miR-155 is essential for proliferation and survival of plasmablast B-cells

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Introduction

Optimal humoral responses against foreign T-dependent antigens requires crosstalk between B-cells and CD4$^+$ T-cells. Following the binding of B-cells to their cognate antigen, B-cells localise to the B:T border, where they receive T cell help. This interaction promotes extensive cell division and the migration of B-cells to the B cell follicles. Later on, the highly proliferative B cell blasts differentiate into germinal centres or antibody secreting plasmablasts (Figure 1).

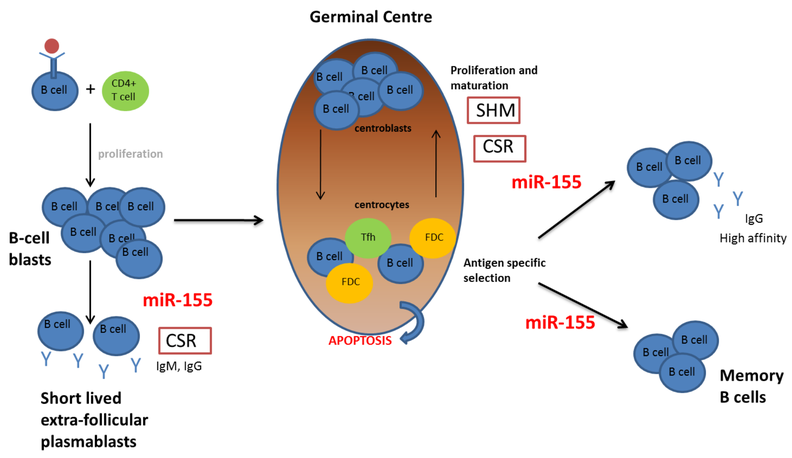

Figure 1: Differentiation of naive B-cells into extrafollicular PBs, GCs or plasma cells

These rapidly emerging plasmablasts are found in the extrafollicular tissue where they continue to expand until they cease proliferation and enter apoptosis [1]. The ability of B-cells to quickly differentiate into short-lived antibody secreting cells to produce neutralising antibodies of different isotypes can be critical to contain the spread of infections [2]. Among the genes that regulate the extrafollicular response in a B-cell intrinsic manner is microRNA-155 (miR-155) [3, 4] . We previously showed that mice lacking miR-155 in B-cells produce a lower number of IgM and IgG secreting plasmablasts relative to their wild type counterparts [4]. Furthermore, we identified PU1 as a key mir-155 target for this process [5]. However, whether the loss of cellularity of miR-155 deficient plasmablasts is due to a differentiation block, impaired proliferation or survival remains to be understood.

Methods

In order to elucidate the effects of miR-155 on plasmablast B-cell differentiation, SW_{HEL} B-cells (CD45.2$^+$) sufficient or deficient in miR-155 were injected into B6SJL (CD45.1$^+$) mice and then stimulated with HEL antigen coupled to sheep red blood cells (HEL-SRBCs, Figure 2).

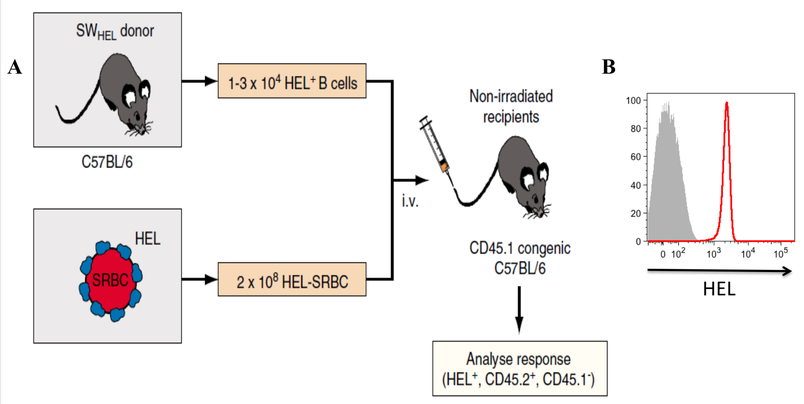

Figure 2: Tracking miR-155 deficient B-cells in vivo using the SW_{HEL} system (A). Flow cytometry plot showing HEL expression on conjugated HEL-SRBCs (B).

SW_{HEL} transgenic B-cells either wild type or mir-155-/- bearing a rearranged hen egg lysosome (HEL)-specific VDJ_H element targeted into the IgH chain locus combined with an HEL-specific k L-chain transgene were adoptively transferred into wild type congenic recipients and immunised with HEL-SRBCs to promote T-dependent responses.

Results

miR-155 is required for the plasmablast response

We analysed B-cell differentiation of antigen activated miR-155 sufficient and deficient B-cells using flow cytometry by staining for the transgenic HEL BCR and B220.

Figure 3: Representative flow cytometric plot showing SW_{HEL} *Mir-155*$^{+/+}$ or SW_{HEL} *Mir-155*$^{-/-}$ donor cells at days 3.5 and 4.5 post immunisation illustrating the defects observed in *Mir-155*$^{-/-}$ B220$^+$ HEL BCR$^+$ B cell blasts (red), B220$^+$ HEL BCR^{lo} germinal centre B-cells (black) or B220^{lo} HEL BCR^{lo} plasmablast B-cells (grey) compared to *Mir-155*$^{+/+}$ B-cells (A). The number of donor-derived HEL-specific plasmablast B-cells was calculated per 10^6 lymphocytes at day 3.5, 4.5 and 6.5 post immunisation in *Mir-155*$^{+/+}$ (black) or *Mir-155*$^{-/-}$ (grey) mice (B). N=16-19 independent *Mir-155*$^{+/+}$ samples and 10-24 independent *Mir-155*$^{-/-}$ samples. Data is representative of at least two independent experiments. The mean ± SEM is shown. An unpaired non-parametric Mann-Whitney T test was used for statistics. HEL-specific antibody titres of the indicated immunoglobulins were measured in the serum of *Mir-155*$^{+/+}$ (black) or *Mir-155*$^{-/-}$ (grey) donors (C).

miR-155 is essential for the proliferation of plasmablast B-cells

After establishing that miR-155 was critical for the plasmablast B-cell response, we next sought to determine the underlying cellular mechanisms. We started by monitoring the proliferation of antigen specific B-cells.

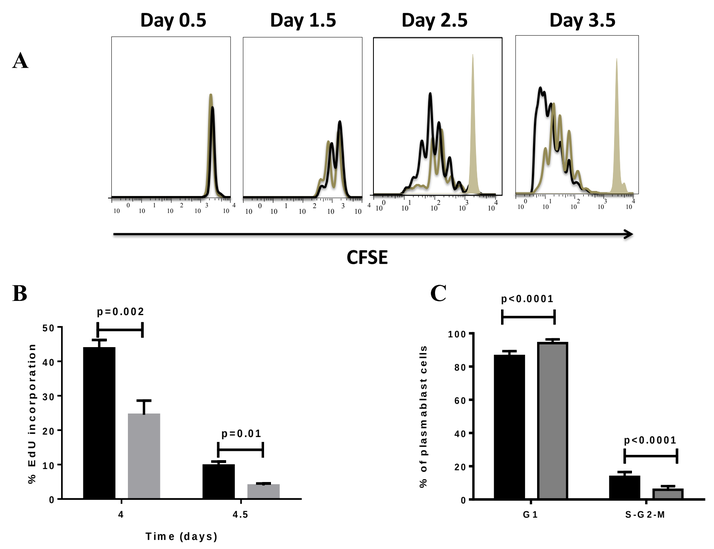

*Figure 4: SW_{HEL} Mir-155$^{+/+}$ or Mir-155$^{-/-}$ B-cells were assessed for CFSE dilution, Mir-155$^{+/+}$ (black line), Mir-155$^{-/-}$ (grey line), negative control (filled grey line) (**A**). EdU incorporation in Mir-155$^{+/+}$ (black bars), Mir-155$^{-/-}$ (grey bars) at the time points indicated. The mean ± SEM is shown. Unpaired T test was used for statistics **(B).** SW_{HEL} Mir-155$^{+/+}$ or Mir-155$^{-/-}$ B-cells were analysed at 4.5 days post immunisation and analysed for cell cycle by DAPI staining. Frequency of G1 or S-G2-M phases in Mir-155$^{+/+}$ (black bars) or Mir-155$^{-/-}$ (grey bars) SW_{HEL} plasmablast B-cells is shown. Unpaired T test was used for statistical calculations. The mean ± SEM is shown. Data is representative of at least two independent experiments.*

B-cells lacking miR-155 showed a reduced number of highly dividing cells compared to wild type at 2.5 days post immunisation which became more pronounced at day 3.5 (Figure 4A). We then measured the amount of cells in S-phase after incorporation of a pulse of the thymidine analogue EdU by plasmablasts at days 4 and 4.5 (Figure 4B). SW_{HEL} *Mir155-/-* plasmablast B-cells exhibited significantly less S-phase cells, suggesting a cell cycle defect. Supporting this observation, we found a significantly increased proportion of *Mir-155*$^{-/-}$ plasmablasts in the G1 phase of the cell cycle and a significantly reduced frequency in the S-G2-M phase compared to wildtype B-cells, when DNA was quantified by DAPI staining (Figure 4C).

miR-155 protects plasmablast B-cells from apoptosis

Next, we asked whether miR-155 deficient B-cells were undergoing increased apoptosis compared to their wild type counterparts. Flow cytometry analysis showed that *Mir155*$^{-/-}$ plasmablasts expressed a significantly increased proportion of active caspases compared to *Mir155*$^{+/+}$ plasmablasts at both 3.5 and 4.5 days following activation (Figure 5A). In an attempt to rescue the number of mir-155-/- plasmablasts by blocking apoptosis, we crossed the SW_{HEL} *Mir155*$^{+/+}$ *or* SW_{HEL} *Mir155*$^{-/-}$ mice with human *Bcl2* (6) transgenic mice (Figure 5B). The presence of the BCL2 transgene increased the frequency and number of plasmablasts (Figure 5C/D) and reduced the frequency of active caspases (Figure 5E), but the altered phenotype was not fully restored.

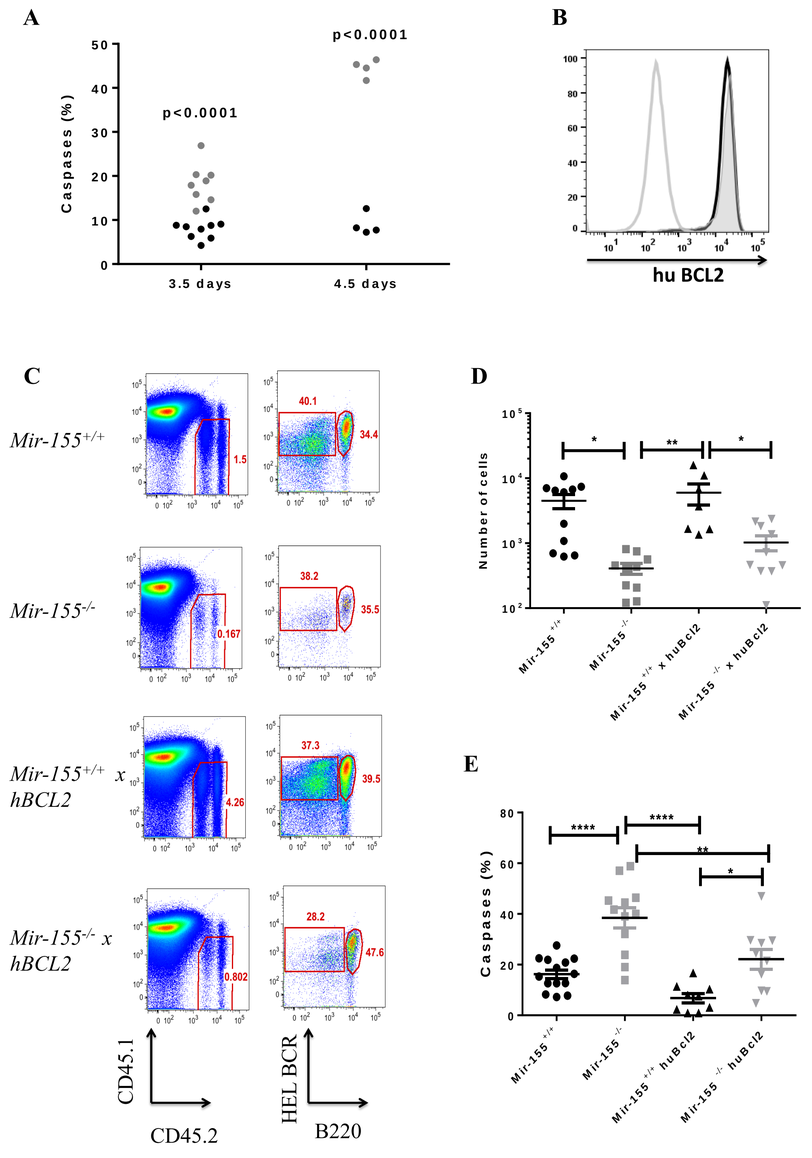

Figure 5: SW_{HEL} HEL BCR$^+$ B220^{lo} *Mir-155*$^{+/+}$ (black) or *Mir-155*$^{-/-}$ (grey) plasmablast B-cells were analysed for activated caspases at the time points shown (A). An unpaired Student's t-test was used for statistics. Expression of the human Bcl2 transgene (huBCL2) in SW_{HEL} x *Mir-155*$^{+/+}$x *Bcl2* (black line) or SW_{HEL} x *Mir-155*$^{-/-}$ x *Bcl2* (solid grey line) plasmablast B-cells, compared to negative control (single grey line, B). Representative FACS plot showing the gating strategy for analysis of adoptively transferred SW_{HEL} *Mir-155*$^{+/+}$ or *Mir-155*$^{-/-}$ B-cells expressing a human *Bcl2* transgene at day 4.5 post immunisation (C). The number of splenic SW_{HEL} *Mir-155*$^{+/+}$ or *Mir-155*$^{-/-}$ B-cells with or without the expression of a human *Bcl2* transgene (D). The frequency of active caspases in miR-155 sufficient and deficient B-cells at day 4.5 post immunisation (E). Data is representative of at least two independent experiments. Statistics calculated using One Way ANOVA where *P<0.05, **P<0.01, ****P<0.001.

miR-155 regulates genes

We next sought to determine the molecular pathways disrupted in *Mir155*$^{-/-}$ plasmablast B-cells by comparing the transcriptome of miR-155 deficient B-cells with their wildtype counterparts. CD45.2$^+$ HEL BCR$^+$ B220^{lo} plasmablast B-cells from miR-155-sufficient or -deficient mice were sorted to over 98% purity and their trancriptome analysed by microarray at 4.5 days post immunisation. We defined differentially expressed genes as those genes with a fold change of at least 1.3 between miR-155 deficient and miR-155 sufficient plasmablasts and a corrected p-value of less than 0.05. We observed 410 upregulated and 652 downregulated genes in mir-155 -/- plasmablasts relative to their wild type counterparts. We then use the gene ontology enrichment analysis tool GOrilla [7] to look for pathway enrichment in the differentially expressed genes. Downregulated and upregulated genes were sorted into functional processes and ranked according to their p value (Figure 6).

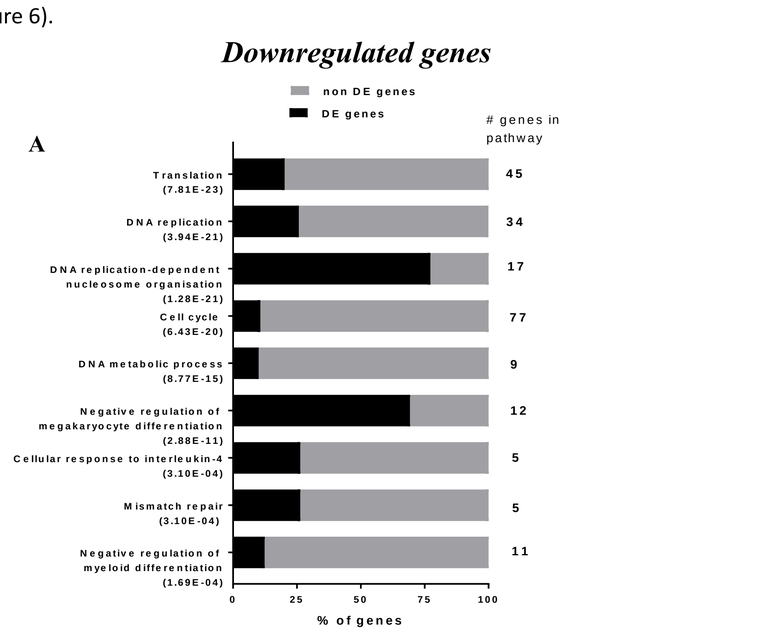

*Figure 6: The groups of genes with known functions were discovered by GOrilla analysis using the genes that were downregulated **(A) and mRNA levels confirmed by qRT-PCR (B)** or upregulated **(C)** in SW_{HEL} Mir-155$^{-/-}$B-cells compared to SW_{HEL} Mir-155$^{+/+}$ B-cells.*

Conclusions

- MiR-155 is required to sustain the plasmablast response and is essential for plasmablast survival and proliferation.
- MiR-155 deficient, HEL-specific plasmablast B-cells showed an increase in apoptosis and defects in cell cycle progression and DNA replication compared to wild type controls.
- Through transcriptome analysis of miR-155 sufficient and deficient SW_{HEL} B cells we determined that miR-155 indirectly regulates genes involved in cellular processes such as the DNA metabolic process, DNA nucleosome assembly, DNA replication initiation and the mitotic cell cycle process which provides new insight into antibody production during the early response to infection and vaccination.

References

1. MacLennan, I.C., et al., Extrafollicular antibody responses. Immunol Rev, 2003. **194**: p. 8-18.
2. Luther, S.A., et al., Early neutralizing antibody response against mouse mammary tumor virus: critical role of viral infection and superantigen-reactive T cells. J Immunol, 1997. **159**(6): p. 2807-14.
3. Vigorito, E., et al., miR-155: an ancient regulator of the immune system. Immunol Rev, 2013. **253**(1): p. 146-57.
4. Vigorito, E., et al., microRNA-155 regulates the generation of immunoglobulin class-switched plasma cells. Immunity, 2007. **27**(6): p. 847-59.
5. Lu, D., et al., The miR-155-PU.1 axis acts on Pax5 to enable efficient terminal B cell differentiation. J Exp Med, 2014. **211**(11): p. 2183-98.
6. Ogilvy, S., et al., Constitutive Bcl-2 expression throughout the hematopoietic compartment affects multiple lineages and enhances progenitor cell survival. Proc Natl Acad Sci U S A, 1999. **96**(26): p. 14943-8.
7. Eden, E., et al., GOrilla: a tool for discovery and visualization of enriched GO terms in ranked gene lists. BMC Bioinformatics, 2009. **10**: p. 48.

Acknowledgements

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