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Original Article

A Comparative Transcriptome and Proteome Analysis in Rat Models Reveals Effects of Aging and Diabetes on Expression of Neuronal Genes☆

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SUMMARY

Background: To understand neuronal molecular changes in senile diabetes we established a rat senile diabetes model and analyzed transcriptome and proteome changes.
Methods: Wistar rats were fed a high sugar, high fat diet for 16 months to induce diabetes. Non-diabetic aged rats and young rats were used as controls. Transcript and protein levels in the liver were then analyzed by microarray and antibody arrays, respectively.
Results: Neuronal genes that were differentially expressed between senile diabetic rats, non-diabetic aged rats, and young rats were distributed across 12 pathways and 23 Gene Ontology (GO) clusters. Among them, 2267 genes were aging-related, 1230 genes were diabetes-associated, and 9 proteins might be associated with neurological disorders.
Conclusion: In this study, we investigated transcriptome and proteome changes in animal models, analyzed the impact of aging and diabetes on neuronal molecules, and confirmed the correlations. Our study provides support for further studies on mechanisms of neuronal diseases.

1. Introduction

Growth in personal income and an increase in average life expectancy have contributed to a dramatic increase in the number of older people with diabetes mellitus (DM) and associated complications, and the mortality rate in this group is much higher than in the general population. Hyperglycemia causes significant damage to the body through the accumulation of metabolic toxins such as superoxides and advanced glycation end products, resulting in ischemia, hypoxia, oxidative stress, and inflammation. It can also cause apoptosis of neurons and gliosis, which may lead to cognitive impairment and even central nervous system disorders such as Alzheimer's disease (AD)[1–3]. Current studies have demonstrated that multiple genes or proteins play important roles in diabetic neuropathy, but there is a lack of data on transcriptome and proteome changes in animal models used in research to investigate the effects of on multiple genetic loci involved in nervous system pathways.

2. Materials and methods

2.1. Animal models

Eighty specific-pathogen-free (SPF) healthy female Wistar rats (6 months of age, weighing 250 ± 20 g) were obtained commercially. Animal experiments were approved by the Ethics

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† The first two authors contributed equally to this study. I certify that all my affiliations with or financial involvement in, within the past 5 years and foreseeable future, any organization or entity with a financial interest in or financial conflict with the participant matter or materials discussed in the manuscript are completely disclosed (e.g., employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, royalties).

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Committee and were performed in accordance with international guidelines[4]. After feeding the rats for 7 days, animals were randomly divided into 3 groups: 10 in the young group, 20 in the aged group, and 50 in the senile diabetes group. Samples from the rats in the young group were immediately prepared as described in the next section. The aged group rats were fed a basic diet (flour 19%, corn starch 23%, sorghum flour 6%, bran 10%, skimmed milk powder 20%, fish powder 10%, starch 7%, glycine 3%, and beer yeast 2%). The senile diabetes group rats were fed a high fat, high sugar diet (sugar 15%, lard 10%, cholesterol 4%, bile salt 0.3%, egg yolk powder 10%, and basic diet 60.7%). The animals were fed continuously for 16 months and the level of blood glucose was monitored. If an animal did not become diabetic, i.e., did not have a blood glucose level $\geq$16.7 mmoL/L characteristic of rats with senile diabetes, a low dose of streptozotocin (STZ) (20 mg/kg) was injected intraperitoneally. Three days later, the blood glucose level was monitored again and animals with a high blood glucose level were selected. The remaining animals received another intraperitoneal injection of STZ and qualified animals were selected after a further 3 days.

2.2. Sample preparation

Rats in each group were weighed and anesthetized with 1% urethane. The abdomen of each rat was then opened, and liver tissues were collected and placed in liquid nitrogen for storage. To obtain RNA for transcriptome analysis, 2 g of liver per animal was obtained from 5 animals chosen at random in each group, and total RNA was extracted from the liver samples using Trizol. For RNA quality control, the value of the RNA integrity number (RIN) measured by the Agilent 2100 Bioanalyzer had to be $\geq$7.0 and the value of 28 S/18 S had to be $\geq$0.7.

2.3. Microarray

Rat RNA and tissue samples were sent to the Shanghai Biotechnology Corporation and analyzed using the Agilent Rat Gene Expression 4 $\times$ 44 K Microarray Kit and the Full Moon Explorer Antibody Array.

2.4. Bioinformatics

SAS software was used for bioinformatics analysis. Differences between means of the 3 groups were analyzed by one-way ANOVA. Differences between pairs of groups were analyzed by Dunnett's test. A P value less than 0.05 was considered statistically significant. Fold change (FC) was calculated, and genes were clustered by Gene Ontology (GO). The effects of aging and diabetes on neuronal genes were analyzed by Pearson's χ^2 test. The *P* values of genes in different signaling pathways were calculated by Fisher's exact test. The genes in the resulted file were then cross-checked and annotated with databases from NCBI Entrez Gene, KEGG, and Biocarta.

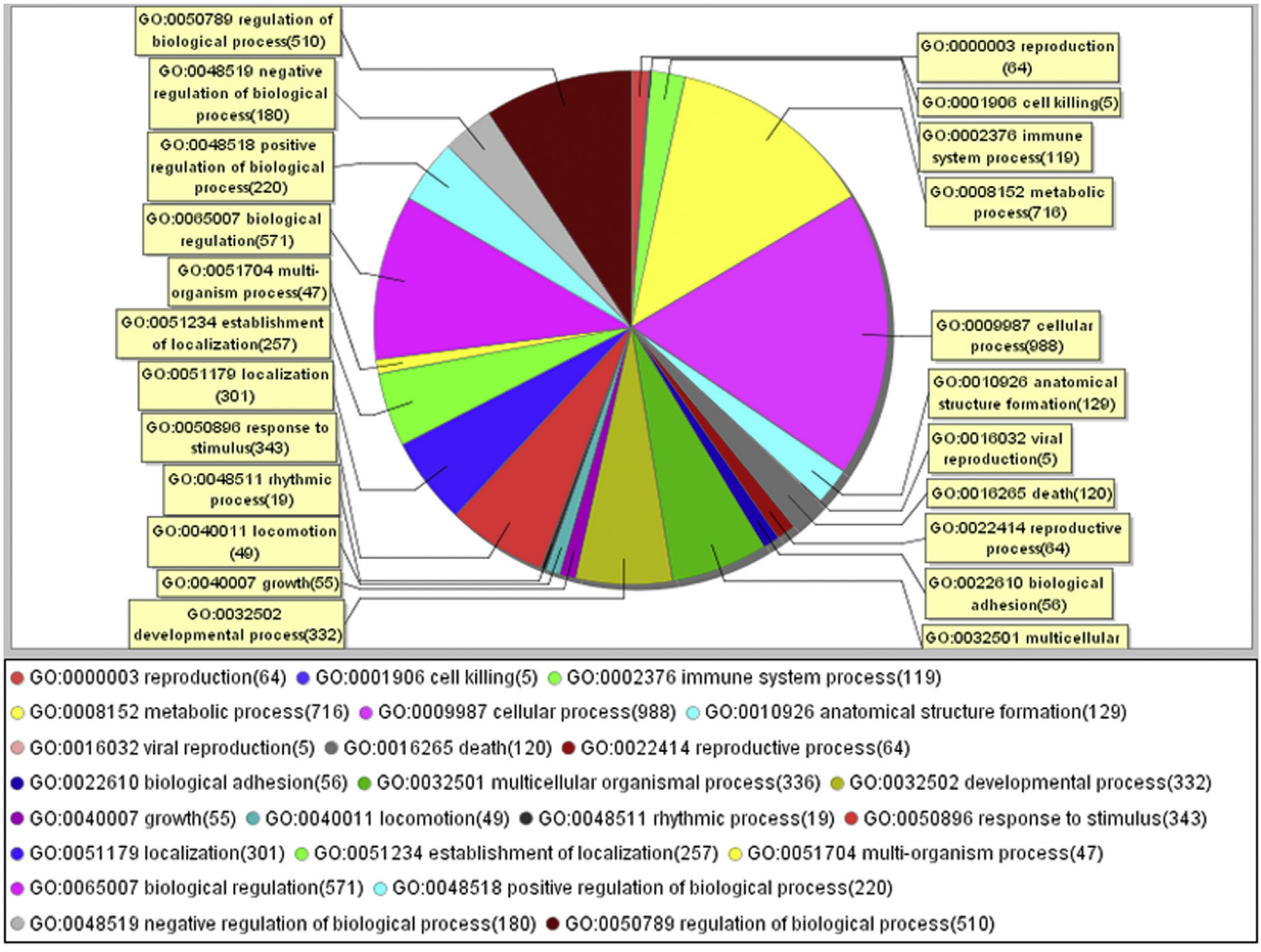

Fig. 1. Pie chart of biological processes of the mRNA.