MACS&more

Excerpt from MACS&more Vol 14 – 2/2012

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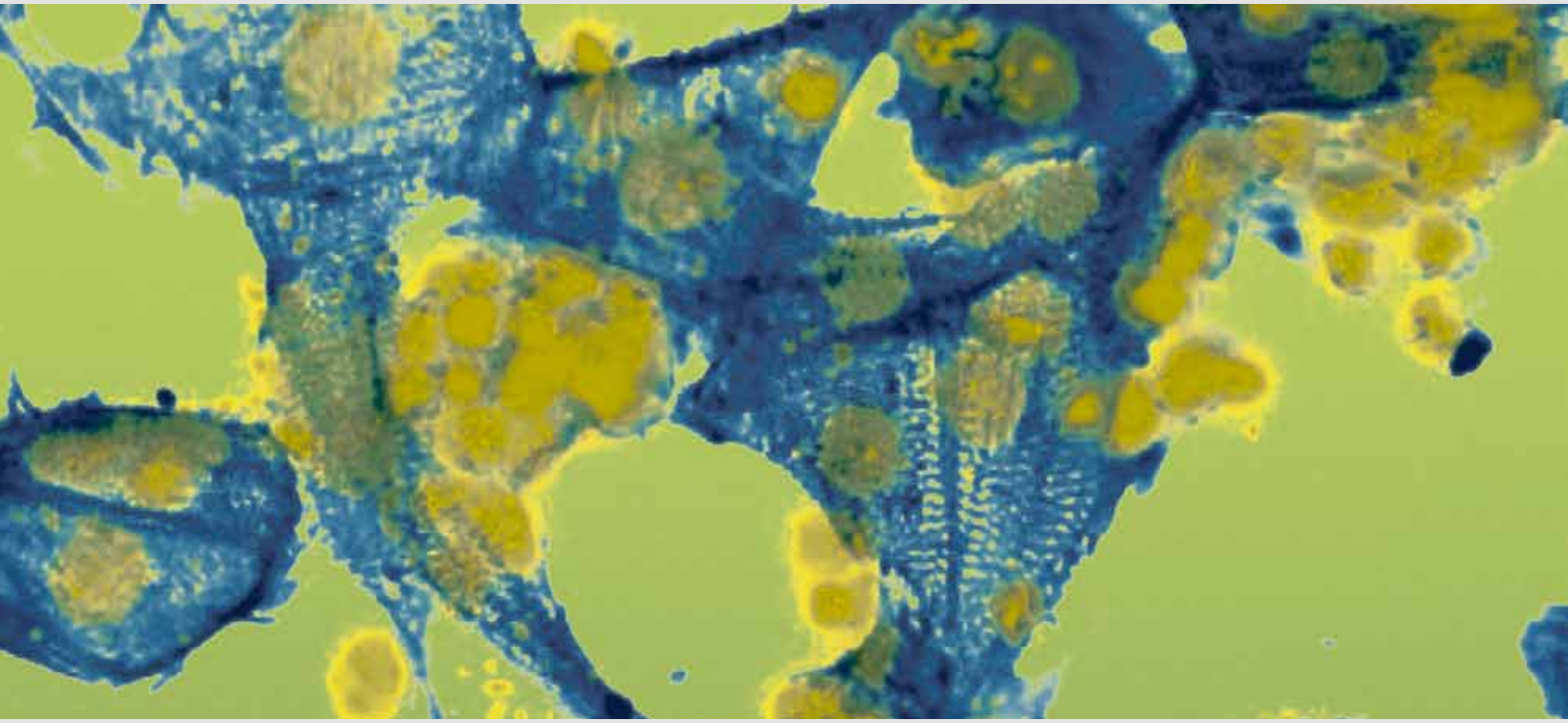
Ute Bissels, Stefan Wild, Stefan Tomiuk, Hartmut Scheel, and Andreas Bosio

Miltenyi Biotec GmbH, Bergisch Gladbach, Germany



Array-based analysis of microRNA and mRNA expression characterizes early differentiation pathways of CD133$^+$ and CD34$^+$ hematopoietic stem and progenitor cells

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Introduction

MicroRNAs, short non-coding RNAs of 20 to 25 nucleotides in length, regulate target mRNAs post-transcriptionally. Each microRNA has the potential to target hundreds of mRNAs and one mRNA can be targeted by multiple microRNAs[1], suggesting a complex network of gene expression regulation. Furthermore, microRNAs have been shown to play an important role in many different cellular, developmental, and physiological processes, including hematopoiesis. To further characterize the role of microRNAs in hematopoiesis, we isolated different subpopulations of hematopoietic stem and progenitor cells expressing CD34 and/or CD133 and performed microRNA expression profiling[2].

Material and methods

Isolation of CD133$^+$ and CD34$^+$CD133$^-$ cells

First, CD133$^+$ cells were magnetically isolated from bone marrow by MACS® Technology. Subsequently, CD34$^+$CD133$^-$ cells were isolated from the negative fraction of the first separation (fig. 1). Purified cells were labeled with CD34-APC and CD133-PE and analyzed by flow cytometry.

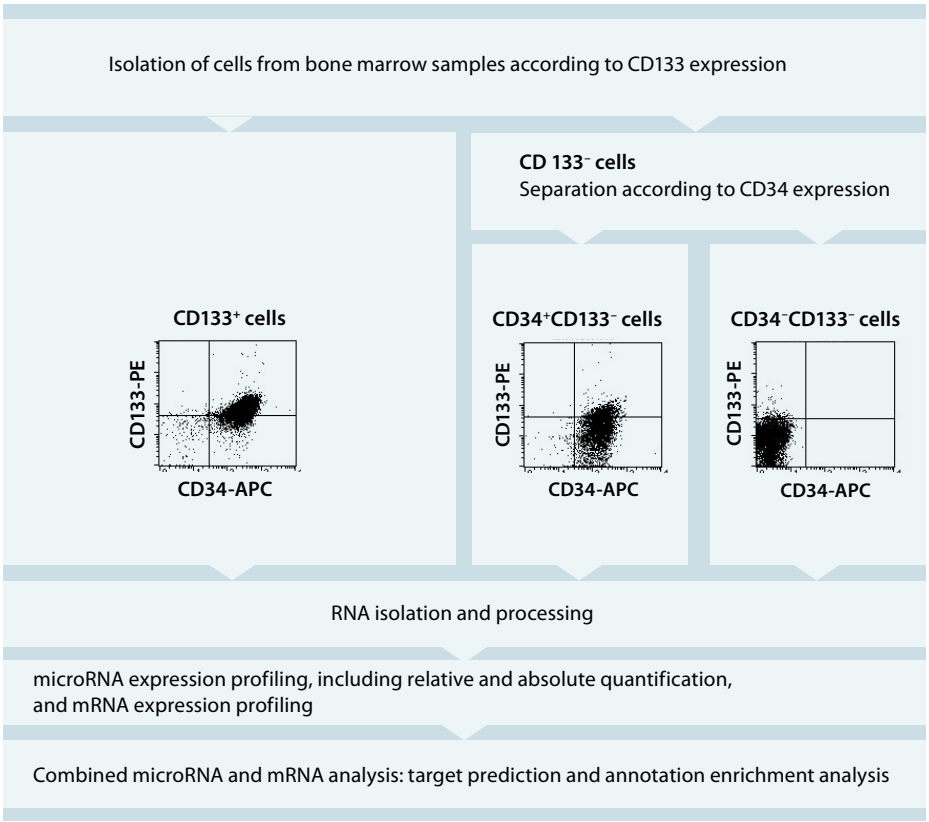

Figure 1 Workflow for the isolation of cell subpopulations from bone marrow by MACS Technology and subsequent microRNA and mRNA expression analysis.

MicroRNA expression analysis using the miRXplore™ Microarray platform

RNA samples from bone marrow cell subpopulations of five different donors were labeled with Cy™5 and hybridized versus the Cy3-labeled universal reference (UR) using the miRXplore™ Microarray Platform. The UR is an equimolar pool of about 1,000 synthetic microRNAs of known concentration that allows direct comparison of microRNA expression across multiple experiments and absolute quantification[3].

Analysis of microRNA-mRNA interactions

Luciferase assays were used to validate some of the microRNA-mRNA interactions. In detail, we cloned the respective microRNA-binding site regions of frizzled5 (FZD5), tropomyosin (TPM1), and CD133 3'-UTRs behind a luciferase reporter gene, resulting in three vectors, pMIR-Luc-FZD5, pMIR-Luc-TPM, and pMIR-Luc-CD133. HEK293 cells were cotransfected with 300 ng vector and oligonucleotides miR-142-3p (50 nM) or miR-29a (100 nM). Relative luciferase activity represents firefly luciferase activity normalized versus *Renilla* luciferase acitivity.

Results

Relative and absolute microRNA expression levels in CD133⁺ and CD34⁺ cells

Significance Analysis of Microarrays (SAM) led to 18 microRNAs that were significantly differentially expressed in CD133⁺ cells and/or CD34⁺CD133⁻ cells versus CD34⁻CD133⁻ cells. Two-dimensional hierarchical average linkage clustering revealed three different expression signatures (groups 1, 2, and 3; fig. 2A). Figure 2B shows microRNA copy numbers of one representative microRNA from each expression group for four different donors: miR-10a was expressed at high levels and miR-425 at low levels in CD133⁺ cells. miR-29a showed high expression levels in both CD133⁺ and CD34⁻CD133⁻ cells.

Verification of microRNA-mRNA interactions via luciferase assay

In order to further analyze the role of the differentially expressed microRNAs in CD133⁺ stem cells, we generated mRNA expression profiles and examined the co-expression of bioinformatically predicted microRNA-mRNA pairs (fig. 3).

We used luciferase assays to validate some of the microRNA-mRNA interactions. Cotransfection of HEK293 cells with miR-29a–mimicking oligonucleotide and pMIR-Luc-FZD5 decreased the luciferase activity by 40%, whereas cotransfection with pMIR-Luc-TPM and miR-29a decreased activity by 33%. Cotransfection with pMIR-Luc-CD133 and miR-142-3p led to a reduction by 30%.

Data are representative of at least five independent experiments. The luciferase assays indicated that FZD5, a receptor of the Wnt signaling pathway, and TPM1, an actin binding protein, which are expressed at lower levels in CD133⁺ cells, are controlled by miR-29a. Our data also suggest that the hematopoietic cell–specific microRNA miR-142-3p regulates expression of CD133.

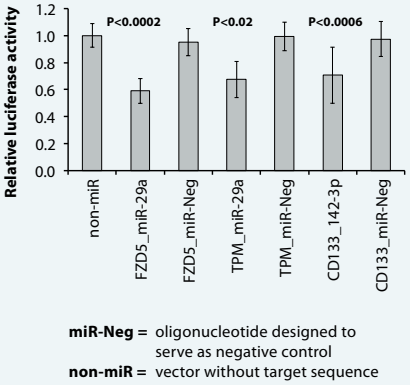

Figure 3 Analysis of microRNA-mRNA interactions by using luciferase assays. HEK293 cells were cotransfected with miR-29a–mimicking oligonucleotide and pMIR-Luc-FZD5 or pMIR-Luc-TPM. An miR-142-3p–mimicking oligonucleotide was used in combination with pMIR-Luc-CD133. miR-Neg: oligonucleotide designed to serve as negative control; non-miR: vector without target sequence.

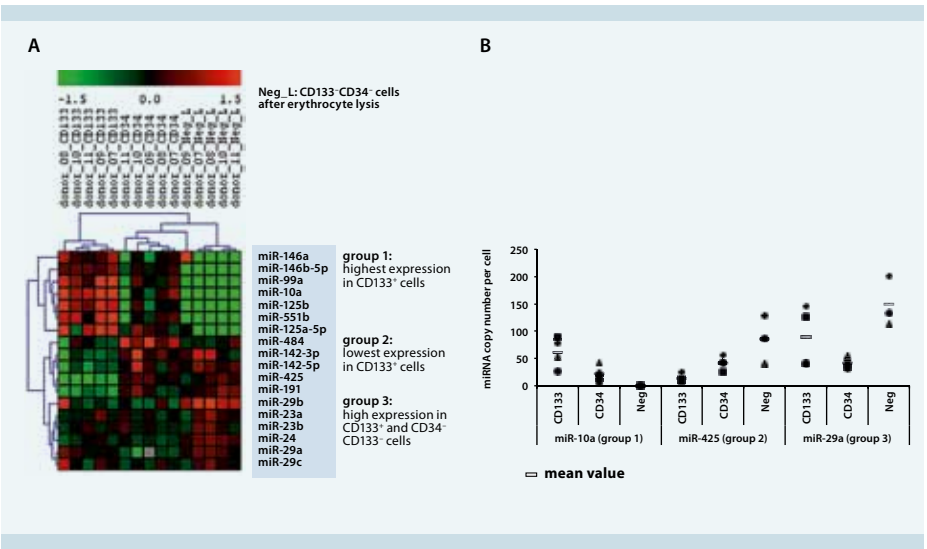

Figure 2 Significance Analysis of Microarrays for microRNA expression in CD34⁺ and CD133⁺ cells. (A) Two-dimensional hierarchical average linkage clustering revealed three different expression signatures. (B) microRNA copy numbers of one representative microRNA from each expression group for four different donors.

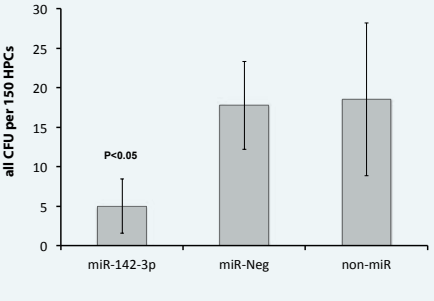

Figure 4 Analysis of colony formation by CD133⁺ cells transfected with an miR-142-3p–mimicking oligonucleotide or control. miR-Neg: oligonucleotide designed to serve as negative control; non-miR: vector without target sequence. HPCs: hematopoietic progenitor cells.

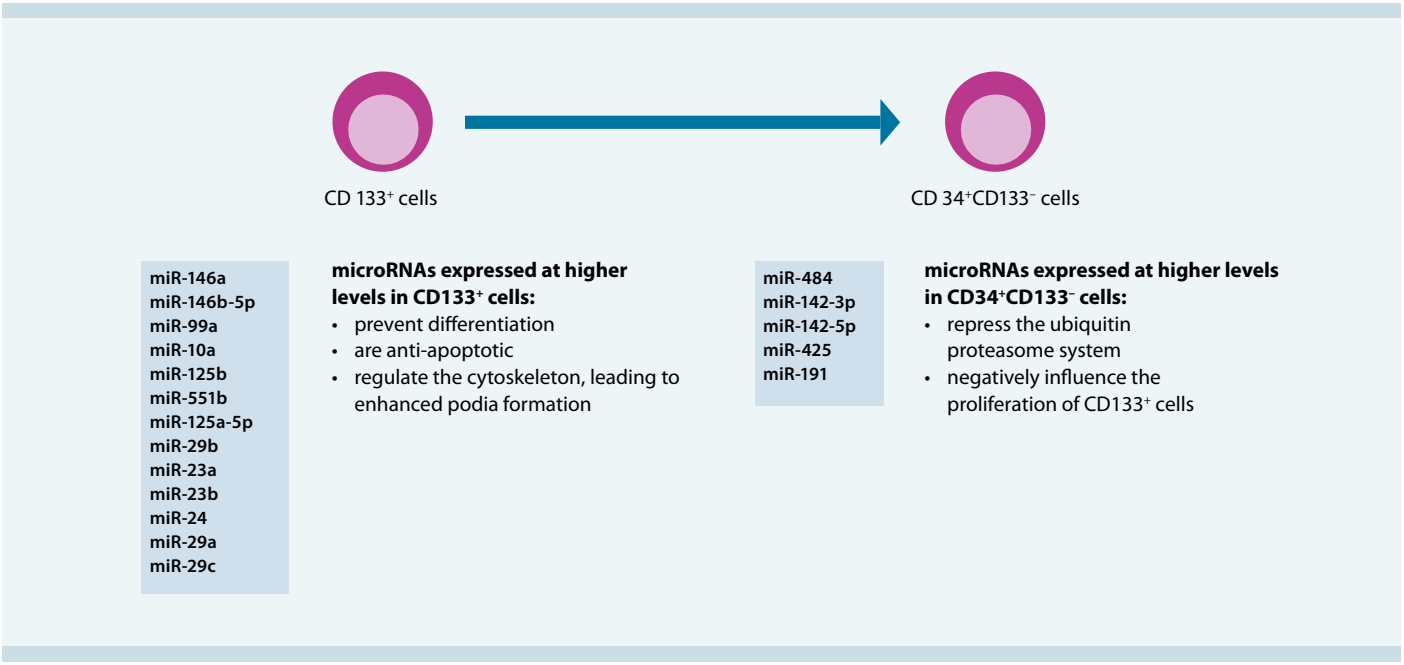

Figure 5 Hypothetical scheme for the biological role of microRNAs in CD34+ and CD133+ cells.

miR-142-3p inhibits CD133+ cells in the formation of colonies

The influence of miR-142-3p on CD133+ cells was analyzed by performing colony-forming unit (CFU) assays. CD133+ cells were transfected with 50 nM of miR-142-3p or control (miR-Neg) and cultivated. After seven days of cultivation, the progeny were seeded at a density of 150 CD34+ cells per one mL in methylcellulose medium. The overall capacity to form colonies was decreased by 71% (fig. 4; n=4; $P<0.05$).

Hypothesis for the biological role of microRNAs in CD133+ cells

A combined microRNA and mRNA analysis, that includes an annotation enrichment analysis, was performed to elucidate the biological function of the significantly differentially expressed microRNAs. The microRNAs that are expressed at higher levels in CD133+ cells prevent differentiation, are anti-apoptotic, and regulate the cytoskeleton, leading to enhanced podia formation and cell polarity. The microRNAs that are expressed at higher levels in CD34+CD133− cells repress the ubiquitin-proteasome system and negatively influence the proliferation of CD133+ cells (fig. 5).

Conclusion

- microRNA expression analysis and comparison of CD133+ and CD34+CD133− cells revealed 18 microRNAs that were significantly differentially expressed.
- FZD5 and TPM1 are controlled by miR-29a; CD133 is controlled by miR-142-3p.
- miR-142-3p inhibits the capacity of CD133+ cells to form colonies.
- The presented model suggests that several microRNAs play a role in preventing apoptosis and keeping the primitive state of CD133+ cells.

References

1. Lim, L.P. *et al.* (2005) Nature 433: 769–773.
2. Bissels, U. *et al.* (2011) Stem Cells 29: 847–857.
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Acknowledgments

This work was supported by the German Federal Ministry of Education and Research, project No. 0312138B. We thank Frank Hübel for support during microarray data analysis.

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