Analysis of *Spermophilus dauricus* Cardiac Hypoxia-Tolerance Gene Pathways ——Unveiling Cardiac Ischemic Stress Response

Junting Zhou

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Abstract

Spermophilus dauricus, is an animal belongs to the Sciuridae family and the Citellus genus, which is well known to the biological community as an animal whose heart resists hypoxia.Although this has been discovered long ago, people are not familiar with its anti-hypoxia mechanism.In this work, we artificially caused varying degrees of damage to the heart of Spermophilus dauricus through surgery, performed RNA sequencing on the cells in the damaged area, and then analyzed the differences in gene expression. Finally, we used GO enrichment analysis to reveal the stress gene expression pathway of hypoxic cardiomyocytes in Spermophilus dauricus. This result may reveal key genes in cardiomyocytes' response to hypoxic conditions, which may have guiding significance for the study of diseases such as myocardial infarction.

Keywords

Spermophilus dauricus; Hypoxia-Tolerance; RNAseq; GO

1 Introduction

Spermophilus dauricus, in mainland China, it is distributed in Shaanxi, Qinghai, Inner Mongolia, Gansu, Hebei, Heilongjiang, Jilin, Shandong, Liaoning, Ningxia and other places, and mostly lives in grasslands and semi-deserts. The type origin of this species is Hulunchi, Inner Mongolia.Most of them live in high latitudes and hibernate when winter comes.

Some studies have revealed that Spermophilus dauricus cardiomyocytes have a very impressive ability to resist hypoxia. However, the mechanism has not revealed yet. This work attempts to reveal the molecular mechanism of resistance to hypoxia of Spermophilus dauricus.

In the wet experiment stage, we used surgery to open the chest cavity of the Spermophilus dauricus and ligated some of its arteries, causing the heart of the Daur chinchilla to become hypoxic. After a period of time, we performed RNA sequencing on cardiomyocytes from different regions to detect their gene expression levels.

After sequencing by illumina, we conducted multiple aspects of analysis, including PCA, GO enrichment analysis, etc. In an attempt to reveal the molecular mechanism of resistance to hypoxia in Daurian squirrels.The results revealed that compared with the sham operation group,

the expression of some genes in the three damaged areas was significantly different.Further analysis showed that most of these genes are related to cell growth and regeneration. Detailed infomation will show in the Result module.

2 Related work

2.1 Analyses of Hypoxia-Tolerance Mechanism of hibernating animals

Biorck et al. (1956) found that hibernating hedgehogs survived in pure nitrogen, cyanide and carbon monoxide for 50 to 120 minutes before developing an accelerated heart rate and prolonged QRS duration, but did not show other fatal symptoms. This shows that hibernating animals are resistant to hypoxia.

D'Alecy et al. (1990) put rats and normal body temperature chinchillas into a 4.5% hypoxic environment. After 1 hour, 100% of the chinchillas survived well, while 80% of the rats died.

If Biorck' s work is weakened by the fact that the body temperature and metabolic rate reduced during hibernation, D' Alecy' s work provides a powerful supplement. Since the brain and heart are the organs that bear the brunt of death from hypoxia, the above fact means that the hearts of hibernating animals have obvious hypoxia tolerance. However, there are no direct reports on this internationally.

A few years ago, Wang Shiqiang's laboratory at Peking University used creatine kinase as an indicator to study ischemic damage in the hearts of hibernating animals. The results showed that the release of creatine kinase in the heart of Spermophilus dauricus during the hypoperfusion and reperfusion periods was significantly lower than that of rats. (Gao Tianli et al.1996) preliminarily proved its resistance to hypoxia.

In addition, hibernating animals also have adaptive changes in response to the insufficient blood supply that may occur during the transition from hibernation to the activation.

Petrovic (1983) measured that superoxide dismutase activity increased significantly in many tissues including the heart when hibernating chinchillas were awakened. This seems to be a corresponding protective measure. Deep hypothermia is an extreme physiological condition for mammals. In the process of interacting with the cold environment, hibernating animals have evolved to have an ectothermic lifestyle. At the same time, they have completed the perfect design of cold-resistant cells and cold-resistant organs.

2.2 Research on the resistance of Spermophilus dauricus of mechanism to ischemia, hypoxia-reperfusion injury and hypothermia

Spermophilus dauricus can adapt to endure multiple hypoxia cycles without sustaining cardiac damage. Yang Y et al.'s work[2]investigates morphological, functional, genetic, and metabolic changes that occur in the heart of ground squirrels in three groups: summer active (SA), late torpor (LT), and interbout arousal (IBA). Morphological and functional changes in the heart were measured using hematoxylin-eosin (HE) staining, Masson staining, echocardiography, and enzyme-linked immunosorbent assay (ELISA).

Results showed significant changes in cardiac function in the LT group as compared with SA or IBA groups, but no irreversible damage occurred.

Transcriptomic analysis showed that differentially expressed genes were involved in the remodeling of cytoskeletal proteins, reduction in protein synthesis, and downregulation of the ubiquitin-proteasome pathway during hibernation (including LT and IBA groups), as compared with the SA group.

Metabolomic analysis revealed increased free amino acids, activation of the glutathione antioxidant system, altered cardiac fatty acid metabolic preferences, and enhanced pentose phosphate pathway activity during hibernation as compared with the SA group.

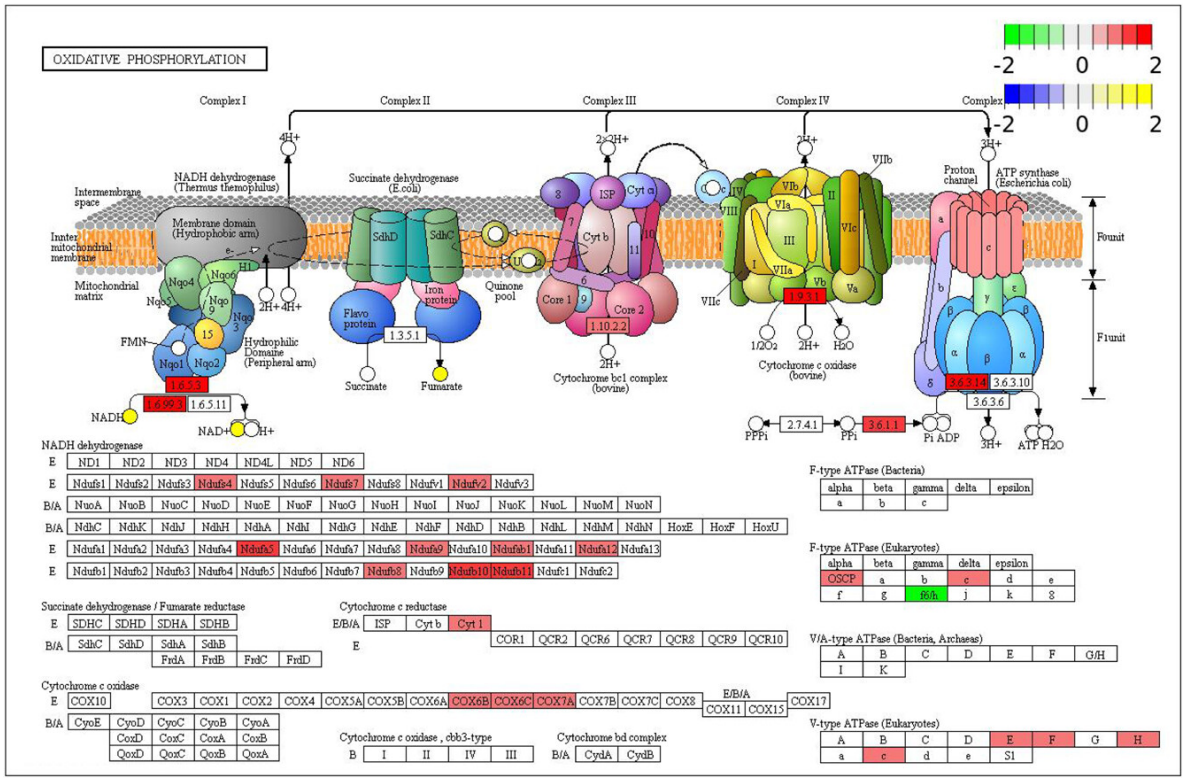

Figure 1: Pathway analysis using the KEGG pathway database. Differentially expressed genes and differentially expressed metabolites enriched in the oxidative phosphorylation pathway of the late torpor group are compared with the summer active group of ground squirrels. Red indicates upregulated expression of genes, whereas green indicates downregulated gene expression. Yellow indicates upregulated metabolite.

Combining the transcriptomic and metabolomic data, active mitochondrial oxidative phosphorylation and creatine-phosphocreatine energy shuttle systems were observed, as well as inhibition of ferroptosis signaling pathways during hibernation as compared with the SA group.

3 Method

3.1 Wet Experiment

In the wet experiment stage, we used surgery to open the chest cavity of the Spermophilus dauricus and ligated some of its arteries, causing the heart of the Daur chinchilla to become hypoxic. According to the research of Prof. Xiao Ruiping, the heart can be divided into injury area, border area and remote area according to the distance from the injury area.After a period of time, we performed RNA sequencing on cardiomyocytes from different regions to detect their gene expression levels. This part of experiment was completed by students of Shiqiang Wang.

3.2 Dry Experiment

This part of experiment was completed by Junting Zhou.After sequencing by illumina, finally we got four groups of Sequencing data:

I: which stands of injury;

B: which stands of border;

R: which stands of remote;

S: which stands of sham;

Each group repeats three times.

To make the results more convincing, We firstly perform quality control testing on the sequencing data. Details of the quality inspection report will be shown in the attachment. The conclusion is that the sequencing effect is good enough to meet the standards, and the results are credible.

Firstly, we constructed genome index. It should be pointed out that because of Spermophilus dauricusd isn't a model animal, so we don't have genome data for it. We ended up using the genome of its closely related species, Ictidomys tridecemlineatu, which is called thirteen-lined ground squirrel also.

We then conducted gene comparisons and tried to quantify gene expression. Quantification means determining the expression level of a gene or transcript. The most direct way is to count the number of reads mapped to this gene/transcript and use the number of reads as the expression level. We call this expression raw count.Based on the raw count and normalized by exon length, the quantitative method of TPM value is obtained. For each gene, divide the raw count by the length of the gene (the sum of exon lengths) to obtain the length-normalized expression amount. The TPM value of a certain gene is a relative abundance calculated using the normalized expression level. The specific calculation formula is as follows.

$$TPM = \frac{\frac{totalexonreads}{exonlength(KB)}}{\frac{GeneAmappedreads(million)}{exonlength(KB)} + \frac{GeneBmappedreads(million)}{exonlength(KB)} + \frac{GeneCmappedreads(million)}{exonlength(KB)} + \cdots} \quad (1)$$

Finally, we found the differentially expressed genes for GO enrichment analysis and searched for their related pathways in KEGG.

4 Result

As can be seen from both of the heatmap and PCA figures, the data we normalized are distributed consistently among each sample, so the samples are comparable. It can also be clearly seen from various cluster visualization diagrams that there are indeed big differences between our two groups. The samples between the groups are separated, and the samples within the groups are clustered together. Then I split the data into two groups which is called treatment and

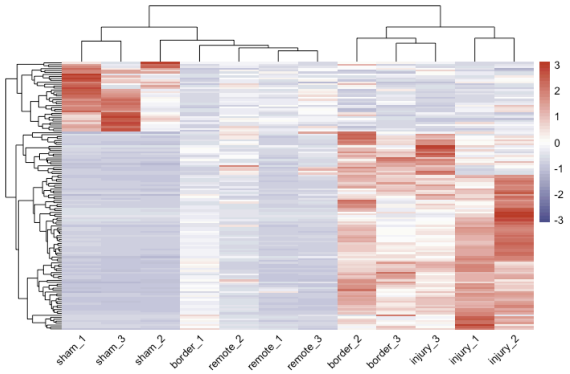

Figure 2: Heat map

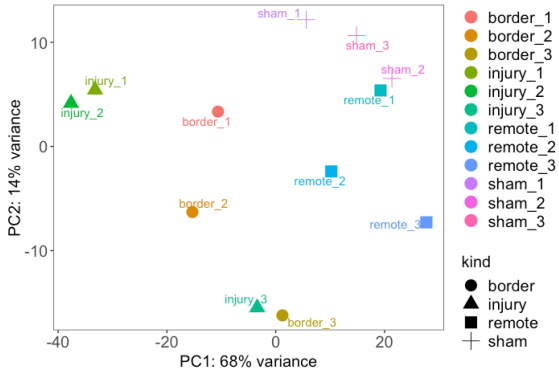

Figure 3: PCA analysis

untreatment. Treatment group includes border, injury and remote groups. They were compared with untreatment group in order to find genes with significantly different expression.

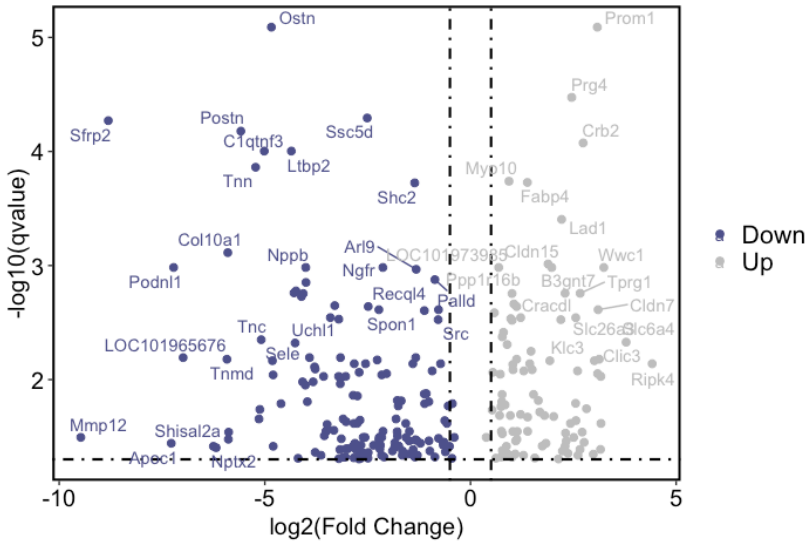

Figure 4: volcano pic. of differential expression genes

Volcano plots provide us with an intuitive visual representation that allows us to quickly identify and understand significant differences in gene expression. In the volcano plot, we observed a large number of gene expression changes, with some genes significantly up- or down-regulated. These significantly different genes are distributed at the top or sides of the graph and represent important biological changes under different experimental conditions. In particular, we focused on and analyzed in depth genes that were significantly different near the top and on the sides of

the plot. These genes may be regulatory genes or key genes associated with specific biological processes.

In order to more intuitively observe what pathways these genes are located in, we conducted GO analyse. The results were shown below.

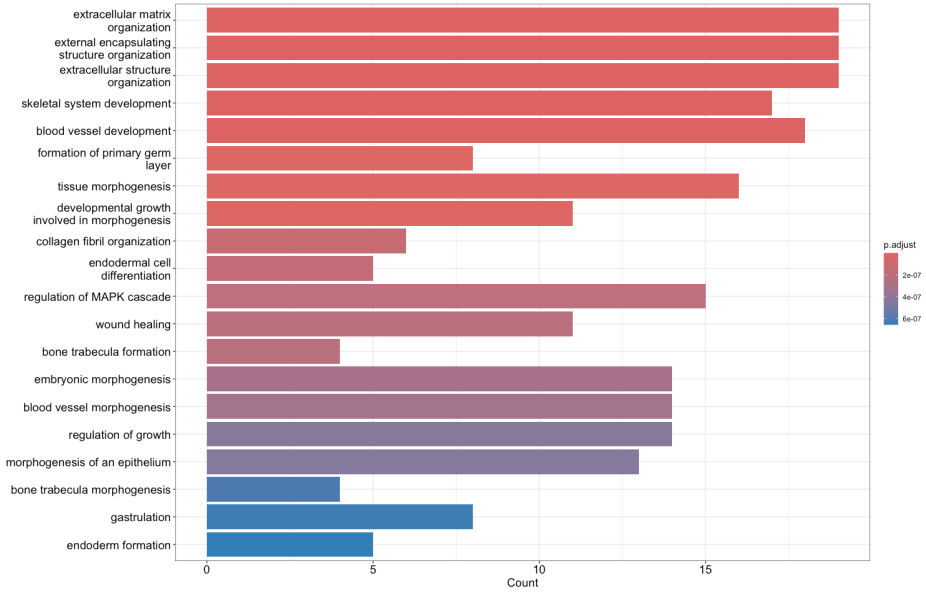

Figure 5: bar plot of the GO analyse

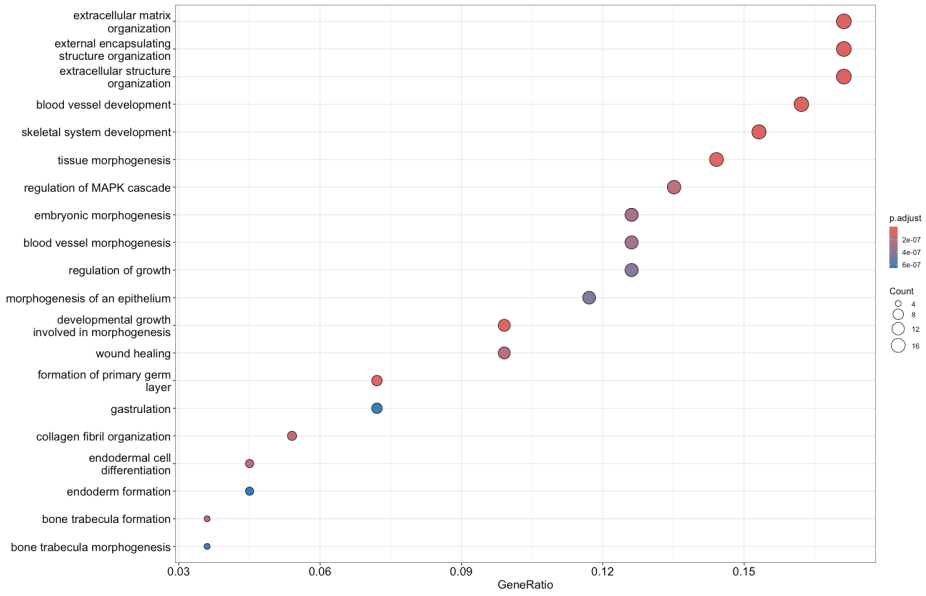

Figure 6: dotplot of the GO analyse

In order to better show the relationship between these genes and the pathways to which they belong, we made directed acyclic graph and cneplot on the GO results to show the gene network under each pathway.

The results shows that genes which differetially expressed are mainly related to cell growth,

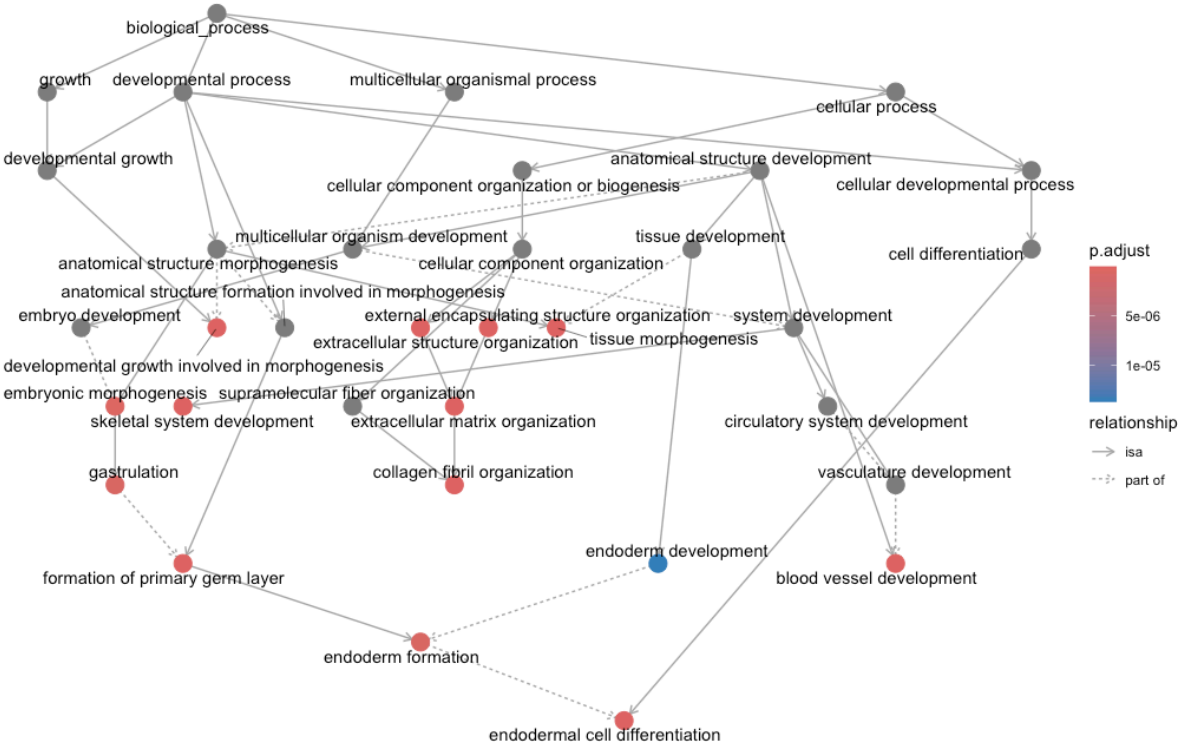

Figure 7: directed acyclic graph

blood vessel development and Anti-apoptosis , which fits our intuition properly. We further studied genes that were significantly differentially expressed in the treatment group.

Secreted Frizzled-related protein 2 (sFRP2) is a key regulatory molecule in the Wnt signaling pathway and plays multiple regulatory roles in cardiac development and cardiac pathology. sFRP2 regulates heart development by bidirectionally regulating cardiomyoblast expansion and myocardial differentiation, and exhibits specific special spatiotemporal specificity. In the process of cardiac fibrosis, sFRP2 shows a concentration dependence. Low concentrations of sFRP2 promote the progression of cardiac fibrosis, while high concentrations of sFRP2 inhibit cardiac fibrosis. In pathological myocardial hypertrophy changes, sFRP2 has a protective effect on pathological myocardial hypertrophy; in terms of vascular regeneration, sFRP2 can inhibit endothelial cell apoptosis caused by hypoxia, promote endothelial cell migration, induce endothelial angiogenesis, and promote vascular regeneration; Previous studies have revealed that secreted frizzled-related protein 2 (SFRP2) is beneficial against apoptosis and oxidative stress.[4] In our study, we also found that the expression of SFRP2 increase significantly.

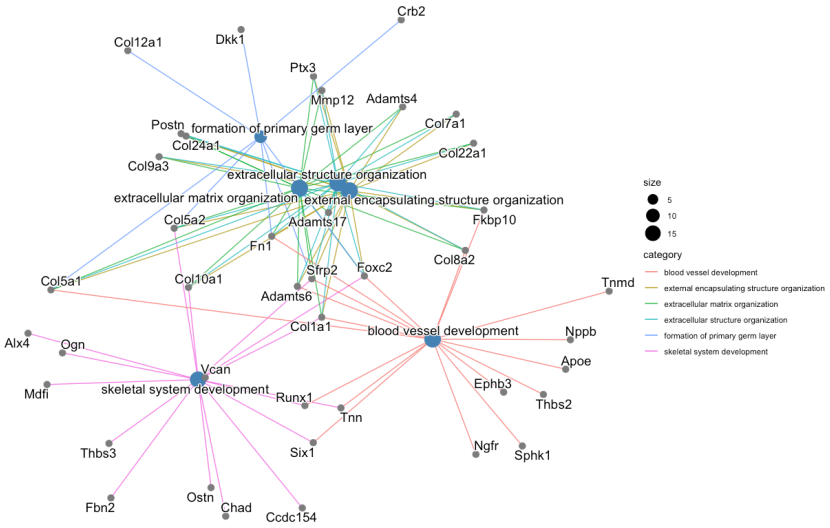

Figure 8: Gene-Concept Network

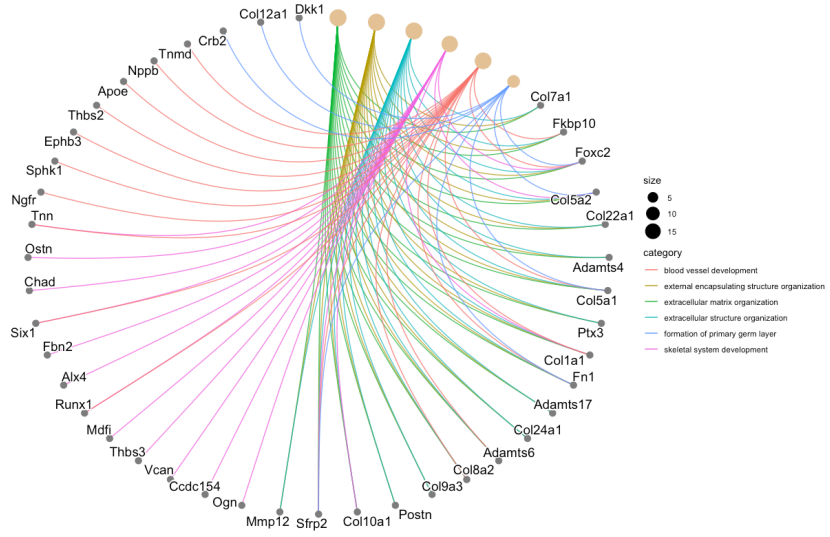

Figure 9: Gene-Concept Network

FABP4 contributes to regulating inflammasome activation in macrophages and, thus, the expression of inflammatory cytokines (interleukins IL-1 and IL-6), chemokines (monocyte chemoattractant protein-1, MCP-1), cyclooxygenase-2, and inducible nitric oxide synthase.The absence of FABP4 or the transplantation of FABP4-deficient bone marrow cells in experimental atherosclerosis has been shown to limit the burden of atherosclerotic plaques with an overall benefit and no observed metabolic side effects[5].

Our results are also in line with expectations. The experiment found that compared with the untreatment group, the expression of this gene in the experimental group was down-regulated. Which may decrease the posibility of inflammation which may cause damage to cardiac tissue.

5 Conclusion

In conclusion,we used RNAseq omics methods to try to explore the gene expression pathways of resistance to hypoxia and damage resistance in the hearts of Spermophilus dauricus. The results shows that genes which differetially expressed are mainly related to cell growth, blood vessel development and Anti-apoptosis， which fits our intuition properly.

Our study also points out the direction for the next step of research. For example, we can knock out the special differentially expressed genes found in this study to observe whether the ability of Spermophilus dauricus to tolerate hypoxia is reduced.These results provide new insights into cardio-protection in hibernators from the perspective of gene and metabolite changes and deepen our understanding of adaptive cardio-protection mechanisms in mammalian hibernators.

References

[1] Yang Y, Hao Z, An N, Han Y, Miao W, Storey KB, Lefai E, Liu X, Wang J, Liu S, Xie M, Chang H. Integrated transcriptomics and metabolomics reveal protective effects on heart of hibernating Daurian ground squirrels. J Cell Physiol. 2023 Nov;238(11):2724-2748. doi: 10.1002/jcp.31123. Epub 2023 Sep 21. PMID: 37733616.

[2] Zhao MJ, Zhao YB, Wei JH. [Cold tolerance of the membrane potentials in cardiac cells of the ground squirrel Spermophilus dauricus]. Sheng Li Xue Bao. 1988 Feb;40(1):36-42. Chinese. PMID: 3388062.

[3] Wei Y, Zhang J, Xu S, Peng X, Yan X, Li X, Wang H, Chang H, Gao Y. Controllable oxidative stress and tissue specificity in major tissues during the torpor-arousal cycle in hibernating Daurian ground squirrels. Open Biol. 2018 Oct 10;8(10):180068. doi: 10.1098/rsob.180068. PMID: 30305429; PMCID: PMC6223210.

[4] Ma T, Huang X, Zheng H, Huang G, Li W, Liu X, Liang J, Cao Y, Hu Y, Huang Y. SFRP2 Improves Mitochondrial Dynamics and Mitochondrial Biogenesis, Oxidative Stress, and Apoptosis in Diabetic Cardiomyopathy. Oxid Med Cell Longev. 2021 Nov 8;2021:9265016. doi: 10.1155/2021/9265016. PMID: 34790288; PMCID: PMC8592716.

[5] Marco Bacigaluppi, Gianvito Martino, FABP4 a novel therapeutic target in ischaemic stroke, European Heart Journal, Volume 41, Issue 33, 1 September 2020, Pages 3181–3183,

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