

读书报告

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胡文攀


Microbiota Modulate Host Gene Expression via MicroRNAs

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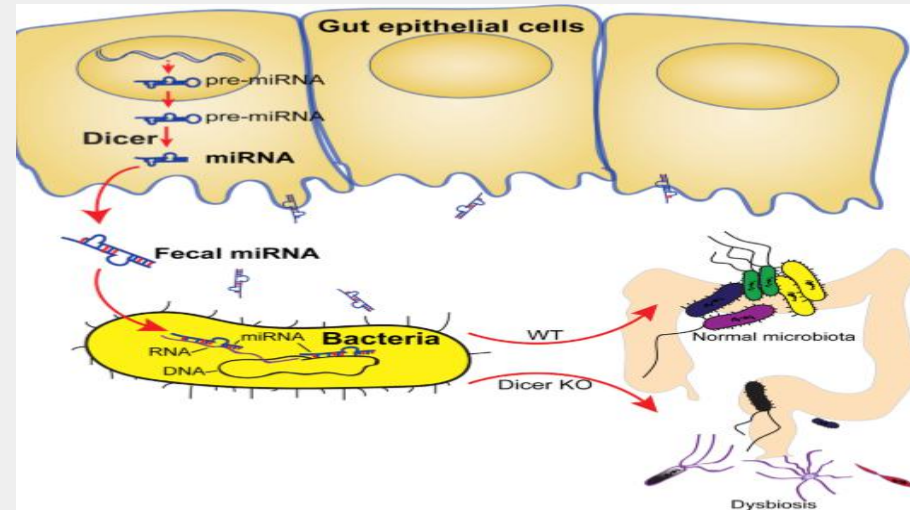
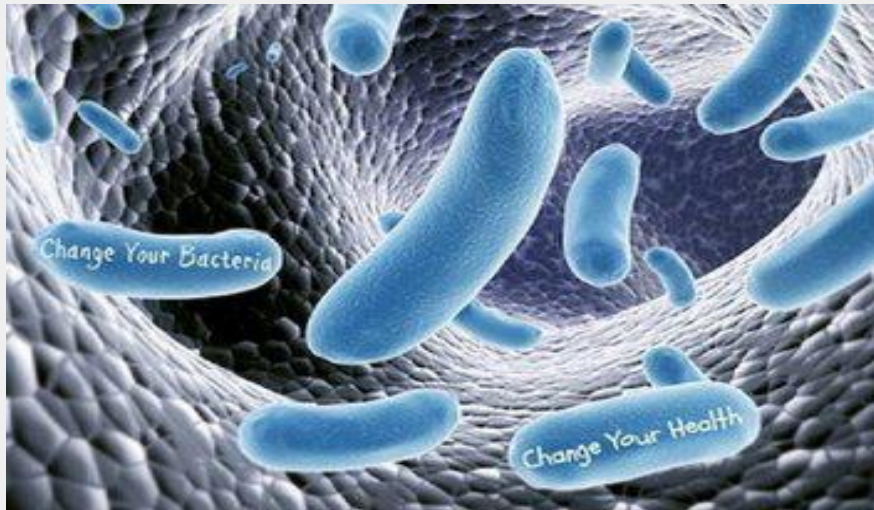


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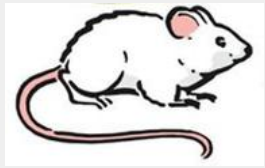
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一、研究背景

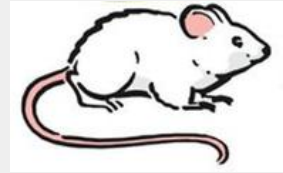
胃肠道中有大量的微生物(10^{14} 个细菌), 是各种微生物的定殖器官。已知微生物群可以调节宿主基因表达, 但其潜在的分子机制仍不清楚。MicroRNAs (miRNAs)通过与靶miRNA的 39-UTRs结合后转录调控基因表达, 在许多细胞功能中起重要作用。然而, miRNA 在微生物-宿主相互作用中的作用尚不清楚。



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amp;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

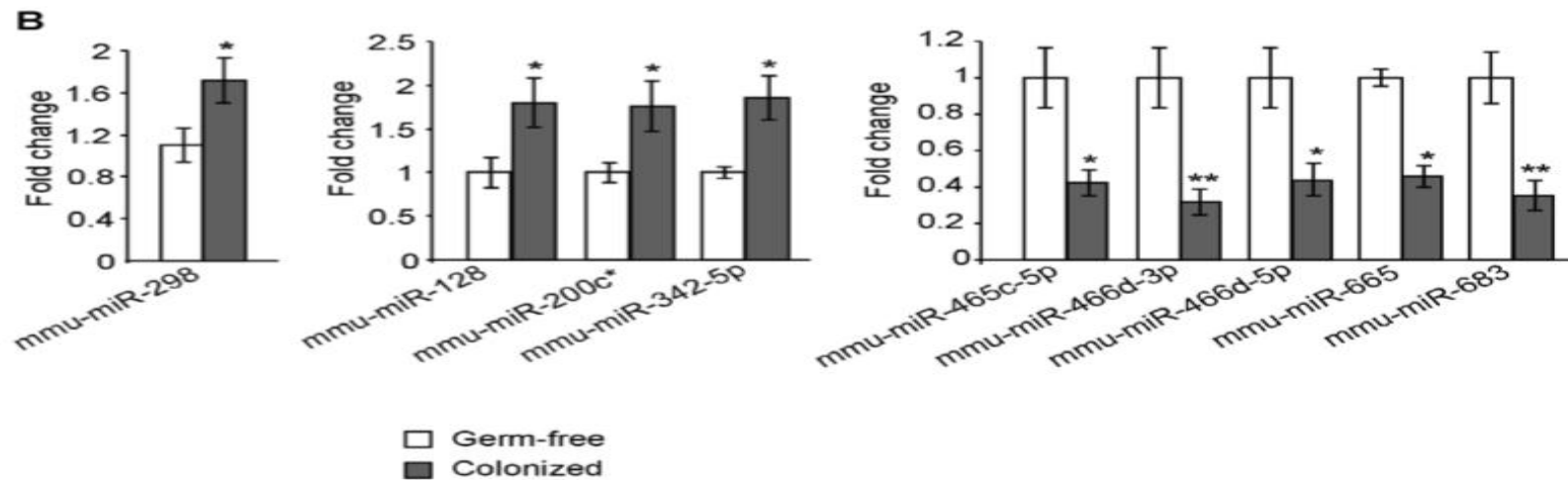


Figure 1. Microbiota modulate host miRNA expression. Germ-free mice were colonized with the microbiota from pathogen-free mice. Total RNAs were extracted from the ileums and colons of germ-free and colonized mice. MiRNAs differentially expressed in the ileum and colon of colonized mice compared to germ-free mice (≥ 1.5 -fold) determined by miRNA array (A) and qRT-PCR (B). Values represent means \pm S.E.M. (n = 6/group; * $P < 0.05$; ** $P < 0.0005$).
 doi:10.1371/journal.pone.0019293.g001

三、研究结果

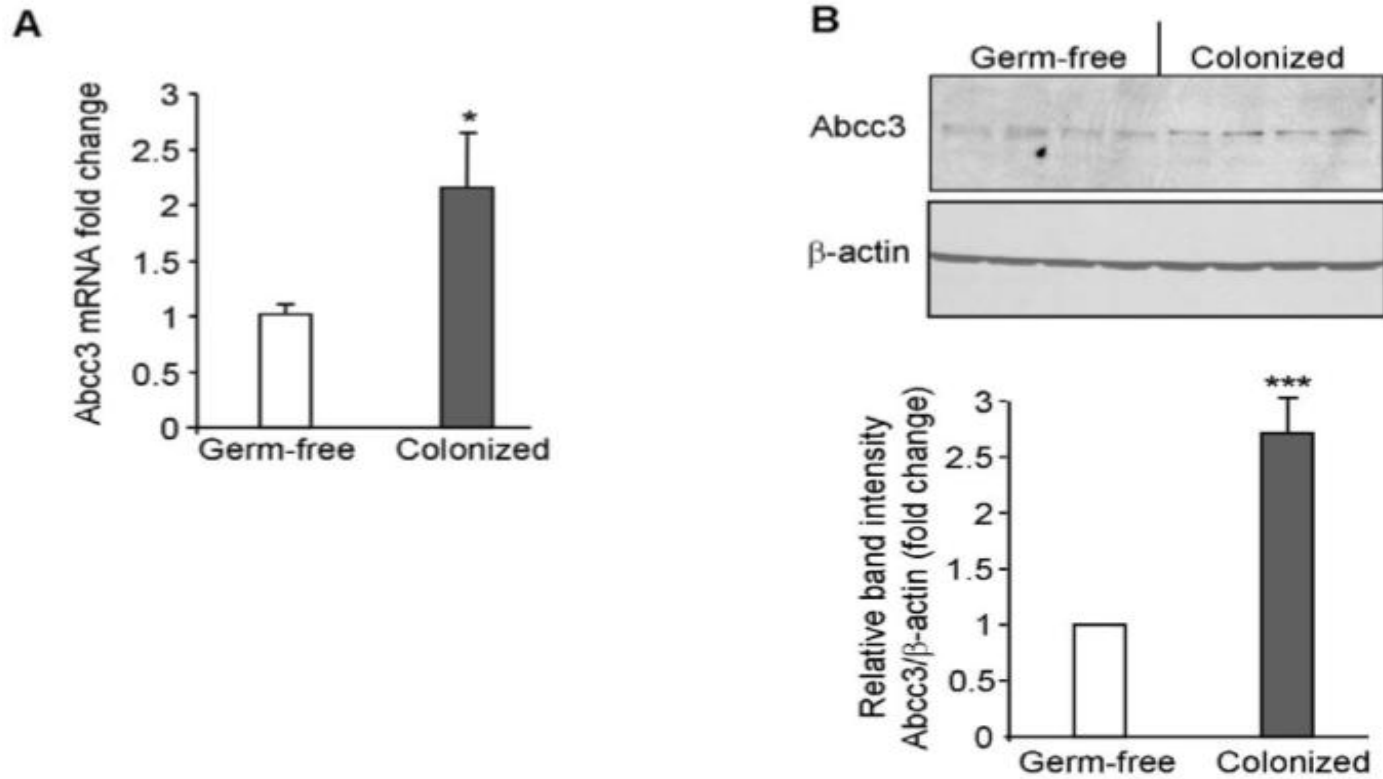
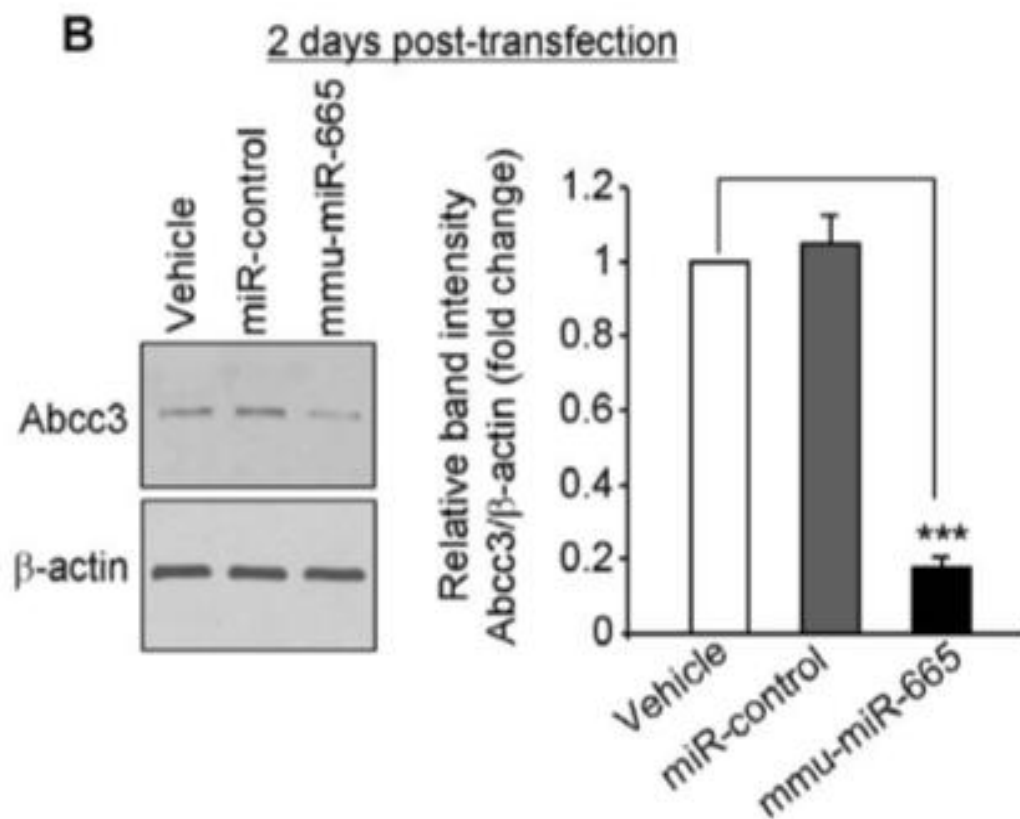
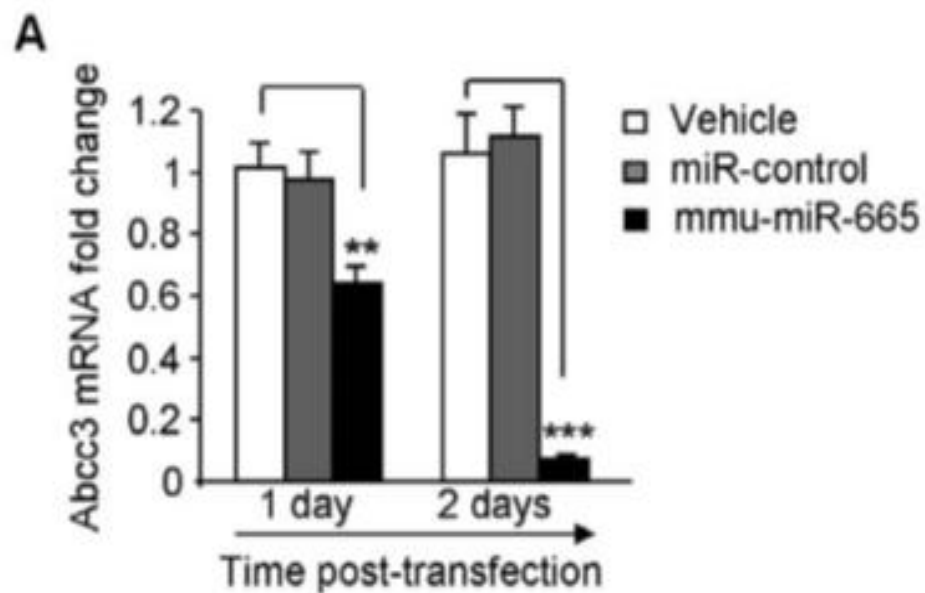


Figure 2. Microbiota up-regulate Abcc3 expression in mouse colon. Germ-free mice were colonized with microbiota from pathogen-free mice. (A) Total RNAs were extracted from the colons of germ-free and colonized mice. Abcc3 mRNA expression levels were assessed by qRT-PCR. Values represent means \pm S.E.M. ($n=6$ /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 \pm S.E.M. $***P<0.01$. doi:10.1371/journal.pone.0019293.g002

三、研究结果



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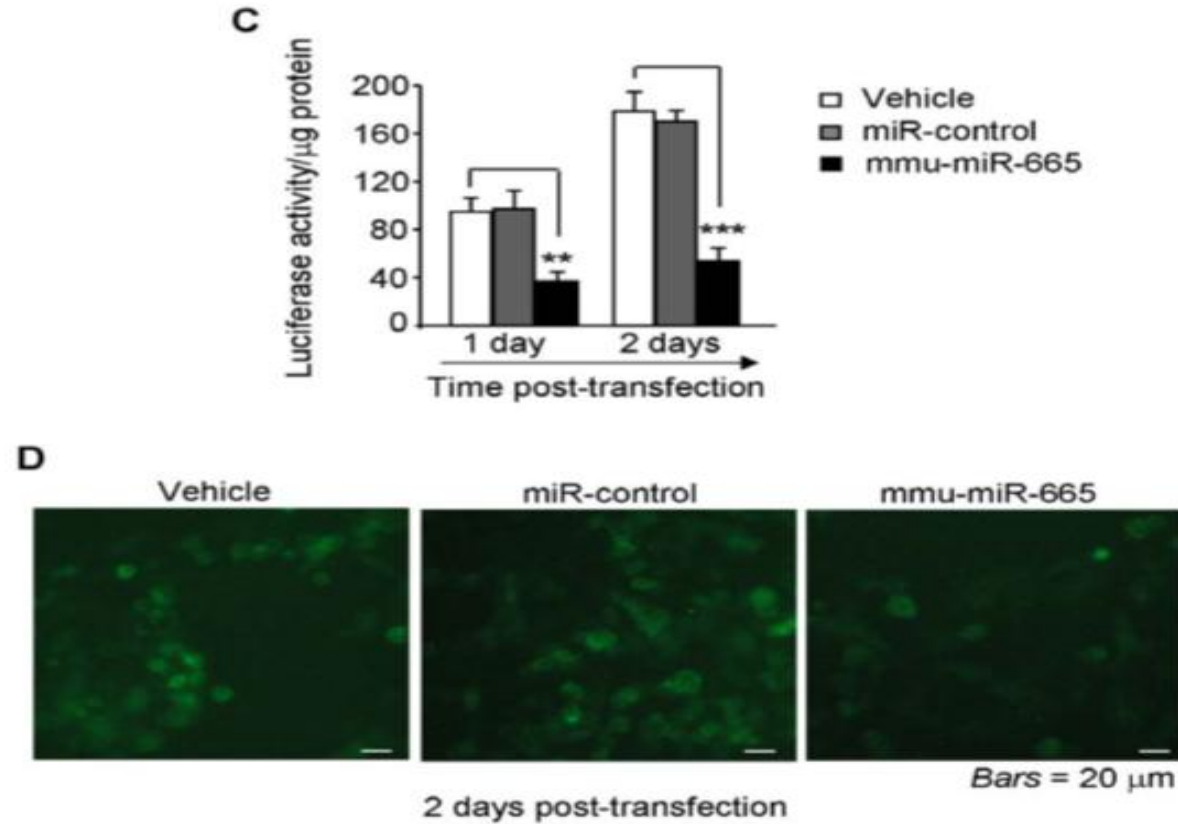
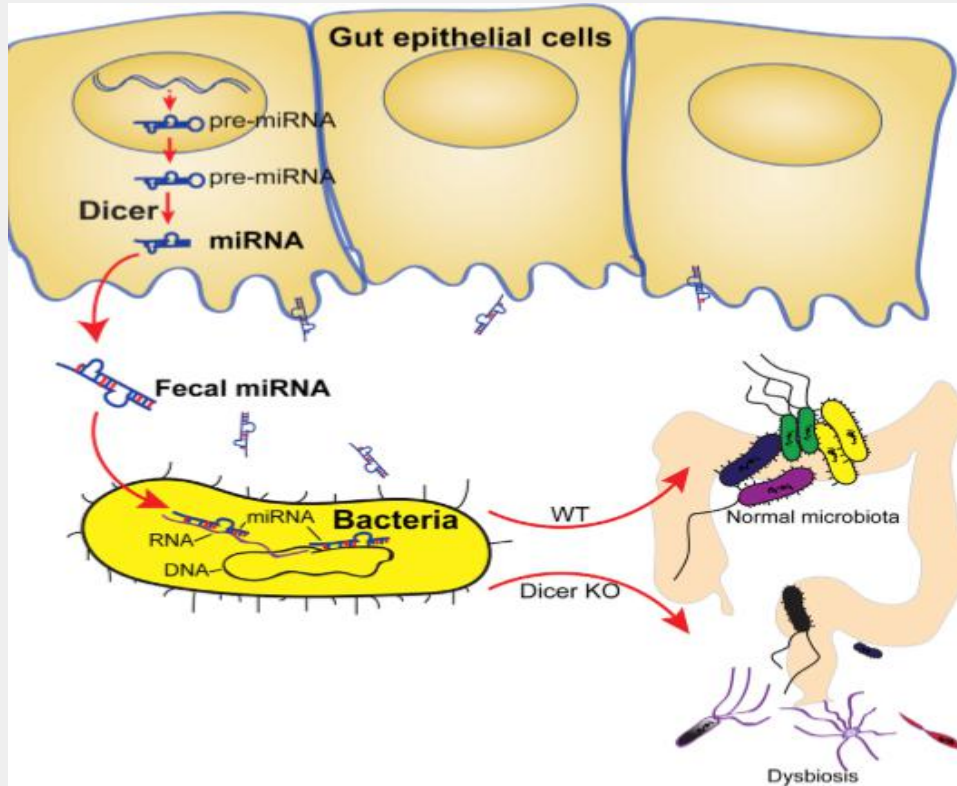


Figure 3. Mmu-miR-665 inhibits Abcc3 expression by directly targeting the Abcc3 mRNA 3'-UTR. (A, B) Mmu-miR-665 represses Abcc3 expression. RAW 264.7 cells were transfected with vehicle or precursors of miR-control or mmu-miR-665, and Abcc3 expression was assessed by qRT-PCR and Western blot. Bar graphs in B show the relative intensity of blots (left panel) from three independent determinations with values represent means \pm 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 \pm 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 <i>in vivo</i> .	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 <i>Dicer1</i> (<i>Dicer1</i> ^{Δgut})	miR-375	KLFS
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

The image features a white background with decorative geometric elements. In the top right corner, there are two overlapping downward-pointing triangles: one in blue and one in red. In the bottom left corner, there are two overlapping upward-pointing triangles: one in red and one in blue. The central text is in a bold, black, sans-serif font.

请各位老师批评指正！