V. 1. PIXE Analysis of a Murine Fibrosarcoma Tumor Treated With a Vascular Disrupting Agent AVE8062

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We have studied therapeutic effects of proton therapy combined with a vascular disrupting agent AVE8062[1,2] using a murine fibrosarcoma (NFSa) tomor. Although AVE8062 treatment causes rapid tumor blood flow interruption leading to extensive necrosis in the tumor tissue, the cells at the tumor periphery survive the treatment and cause tumor regrowth. As a result, the tumor receiving AVE8062 administration consists of inner necrotic-cell and outer viable-cell regions. Thus, spatial distributions of elements in the AVE8062-treated tumor are expected to be different from those in an untreated tumor. The aim of this study was to evaluate spatial distributions of principal elements in a solid tumor treated with AVE8062 on the basis of particle-induced x-ray emission (PIXE) analysis using a submillimeter-sized beam (submilli PIXE).

NFSa fibrosarcoma cells[3] (5×10^6/50 mL) were transplanted into hind limbs of C3H/HeSlc male mice aged 11-14 weeks old. When tumor diameters reached around 10 mm, AVE8062 dissolved in 0.9% saline at a concentration of 5 mg/mL was injected i.p. at a single dose of 40 mg/kg to the mice. The mice were sacrificed by cervical dislocation 24 hours after AVE8062 administration, and the tumors were excised and immediately frozen with powdered dry ice. The tumors were stored at -80°C until sample preparation.

In order to evaluate the spatial distributions of principal elements in the tumor tissue treated with AVE8062 using the submilli PIXE analysis, we obtained tissue section samples by cutting the frozen tumors in a cryostat (-20°C) (MICROM HM 500) and mounted them on 4 μm-thick prolene films attached on target holders. We used thick

tissue sections to obtain statistically improved elemental maps. When cutting the frozen tumor, we determined thickness of the tissue section so that the X-ray yield rates of heavier elements such as Fe and Zn was maximized. As a result, the thickness of the tissue section was 250 μm. The tissue sections were dried and stored at -80°C until the PIXE analysis was performed.

A 3-MeV proton beam was provided from a single-ended Dynamitron accelerator. The tissue section samples in dry state were irradiated in air by a beam scanning technique using two dipole magnets. The proton beam was delivered to the target through a 12.5 mm Kapton window. The beam spot size was about 0.5 mm (FWHM) and the beam intensity was 2nA on target. Energies of X-rays from the samples were measured using a Si(Li) detector (10 mm^2 active area) with a 7.5 mm Be window for low energy measurements and a Si(Li) detector (60 mm^2 active area) with a 12.5 mm Be window for high energy measurements. The detectors for low-energy and high-energy X-ray measurements were located at -135° and 135°, respectively, with respect to the beam direction. In addition, the high energy detector was mounted with a sheet of 700 mm thick Mylar film to remove low energy X-rays. Elemental maps of principal elements in the tissue section were obtained from the least-squares fitting analysis of energy spectra using the quantitative PIXE imaging and analysis software GeoPIXE II[4]. Details of the submilli-PIXE system used in this work have been described in ref. 5.

We could evaluate the distributions of phosphorus, sulfur, potassium, calcium, iron and zinc in the tumor samples, as shown in Figs. 1 and 2. When compared to the results of the control tumor, it was found that AVE8062 treatment made significant differences in their spatial distributions between the untreated and treated tumors as is the case in the cross-sectional images. Although the principal elements appear to be distributed uniformly in the tissue section of the control tumor, potassium, calcium and sulfur concentrations can be seen at the periphery of the treated tumor. The analysis is currently in progress.

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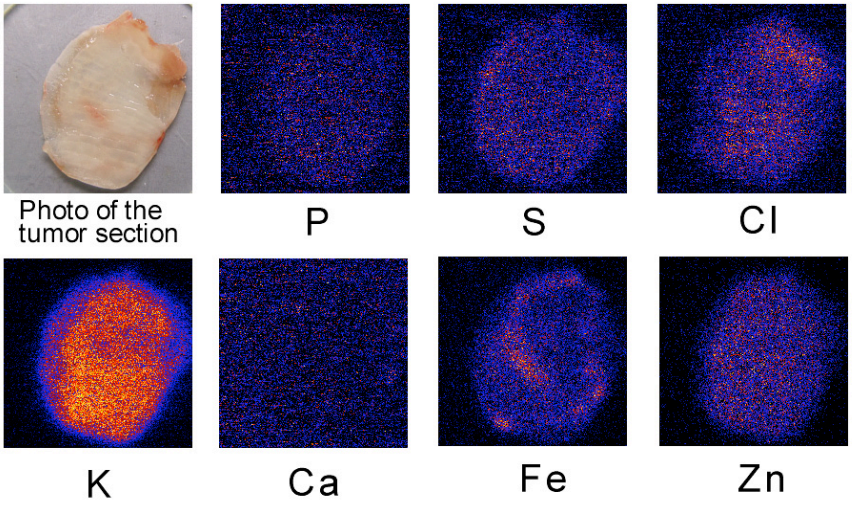

Figure 1. Distributions of principal elements in the tissue section of the untreated NFSa tumor evaluated from in-air submilli-PIXE analysis.

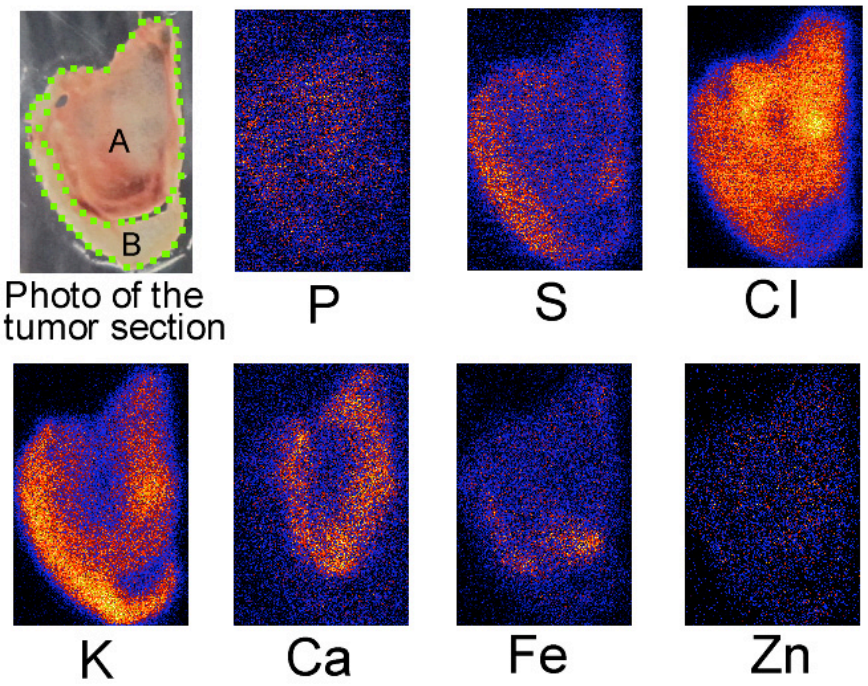

Figure 2. Distributions of principal elements in the tissue section of the AVE8062-treated NFSa tumor evaluated from submilli-PIXE analysis. The photograph of the section also indicates tissue regions (inner region (A) and peripheral region (B)) used as samples for conventional PIXE analysis.