

Gene Expression Analysis of Heat Shock Response Using Fuzzy ART

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1 Introduction

Hyperthermia is an effective modality for cancer therapeutics, particularly in conjunction with radiotherapy, to obtain greater therapeutic benefits [1]. When cells were exposed to heat shock by hyperthermia, heat shock response starts to restore injured molecules and protect cell death, which is including expression of gene such as *hsp70* (heat shock protein 70). Although calefactory appliances have been developed well up to the present, the mechanisms of cell death by hyperthermia have not been elucidated. The effectiveness of hyperthermia will definitely improve if the mechanism of heat shock response is clearly and heat shock response of cells will be deranged. In the present study, to assess the comprehensive genetic response following heat shock, we used microarrays containing 12,814 clones, and clustered expressed genes by Fuzzy ART [2, 4]. We discovered a gene that plays an important role when cells are exposed to heat shock and the effect of inhibitor to the protein encoded by the gene was demonstrated.

2 Methods

2.1 Acquisition of Array Data

For gene expression analysis, HeLa cells seeded in 100mm petri dish were dipped in water bath for 1 h at 44°C(±0.03°C). RNA was isolated from cells 0, 3, 6 and 12 h after heating. The cells just before heat shock treatment were used as control. Labeled probe was hybridized to a Human 1 cDNA microarray (no.G4100A; Agilent Technologies). The gene expression experiment was repeated two times.

2.2 Data Analysis

Signal intensities of Cy3 and Cy5 from the 12,814 spots were quantified and analyzed by GenePix (Axon Instruments, Foster City, CA). Previously fagged spots by GenePix and 60% of spot pixels with intensities more than one standard deviation above the background pixel intensity were excluded. Residual spot signals were normalized so that median of all signal ratio (Cy3/Cy5) would be 1.0. We extracted the genes that showed Cy3/Cy5 signal ratio > 2.0 or 0.5 < at both two times experiment.

3 Results

3.1 Analysis of Gene Expression Profiles from Data Preprocessing

752 genes were up or down-regulated after heat shock. The temporal pattern of expression for 752 genes is more easily recognized through clustering. Using Fuzzy ART, those genes were separated

into 8 clusters (Fig. 1). Up-regulated genes at 0h would play an important role in repair of injured cells. “Cluster 1” and “Cluster 2”, containing 53 genes were selected and “Cluster 2” included HSP70 which was well known as heat shock response gene. Among these genes, we focused on Matrix metalloproteinase 3 (MMP-3), which was included in “Cluster 2” and conducted next experiment.

3.2 Inhibitory Effect Using MMP-3 Inhibitor

MMP-3 inhibitor (no. 444225; CALBIOCHEM) was dissolved in DMSO. The final concentration of MMP-3 Inhibitor in each culture medium was $13\mu\text{M}$. With the same concentration DMSO was used as control. MMP-3 inhibitor was added 1h before heat shock and dishes were dipped in water bath at 44°C for 60, 75 and 90 min to make the surviving curve. Surviving cells were counted by trypan blue dye exclusion method after 3 days. (Fig. 2) Obviously, MMP-3 Inhibitor induced much more cell death than DMSO. This data indicates that MMP-3 may play an important role in restoration of injured cells.

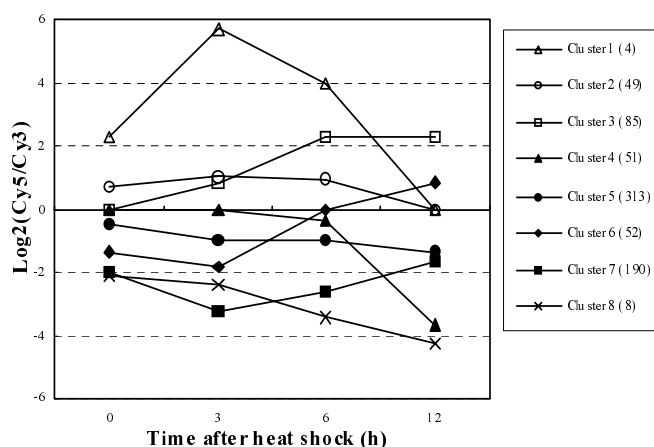


Figure 1: Result of Fuzzy ART clustering. Figures in parenthesis show the number of genes included in each cluster.

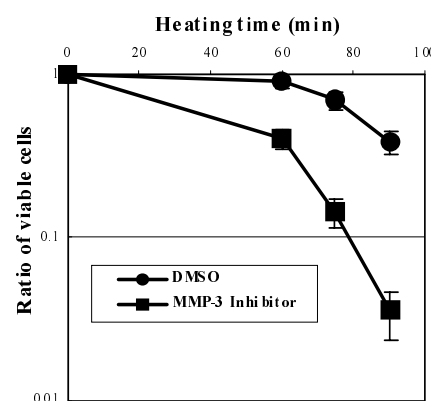


Figure 2: Surviving curve of HeLa cells after heat shock in the response of MMP-3 inhibitor.

4 Discussion

We indicated that MMP-3, which was extracted by our clustering method Fuzzy ART, may play an important role in restoration of injured cells. Until now, it has been known that HSP70 expression inhibitor induces much more cell death by heat shock [3]. We discovered that MMP-3 inhibitor also show the same effect. We selected “Cluster 1” and “Cluster 2” in this study, but the genes that were included in “Cluster 3” were also attractive, because the stress response genes such as GADD45 and HSP40 were included. Now, we are further examining not only the 53 genes of “Cluster 1” and “Cluster 2” also the genes of “Cluster 3”.

References

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- [4] The program is available at <http://www.nubio.nagoya-u.ac.jp/proc/ENGLISH/Fuzzy.html>