The influence of miRNAs on HLA-DR cell surface expression of human monocytes and monocyte-derived dendritic cells

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1. Abstract

An immunodepression is strongly associated with a loss of function by monocytes and dendritic cells. The cell surface expression of Human Leukocyte Antigen – DR related (HLA-DR) correlates with the depression of the adaptive immune system. The purpose of the present study was to investigate the influence of microRNAs (miRNAs) on cell surface HLA-DR expression of primary human monocytes and monocyte-derived dendritic cells (MDDCs). Monocytes and MDDCs were transfected or transduced with miRNAs and the change of HLA-DR expression was characterized by Fluorescence activated cell sorting (FACS). Different miRNA transfections and transductions were characterized and revealed an influence on HLA-DR surface expression for certain miRNAs. Nine miRNA transfection in monocytes and three miRNA transfection in MDDCs did lead to a significant change in HLA-DR surface expression. Certain miRNAs play a role in the complex network of up- and downregulation of surface expression HLA-DR in monocytes and MDDCs. This may lead to new insight in the treatment of immunodepression diseases.

2. Introduction

Clinical studies revealed that HLA-DR cell surface expression is being downregulated in patients after surgery, burn and/or trauma, which is thought to be strongly associated with immunodepression [1]. It is important the cells in the human body have a good HLA-DR expression Moreover self-protection mechanisms lead to a reduction of proinflammatory cytokine production to protect the body from cytokine induced tissue damage. Therefor secondary infections can easily happen such as postoperative sepsis. The down-regulated amount of HLA-DR on monocytes, which presents antigens to T cells, is an indicator for a reduced probability of survival. Therefor a better understanding of HLA-DR surface expression is of interest.

3. Aims/ Leading Question

Aims:
- Establishing of a method to transfec transfected / transduce miRNA mimics into human Monocytes/MDDCs
- Determination whether transfected / transduced miRNAs have an influence on HLA-DR surface expression in human Monocytes/MDDCs

Leading questions:
- Which transfection method suits best to introduce “miRNA mimics” into Monocytes/MDDCs?
- Is a use of lentiviral particles necessary/advantageous to express miRNAs in Monocytes/MDDCs?
- Have the selected miRNAs an influence on HLA-DR surface expression of Monocytes/MDDCs?
- Is the HLA-DR surface expression pattern of the miRNA transfection / transduced Monocytes/MDDCs comparable to experiment with MelJuSo cells line?

4. Material and Methods

The approval of the study was obtained from the institutional ethics committee. Healthy donors aged ≥ 18 years were recruited and blood was drawn after written consent.

- Monocytes were isolated of Peripheral Blood Mononuclear Cells (PBMC) by magnetic cell isolation and differentiated to MDDCs with GM-CSF and IL-4
- Characterized by Fluorescence activated cell sorting (FACS)
- Cells were controlled with the Mo-DC Differentiation Kit (Miltenyi Biotec)
- Viability were checked with alamarBlue® cell viability reagent
- miRIDIAN microRNA mimic transfection control Dy547 was used as positive control
- Functionality / correct localization of transfected miRNAs were controlled with a positive control for HLA-DR by downregulation of CIITA by a [2], [3] and a negative control with two siRNAs Hs-4487 and Hs_HLA-DMB 6.

5. Results

The HLA-DR cell surface expression of transfected monocytes and MDDCs with and without LPS activation. The MFI of the HLA-DR expression were normalized to the control miRNAs. Monocytes and MDDCs were transfected with miRNAs that lead to a down- or upregulation of MHC class II molecules in MelJuSo cells. The original box and whiskers plots show normalized MFI and 5-95 percentiles. n=7 independent donors in each group. Table 5.1 shows a overview of the outcome.

6. Discussion

In summary, the results demonstrate that HLA-DR expression on the cell surface of primary human monocytes and dendritic cells is influenced by certain miRNAs. Transfections may have an influence on the viability of the cells. In addition, LPS may activate gene expression pattern, which may disturb the functionality of the transfected miRNAs. In turn, these miRNAs are predicted to have a key role in the HLA-DR surface expression that is linked to immunity, inflammation and pain. This indicates that miRNAs plays an important role in the human immune response by monocytes and MDDCs.

- Chemical techniques works for transfection and are more gently to the cells than physical methods
- The innovative polymeric system that delivers DNA and RNA out of the endosome and into the cytoplasm suits the best (Mirus TranIT-X29)
- Lentiviral particles will not be necessary to introduce miRNAs into monocytes and MDDCs, at least to answer the question of this project
- The influence of the tested miRNAs in monocytes and MDDCs are different and varies depending on LPS activation or not

The outlook of this research may be literature search of the miRNAs with a significant change in HLA-DR surface expression to analysis of the miRNA expression pattern under the influence of LPS. Are they linked to any key cell signaling cascade or not? Additionally, it could be tested in patient material if these miRNAs are of importance in a clinical situation as well e.g. a whole genome wide approach could be chosen to verify that and to integrate the data in a more complete setting.

References

Tables
Table 5.1 Phour, J. (2017), HLA-DR cell surface expression of monocytes and MDDCs. Bern, own table

Figures
Cover photos Phour, J. (2017), Fluorescence capture of transfected MDDCs with Dy547 and Hoechst 33342. Bern, own figure