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Increase in Dual specificity phosphatase 1, TGF-beta stimulated gene 22, domain family protein 3 and Luc7 homolog (*S. cerevisiae*)-like messenger RNA after mechanical asphyxiation in the mouse lung

Hiroyuki Takahashi [a], Kazuya Ikematsu [b,*], Ryouichi Tsuda [b], Ichiro Nakasono [b]

[a] School of Medicine, Nagasaki University, 1-12-4 Sakamoto, Nagasaki City, Nagasaki 852-8523, Japan
[b] Division of Forensic Pathology and Science, Unit of Social Medicine, Course of Medical and Dental Sciences, Graduate School of Biomedical Sciences, Nagasaki University 1-12-4 Sakamoto, Nagasaki City, Nagasaki 852-8523, Japan

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ABSTRACT

We investigated the transcriptome profile of mechanical asphyxia and decapitation at 60 min after death using serial analysis of gene expression. After comparing the results, 11 genes were significantly increased by the mechanical asphyxia treatment in the mouse lung. Of those genes, quantitative real-time PCR revealed that Dual specificity phosphatase 1 (Dusp1), TGF-beta stimulated gene 22, domain family protein 3 (TSC22d3) and Luc7 homolog (*Saccharomyces cerevisiae*)-like (Luc7l) after asphyxia were more significantly increased than those after decapitation. Dusp1 inactivated mitogen activated protein kinase, which functions in cell proliferation. However, the consumption of oxygen had a disadvantageous effect on survival, because tissue or cells were not able to produce energy by internal respiration under the suddenly hypoxic condition following asphyxia. The increased transcripts of Dusp1 following asphyxia suppressed oxygen consumption. TSC22d3 was isolated as a TGF-beta-inducible gene and it is also identified as a glucocorticoid (GC)-induced leucine zipper (GILZ). GC was released from the adrenal gland via HPA axis under the hypoxic condition. Especially in acute suffocation, GC rapidly increased. Therefore, the increase in TSC22d3 may be induced by the increased GC following asphyxia. We were unable to clarify the Luc7l increase, because there are no reports in relation to asphyxia. In addition, GILZ mediates the antiproliferative activity of glucocorticoids. We thought that the increasing TSC22d3 may lead to the suppression of oxygen consumption to avoid wasting energy, as in proliferation, the same as the increase in Dusp1. Our data indicated that the determination of the protein product level in the lung could help in diagnosing asphyxia. In addition, these data may contribute to revealing the pathophysiology of asphyxia and to help diagnose asphyxia, including hanging.

1. Introduction

In forensic practice, the diagnosis of suffocation is mainly based on autopsy observations, such as the objective external and internal findings. The diagnosis is sometimes complex and controversial, because findings at autopsy are sometimes lacking and the absolutely biological and immunological technique to diagnose asphyxia is still undeveloped. For this reason, as in the case of discrimination between sudden infant death syndrome and asphyxia in the prone position in sleep, there are certain cases where the diagnosis of suffocation is very difficult.

To date, the molecular response to suffocation has been little studied in autopsy cases and animal experiments. Our previous study [1] suggested that certain genes may change the expressional transcript level after hanging in mouse brain.

At autopsy, pulmonary oedema is often noted in mechanical asphyxia cases such as strangulation or hanging [2,3]. However, few papers are available on the gene expression concerning asphyxia in the lungs. Maeda et al. [4] evaluated pulmonary surfactant-associated protein A transcripts in asphyxia in forensic autopsy cases. In addition, our study revealed that the immediate early genes (IEGs) transcripts increased after mechanical asphyxiation in the experimental mouse lung [5]. IEGs belong to a class of genes that are rapidly activated, usually in a transient fashion, in response to intracellular signaling cascades. Fos proteins, including c-fos and fos-B, can heterodimerise with Jun family proteins such as c-jun to form the AP-1 transcriptional factor. AP-1 binds to the specific DNA sequence and this leads to the expression of various effector genes [6]. Therefore, it was more likely that the expression of some genes in the lungs changed with mechanical asphyxiation.

Serial Analysis of Gene Expression (SAGE), described by Velculescu et al. in 1995, is now an established method of gene expression profiling [7], and Long SAGE was modified from the original tech-

* Corresponding author. Tel.: +81 95 819 7076; fax: +81 95 819 7078.
E-mail address: k-ikema@nagasaki-u.ac.jp (K. Ikematsu).

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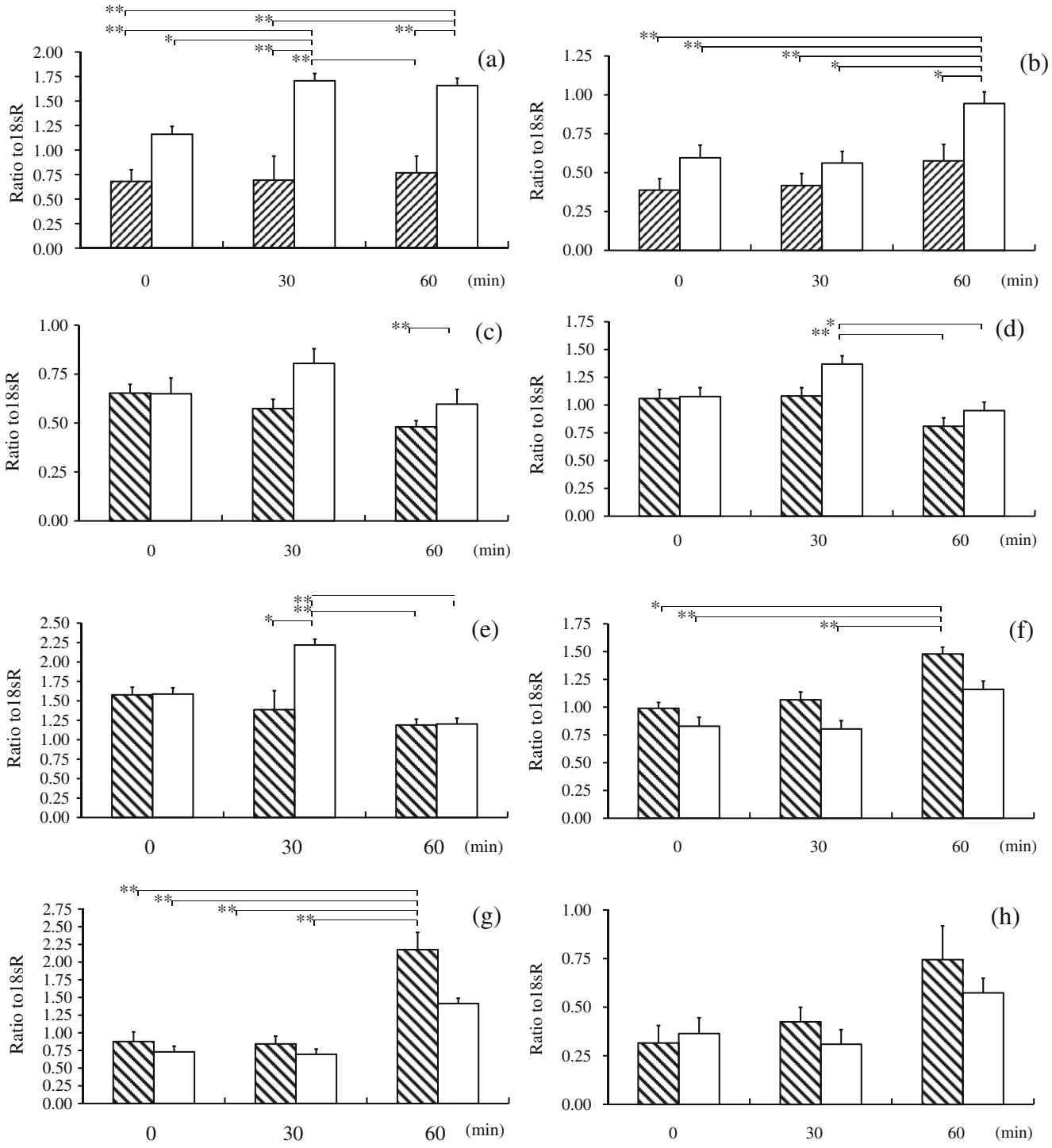

Fig. 1. Time course expression of the transcript levels to ribosomal protein S18 (18sR) from 0 min to 60 min after death with mechanical asphyxia and decapitation treatment. Data are means + SEM. ◎; decapitation and □; mechanical asphyxia. Dusp1 (a), TSC22d3 (b), Luc7l (c), Stab1 (d), Akr1a4 (e), Ppp1cc (f), Rik0710008K08 (g), Atp5d (h), Capn1 (i), Trappc6b (j) and Fryl (k). $^{*}p < 0.05$, $^{**}p < 0.01$. Six mice in each group.

nique to increase efficiency. The Long SAGE method is based on the principle that tags 17–21 nucleotides in length are sufficiently instructive for the identification of each transcript in a collection of cDNA clones. Long SAGE is a technique that allows rapid, detailed analysis of thousands of transcripts simultaneously. This method can be used not only to characterize the quantitative information on an abundance of known transcripts, but also to identify novel expressed genes.

As mentioned above, the kind of expressed gene with mechanical asphyxia is completely unknown. The aim of this study was to elucidate the characteristic gene expression in the lungs influenced by mechanical asphyxiation with Long SAGE.

2. Materials and methods

2.1. Animal treatment and RNA isolation from mouse tissue

Nine-week-old male BALB/c mice were obtained from SLC, Inc (Shizuoka, Japan). These mice were anaesthetized, and compression was applied to the neck by the ligation with a string for