TRANSCRIPTIONAL BIOMARKERS OF MITOCHONDRIAL AGING AND MODULATION BY CORDYCEPS SINENSIS CS-4

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ABSTRACT

One of the earliest manifestations of human aging is a decline in energy, which begins as early as 30 years of age. The source of this decline is multi-factorial, yet changes in mitochondria (function, size, and number) have been implicated as an integral component of the age-associated decline in humans. Therefore, we set out to identify mitochondrial-related nuclear genes that consistently change in expression with aging. Cordyceps sinensis Cs-4 (Cs-4) is a natural ingredient that has been shown to have anti-aging properties and positive effects on energy, including maximal oxygen consumption (VO_{2max}). Therefore, we examined whether age-related gene expression changes could be opposed by Cs-4.

METHODS: Mice (C57Bl/6), aged 5 (n=5; young control-YC) and 22–25 (n=10; old-O) months of age were fed an AIN 93M diet. The old group was divided and fed the diet alone (old control-OC) or supplemented (old supplemented-OS) with Cs-4 (30 mg/kg body weight) for three months. Tissues were collected from skeletal muscle (gastrocnemius) and brain (cerebral cortex); gene expression was analyzed by microarrays. Gene expression profiling was used to identify mitochondrial-related transcripts that consistently changed with age in brain and muscle. Gene ontology terms were used and Parametric Analysis of Gene set Enrichment (PAGE) performed to determine effects of age (YC vs. OC) and supplementation with Cs-4 (OC vs OS).

RESULTS: We identified 393 out of 1,241 mitochondria-related nuclear transcripts in the muscle and brain that changed in expression with age. Cs-4 opposed the age-related changes in 52 of the genes (P<0.05). In addition, Cs-4 opposed the effects of aging in several gene ontology pathways.

CONCLUSION: We identified mitochondrial-related nuclear genes that consistently change in expression with age. Using this methodology, we found that Cs-4 opposed many of these changes in aging brain and muscle. Ongoing studies are utilizing this technique to investigate the effects of a variety of natural ingredients in brain, muscle, and other tissues.

INTRODUCTION

- Mitochondrial changes occur with aging (function, size, and numbers).
- Skeletal muscle and brain tissue show signs of aging and contain high concentrations of mitochondria.
- Cordyceps sinensis is traditionally believed to have anti-aging activities and to promote longevity:
  - Improvements in endurance, anti-fatigue, maximal oxygen consumption (VO_{2max}), glucose, and lipid metabolism (Dai et al. J Altern Compl Med. 7:231, 2001; Zhao et al. J Altern Compl Med. 8:309, 2002; Xiao et al. Chin J Integrat Med. 10:187, 2004; Li et al. Chin J Clin Pharmacy 16:274, 2007; Li et al. Shanghai J Prevent Med. 20:367, 2008; Wang et al. 2008 Symposium Chin Assoc Med Mycol. pp157–164)
- **The purpose of this study is to examine whether age-related gene expression in mitochondrial-related nuclear genes can be opposed by Cs-4**

冬虫夏草
Cordyceps sinensis

***Cordyceps sinensis* (Berk.) Sacc. 冬虫夏草**
(Collected from Qinghai-Tibetan plateau of China)

↓ Isolation & Purification

Cs-4 (A fungal strain of *Paecilomyces hepiali* Chen et Dai)

↓ Industrial Fermentation

Cs-4

ABSTRACT

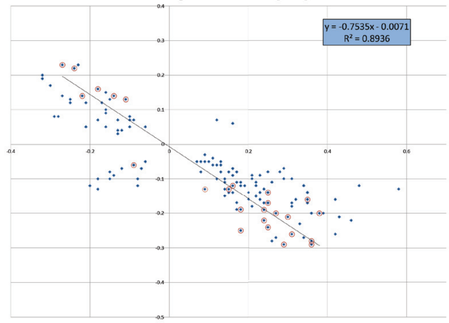
C57Bl mice fed AIN 93m diets (n=5/group)
- Fed for three months
- Skeletal muscle (gastrocnemius) and brain (cerebral cortex) tissue harvested
- Gene Expression (Affymetrix Chips)
- Parametric Analysis of Gene set Enrichment (PAGE)

GROUPS:
1. Young Control (YC); 5 months of age
2. Old Control (OC); 22–25 months of age
3. CS-4 Supplemented (OS) with 30 mg/kg body weight; 22–25 months of age

RESULTS

RESULTS

Muscle Mitochondrial-Related Nuclear Gene Changes from Aging and CS-4



Figures of Gene Expression (fold change) as influenced by aging (x-axis) vs. CS-4 (y-axis). Each point represents one gene. Those that are statistically different (P<0.05) with CS-4 supplementation are circled in red.

PAGE OVERVIEW:

MUSCLE

GO Term	Z-Age*	Z-OS
RNA metabolic process	10.63	-8.35
RNA processing	9.58	-7.04
RNA binding	9.01	-5.92
Nuclear part	7.95	-3.48
Protein transport	7.94	-4.08
Establishment of protein localization	7.93	-4.02
mRNA metabolic process	7.66	-4.24
Protein localization	7.46	-3.47
Intracellular transport	7.23	-3.55
Organelle organization	7.16	-2.36
Macromolecule localization	6.94	-2.94
Chromatin modification	6.76	-4.30
ncRNA metabolic process	6.58	-7.62
Serine hydrolase activity	-4.14	3.80
Structural constituent of eye lens	-4.16	3.24
Serine-type peptidase activity	-4.20	3.90
Wnt receptor signaling pathway	-4.24	4.36
Substrate-specific transporter activity	-4.47	3.26
Cation transmembrane transporter activity	-4.49	2.94
Ion transport	-4.49	3.26
Ear morphogenesis	-4.51	3.79
Cation channel activity	-4.56	2.88
Amine receptor activity	-4.70	4.49
Ear development	-4.71	4.02
Inner ear morphogenesis	-4.73	3.69
Ion tranmembrane transporter activity	-5.03	3.19
Ion channel activity	-5.11	2.91
Keratin filament	-5.12	6.43
Cell-cell signaling	-5.22	4.30

BRAIN

GO Term	Z-Age	Z-OS
Defense response	7.64	4.22
Immune response	7.19	5.02
Antigen processing and presentation of peptides	6.53	2.71
Response to biotic stimulus	6.35	3.38
Response to other organisms	6.25	4.31
Defense response to bacterium	6.12	3.86
Antigen processing and presentation	5.98	2.85
Response to wounding	5.97	3.48
Inflammatory response	5.78	3.45
Positive regulation of immune system process	4.99	3.58
Response to bacterium	4.94	3.89
Regulation of response to stimulus	4.93	4.31
Regulation of immune response	4.89	3.75
Response to external stimulus	4.88	3.79
Cell surface	4.69	4.65
Amino acid ligase activity	-4.21	-2.85
RNA binding	-4.35	-4.81
Translation	-4.41	-3.43
RNA metabolic process	-4.48	-6.77
Chromosome organization	-4.69	-3.75
mRNA processing	-4.72	-3.51
RNA processing	-5.21	-5.54

*Z-scores from PAGE are highlighted in **RED** or **GREEN** to indicate pathways that are up- or down-regulated by age and treatment (P<0.01)
Age refers to YC vs OC; OS refers to OC vs OS

Brain Mitochondrial-Related Nuclear Gene Changes from Aging and CS-4

SUMMARY & CONCLUSION

1. CS-4 opposed age-related changes in gene expression pathways in skeletal muscle tissues; different pattern in brain tissue.
2. Age-related mitochondrial nuclear gene expression changes were opposed in the brain and muscle by CS-4.
3. Of the genes that were changed in expression by CS-4, there were 92% that changed statistically significantly in the direction of a more youthful profile.
4. Using both gene expression and PAGE analysis provides insights into CS-4 effects.
5. Since different information is provided by both gene expression and PAGE analysis, it is recommended to examine results from both methods to determine effects.
6. Ongoing studies are utilizing gene expression profiling in brain, muscle, and other tissues to investigate potential effects of natural ingredients in opposing the effects of aging.