Investigation of differentiation of mouse ES cells

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Introduction

Embryonic stem (ES) cells are expected to bring the breakthrough in the therapy for progressive neurodegenerative disorders such as Parkinson's disease, Alzheimer disease and Huntington disease. ES cells are generally called pluripotent stem cells and are unique in that they have the capacity for unlimited self-renewal along with the ability to produce multiple different types of terminally differentiated descendants. The differentiation of ES cells can be controlled in vitro by choosing the configuration of culture conditions. Although the procedure to induce neuronal differentiation is partly revealed, the details of mechanism are unknown. Therefore it remains quite difficult to culture neurons efficiently for therapeutic application.

In this study, x-ray fluorescence (XRF) analysis was applied to investigate the mechanism of differentiation by dissecting the change of distributions and concentration of intracellular trace elements in mouse ES cells [1,2]. It is considered that trace metal elements and metalloproteins are deeply related to the orientation of differentiation as active centers as well as the neural cell death in neurodegenerative disorders.

Experimental Set ups and Sample Preparation

The SR-XRF analyses in this investigation were performed at Photon Factory in beam line 4A. The incident x-ray energy was 14.3 keV and the beam size was approximately 7 x 5 μm^2 . The analyses were carried out in air.

The mouse ES cells (129/Sv) were purchased from Cell & Molecular Technologies, Inc. and the passage number (the age of cell line) was 15 in the beginning of the cell culture. Samples for the elemental analysis were prepared by fixing colonies that had been cultured on Mylar films with 20% formalin solution. Three and two samples are made at the passage number 16 and 17.

Results and Discussions

The elemental distribution were obtained in the three or four areas that contained colonies in each sample. The typical image of Zn of the sample at the passage number 17 is shown in Fig. 1. The scale on right side of the image shows the count of the x-ray intensity. The typical spaectra obtained in the colonies are shown in fig. 2. It is suggested that chlorine and zinc had increased according to the differentiation. The concentration of Zn had increased from 0.07 to 0.09 and those of Cl had decreased from 0.67 to 0.14 in the average of relative amounts to P during the change of the passage number. The difference among concentration of these elements is considered to be deeply related to the biological functions such as proliferation, gene transcription and cell excitability in the process of the differentiation.

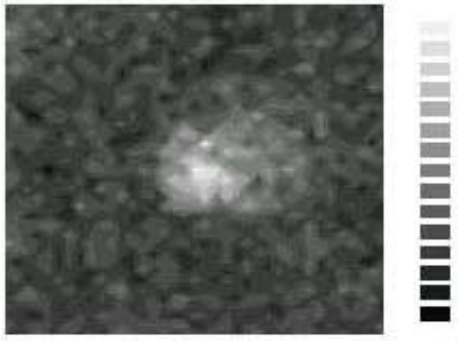

Fig. 1. The typical image of Zn obtained in the mouse ES cell colony at the passage number 17. Measurement area was 144 x 144 μm^2 and the measurement time was 6sec/point.

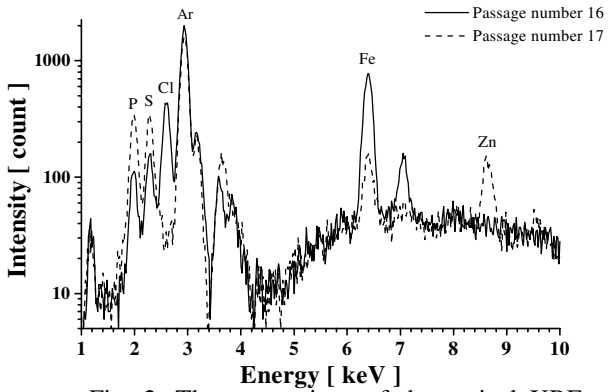

Fig. 2. The comparison of the typical XRF spectra obtained in the mouse ES cell colonies at the passage number 16 (solid line) and 17 (dotted line). The measurement time was 200sec.

References

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