Element Array Analysis by Scanning X-ray Fluorescence Microscopy After *Cis*-diamminedichloro-platinum (II) Treatment

Minerals are essential for many cellular functions, and iron, magnesium, cobalt, and manganese are well characterized with respect to their roles in enzymatic catalysis. Zinc (Zn) is also a requirement for cell growth and survival, and essential for the activity of zinc-metalloenzymes that participate in cell metabolism. To clarify the biological roles of these ions it is important to characterize elements in individual cells. Highly coherent X-rays from third-generation synchrotron sources are opening up various new possibilities of nano-imaging for application in cellular biology. Although recently developed scanning X-ray fluorescence microscopy (SXFM) enable the detection of elements at cellular the level [1,2], higher resolution is still expected for imaging intracellular elements. Yamauchi *et al.* have developed a Kirkpatric-Baez (K-B)-type X-ray focusing system [3] in which, using the 1 km beamline of SPring-8, elliptically figured mirrors focus coherent X-rays to various spot sizes selectively on the demand of spatial resolution, sensitivity and time. As the first application of this method, we visualized platinum (Pt) alongside other cellular elements in mammalian cells after treatment with *cis*-diamminedichloro platinum (II) (CDDP) [4].

CDDP is an effective anti-cancer agent, but tumor cells can become resistant after CDDP-based therapy [5]. We measured intracellular elements by SXFM before and after treatment with CDDP and compared the element profiles of PC-9 cells (PC/SEN) which are originally derived from a lung carcinoma and PC-9 sublines resistant to CDDP (PC/RES). SXFM was set up at the undulator beamline **BL29XU** by combining the K-B-type X-ray focusing system [3], an xy-scanning stage for sample mounting, and an energy-dispersive X-ray detector. Monochromatic X-rays at 15 keV for Pt *L*-line excitation were focused into a 1.5 μm (H) × 0.75μm (W) spot with a measured flux of ~1 $\times 10^{11}$ photons/s. The focused X-rays simultaneously yielded the fluorescence of various chemical species in a small volume of sample cells (Fig. 1(a)). After counts were collected for 4.0-8.5 sec at each pixel of scanning, the counts were normalized with incident beam intensity. Elemental concentration per cell was also calculated from integrated elemental intensity over the entire mapping image.

Twelve hours after treatment with CDDP, the level of Pt increased in PC/SEN cells, whereas a slight increase in Pt level was observed in PC/RES cells (Fig. 1(b)). The signal intensity of Pt in PC/RES cells was 2.6-fold less than that in PC/SEN cells [4]. The

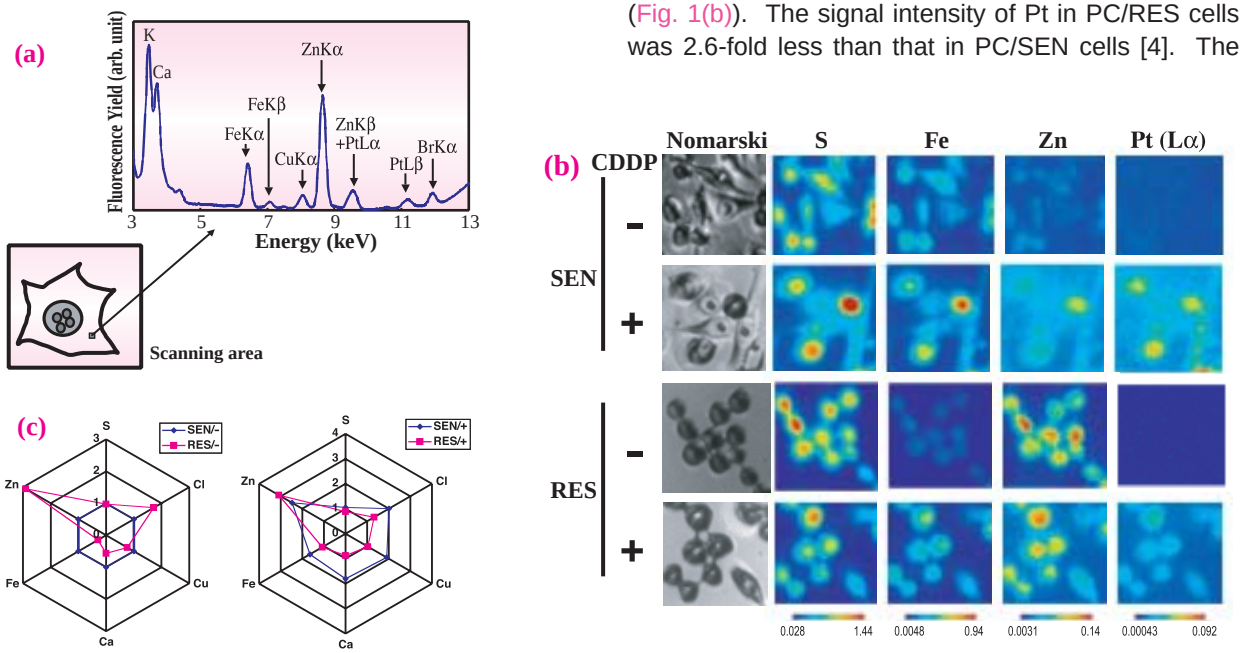

Fig. 1. Element array analysis. **(a)** Scheme of imaging cellular elements by SXFM. **(b)** SXFM analysis of original PC-9 cells (SEN) and subline cells (RES) resistant to CDDP after CDDP treatment (+) and under CDDP free condition (−). Brighter colors indicate a higher signal intensity of each element. **(c)** Element array based on SXFM analysis.

decreased accumulation of CDDP is therefore likely responsible for resistance in PC/RES cells, which is consistent with the finding that the excretion of CDDP may be one of the major factors contributing to CDDP resistance [5]. On the base of the mean signal intensity obtained by SXFM, element array analysis was carried out (Fig. 1(c)). We noted that the Zn content of untreated PC/RES cells was approximately 3-fold that of PC/SEN cells (Fig. 1(c), left panel). When CDDP was used for treatment, the high content of Zn was observed most markedly in both cell lines (Fig. 1(c), right panel).

We next focused on a zinc-related excretion system to clarify the role of Zn in a mechanism underlying resistance to CDDP. We found that the level of intracellular glutathione (GSH) was constitutively high in PC/RES cells (Fig. 2(a)), showing a significant correlation with the levels of Zn detected by both ICP-MS and SXFM (Pearson product-moment correlation coefficient r: 0.794, $P < 0.05$ and r: 0.533, $P < 0.05$, respectively) (Fig. 2(b)). Consistent with these observations, a Zn (II) chelator, N,N,N',N'-tetrakis-(2-pyridylmethl)-ethylenediamine (TPEN), decreased both cellular Zn and GSH level in PC/RES cells [4], making PC/RES cells sensitive to CDDP (Fig. 3).

In the current study, we demonstrated the use of element array analysis by SXFM for examining a mechanism of CDDP resistance. We propose that element array analysis is a powerful tool contributing to a better understanding of cancer biology as well as other fields of medical science. We expect that researchers in various life science fields would join in this promising project.

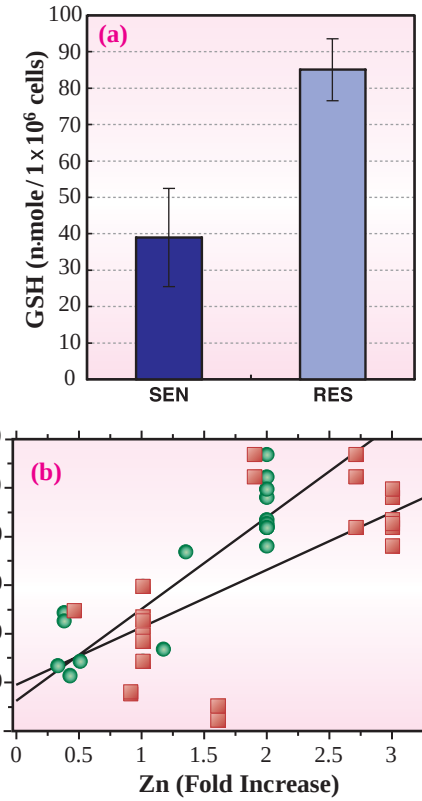

Fig. 2. **(a)** Basal level of intracellular GSH. **(b)** Correlation between Zn and intracellular GSH levels. A scatter diagram for Pearson product-moment correlation coefficient is shown. Zn level, measured by SXFM (red squares, $n = 27$) and ICP-MS (green circles, $n = 29$), was plotted against intracellular GSH level.

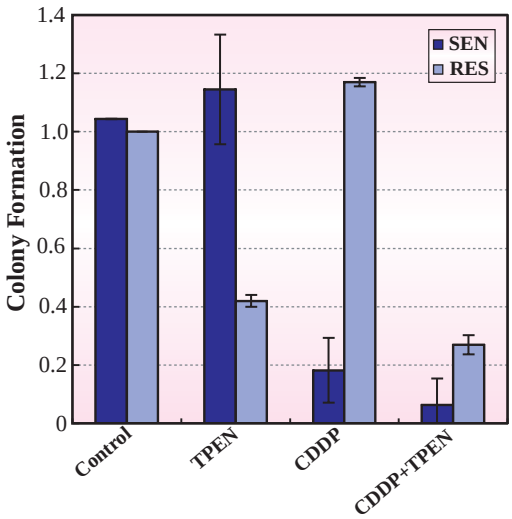

Fig. 3. Colony formation after treatment with CDDP and/or TPEN (Zn (II) chelator), which shows the effectiveness of the treatments to the cell viability. Control shows the result for treatment-free cells for comparison. Cells entitled by TPEN or CDDP + TPEN were pulse-treated for 2 h with TPEN for 5 consecutive days. The ability for colony formation, the aggregation of more than 50 cells arising from a single surviving cell, was examined.

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