Functional validation of microRNA-126-3p as a platelet reactivity regulator using human haematopoietic stem cells

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**Abstract**

BACKGROUND: Platelets are an abundant source of micro-ribonucleic acids (miRNAs) that may play a role in the regulation of platelet function. Some miRNAs, such as miR-126-3p, have been noted as potential biomarkers of platelet reactivity and the recurrence of cardiovascular events. However, the biological relevance of these associations remains uncertain, and the functional validation of candidate miRNAs on human-derived cells is lacking. OBJECTIVE: This article functionally validates miR-126-3p as a regulator of platelet reactivity in platelet-like structures (PLS) derived from human haematopoietic stem cells.

MATERIALS AND METHODS: CD34+-derived megakaryocytes were transfected with miR-126-3p and differentiated in PLS. PLS reactivity was assessed using perfusion in a fibrinogen-coated flow chamber. miR-126-3p's selected gene targets were validated using quantitative polymerase chain reaction, protein quantification and a reporter gene assay.

RESULTS: CD34+-derived megakaryocytes transfected with miR-126-3p generated PLS exhibiting 156% more reactivity than the control. These functional data were in line with [...]
Supplementary Fig. S1 Characterization of human haematopoietic stem cells during differentiation. (A) CD34⁺ culture and transfection procedure. CD34⁺ cells were cultured for 7 days in StemSpan megakaryocyte expansion supplement (CC220 containing stem cell factor [SCF], interleukin [IL]-6, IL-9 and thrombopoietin [TPO]). An additional 8 days of culture was carried out in presence of TPO. The transfection procedure was performed at day (D) 13, and analysis were performed at D15. (B) Percentages of cells positive for CD34, CD41, CD42b and CD42d throughout the differentiation process. Expression levels of specific markers were measured using fluorescence-activated cell sorting (FACS) at D7, D13 and D15 (n = 3).
**Supplementary Fig. S2** Impact of miR-126-3p transfection. (A) Evaluation of transfection efficiency in megakaryocytes using Alexa Fluor 488, 5 hours after the procedure at day (D) 13 (n = 3). *p < 0.05. (B) miR-126-3p level in platelet-like structure (PLS) after transfection (n = 3). Results are expressed relative to the mock condition. **p < 0.01. (C) Quantification of the expression of selected markers (CD34, CD41, CD42b and CD42d) at D15 (n = 3). (D) Proportion of PLS in the cell culture (compared to the number of megakaryocytes and PLS) at D15 (n = 7). (E) miR-223-3p expression level after miR-126-3p transfection (n = 3).