Milk fat fraction as a non-invasive source of mammary microRNA


INRA, UMR1213 Herbivores, VetAgroSup, F-63122 Saint-Genès-Champanelle, France
INRA, UMR 1313 GABI, AgroParisTech, F-78352 Jouy-en-Josas, France
HRMU gathers 145 peoples in 5 research teams, including a permanent staff of over 75 scientists.

And with a transcriptomic PF

with 19 peoples in the BIOMARKERS team

One of our team aims: Assess the effects of nutrition on lactation, body reserves and milk quality of dairy ruminants.
Bovine milk

- Fat (37g/L)
- Proteins (34g/L)
- Lactose (46g/L)
- Minerals (7g/L)

Water (87%)

Milk lipids are secreted in Milk Fat Globules

- Triglycerides (98% of fat)
- Phospholipids (0.2-1.0% of fat)
- Sterols (0.2-0.4% of fat)
Milk fat globules (MFG)

Cellular heterogeneity of MG

Mammary epithelial cell

Myoepithelial cell

Milk fat globules are secreted with cytoplasm crescent

[Argov-Argaman et al., 2010]

[Linzell JL & Peaker M; 1971]

[Robenek et al., 2006]
Milk fat globules (MFG) as a source of mRNA

MFG contains mRNA representative to MEC
Bovine milk

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Many other less known components (e.g.: oligosaccharides, immune-related substances or microRNA) are present in milk in small amounts.
MicroRNAs are short, ~22-nucleotides, single-stranded, non-coding RNAs

Today more than 800 miRNAs encoded by the bovine genome have been characterized
MicroRNA (miRNA)

miRNAs are inhibitors of gene expression through Watson–Crick base pairing with complementary sequences within mRNA molecules.

miRNA induce mRNA degradation or inhibit protein synthesis.

Each miRNA can regulate the expression of multiple genes and many genes can be regulated by multiple miRNAs.

miRNAs control the post-transcriptional regulation of 60% of genes.
MiRNomes (NGS) in the mammary gland in different species.

- Species-specific miRNA
  - Caprine mammary miRNome
    - [Li et al., 2012, Wang et al., 2012, Le Guillou,...Leroux, et al., 2014]
  - [Mobuchon,...Leroux, et al., 2015]

Nutri-regulation of mammary miRNome in ruminants.

- 30 miRNA affected by 48h food-deprivation
  - [Mobuchon,...Leroux, 2015]
- 2 miRNA affected sunflower supplementation
  - [Mobuchon,...Leroux, submitted]

miRNA in milk:

- miRNA in raw milk: difference between colostrum & mature milk
  - [Chen et al., 2010]
- miR-223 is potential biomarker of mammary Staphylococcus infection from milk exosomes
  - [Sun et al., 2015 & Li et al., 2015]
  - (which need a large volume of milk and not easy to obtained)
OBJECTIVES: To identify early and easily accessible biomarkers of mammary inflammation

To assess MFG as a source of miRNAs and whether the latter are representative of MG miRNA expression

Comparison miRNA profiles in MFG and MG sampled from mid-lactation Holstein cows.
Comparison of mammary & milk miRNomes in inflammatory conditions

Post milking
Milk fat (MFG)

Biopsy
Mammary gland (MG)

N=4

Intramammary injection of 50 µg LPS

RNA extraction using Trizol

miRNA microarray analyses
(bos taurus-capra hircus-ovis aries 60 K chip ; Agilent)

[Brenaut et al., 2012]
Mammary and milk miRNomes

Preliminary results

A large majority were similar in MG and MFG.

miRNAs from MFG are not fully representative of mammary biopsies.

MFG are a good source of miRNA.
Bioinformatic & Network analyses

Using Metacore

Hierarchic clustering

Data analyses in progress
What is happening in healthy mammary gland?

Post milking

Milk fat (MFG)

N=6

Biopsies

Mammary gland (MG)

N=6

RNA extraction

9 candidate miRNAs
miR-29a, miR-125, miR-126, miR-141, miR-148a, miR-204, miR-223, miR-320 and miR-494

RT-qPCR

[Brenaut et al., 2012]

[Le Guillou et al., 2014; Mobuchon et al., 2015]
Mammary and milk candidate miRNA

RT-qPCR on 9 miRNA in milk fat (MFG) and in mammary gland (MG)

2 miRNAs are below detection threshold: miR-126 and miR-204

Similar differences between MFG and MG miRNA profiles excepted for 2

[Lago-Novais, Pawlowski et al., submitted]
Conclusions

• Milk fat an « easy » non-invasive source of miRNA

• Mammary and milk fat miRNomes are not exactly the same but the majority presents the same abundance

• Give rise to questions on miRNA secretion in MFG
Perspectives

• Identify MFG miRNA as Biomarkers:

  ➔ miRNA as biomarkers of health, physiological or metabolic status:

  miRNomes analyses before and after LPS challenge

• Determine the secretion mechanism of miRNA in MFG:

  1. Are they synthesized by other mammary cell-types?
  2. Are miRNA lacking in MFG synthetized in mammary epithelial cells but sorted and not packaged in MFG?

  As reported for miRNA in exosomes, are miRNA submitted to a sorting before to be package in MFG?  
  
  [Janas et al., 2015; Bas et al., 2015]
• **Their role in healthy milk**: controversial

• miRNA detected in commercial cows milk [Izumi et al., 2012; Pieters et al., 2015] even some of them were eliminated by technological processes [Howard et al., 2015]

• the biological effects of extracellular vesicles containing miRNA demonstrated both *in vitro* [Pieters et al., 2015] and in mice models [Arntz et al., 2015]

• the action of milk miRNA could be modulated by their packaging within “transporting vehicles” [Alsaweed et al., 2015]: in MFG?

• **BUT** after overconsumation of miRNA, they were not detected in mice tissues [Laubier et al., 2015; Title et al., 2015]

More studies are necessary!
UMRH near Clermont-Ferrand

Lago-Novais D.
(PhD student from Brazil)

Pawlowski K.
(Post-Doc from Poland)

Boby C.

Soulard C.
(tempory)

Mobuchon L.
(PhD student)

Pires J.

Bes S.

Faulconnier Y.

GABI unit near Paris

Martin P.

Bevilacqua C.

Funded by INRA