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**Title of abstract in Arial font: size14 point**

Yanlan Li1\*, Zheyu Pang2, Zhiwei Wang3,Yubao Li4

\*lead presenter

117663530765@163.com, College of Agronomy and Agricultural Engineering, East Campus of Liaocheng University, Liuyuan Street, Dongchangfu District, Liaocheng City, Shandong Province 252059, China

2 College of Agronomy and Agricultural Engineering, East Campus of Liaocheng University, Liuyuan Street, Dongchangfu District, Liaocheng City, Shandong Province 252059, China

3 College of Agronomy and Agricultural Engineering, East Campus of Liaocheng University, Liuyuan Street, Dongchangfu District, Liaocheng City, Shandong Province 252059, China

4 College of Agronomy and Agricultural Engineering, East Campus of Liaocheng University, Liuyuan Street, Dongchangfu District, Liaocheng City, Shandong Province 252059, China

**Abstract:**

**Background/Objective:** [Heat shock proteins (HSPs), a class of evolutionarily conserved proteins widely found in prokaryotic and eukaryotic cells, are mainly involved in protein synthesis, folding, accumulation, assembly, transport and degradation as molecular chaperones. Heat shock proteins are abnormally expressed in a variety of tumor tissues and their cellular localization is clearly correlated with tumor cells, but their specific biological roles need to be further investigated.]

**Methods:** [In this project, DF-1 cells were used as the research object, and HSP60 overexpression cell lines, HSP60 knockdown cell lines, HSP60 knockout cell lines, HSP60 MIT mutant cell lines were constructed. The apoptosis level of cells was detected after the change of the expression of HSP60 by using ELISA and flow cytometry, and the transcripts and expression level of apoptotic factors, such as BAX, BAK, Bcl-2, P53, and Caspase 3 were detected by using qPCR, and western blot, so as to dynamically investigate the relationship between HSP60 and cell apoptosis.]

**Results:** [**1. Effect of overexpression of HSP60 on apoptosis.** Based on the HSP60 sequence

published by NCBI, combined with the sequence of pEGFP-C1 vector, primers were designed to construct pEGFP-HSP60 recombinant vector. pEGFP-HSP60 recombinant vector with correct sequencing and PEGFP-C1 empty vector were transfected into DF-1 cell line. The results showed that DF-1 cells overexpressing HSP60 had a 9.57-fold increase in HSP60 transcripts and significantly higher expression compared with the control group.ELISA and flow cytometry results showed that apoptosis was significantly reduced by overexpression of HSP60.HSP60 overexpression inhibited the cellular death of DF-1 cells by down-regulating the expression of BAX and BAK, and by up-regulating the expression of Bcl-2, and by down-regulating the expression of Caspase 3, and inhibiting the cellular death of DF-1 cells by overexpression of HSP60. HSP60 overexpression inhibited apoptosis by down-regulating BAX and BAK expression, up-regulating Bcl-2 expression and down-regulating Caspase 3 expression.

**2. Effects of RNA interference to knock down HSP60 on apoptosis.** Three HSP60-SiRNA

sequences and control sequences were designed and transfected into DF-1 cell line to construct HSP60 knockdown cell line. The results showed that the HSP60-SiRNA-1087 sequence had the highest interference efficiency, and the HSP60 protein expression level was reduced by 40% compared with the HSP60-SiRNA-NC sequence. Knockdown of HSP60 resulted in a significant decrease in the expression of BAX and Bcl-2, a highly significant increase in the expression of BAK and Caspase 3, and a highly significant increase in the level of apoptosis.

**3. Effect of knockdown of HSP60 on apoptosis.** The CRISPR-Cas9 system was utilized to

knock down HSP60 in DF-1 cells, and Guide RNA was designed to construct PX459M-1-2

double knockdown recombinant plasmid, and the sequenced correct PX459M-1-2 double

knockdown recombinant plasmid was transfected into the DF-1 cell line to construct the

DF-1-HSP60-KO cell line with knockdown of HSP60. The results showed that compared with

the DF-1 cell line, knockdown of HSP60 resulted in a highly significant increase in apoptosis

level, a decrease in the expression of BAX and Bcl-2; and an increase in the expression of BAK and Caspase3. It indicates that HSP60 knockdown promotes apoptosis by down-regulating BAX and Bcl-2 expression, up-regulating BAK expression, and up-regulating Caspase 3 expression.

**4. Effect of HSP60 MIT mutation on apoptosis.** Using Pymol for protein structure prediction, the key nucleotide sequence CAC at position 61-63 after the start codon of HSP60

MIT was replaced with AAG, and the corresponding amino acid His at position 21 of MIT was replaced with Lys.After the mutation of HSP60 MIT, HSP60 could not enter the mitochondria to play the role of anti-apoptosis, and apoptosis level was significantly elevated, and the level of BAX The expression level of BAX was significantly increased; BAX expression level was significantly decreased from 0-2 h and significantly increased from 3-6 h; BAK expression level showed a trend of increasing and then decreasing; Bcl-2 expression level was significantly decreased (*p* < 0.01); Caspase3 expression level was significantly increased from 0-1 h and significantly decreased after 2 h. We hypothesized that HSP60 was altered by altering the mitochondria to play an anti-apoptotic role, and that HSP60 could not enter the mitochondria to play an anti-apoptotic role. We hypothesized that HSP60 regulates apoptosis by altering the expression levels of apoptotic factors such as BAX/BAK/Bcl-2/Caspase 3 in the mitochondrial apoptotic pathway.]

**Conclusion:** [In this study, we demonstrated that HSP60 plays a role in inhibiting apoptosis in DF-1 cell line, and that changes in the transcription and expression of HSP60 cause changes in BAX, BAK, Bcl-2, Caspase 3 and other factors in the mitochondrial apoptotic pathway, and that amino acid at position 21 in the MIT of HSP60 is a key site for HSP60 to enter the mitochondria to play an anti-apoptotic role.]

**Keywords:** [DF-1 cells; HSP60; apoptosis; flow cytometry; mechanism]