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Abstract

The Effects of Autophagy Related Atg6 Gene on Cellular Apoptotic Process

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Abstract

Background and aim: The *Bombyx mori* silk gland is degenerated through apoptosis and autophagic cell death mechanisms during larval-pupal metamorphosis. In this process, programmed cell death mechanisms are genetically in tight interaction with each other. However, information about this interaction is contradictory and insufficient. In this study, we aimed to investigate the effects of atg6 gene, which has a significant role in the nucleation of the isolation membrane in autophagy, on the apoptosis mechanism during the larval pupal metamorphosis.

Materials and methods: On the third day of cocoon spinning activity, RNA interference was used to silence the Atg6 gene. Measuring Atg6 mRNA levels after treatment was used to assess the method's effectiveness. Morphological observations were analysed by Hematoxylin-eosin staining. TUNEL assay was used to determine DNA fragmentation. Western blot analysis was used to evaluate the proteins sitc, caspase3 and p53 which are the apoptotic process's triggers. The findings were compared with the results of the control groups. The decrease in mRNA levels of Atg6 gene after dsRNA application was determined. Apoptotic degenerative changes in the groups were analyzed morphologically. The data were analysed using independent sample t-test by SPSS program.

Results: The dsRNA-treated group didn't show any degeneration. There was a noticeably lower tunnel signal in the dsRNA-treatment group compared to the control group in the apoptosis-specific DNA fragmentation. In the dsRNA-treatment group, an increase in the amount of apoptotic stimulating proteins cytosolic cytochrome-c, cleaved caspase-3 and p53 protein were observed compared to the control group. The findings indicated that during metamorphosis process, apoptosis and autophagy together caused the silk gland to degenerate. Because of silencing of atg6, autophagy mechanism is interrupted, together with an increase in proapoptotic proteins. Despite the strong stimulation of apoptosis, TUNEL and morphological analysis results showed that apoptotic cell death mechanism alone is not adequate for the degeneration of this tissue.

Conclusion: As a result, in addition to playing a crucial function in autophagy, Atg6 may also importantly play a part in the molecular connection between autophagy and apoptosis.

Keywords: *dsRNA, Atg6, Apoptosis, Autophagy, Bombyx mori*

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