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Knockout of *Hsp70* genes causes changes in the transcriptome of leg skeletal muscles and locomotion speed in *D. melanogaster* comparable to age-related changes

Kurochkina N.S.[1]*, Makhnovskii P.A.[1], Kukushkina I.V.[2], Popov D.V.[1]

[1] *Institute of Biomedical Problems, RAS, Moscow, Russia*

[2] *Moscow State University, Moscow, Russia*

* *nadia_sk@mail.ru*

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Motivation and Aim: Aging is associated with complex changes, including a decrease in physical activity, mass and functional capabilities (insulin sensitivity, performance and muscle strength) of skeletal muscles. These changes are based on various molecular and cellular processes, among which the disturbance of proteostasis plays an important role. Heat shock proteins 70 (HSP70) play a key role in the regulation of proteolysis. The association of *Hsp70* (*Hspa*) genes expression in skeletal muscle with primary aging has recently been discovered [1], but their role in age-related changes in skeletal muscle functions is not clear [2]. The aim of the work was to study the effect of knockout of *Hsp70* genes on transcriptome of leg skeletal muscle and their performance and compare these changes with age–related changes in the control group (without knockout).
Methods and Algorithms: Knockout is a classic approach for studying the function of a gene. The *Hsp70* family consists of more than 10 genes; knockout of one of them can be compensated by other genes of this family, which makes it difficult to interpret the results of such experiments. Therefore, we used the *D. melanogaster* line with knockout of 6 of the 13 genes in this family. The study was performed on males of *D. melanogaster* control line w^{1118} and line $Hsp70^{-}$ with knockout of *Hsp70Aa, Hsp70Ab, Hsp70Ba, Hsp70Bb, HSP70BB, Hsp70Bc* genes (orthologs of the *Hspa1a, Hspa1b, Hspa2* and *Hspa8* mammalian genes). Limbs consisting mainly of skeletal muscles were separated from 7-, 23- and 47-day-old flies of both lines, then RNA was isolated to assess the transcriptomic profile of protein-coding genes by RNA sequencing in single-end mode with a depth of 25 million reads/sample (NextSeq 550, Illumina, USA). Differentially expressed genes were defined as genes with an expression level >1 TPM, *Padj* <0.01 and fold change >1.25 times. The performance of flies was assessed by their locomotion speed (climbing a tube wall) in a negative geotaxis test, continuously determining the position and vertical speed of each fly (FreeClimber tool).
Results: In old w^{1118} flies (47 days vs. 7 days), the locomotion speed decreased significantly and the leg transcriptomic profile changed (more than 3,500 genes). Functional enrichment analysis showed that up-regulated genes belonged to functional categories related to nuclear proteins, proteolysis/proteasomes, protein folding, translation/ribosomes and extracellular matrix. Down-regulated genes were associated with mitochondrial proteins (oxidative enzymes, etc.), membrane proteins and synaptic proteins of motor neurons, enzymes of glucose/glycogen and amino acid metabolism, proteins of sarcomeres and extracellular matrix. These changes are in good agreement with age-related changes in the transcriptome of skeletal muscles in rodents and humans [3, 4].

Knockout of six genes of the *Hsp70* family led to a decrease in the locomotion speed in young (7-day-old) flies by 2 times, relative to young control flies (w^{1118} line), which was accompanied by noticeable changes in the transcriptomic profile of leg muscles (more than 1,500 genes). Interestingly, about a third of these genes also changed expression during aging in the control group. Among them, up-regulated genes enriched the functional category "extracellular space", and down-regulated genes – "plasma membrane", "metabolic pathways", "carbohydrate metabolism".

Conclusion: Knockout of six genes of the *Hsp70* family causes pronounced changes in the transcriptome of leg skeletal muscles and performance (locomotion speed) in young (7-day-old) flies. A third of these transcriptomic changes overlapped with age-related changes in the control line (47 days vs. 7 days), which partially explains the decrease in locomotion speed in young $Hsp70^{-}$ flies relatively young w^{1118} flies.

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