Conservation of Bovine Milk Exosome miRNAs

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Introduction

miRNAs bind to target mRNAs, resulting in changes to gene expression. Some are highly conserved across species.

Bovine milk exosomes contain miRNAs and most of these miRNAs are identical to human miRNAs. Further, treatment with bovine milk exosomes alters mammalian gene expression and metabolism.

Based on this, it is hypothesized that bovine milk exosome miRNAs regulate host gene expression by becoming incorporated into miRISC complexes.

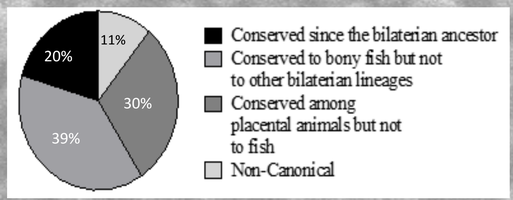
Here we present a comparative genetic analysis of bovine milk exosome miRNAs from the Dietary MicroRNA Database and their recognition elements. We also examine association of these miRNAs with human disease risk.

Results

The majority of miRNA in the Dietary MicroRNA Database are highly conserved

Classification	Number of bovine milk exosome miRNAs
High confidence miRNAs	137
Low confidence miRNAs	84
miRNAs* (passenger strand)	21
Small RNA	1

Most of the bovine milk exosome high confidence miRNAs can be classified into conserved canonical gene families

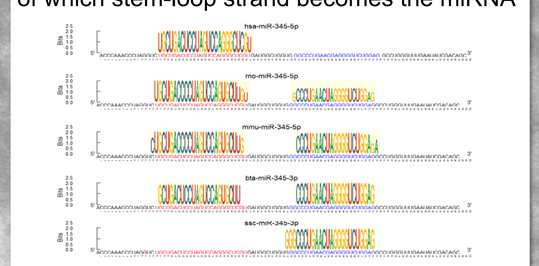


Bovine milk exosome miRNAs are broadly conserved in Bilateria

Alignment of representative miRNAs from multiple species

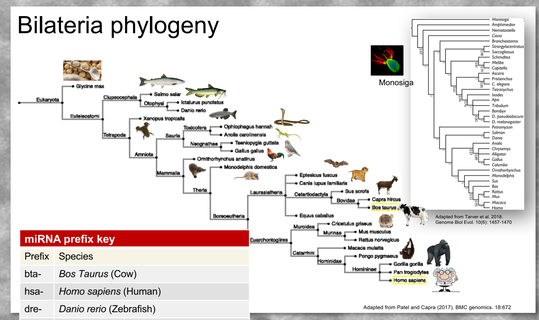
Sequence alignment of representative bovine and human pre-miRNA stem-loop sequences

miR-345 is undergoing a change in the choice of which stem-loop strand becomes the miRNA

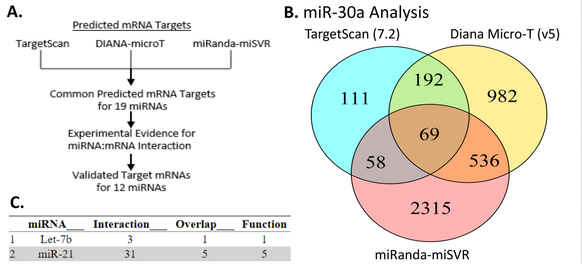


Alignment of the sequence reads with the miR-345 stem-loop sequence for humans, rats, mice, cows and wild boar

Bilateria phylogeny



Conservation of MREs in predicted target mRNAs

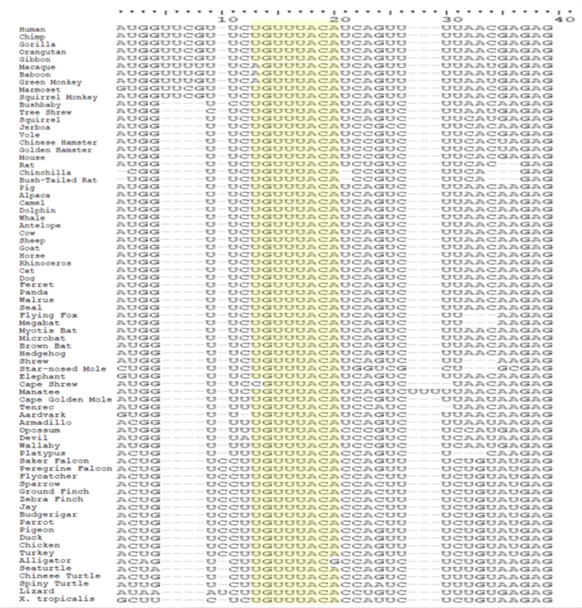


C.

	miRNA	Interaction	Overlap	Function
1	Let-7b	3	1	1
2	miR-21	31	5	5
3	miR-26a	140	2	2
4	miR-26b	79	1	1
5	miR-29b	217	17	6
6	miR-30a	27	3	3
7	miR-30d	410	5	2
8	miR-99a	2	2	2
9	miR-146a	7	1	3
10	miR-148a	136	2	4
11	miR-200c	181	16	11
12	miR-345-5p	8	1	1
13	miR-503	28	2	1

Verified Targets of Selected Bovine Milk Exosome miRNA

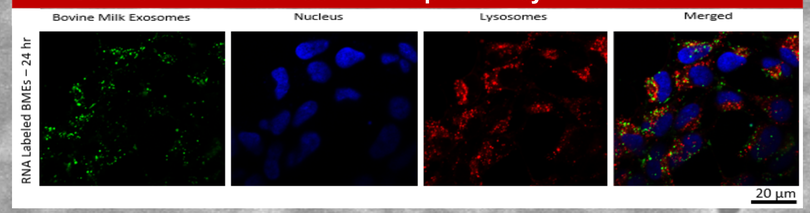
miR-30a ATP2A2 validated Target is highly conserved



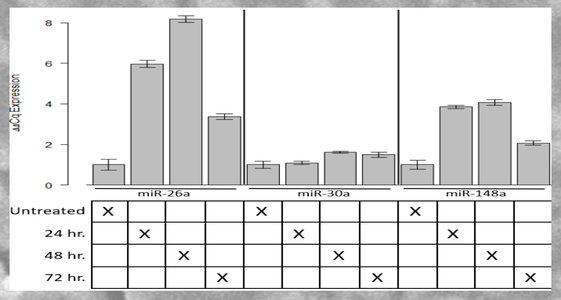
High confidence bovine milk exosome miRNAs are consistently present in BMEs

	09/04/2019	10/18/2019	12/05/2019	01/20/2020	01/22/2020	03/19/2020	09/01/2020	11/05/2020
miR-26a	NA	NA	NA	NA	X	X	X	X
miR-29b	X	X	NA	X	X	X	X	NA
miR-30a	NA	NA	NA	NA	X	X	X	X
miR-99a	NA	NA	NA	NA	X	X	X	X
miR-148a	NA	NA	NA	NA	X	X	X	X
miR-200c	NA	X	X	NA	X	X	NA	NA

Bovine Milk Exosomes RNAs Up-taken by HEK-293 at 24 hr.



miRNAs increase in HEK-293 with bovine milk exosomes and levels are time dependent



Casual association of high confidence bovine milk exosome miRNAs with disease risk

Disease	Better Outcome	Worse Outcome
Obesity	NA	miR-148a, miR-26b, miR-21
Diabetes Mellitus, Type 2	NA	miR-21
Coronavirus Infections	NA	miR-146a
Inflammation	Let-7b, miR-29b, miR-148a, miR-146a	miR-21
Pancreatic Neoplasms	miR-200a/b/c	miR-200c

Conclusions

Of the 243 bovine milk exosome small RNAs in DMD, 221 are annotated miRNAs. These annotated miRNAs are members of the canonical miRNA gene families. Their sequences are highly conserved. Only 7 miRNAs have differences in 1-2 nucleotides outside of seed region.

Bioinformatic analysis of experimentally validated mRNAs targets for miR-29b, -200c and -30a bovine milk exosome miRNAs suggests the MREs are highly conserved.

Conservation of these miRNAs and their target sites suggests that bovine miRNAs delivered into human cells by exosomes through the consumption of cow's milk have the potential to regulate human gene expression.

Homologs of high confidence bovine miRNAs are associated with changes to disease risks in humans.

Acknowledgements

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