant cause of mortality worldwide.¹ Overexpression of miR-18a-5p was demonstrated to be pro-atherogenic in animal studies.²
- Type 1 diabetes (T1DM) offers an ideal model of subclinical CVD as characterized by endothelial dysfunction, elevated inflammatory markers, and reduced levels circulating endothelial progenitor cells and colony forming unit Hill's (CFU-Hill's).³⁻⁶
- CFU-Hill's are hematopoietic-derived cells that have been suggested to serve as a biomarker for vascular health.⁷ We explored miR-18a-5p expression in CFU-Hill's in T1DM/subclinical CVD, with additional intervention metformin, a promising cardio-protective drug candidate for CVD.

Aims

To explore the role of miR-18a-5p as a sensitive biomarker in a model of subclinical CVD (T1DM patients) and investigate the cardio-protective role of metformin in CVD.

Methods

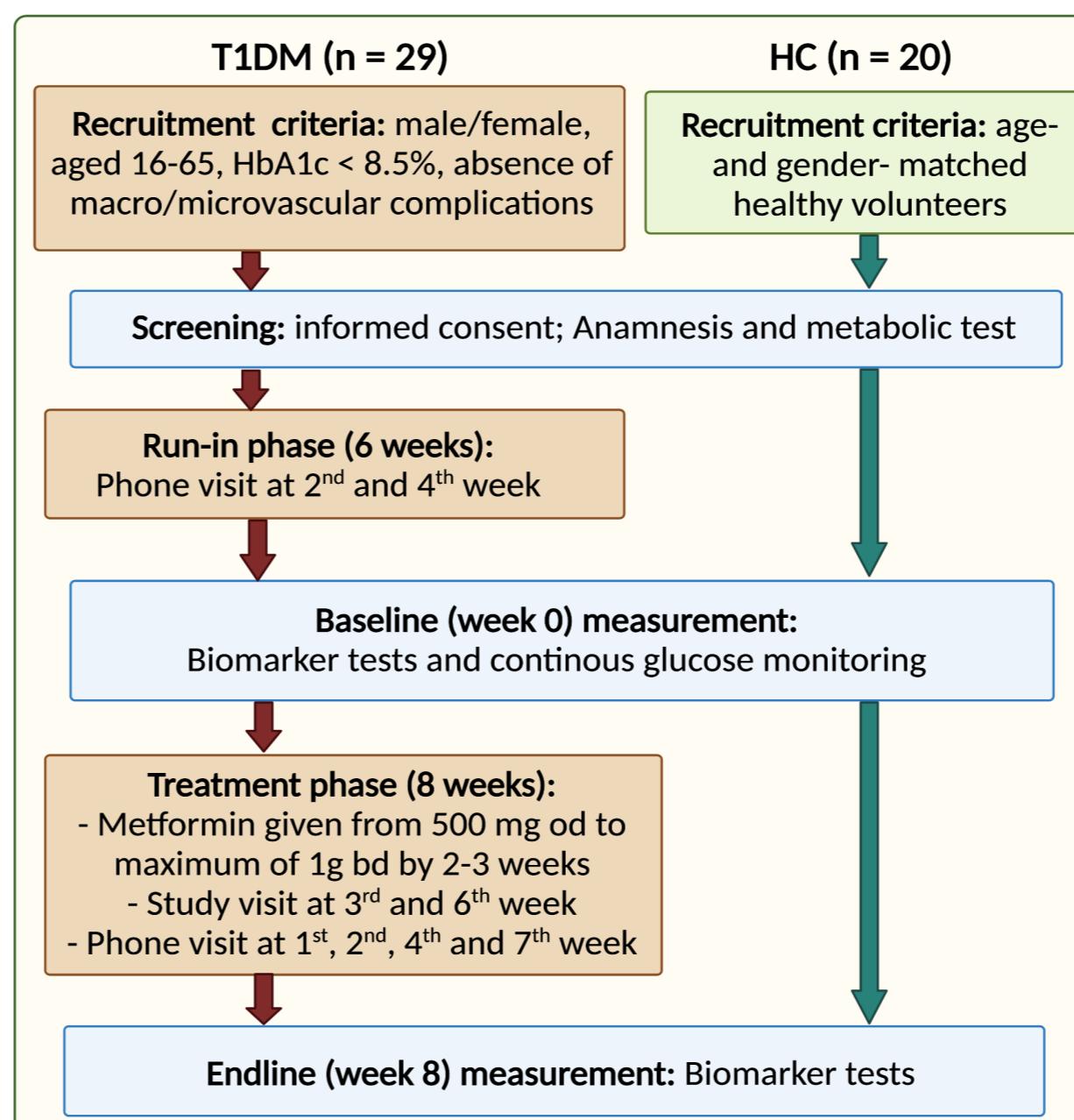


Figure 1. Flow diagram of study design. T1DM: type 1 diabetes; HC: healthy control.

- CFU-Hill's were cultured according to the method described by Hill et al.⁷
- Meso scale discovery assay was used to measure the cytokines level.
- Flow cytometry procedure was performed to evaluate circulating progenitor cells.
- MiR-18a-5p expression in CFU-Hill's were evaluated using real-time quantitative PCR.
- MiR-18a-5p expression, cytokines, inflammatory and vascular health markers were measured before/after metformin.
- Ingenuity Pathway Analysis (IPA) software predicted target genes and pathways regulated by miR-18a-5p.

Results

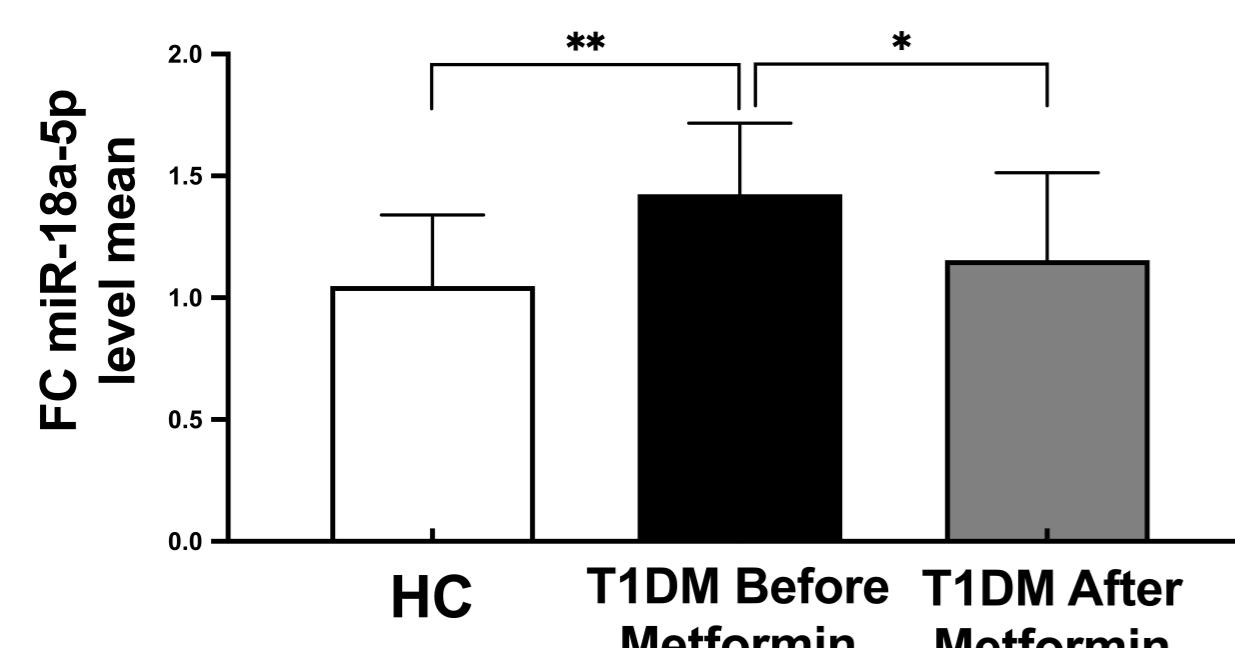


Figure 2. Comparison of miR-18a-5p level in HC, T1DM before and after metformin. FC: fold change; HC: healthy control; T1DM: type 1 diabetes. *p<0.05; **p<0.01

- In T1DM, miR-18a-5p was upregulated by 1.4-fold compared to HC ($p = 0.008$).
- Following metformin, miR-18a-5p was downregulated by 1.3-fold ($p = 0.044$), normalizing its expression to HC ($p = \text{NS}$).

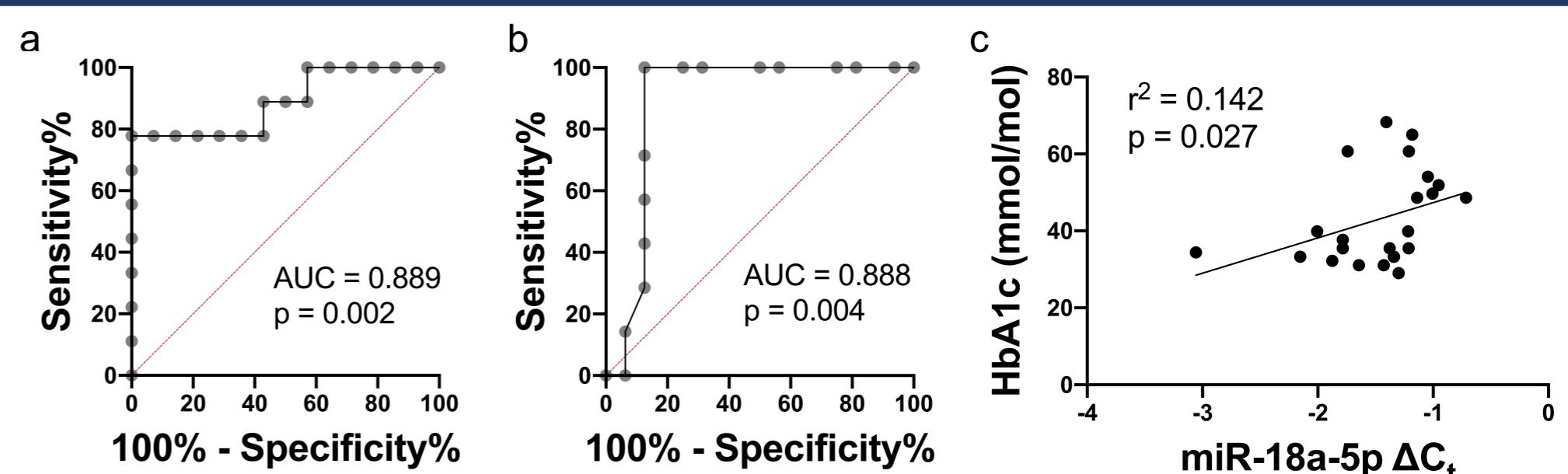


Figure 3. ROC curve analyses of (a) miR-18a-5p in discriminating T1DM from HC ($AUC=0.889$, sensitivity=77.78%, specificity=100%), (b) HbA1c indicating upregulated miR-18a-5p ($AUC=0.888$, sensitivity=100%, specificity=87.5%); (c) Significant correlation between HbA1c and miR-18a-5p.

- Upregulated miR-18a-5p defined subclinical CVD at HbA1c of 44.5 mmol/mol (pre-diabetes).

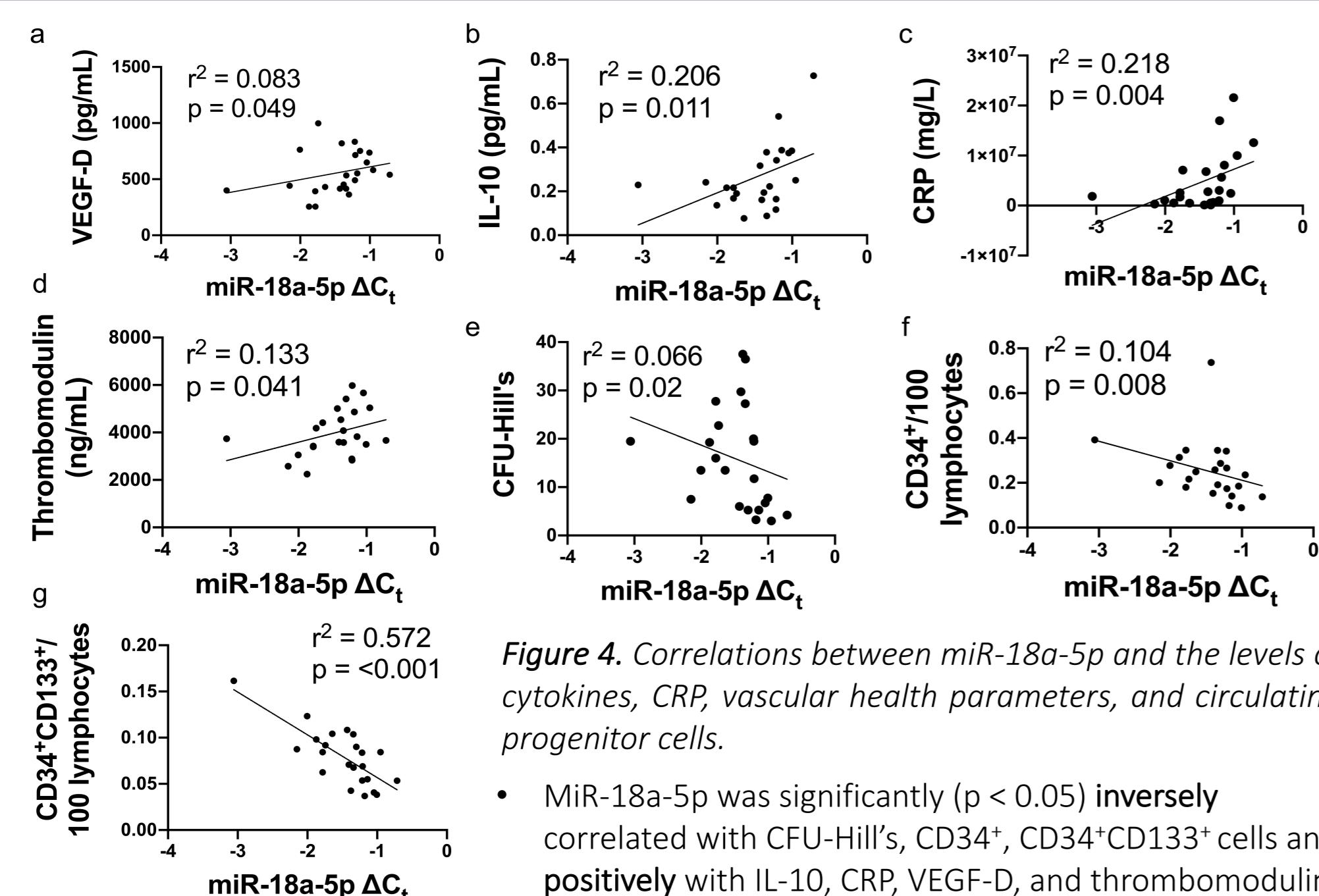


Figure 4. Correlations between miR-18a-5p and the levels of cytokines, CRP, vascular health parameters, and circulating progenitor cells.

- MiR-18a-5p was significantly ($p < 0.05$) inversely correlated with CFU-Hill's, CD34⁺, CD34⁺CD133⁺ cells and positively with IL-10, CRP, VEGF-D, and thrombomodulin.

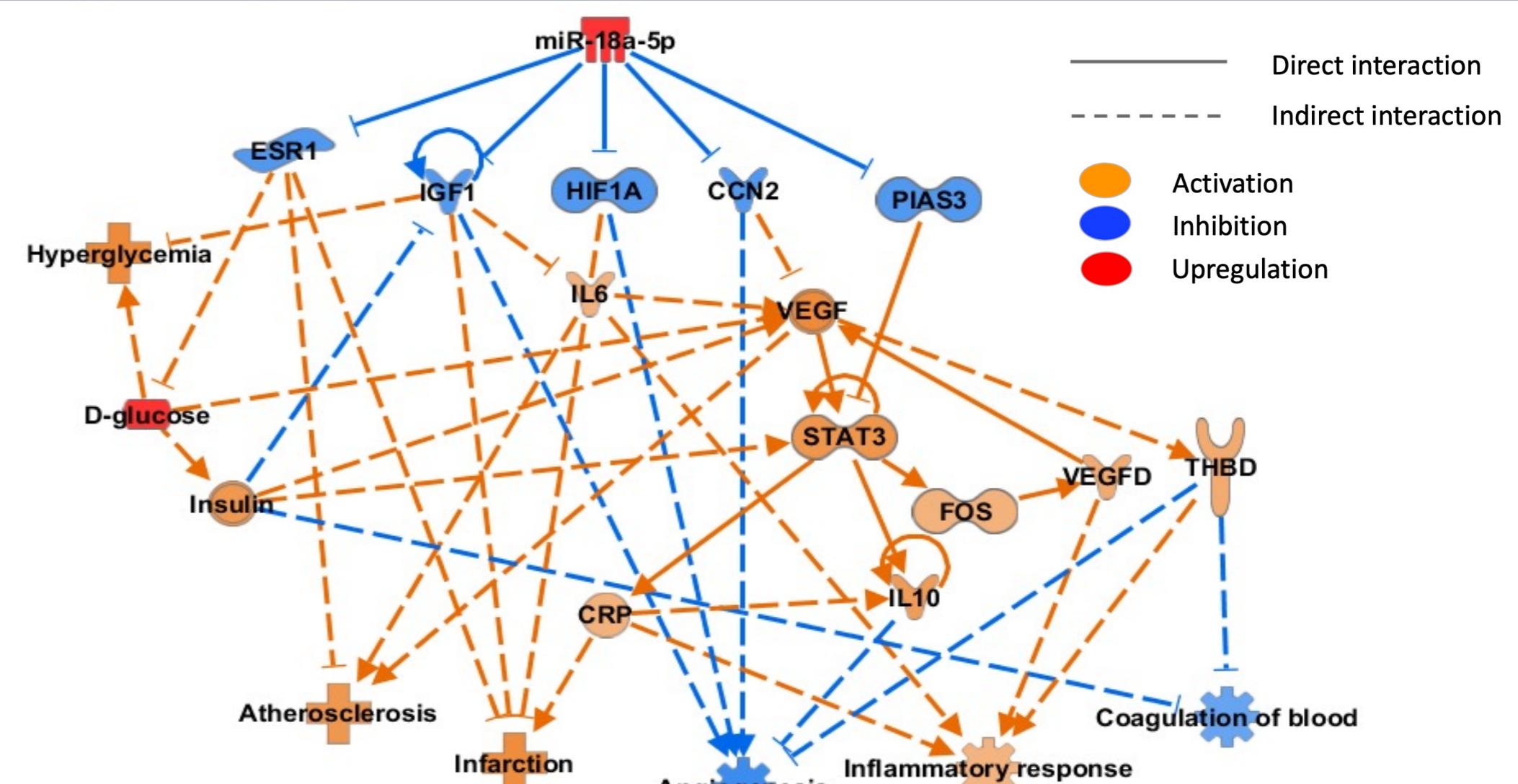


Figure 5. IPA prediction network of miR-18a-5p and its mRNA targets and cytokines supporting its involvement in CVD.

- Predicted downstream target genes involved in CVD: ESR1, IGF1, HIF1A, CCN2, and PIAS3.

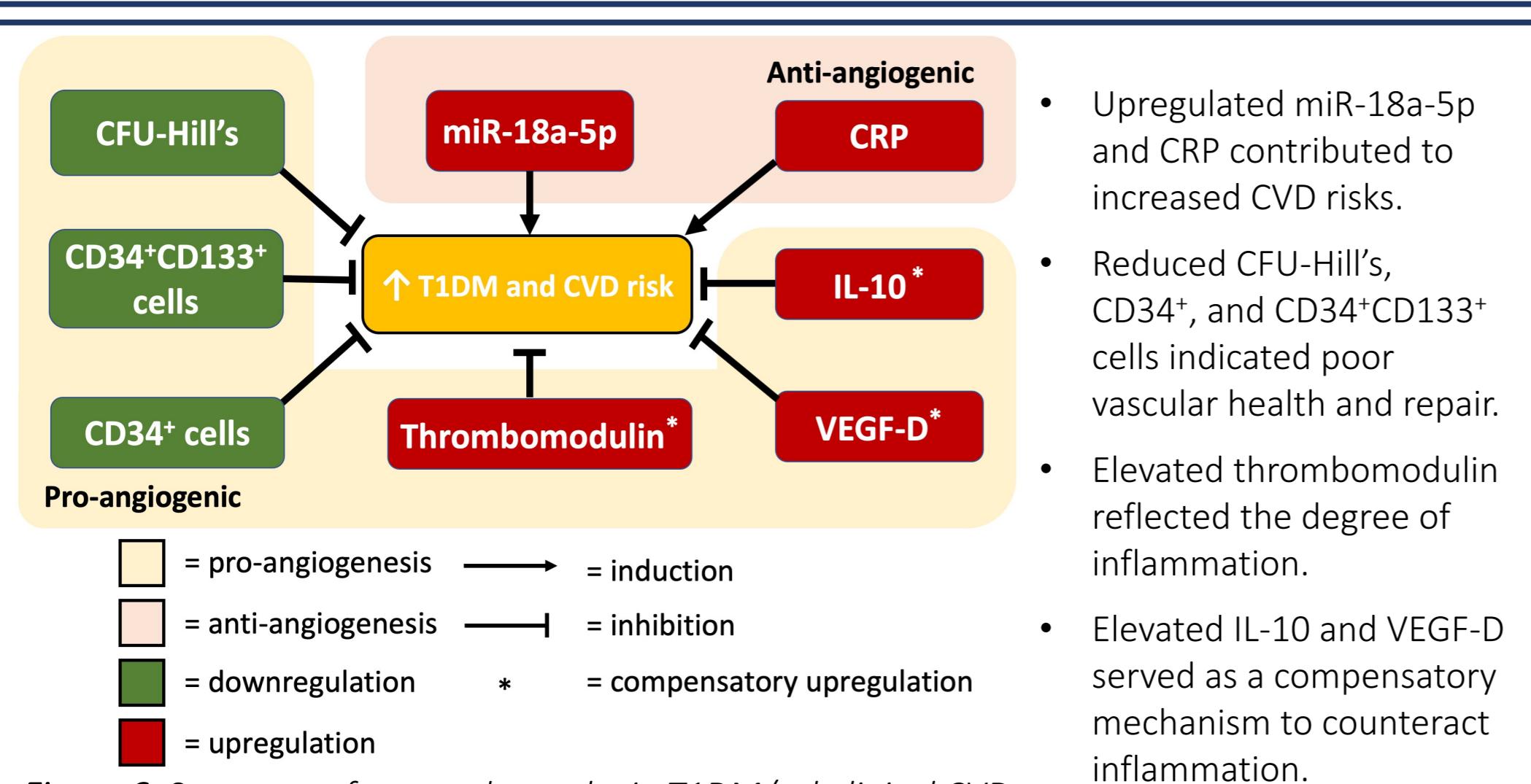


Figure 6. Summary of our study results in T1DM/subclinical CVD.

- Upregulated miR-18a-5p and CRP contributed to increased CVD risks.
- Reduced CFU-Hill's, CD34⁺, and CD34⁺CD133⁺ cells indicated poor vascular health and repair.
- Elevated thrombomodulin reflected the degree of inflammation.
- Elevated IL-10 and VEGF-D served as a compensatory mechanism to counteract inflammation.

Conclusions

- MiR-18a-5p in CFU-Hill's is upregulated in T1DM and downregulated by metformin.
- We validated animal research on anti-angiogenic miR-18a-5p with several target genes for future CVD therapies.
- Upregulated miR-18a-5p defined subclinical CVD at pre-diabetes (HbA1c of 44.5 mmol/mol) and may act as sensitive biomarker and prognostic indicator of CVD.

Future Work

- Validate our findings in larger cohorts of longer duration.
- Develop animal models of T1DM to examine their miRNA profiles and affirm the cardio-protective role of metformin.
- Explore miRNA-based, cytokine-based, and downstream target gene therapies for CVD.

