

# microRNAs are key regulators of the development and functional differentiation of $v\delta$ T cell subsets

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## Introduction

The ability of murine γδ T cells to rapidly produce the pro-inflammatory cytokines interleukin-17 (IL-17) or interferon-γ (IFN-γ) underlies their crucial and non-redundant roles in several (patho)physiological contexts, such as tissue homeostasis, infection, autoimmunity and cancer. This capacity stems from a complex process of 'developmental pre-programming' in the thymus, after which a large fraction of γδ T cells migrate to peripheral sites already committed to producing IL-17 or IFN-γ, unlike their αβ T cell counterparts<sup>1</sup>. So far, several miRNas have been implied in the control of the differentiation and IFN-y and IL-17 levels by  $\alpha\beta$  Th1 and Th17 cells, respectively<sup>2</sup>. However, little is known about the action of these post-transcriptional regulators on  $\gamma\delta$  T cell differentiation. Schmolka *et al.* showed that miR-146a is selectively enriched in IL-17-biased CD27  $\sqrt{\delta}$  T cells and restricts their co-production of IFN-y by targeting *Nod1* mRNA, therefore regulating  $\sqrt{\delta}$  T cell plasticity<sup>3</sup>. This isolated work illustrates the need of a more comprehensive study of the miRNA repertoires of  $\gamma\delta$  T cells and of the regulatory networks they take part in the control of IFN- $\gamma$  and IL-17 production by these cells.

### Aims

- To characterize the miRNA:mRNA regulatory networks that regulate IFN-γ and IL-17 expression in γδ T cells subsets in vivo, we will:
  - 1. Identify the miRNA and mRNA repertoires of pure IL-17- and IFN-γ-producing γδ T cells;
  - Determine the functional impact of specific miRNAs on  $\gamma\delta$  T cell differentiation; 2.
  - Analyse the regulation of candidate miRNA expression in IFN- $\gamma^*$  and IL-17\*  $\gamma\delta$  T cells з Identify mRNA networks controlled by candidate miRNAs.

### **Results**





reperforms of thr<sup>1</sup>-th<sup>2</sup>(γ), btr<sup>2</sup>(LL<sup>T</sup> and LL<sup>T</sup> and Lt<sup>2</sup>(γ) of Cells. (μ) resumps depicing unconvergence expressed miRNAs between IFN-γ<sup>2</sup> and IL-17<sup>+</sup> γδ T cells. Colors indicate the direction and magnitude of relative expression, with red representing miRNAs at higher expression (Log2CPM). Values inside squares refer to calculated 2-scores.

#### 2. miRNA:mRNA interaction networks



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Figure 2. miRNAs predicted to target key determinant mRNAs of the IL-17 and IFN-y gene expression networks of yo T cells. (A) miRNAs differentially spressed in  $(H_V^+ \gamma r L_3^- 2\gamma r h_5^- T_2)$  for believerb bioinformatically integrated, via 2 UTR analysis, with mRMs that are key determinants for the expension of these cytokines by 61 cells and abilitin an expression pattern opposite of the respective mRMs. mRMs-mRMs bioinformatic targeting prediction analysis resulted in the L-17 (B) and iFN-Y (C) gene expression networks. Candidate mRNAs (identified in the figure) were selected for further functional characterization based on the level of its differential expression and the relevance of its predicted target(s) for the expression of the respective cytokine, as well as the number of targets.

3. Candidate miRNA overexpression during in vitro yo T cell expansion IL-17"IFN-7 0 0 6 -#NA Transduction "<mark>į±tįt<sub>i</sub>ŧ∮</mark>t ć 444411 VIIIIII G IL-17'IFN-7 0.17 \_<del>Ittilliti</del> <u>İ</u>İjitiliji 77/11/1/ Willing . 2111111 ssion during in vitro vo T cell expansion. Workflow (A) and results (B to H) of retroviral (RV) overexpression Figure 4. Candidate miRNA ove right e.4. canonate minitor overexpression ouring in write yor Len expansion. Worklow (a) and results (b. Ori y) trendension (b) write pression or minita21, mik-320, mik-320, mik-320, mik-323, mik-333 and mik-3949 in peripheral y6 T cells. For vy chornetry analysis of intracellular LL12 and IFNy expression in LL-17, IFNy" and LL-17/IFNy" y6 T cells and frequency of LL-17, IFNy" and LL-17/IFNy" in GFP retrovirally transduced y6 T cells expressing either a control vector (WC-ontrol) or each candidate mikiNA (R-miki) (C-E). MFI (F-H) stands for mean fluorescence intensity. Data are representative of three independent experiments. \*P < 0.05 and \*\*P < 0.01.

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## References

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#### 4. miR-128-3p and miR-139-5p restrict IFN- $\gamma$ production in peripheral $\gamma\delta$ T cells



Figure 4. mIR:232 and miR:139 restrict IFN-y production in peripheral y0 T colls. Workflow (A) and results (B to C) of retroviral (RV) overexpression of mIR:322, mIR:326, mIR:450, mIR:73, mIR:238, mIR:138 and mIR:349 in peripheral CD27+y0 T cells. Flow cytometry analysis of intracellular each candidate mIRMS (NrvmR) (D) (E) (Effects of candidate mIRMA overexpression on cytokine expression of peripheral y0 T cells cultured in vitro; data from Figure 3 and 4. Data are representative of three independent experiments. \*P < 0.05 and \*\*P < 0.01.



## 6. miR-128-3p and miR-181a-5p control thymic γδ T cell commitment and differentiation in vivo



Figure 6. miR-128-3p and miR-131a-5p control thymic võ T cell commitment and differentiation *in vivo*. miR-128-3p Workfow (A) and results (B) of overnight TCR-stimulation with plate-bound anti-CD2 and anti-CD28 of peripheral CD27<sup>-</sup> võ T cells sorted from the lymph nodes of a hematopositic-restricted miR-128-2 deficient mouse strain and litermate controls. How cytometry analysis of intracellular IFA-y expression. (C) Flow cytometry analysis of mature CD24<sup>+</sup> võ T cell for IL-72 or IFA-y committement in the thymus of aduut miR-128-2 deficient mice versus litermate controls. miR-1315-p(D) RT-qPCR analysis of miR-181-5p expression in immature CD24<sup>+</sup>, uncommitted CD44CD45R8, IL-17 committed CD44<sup>thy</sup>CD45R8 and IFA-y-committed CD45R8<sup>+</sup> võ T cell subste from embryonic E17.5 thymus, neonatal D3 thymus and adult peripheral lymph nodes. (E) Frequency of võ

\*Commute UCMARY to ten sources from entroyonic E17.3 upmos, neonato 3 upmos and adout peripheral mpmin hodes, Ef Prequency or yo realisin the thymus, peripheral hymph nodes and splence of adult miR-181a deficient mice and litermate controls identified as CL32\*(RC6)\* among live cells by flow cytometry, IF) Flow cytometry analysis of mature CD24\* yo T cell for L1-12\* or IFN+y commitment in the peripheral hymph nodes of adult miR-181a's of mature CD24\* yo T cells for L1.2\* or FIN+y commitment in the thymus of embryonic or neonatal miR-181a deficient mice versus litermate controls. (B) Flow cytometry analysis of mature CD24\* yo T cells for L1.2\* or IFN+y commitment in the thymus of embryonic or neonatal miR-181a deficient mice versus litermate controls.

miR-128-3p working model (based on results 5B and 6B, C): in the thymus, where miR-128-3p is highly expressed in yô T cell precursors, miR-128-3p limits commitment of yố T cells to the IFN-y pathway, promoting IL-17 commitment instead. In the periphery, miR-128-3p also inhibits differentiation of yố T cells into IFN-ty producers in response to TCR stimulation. miR-131-5p working model: in the thymus, miR-131-5p limits commitment of yố T cells to the IFN-y pathway, promoting IL-17 commitment instead, posibly by inhibiting strong TCR signals that promote IFN-yố commitment.

#### 7. Modulation of candidate miRNA levels by extracellular cues



Fig.7. Expression levels of the 4 studied miRNAs. These graphs represent data from 6 independent experiments, that were pooled posteriorly, n=3.2 fact synthemic represents a biological replicate. A miR-1810-5p expression in CD27 cells from the peripheral lymph nodes. C miR-128-3p expression in CD27 cells from the peripheral lymph nodes. The dotted lines represent the median of the corridor grant and the median of the corridor grant and the median of the corridor grant.

# **Open questions/future work**

es miR-139 function as an IFN-y auto-repressor in vivo? What is the  $\gamma\delta$  T cell phenotype of embryonic/neonatal miR-128 KO mice? What are the relevant mRNA targets of the candidate miRNA?