

Circular RNA *ame_circ_000115* regulates gene expression immune response of European honeybee larvae to *Ascosphaera apis* infection

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Circular RNAs (circRNAs) are a new class of non-coding RNAs, which have been confirmed to regulate insect gene expression and immune response through multiple manners such as competing endogenous RNA (ceRNA) regulatory network. Currently, function of circRNA in the immune response of *Apis mellifera ligustica* to *Ascosphaera apis* infection remains unclear. PCR and Sanger sequencing confirmed the existence of back splicing (BS) site within *ame_circ_000115* (*ac115*). RT-qPCR assay showed that as compared with that in the un-inoculated group, the expression level of *ac115* in 4-day-old larval gut of the *A. apis*-inoculated group was significantly up-regulated, while that in 5- and 6-day-old larval guts were significantly up-regulated. The brightness of specific band for *ac115* in 4-, 5-, and 6-day-old larval guts of the siRNA-*circ_000115*-fed group gradually became weak, whereas that of the siRNA-scramble-fed group was pretty high without obvious variation. Compared with that of the siRNA-scramble-fed group, the expression of *ac115* in 4-day-old larval gut of the siRNA-*circ_000115*-fed group was significantly down-regulated, whereas that of the 5- and 6-day-old larval guts were significantly down-regulated. Dual-luciferase reporter gene assay suggested that *ame-miR-13b* was truly existed and expressed in the larval guts, and there was true binding relationship between *ac115* and *ame-miR-13b*. In comparison with that in the siRNA-scramble-fed group, the expression levels of antimicrobial peptide genes hymenoptaecin and abaecin in 6-day-old larval gut of the siRNA-*circ_000115*-fed group were significantly up-regulated, while that of ecdysone receptor (*Ecr*) was significantly down-regulated. These results indicate that *ac115* is truly expressed in *A. m. ligustica* larval guts, BS site truly exists within *ac115*, and effective interference of *ac115* in *A. m. ligustica* larval guts can be achieved via feeding siRNA. Moreover, *ac115* potentially regulates *Ecr* expression through adsorption of *ame-miR-13b* and expression of hymenoptaecin and abaecin using a non-ceRNA manner, further participating in host response to fungal invasion.