

Mengyi Wang¹, Xiaoxue Fan¹, He Zang¹, Dafu Chen², Rui Guo³

¹College of Bee Science and Biomedicine, Fujian Agriculture and Forestry University, Fuzhou 350002, China

²National & Local United Engineering Laboratory of Natural Biotxin, Fuzhou 350002, China

³Apitherapy Research Institute of Fujian Agriculture and Forestry University, Fuzhou 350002, China

piRNA is a kind of small non-coding RNA which plays an essential part in genome stabilization by suppressing transposons and modulation of biological processes through regulating target gene expression. Currently, little is known about the regulatory role of piRNAs in honeybees due to a lack of functional study. Here, molecular validation of piR-ame-1186994 in the *Apis mellifera* worker's midgut, drone's testis, and queen's ovary was conducted, and followed by prediction and annotation of target mRNAs of piR-ame-1186994. Additionally, regulatory networks relative to developmental signaling pathways, energy metabolism-associated pathways, and cellular and humoral immune-relevant pathways were analyzed. Further, functional investigation of piR-ame-1186994 was performed via feeding-based overexpression and knockdown. The specific fragment of piR-ame-1186994 was amplified from the *A. mellifera* worker's midgut, drone's testis and queen's ovary. piR-ame-1186994 targeted 1097 mRNAs annotated to 30 GO terms such as metabolic process, binding and cell as well as 182 KEGG pathways including Wnt signaling pathway, endocytosis and oxytocin signaling pathway. Thirty-six and 16 target mRNAs were respectively involved in 5 developmental pathways (mTOR, Wnt, Hippo, AMPK and Notch signaling pathways) and 4 pathways relative to energy metabolism. Additionally, 29 and 8 target mRNAs were engaged in 4 cellular immune pathways (lysosome, endocytosis, phagosome and ubiquitin-mediated protein degradation) and 3 humoral immune pathways (PI3K-Akt, MAPK and FoxO signaling pathways), respectively. As compared with that in the mimic-NC group, the expression level of piR-ame-1186994 was significantly up-regulated in the 1, 2, 3, 4, and 5-day-old workers' midguts in the mimic-piR feeding group, the expression level of target gene YAP1 was significantly downregulated in the 1, 3, 4, and 5-day-old workers' midguts and downregulated in the 2-day-old worker's midgut, the expression level of target gene PLD2 was significantly downregulated in 2, 3, 4, and 5-day-old workers' midguts. These results suggest that piR-ame-1186994 expresses in the *A. mellifera* worker's midgut, drone's testes, and queen's ovary, piR-ame-1186994 potentially modulates the development and immunity of worker's midgut through targeting and negatively regulating the expression of YAP1 and PLD2. Our findings reveal the function of piR-ame-1186994 at molecular level and deepen the understanding of modulatory role of bee piRNAs.