读书报告

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Microbiota Modulate Host Gene Expression via MicroRNAs

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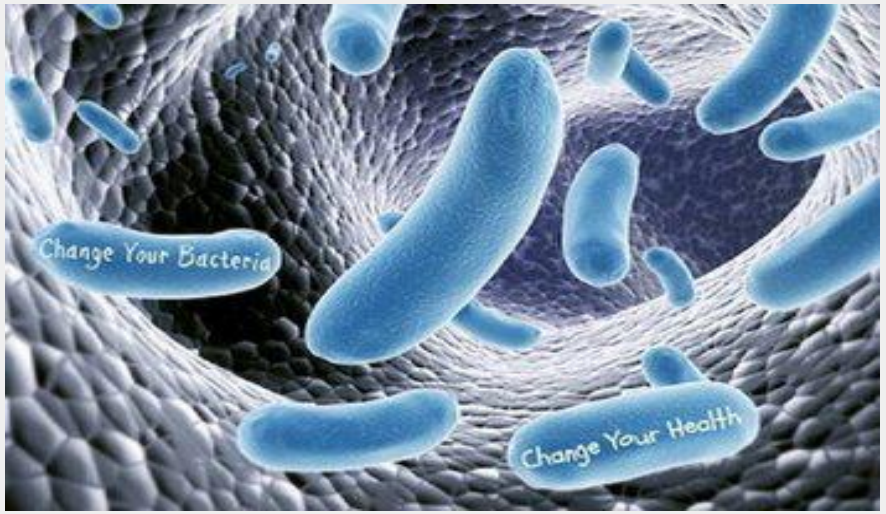
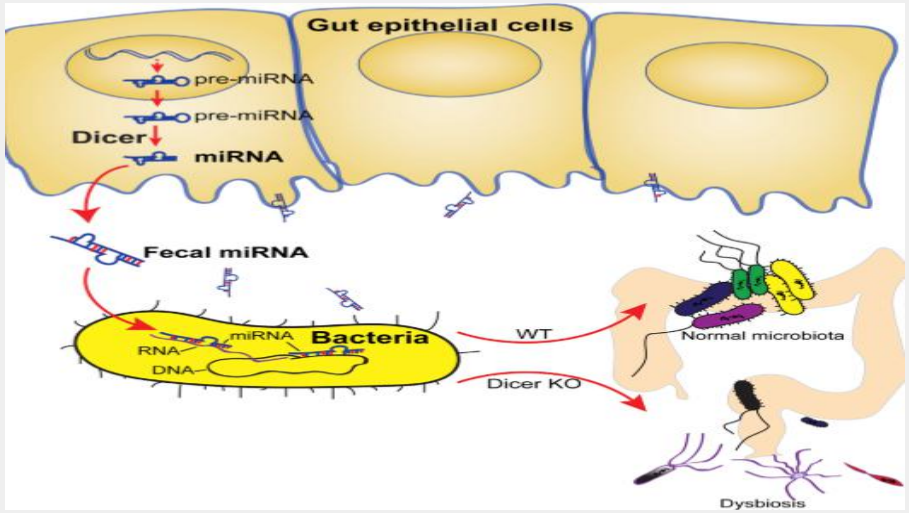
目录：

一、研究背景

胃肠道中有大量的微生物(10^{14}个细菌)，是各种微生物的定殖器官。已知微生物群可以调节宿主基因表达，但其潜在的分子机制仍不清楚。MicroRNAs (miRNAs)通过与靶miRNA的 39-UTRs结合后转录调控基因表达，在许多细胞功能中起重要作用。然而，miRNA 在微生物-宿主相互作用中的作用尚不清楚。





实验思路

Germ-free

Colonized

利用miRNA芯片筛选差异表达的miRNA

利用Target scan…等预测miRNA靶基因

miRNA潜在靶基因与微生物诱导失调的基因交叉

若干miRNA—靶基因

挑选Abcc3基因—mmu-miRNA-665

靶基因与目标miRNA共培养，mRNA水平与蛋白水平检测

微生物群可以调节宿主microRNA的表达，进而调控宿主基因的表达

二、材料与方法

- Cell culture
- cDNA and miRNA expression analysis
- MiRNA target prediction
- Quantitative real-time PCR (qRT-PCR)
- Luciferase and GFP repression experiments
- Western blot

三、研究结果

A

	Up-regulated miRNAs		Down-regulated miRNAs	
	Name	Fold change	Name	Fold change
Ileum	mmu-miR-298	1.57		
Colon	mmu-miR-128	1.53	mmu-miR-465c-5p	-1.56
	mmu-miR-200c*	1.62	mmu-miR-466d-3p	-1.56
	mmu-miR-342-5p	1.69	mmu-miR-466d-5p	-1.54
			mmu-miR-665	-1.54
			mmu-miR-683	-1.64

B

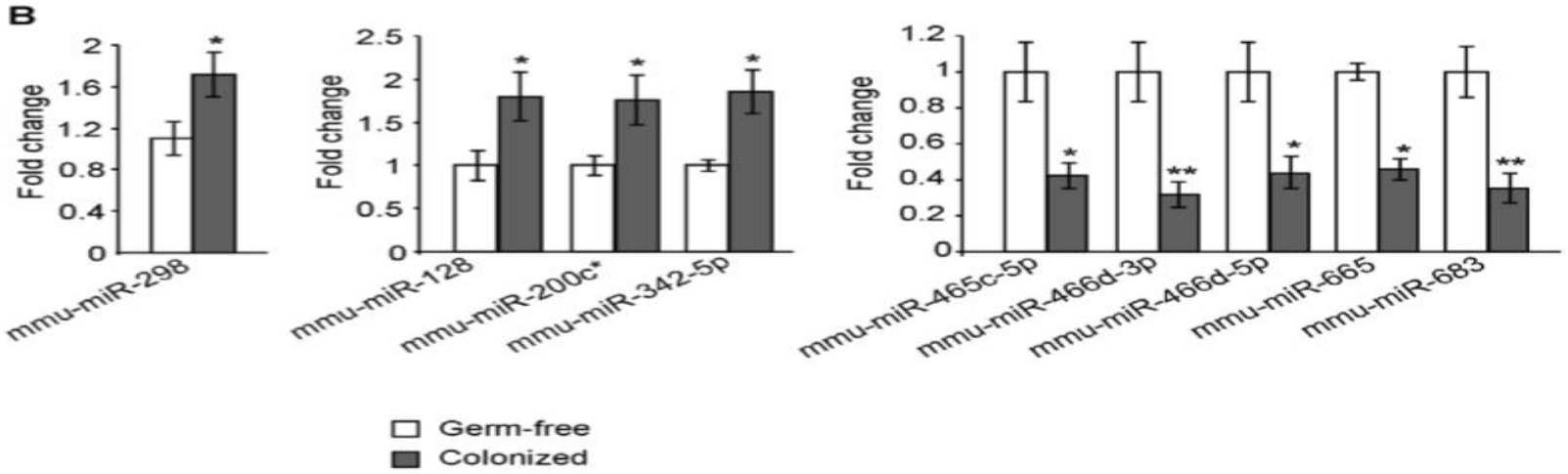


Figure 1. Microbiota modulate host miRNA expression. Germ-free mice were colonized with the microbiota from pathogen-free mice. Total RNAs were extracted from the ileums and colons of germ-free and colonized mice. MiRNAs differentially expressed in the ileum and colon of colonized mice compared to germ-free mice (≥ 1.5-fold) determined by miRNA array (A) and qRT-PCR (B). Values represent means ± S.E.M. (n = 6/group; *$P<0.05$; **$P<0.005$).
doi:10.1371/journal.pone.0019293.g001

三、研究结果

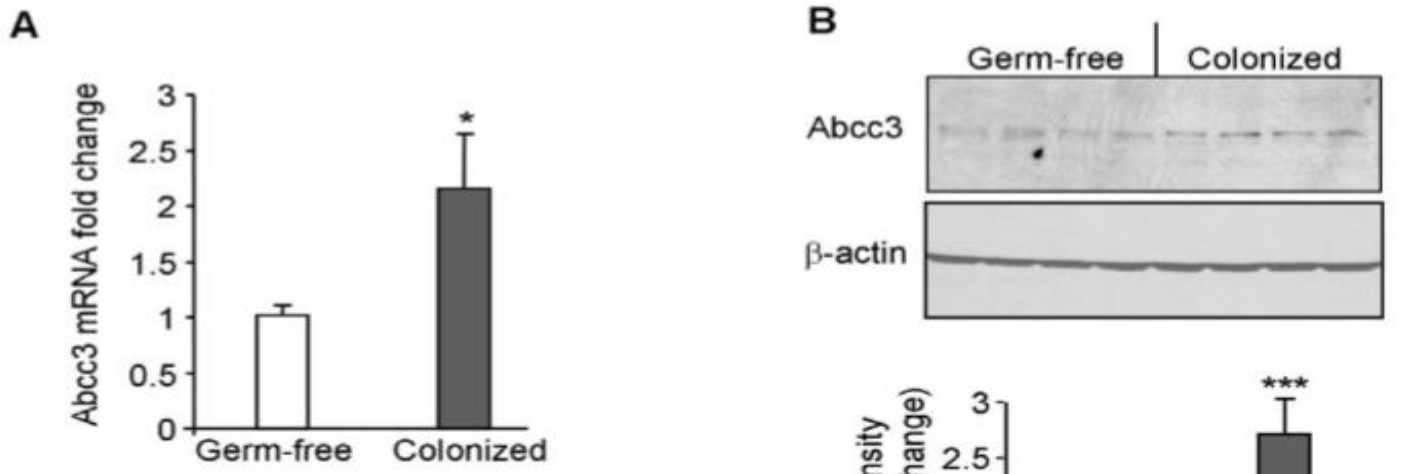
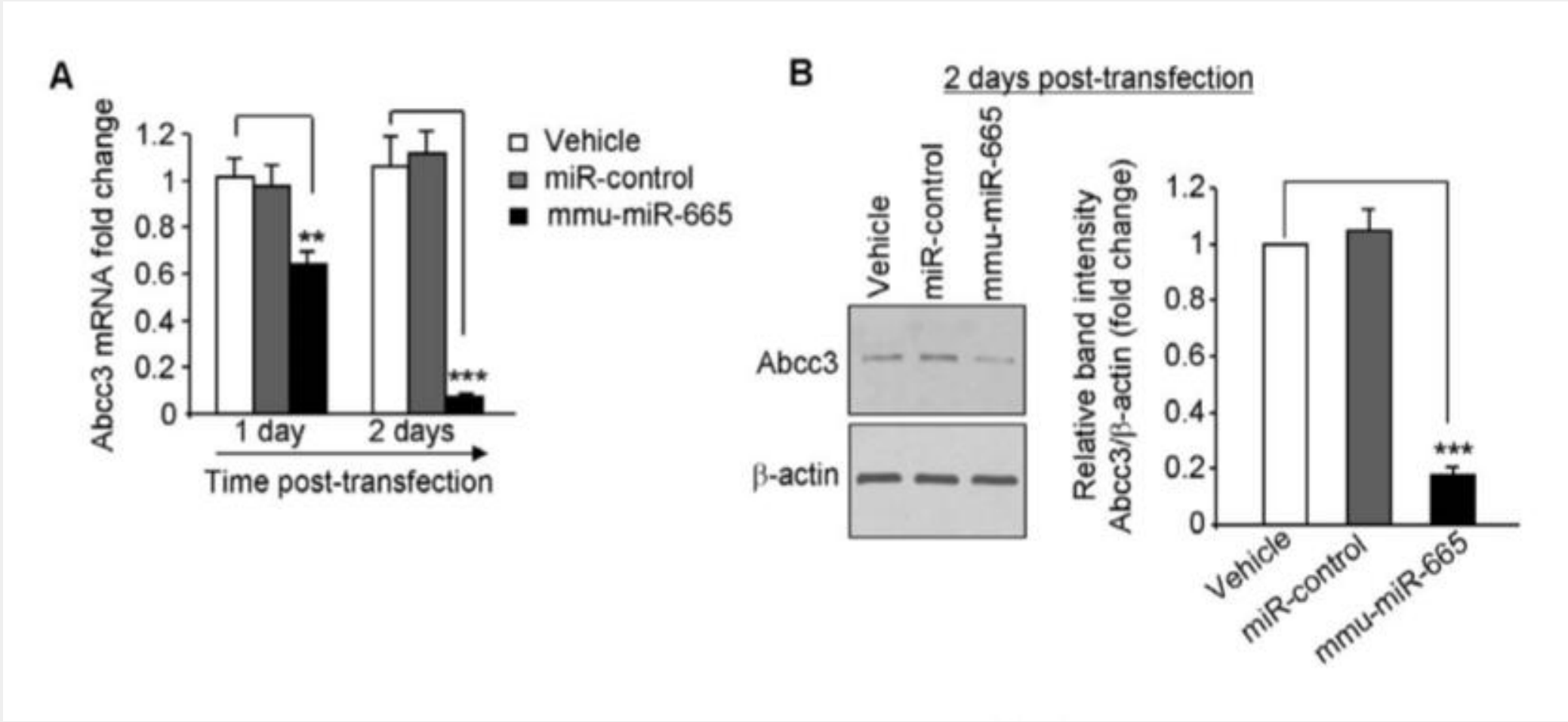


Figure 2. Microbiota up-regulate Abcc3 expression in mouse colon. Germ-free mice were colonized with microbiota from pathogen-free mice. (A) Total RNAs were extracted from the colons of germ-free and colonized mice. Abcc3 mRNA expression levels were assessed by qRT-PCR. Values represent means ± S.E.M. (n = 6/group; *$P<0.05$). (B) Abcc3 protein expression levels in the colons of germ-free and colonized mice were assessed by Western blot. Bar graphs in *B* show the relative intensity of blots (upper panel) with values represent means ± S.E.M. ***$P<0.01$.
doi:10.1371/journal.pone.0019293.g002

三、研究结果


A
Abcc3 mRNA fold change
1.2
1
0.8
0.6
0.4
0.2
0
**
***
1 day
2 days
Time post-transfection
Vehicle
miR-control
mmu-miR-665
B
2 days post-transfection
Vehicle
miR-control
mmu-miR-665
Abcc3
β-actin
Relative band intensity
Abcc3/β-actin (fold change)
1.2
1
0.8
0.6
0.4
0.2
0
***
Vehicle
miR-control
mmu-miR-665

三、研究结果

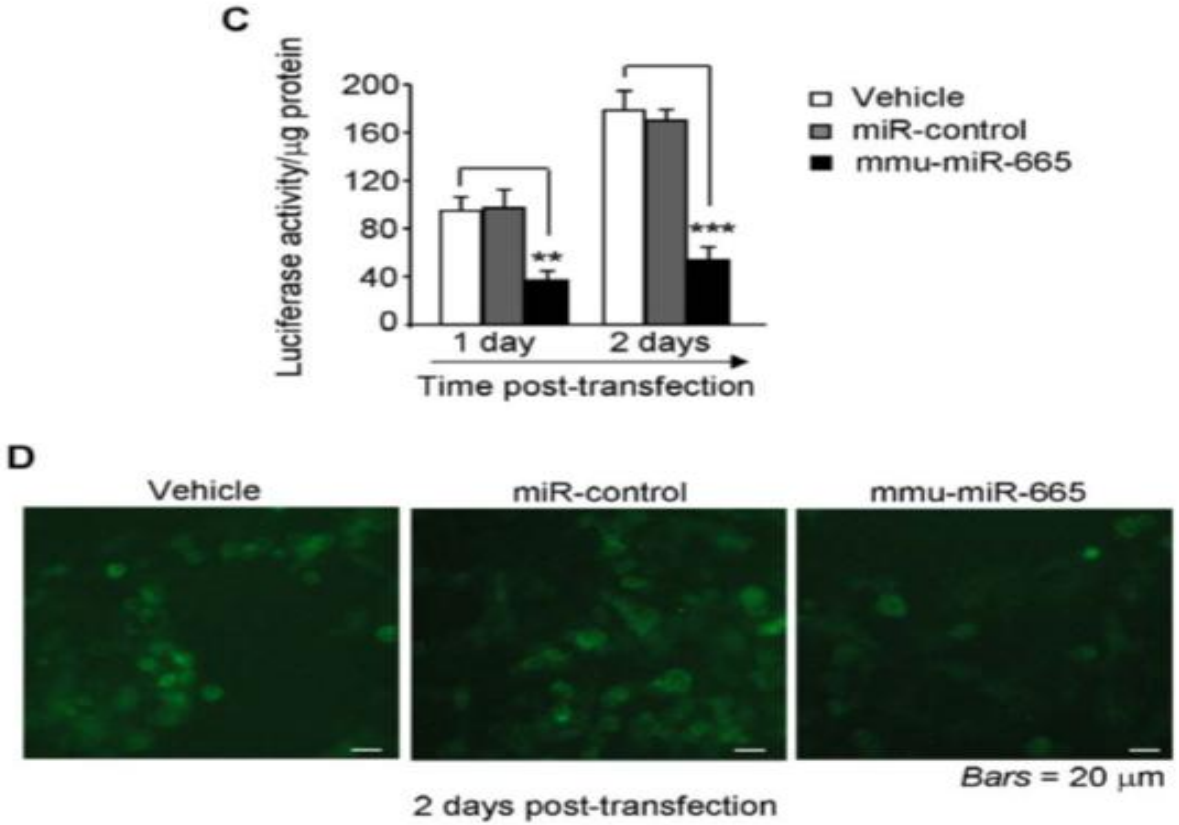
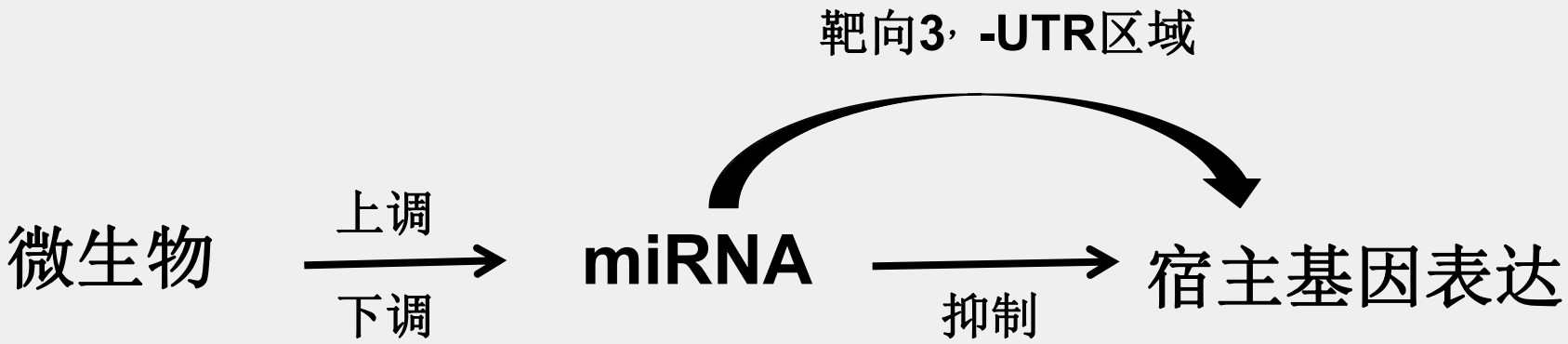
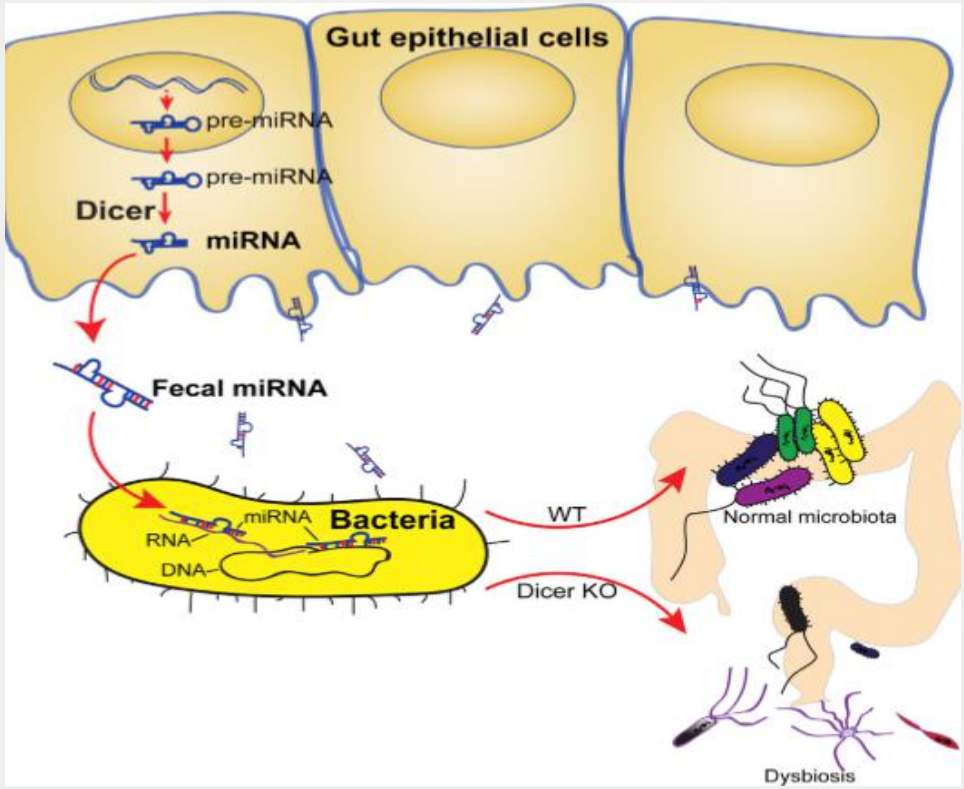


Figure 3. Mmu-miR-665 inhibits Abcc3 expression by directly targeting the Abcc3 mRNA 3′-UTR. (A, B) Mmu-miR-665 represses Abcc3 expression. RAW 264.7 cells were transfected with vehicle or precursors of miR-control or mmu-miR-665, and Abcc3 expression was assessed by qRT-PCR and Western blot. Bar graphs in *B* show the relative intensity of blots (left panel) from three independent determinations with values represent means ± S.E.M. (C, D) Mmu-miR-665 directly targets the Abcc3 mRNA 3′-UTR. RAW 264.7 cells were transfected with a luciferase or a GFP vector containing the Abcc3 3′-UTR in the presence or absence of mmu-miR-665. Luciferase activity (C) and fluorescent intensity (D) were determined. Values represent means ± S.E.M. (n = 6/group; *$P<0.05$; **$P<0.005$; ***$P<0.001$).
doi:10.1371/journal.pone.0019293.g003

四、结果与讨论



五、借鉴之处



- 之前读书报告阐述了，宿主通过miRNA调控微生物群落。
- 本文阐述了，微生物通过miRNA调控宿主的基因表达。
- 揭示了宿主、微生物和miRNA三者之间的关系。

肠道菌群与miRNA相互作用的研究：

Aim of the study	Experimental model	Investigated miRNAs	Target genes
To study whether miRNAs are involved in microbiota-mediated regulation of host gene expression.	Germ-free mice colonized with the microbiota from pathogen-free mice	miR-298; miR-128; miR-200c*; miR-342-5p; miR-465c-5p; miR-466d-3p/5p; miR-665;miR-683	Abcc3
To study the impact of the endogenous microbiota on the global expression of caecal miRNAs *in vivo*.	Germ-free and conventionally raised mice	miR-21*; miR-351; miR-487b; miR-467a; miR-27b; miR-148a; miR-145; miR-183; miR-133a; miR-150; miR-672; miR-181a; miR-664; miR-455; miR-138*; let-7g*	34 genes among glycosylation enzymes, junctional proteins, proteins found in the mucus layers and in the immune regulation.
To study miRNAs affecting the intestinal epithelial monolayer.	Mice with an inducible intestinal epithelial cell–specific deficiency in *Dicer1* (*Dicer1* $^{\Delta gut}$)	miR-375	KLF5
To study the TLR-4-mediated transcriptional activation of intestinal epithelial cells (IECs).	Mice immediately after birth	miR-146a	IRAK-1
To study microbiota regulation of miRNA expression and intestinal homeostasis.	C57BL/6 (B6), B6.IL-10$^{-/-}$, B6.MyD88$^{-/-}$ and B6.RAG$^{-/-}$ mice	miR-10a	IL-12/IL-23p40

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