MiRNA-155 regulates IL-12 expression by targeting SOCS-1 in human dendritic cells

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Dendritic cell and miRNA regulation and processing

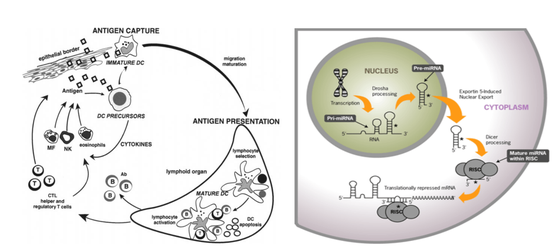


Figure 1: Role and regulation of dendritic cells and miRNAs. Dendritic cells (DCs) are the most potent type of white blood cells that regulate the immune response. DCs' antigen processing activities are controlled in response to inflammatory stimuli. DCs play a unique role in the immune activation to pathogens and transfomed cells. MicroRNAs (miRNAs) are small, single-stranded non-coding RNAs that function through stem-loop binding to the 3' untranslated regions (UTRS) of target mRNAs, usually silencing its protein's production and degrading the mRNA itself.

Aims of the study

1. Understand miR-155's role during monocyte-derived dendritic cell maturation
2. Understand mechanism by which miR-155 functions during dendritic cell development

Experimental design

Step 1: Isolate CD14+ monocytes

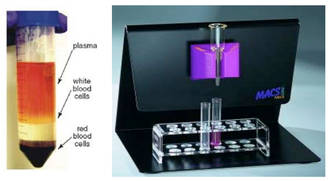


Figure 2: Isolating CD14+ monocytes. Monocytes are isolated using lymphocyte separation media, (Ficoll). Peripheral blood monocytes are taken from healthy donors or Memorial Blood Centers and are separated using MACS© magnetic cell sorters. Cells are cultured in serum free AIM-V media.

Step 2: Culture dendritic cells

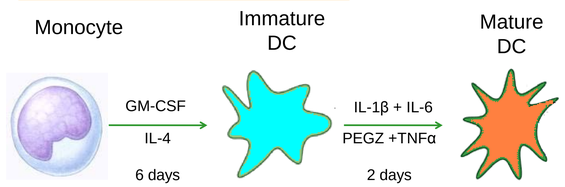


Figure 3: Monocyte derived dendritic cell maturation. A classic cytokine cocktail is added at specific intervals to the cultured cells to induce maturation.

Step 3: Microarray

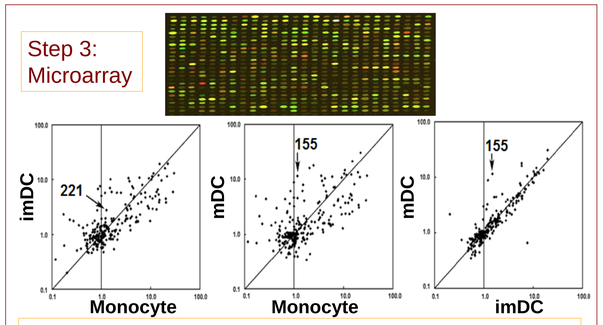


Figure 4: Microarray data displaying miR-155 expression throughout monocyte differentiation and maturation of DCs. Each dot on the array represents a different miRNA; miR-155 is dramatically up-regulated during the mDC stage.

Step 4: Real-Time PCR

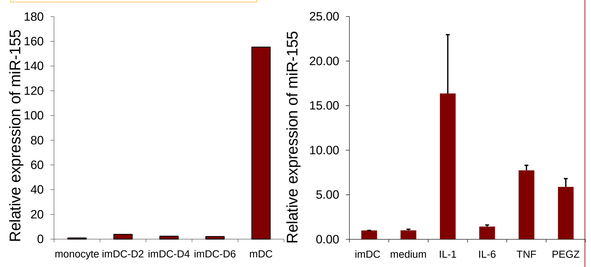
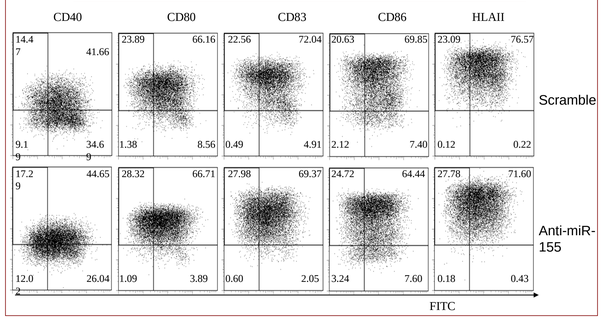


Figure 5: Time course of miR-155 expression. Results demonstrate that miR-155 is highly up-regulated in mature dendritic cells as well as strongly promotes the activation of cytokine signaling.

Step 5: Functional Study

miR-155 does not regulate DC maturation



miR-155 regulates IL-12

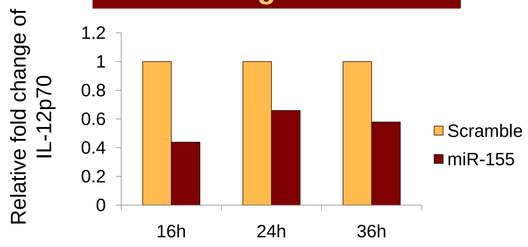


Figure 6: Relative IL-12p70 fold change. Silencing of miR-155 reduced the production of IL-12p70 during dendritic cell maturation.

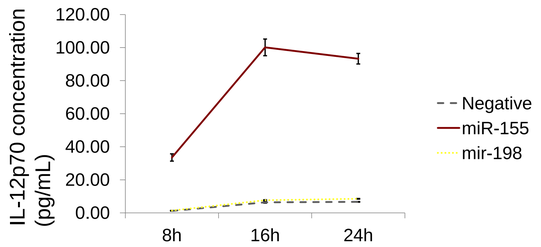


Figure 7: Over-expression of miR-155 up-regulates IL-12 production.

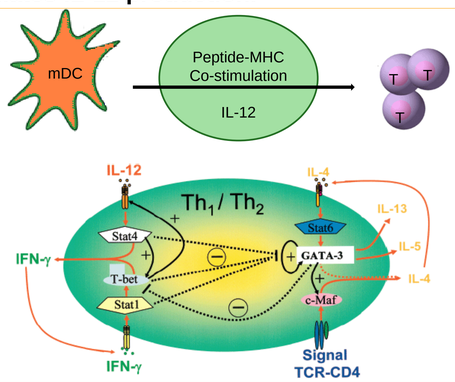


Figure 8: IL-12 signaling cascade. IL-12 is a potent cytokine that stimulates naïve T cells to differentiate into helper T cells. IL-12 mediates the cytotoxic effect of Natural Killer and CD8+ T lymphocytes that are involved in inducing death of tumor cells. The T cells posses a T cell receptor that specifically recognizes antigenic peptides bound to Class I MHC molecules to promote the death of infected cells.

miR-155 targets SOCS-1

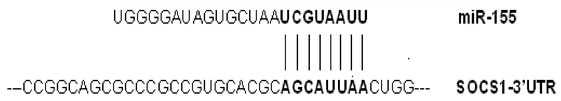


Figure 9: The alignment of miR-155 with SOCS-1 through 3' UTR. miR-155 binds to and inhibits SOCS-1 activity; inhibiting IL-12 cytokine signaling.

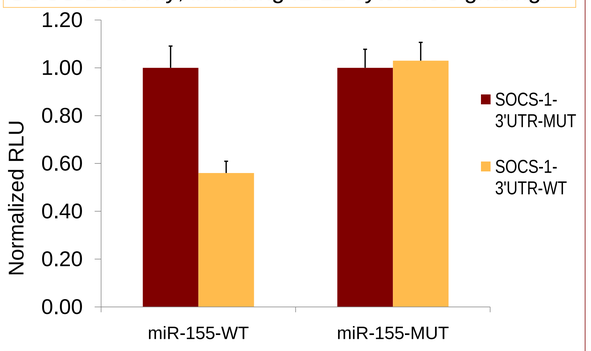


Figure 10: Luciferase assay results displaying transcriptional activity in dendritic cells. Upon transfection with miR-155-WT or –MUT along with the luciferase gene, photo emitted luminescence is quantified using a luminometer. miR-155-WT is successfully able to target and repress full transcriptional activity of SOCS-1, thereby inhibiting its cytokine suppressing activity.

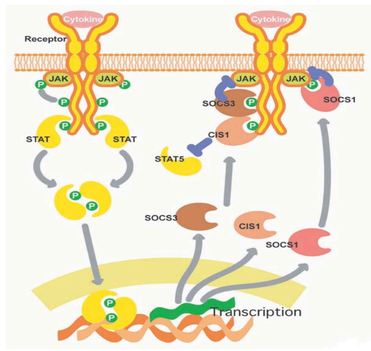


Figure 11: Molecular mechanism of the inhibition of IL-12 signaling by SOCS-1. Cytokine stimulation activates the JAK/STAT pathway which induces SOCS-1 to bind to and inhibit the JAK/STAT catalytic transcription of IL-12 genes.

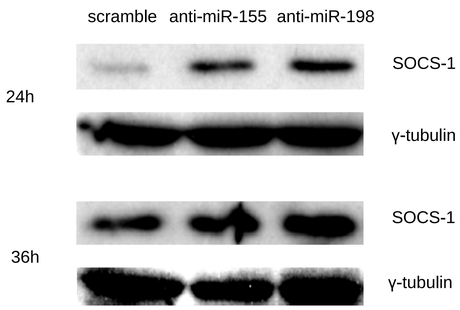


Figure 12: Western blot displaying SOCS-1 expression when silencing miR-155. Silence of miR-155 in mDCS results in an increase of SOCS-1 protein concentration with time. miR-155 is not able to bind and inhibit SOCS-1 resulting in an increase of protein expression.

Future directions

1. Begin an *in vivo* experiment in mice over-expressing miR-155.
2. Determine possible cooperation of miR-155 with other miRNAs during dendritic cell maturation.

Acknowledgments

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