Deregulation of microRNAs in a mouse model of *Helicobacter pylori*-associated gastric lymphomaganesis.

Floch Pauline[1], Capdevielle Caroline[1], Staedel Cathy[2], Mégraud Francis[1], Lehours Philippe[1].
[1]Univ. Bordeaux, Bacteriology Laboratory, F-33000 Bordeaux, France; INSERM U1053, F-33000 Bordeaux, France; [2]Univ. Bordeaux, INSERM U1212, F-33000 Bordeaux, France;

Introduction and aim

Helicobacter pylori **(*H. pylori*) infection is considered an excellent model of chronic inflammation-induced tumor development. Our project focuses on gastric MALT lymphoma (GML), a severe disease which originates from a chronic inflammatory process initiated by *H. pylori*. Recently, microRNAs (miRNAs) have emerged as a new class of gene regulators and play a key role in inflammation and carcinogenesis acting as oncogenes or as tumor suppressors. The precise characterization of the role of miRNAs in the development of inflammation and their contribution in regulating responses of host cells to infection by *H. pylori* have been little explored. Our goal was to analyze the miRNAs specifically induced in a mouse model of GML previously described by the laboratory using BALB/c mice thymectomised at day 3 post-birth (d3Tx model) (Chrisment D *and al*., Am J Pathol 2014) and to clarify their implication in GML pathogenesis.**

Material and Methods

Material

(Chrisment D *et al*, Am J Pathol 2014)

Neonatal thymectomy (day 3)

BALB/c d3Tx — BALB/c NTx

H. pylori Infection

Non infected mice (NI)
Infected mice (I)

12 month post-infection

Mice stomachs (-80°C and paraffin embedding (FFPE))
Sera (-80°C)

miRNAs extraction (stomachs) and PCR array

Stomachs (-80°C) → miRNeasy mini Kit → miRNA ← miRNeasy FFPE Kit ← FFPE stomachs

4 I d3Tx
3 NI d3Tx
(I d3Tx : GML mice without RNAs degradation)
(NI d3Tx : mice without inflammation)

2 I d3Tx
2 NI d3Tx
(I d3Tx : GML mice not studied in PCR Array 1 due to excessive degradation of RNAs)
(NI d3Tx : mice without inflammation)

miRNA → RT-PCR miScript II RT kit → ADNc

SYBR Green 2x QuantiTect® PCR Master Mix + 10x miScript Universal Primer

PCR Array 1 « Mouse Cancer Pathway Finder 384HC» PCR Array 2

ΔΔCt analyses (pcrdataanalysis.sabiosciences.com/mirna/arrayanalysis.php)

Real-time PCR (Bio-Rad CFX384TM, plateforme TBM- Core)

→ qRT-PCR (Confirmation of some targets)

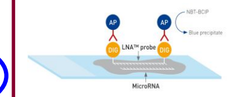
→ analysis of the data according to the inflammatory scores previously obtained

miRNAs extraction (sera) and qRT-PCR

Sera (80°C)
12 d3Tx I
7 d3Tx NI

→ TRIzol® LS Reagent → miRNA → RT-PCR miScript II RT kit → cDNA → qRT-PCR SYBR Green® Premix Ex Taq™ Takara

in situ hybridization



-sections (3 µm thick) from stomachs of Id3Tx and NId3Tx mice
-miR-142a (LNA™ , Exiqon)

Bioinformatic analysis of predicted targets

- online prediction site (TargetScan)
  http://www.targetscan.org
- overexpressed miRNAs common targets?

Results

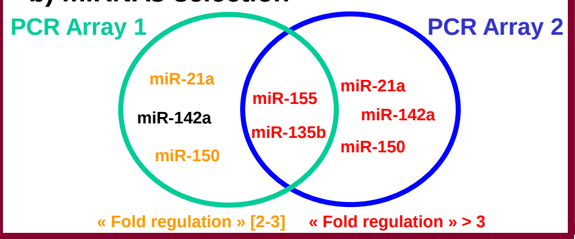
1) PCR array

a) miRNAs expression in stomachs

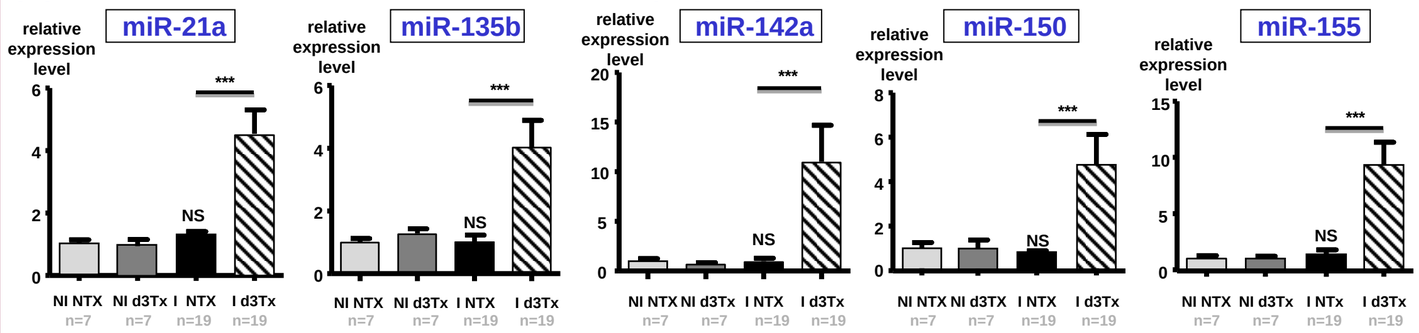
PCR Array 1 : stomachs (-80°C)	PCR Array 2 : FFPE stomachs
2 overexpressed miR*	21 overexpressed miR*
5 underexpressed miR*	13 underexpressed miR*

*« Fold regulation » > 3

b) miRNAs selection



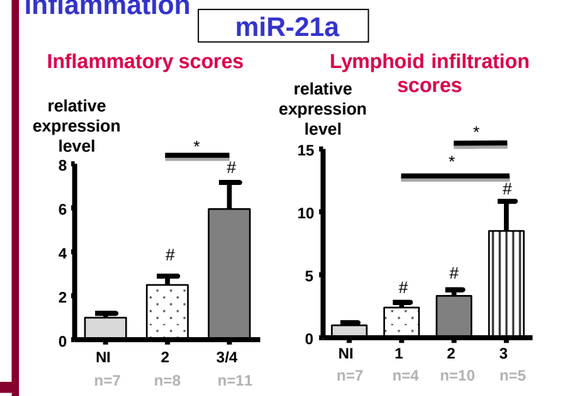
2) qRT-PCR confirmation in all NTx and d3Tx mice (I and NI)



→ overexpression of 5 miRNAs in gastric MALT lymphoma

(normalization of expression levels with SNORD72)

3) Relative expression *vs* gastric inflammation

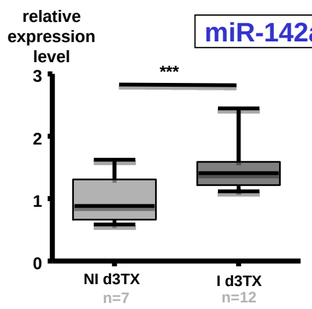


→ miR-21a : inflammation intensity marker

→ no influence with miR-135b, miR-142a, miR-150 et miR-155

(# : significant expression level vs NI mice)

4) And in sera?

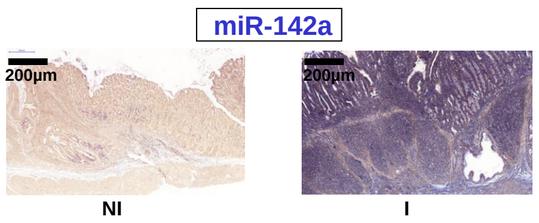


→ miR-142a could be a non invasive diagnostic marker

(normalization of expression levels with miR-16a)

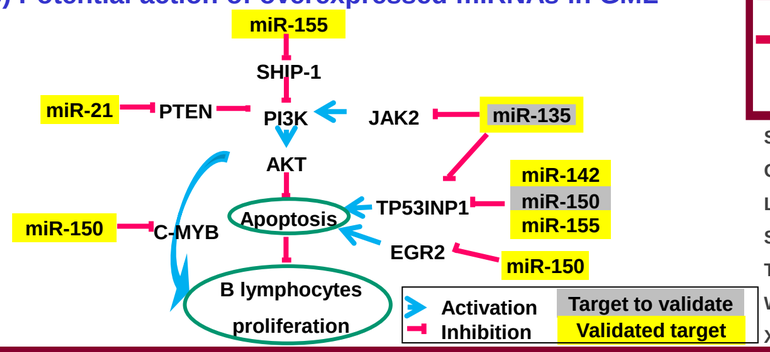
5) What is the origin of overexpressed miRNAs?

I and NI d3Tx mice stomachs



→ miR-142a is expressed by lymphoid infiltrates and tumor environment

6) Potential action of overexpressed miRNAs in GML



Saito *et al*., 2012
Gebauer *et al*., 2014
Lawrie *et al*., 2012
Sheedy *et al*., 2015
Thorns *et al*., 2012
Wu *et al*., 2010
Xiao *et al*., 2007

Conclusion-Discussion

Overexpression of these 5 miRNAs could play a critical role in the pathogenesis of GML. The analysis of these miRNAs in human biopsies or histological sections would validate their deregulation at GML stage and define potential new therapeutic targets.