Identification of Major Milk Fat Globule Membrane Proteins from Pony Mare's Milk highlights the Molecular Diversity of Lactadherin across Species

Christelle Cebo, Emmanuelle Rebours, Céline Henry, Samira Makhzami, Pascal Cosette, Patrice Martin

Identification of Major Milk Fat Globule Membrane Proteins from Pony Mare's Milk highlights the Molecular Diversity of Lactadherin across Species

C. Cebo*, E. Rebours*, C. Henry#, S. Makhzami*, P. Cosette[$] and P. Martin*

[*]INRA, UMR1313 Unité Génétique Animale et Biologie Intégrative, F-78350 Jouy-en-Josas, France
[#]INRA, Plateforme PAPSSO (Plateforme d'Analyse Protéomique Paris Sud Ouest), F-78350 Jouy-en-Josas, France
[$]UMR6270 CNRS, Université de Rouen, Plateforme Protéomique de l'IFRMP23, F-76821 Mont-Saint-Aignan Cedex, France

Although numerous studies have been devoted to the soluble fraction of mare's milk, namely caseins and whey proteins, to date, little is known about the Milk Fat Globule Membrane (MFGM) fraction from mare's milk. The objective of this study was thus to describe MFGM proteins from *Equidae* milk. Most of MFGM already described in cow or goat milks were identified in mare's milk using mass spectrometry. Prominent differences through species were highlighted for lactadherin. Indeed, whereas one or two polypeptide chains are respectively identified by peptide mass fingerprinting Matrix-Assisted Laser Desorption/Ionisation- Time of Flight (PMF MALDI-TOF) analysis for caprine and bovine lactadherin, lactadherin from mare's milk appears as three polypeptide chains in 6% SDS-PAGE. Digestion of MFGM proteins from mare's milk with Peptide N-glycosidase F (PNGase F) revealed that the existence of three distinct polypeptide chains for equine lactadherin could not be solely explained by differential N-glycosylation of a single polypeptide chain. On the other hand, Polymerase Chain Reaction (PCR) experiments on lactadherin transcripts isolated from milk fat globules revealed that splicing events occur on lactadherin from mammary gland with the existence of two distinct lactadherin transcripts in the horse species. Cloning and sequencing of both transcripts for lactadherin revealed the existence of a cryptic splicing site located at the end of exon 5 of equine lactadherin and Liquid Chromatography coupled to tandem Mass Spectrometry (LC-MS/MS) analyses confirmed the existence of both lactadherin variants in the MFGM from mare's milk. Interestingly, this additional splicing event led to the suppression of a putative N-glycosylation site in the protein. Whatever, expression of lactadherin is thus species-dependent, therefore questioning about of the precise function of these different isoforms in mammary gland biology across species.