Introduction

Over the past decade microarray-based gene expression profiling has become a valuable and powerful tool for the identification of prognostic and therapeutic biomarkers. Microarray-based gene expression signatures have also been used for, e.g., improved classification of clinically relevant disease subgroups, as well as for the prediction of treatment response or risk of relapse/transition. While in cancer research usually the tumor and/or metastasis is subjected to gene expression profiling, the choice of appropriate tissue in other settings, e.g., in transplantation or autoimmune diseases, is more challenging. In many disease states a majority of pathologic transcriptomic changes are made not by tumor cells, but rather by surrounding stromal cells. It is therefore highly preferred for improved reproducibility, this might be difficult to be realized in multicenter clinical trials. Therefore, we established standard operating procedures (SOP) for manual and automated magnetic separation of CD14+ monocytes for MACS Technology. Our protocol with automated procedures has recently been successfully applied in a clinical phase I study.

Methods

Experimental cell separation, and gene expression analysis

In the study described here, we investigated whether CD14+ monocytes purified from whole blood of five different healthy volunteers were compared to profiles of stabilized whole blood or Ficoll-derived monocytes². A major advantage of using blood as the starting material is its easy accessibility and the possibility of repeated sampling. Furthermore, it is a realistic choice that the stained gene expression profiles reflect the disease or progression stage, even blood cells are permanently in contact with the blood stream. However, one major drawback of this approach is that the detected transcriptional changes are mainly attributed to changes in the cell population rather than due to disease-related biology.

Results

1 Highly pure CD14+ monocytes isolated directly from whole blood

An overview of the experimental setup and workflow is shown in Figure 1. Gene expression profiles of isolated CD14+ monocytes from five healthy volunteers were compared to profiles of stabilized whole blood and MACS from the same donor. The average purity of CD14+ monocytes was >98%. The average recovery was >90% with PBMCs. Figure 1A shows representative flow cytometric analysis of a single representative donor. Monocytes represent circulating cells in the blood stream and play pivotal roles in the immune system. Several studies demonstrated altered flow and function in patients with rheumatic diseases. Monocytes derived from patient blood, either as established whole blood cultures or as PBMC derived monocytes, are less well characterized.

2 High concordance between manual and automated separation methods

Monocyte-intrinsic differences provide deeper insights into the pathology of immune-related diseases and offer a greater chance to detect clinically relevant biomarkers.

• Our approach, also referred to as “MACS-4-omics,” can easily be expanded towards direct isolation of other immune cell subsets, such as granulocytes, B cells, T cells, or NK cells.

• “MACS-4-omics” is not restricted to genomics but may serve all areas of a biological approach.

3 Analysis of isolated CD14+ monocytes revealed superior sensitivity

In order to prove the enrichment of monocyte-specific genes in isolated CD14+ monocytes versus whole blood/PBMCs, a “between-subject” t-test was performed. More than 3,700 gene probes showed an enrichment factor of 2 to 4, and >100 probes were expressed at levels that were 4-500 fold higher in monocytes, among them, e.g., the CD4 gene as expected (Fig. 4c, 4d). Additionally, numerous genes were fixed to be strongly upregulated in whole blood and/or Ficoll, in particular “contaminating” genes, such as HBA, which is mainly derived from erythrocytes, with more than 10,000-fold higher monocyte signal intensities in whole blood versus purified CD14+ monocytes, or 8 plasminogen factor, which is mainly derived from platelets, with more than 100-fold higher monocyte signal intensities in whole blood in PBMCs versus CD14+ monocytes. These genes were negligible in purified CD14+monocytes, leading to a higher overall sensitivity and detection of monocyte-specific genes (Fig. 4f).

Conclusion and outlook

The protocols required minimal handling steps, took less than 30 min, and resulted in sufficient yields and excellent RNA quality for gene expression profiling experiments. Expression profiles demonstrated high concordance between manual and automated separation methods. Compared to whole blood or PBMCs, the highly enriched monocyte preparations showed negligible signs of contaminating erythrocyte- or platelet-derived transcripts, leading to improved sensitivity for the detection of CD14+ cell-specific transcripts. Monocyte-intrinsic differences provide deeper insights into the pathology of immune-related diseases and offer a greater chance to detect clinically relevant biomarkers.

References