

# DNA Hypermethylation and Cancer

## Junior Group

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Almudena R. Ramiro

Junior Group Leader

Almudena R. Ramiro was born in Madrid in 1971 and obtained her BSc degree in Biochemistry and Molecular Biology from the *Universidad Autónoma de Madrid* in 1994. She received her PhD with First Class Honours from the same University in 2000.

In 2001 she joined M. Nussenzweig's Laboratory at Rockefeller University in New York (USA) as a Postdoctoral Fellow funded by the *Ministerio de Educación y Ciencia*. During this time she focused on the function of Activation Induced Deaminase (AID) in the diversification of antibodies and established the role of AID in the initiation of lymphomagenic chromosome translocations. This work was published in leading journals and is widely recognised within the field.

Ramiro has been a *Ramón y Cajal* investigator since 2004. She received the 2006 Biogen Idec Award for Young Investigators for her work on the role of genomic instability and p53 in AID-induced *c-myc/IgH* translocations. In 2007 Ramiro was awarded a European Research Council Starting Grant.

She joined the CNIO as a Junior Group Leader in 2006.

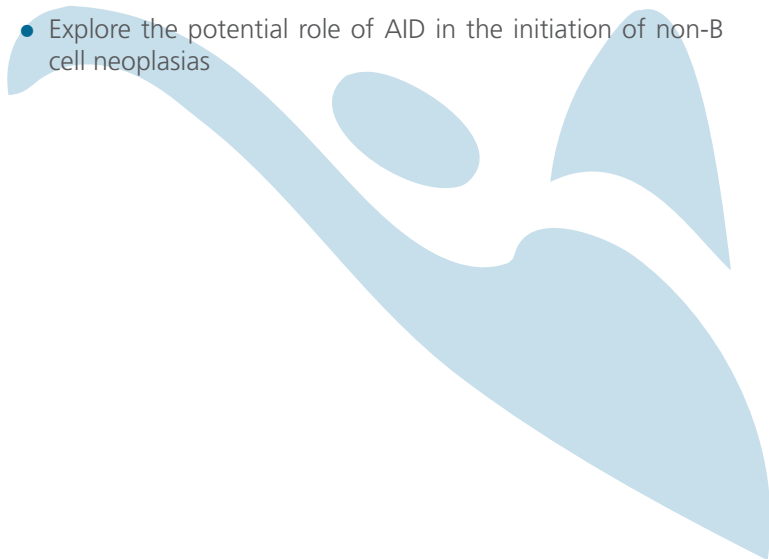
## Summary

Most lymphomas originate from mature B lymphocytes and are characterised by the presence of recurrent chromosome translocations. Mature B cells are unique in that they can diversify their antibody repertoire by somatic remodelling of immunoglobulin genes in germinal centres. These remodelling events are initiated by Activation Induced Deaminase (AID). While critical for immune response, we have previously shown that AID can also initiate lymphomagenic chromosome translocations.

We aim to study the regulation of the germinal centre reactions and their role in lymphomagenesis from two different perspectives: on the one hand we are identifying microRNAs responsible for regulating mature B cell function; on the other, we will explore the molecular mechanisms that regulate AID activity, including transcriptional and post-transcriptional regulation and target specificity in the context of B cell neoplasias.

## Strategic Goals

- Study the mechanisms that regulate AID activity and its role in B cell transformation
- Analyse the regulation of mature B cell function by microRNAs
- Explore the potential role of AID in the initiation of non-B cell neoplasias





**Staff scientists:** M. Pilar Delgado and Virginia G. de Yébenes. **Post-doctoral fellow:** Thomas Wossning. **Graduate students:** Nahikari Bartolomé (since March), Pablo Pérez and Isora Vidal. **Technicians:** Laura Belver and Sonia M. Mur (since August).

## Highlights

MicroRNAs are non-coding small RNA molecules (21-22 nucleotides long) that regulate gene expression post-transcriptionally. These small RNA molecules have been shown critical for several aspects of mammalian immune system regulation and maintenance.

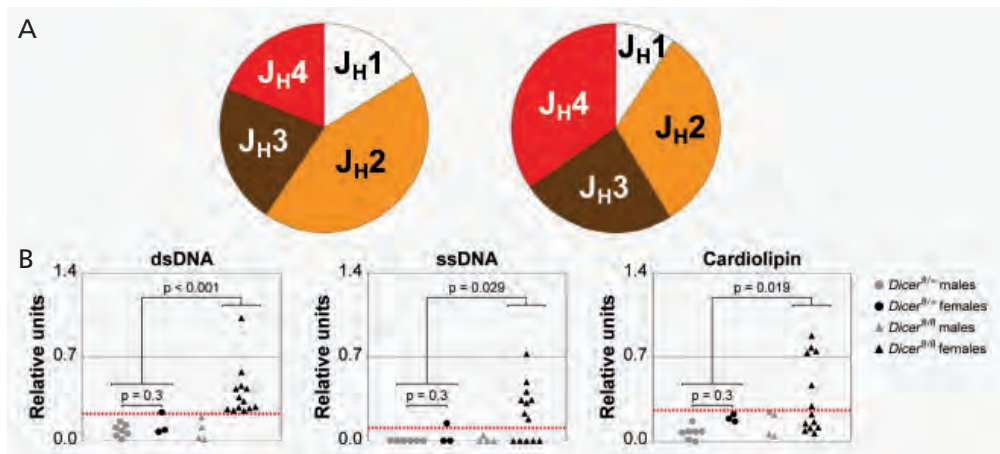
We used two complementary approaches to examine the role of microRNAs in the regulation of mature B cell function and B cell lymphomagenesis. Firstly, we addressed the overall function of microRNAs in late B cell differentiation and function by investigating the impact of B cell specific microRNA deficiency in *Cd19-Cre<sup>ki/+</sup>Dicer<sup>fl/fl</sup>* mice.

We found that in the absence of Dicer the marginal zone (MZ) B cell compartment is over-represented and

follicular (FO) B cells exhibit an impaired response to BCR signalling. We observed that the microRNAs that are differentially expressed in FO versus MZ compartments are downregulated in the MZ B cells and that *Btk* is a potential target of one of these microRNAs.

Importantly, Dicer deficient B cells have a skewed BCR repertoire (Figure, A) with hallmarks of autoreactivity, which correlates with high titers of autoreactive antibodies in serum (Figure, B) and immune complex deposition in the kidney.

Collectively, our results reveal a crucial role of microRNAs in both late B cell differentiation and the establishment of B cell tolerance. To identify individual microRNAs playing a role in B cell function and transformation we combined functional and expression screening approaches. These analyses allowed us to single out miR181b as a direct negative regulator of AID expression.



**Figure:** microRNAs prevent the generation of autoreactive antibodies. (A) Skewed immunoglobulin repertoire in the absence of microRNAs. Immunoglobulin heavy chain repertoire of *Cd19-Cre<sup>ki/+</sup>Dicer<sup>fl/+</sup>* (left) and *Cd19-Cre<sup>ki/+</sup>Dicer<sup>fl/fl</sup>* (right) was analysed after PCR cloning and sequencing. Pie charts represent the percent of cells harbouring the indicated rearranged J<sub>H</sub> region. (B) Dicer deficient females contain high titers of autoreactive antibodies in serum. Serum from *Cd19-Cre<sup>ki/+</sup>Dicer<sup>fl/+</sup>* (left) and *Cd19-Cre<sup>ki/+</sup>Dicer<sup>fl/fl</sup>* animals was collected and titers of anti-dsDNA, anti-ssDNA and anti-cardioliipin antibodies were determined by ELISA.

Finally in recent studies, we have identified a microRNA whose expression is regulated during the germinal centre reaction and which displays mild transforming activity *in vivo*. We have generated *in vivo* overexpression mouse models to investigate its functional role in germinal centres and B cell transformation.

## Publications

Belver L, de Yébenes VG, Ramiro AR (2010). MicroRNAs prevent the generation of autoreactive antibodies. *Immunity* 33, 713-722.

de Yébenes VG, Ramiro AR (2010). MicroRNA activity in B lymphocytes. *Methods Mol Biol* 667, 177-192.