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Introduction

The potential of carbon-11 labeled dopa and tyrosine has been investigated at 1 hr and the incorporation of L-dopa into melanin was assessed in TCA non-extractable fraction ¹⁾. ¹⁸F-Labeled dopa, which was originally developed for imaging of dopamine containing structures in the brain, has been applied for melanoma specific imaging because of a preferably longer half life of ¹⁸F than that of ¹¹C for PET study ²⁾. The selectivity and superiority of 3,4-dihydroxy-2-[¹⁸F]fluoro-L-phenylalanine (2-[¹⁸F]FDOPA) for incorporation into melanin in 6 hr has been shown to be a better tracer for melanoma imaging than the 6-fluoro analog ³⁾. However, both ¹⁸F- and ¹¹C-labeled dopa and tyrosine were accumulated into not only melanomas but non-melanomas by accelerated amino acid transport and/or enhanced amino acid demand in the malignant tumour proliferation. The differentiation of melanogenesis from the cell proliferation seems to be an important approach to exploit the melanoma specific imaging by PET. In this study, we have newly developed a double-tracer microautoradiography method which allows the simultaneous investigation of two distinct metabolic processes in one system of experimental model in vivo. With this technique, we detected the different distribution of 2-[¹⁸F]FDOPA from [6-³H]thymidine ([³H]Thd), a DNA precursor.

Materials and methods

Double-tracer experiment and tissue sampling

Clean gelatinized glass slides were dipped in NTB2 nuclear emulsion (Kodak, USA) at 40°C, dried in clean air and stored with silica-gel in a dark box at 4°C until use. C57BL/6 male mice with subcutaneously transplanted B16F1 tumors were injected with 1 mCi of 2-[¹⁸F]FDOPA and 20 μCi of [³H]Thd intravenously through the tail vein. Both tracers were mixed immediately before injection, and the total volume given per mouse was adjusted to 0.2 ml with saline. The mice were killed 1 hr later, and the tumours were quickly removed and prepared for frozen sectioning as reported previously ⁴⁾. In brief, the trimmed tumour

samples were embedded in the O.C.T. compound (Miles Inc., USA) and frozen by isopentane cooled with liquid nitrogen. The frozen sample block were sectioned on a cryostat at -25°C .

First ARG process for 2-[^{18}F]FDOPA

Under a safety light, the frozen $5\mu\text{m}$ -sections were directly mounted on the slides coated with NTB2 emulsion cooled to -15°C . They were immediately deep-frozen on a flat dry-ice block and placed in exposure boxes cooled with dry-ice. After 4 hr exposure, they were transferred to ethanol with 5 % acetic acid at -70°C and 18.5°C for 1 min each. The acid ethanol was washed out in water for 2 min and the autoradiograms were developed in Konidol-X (Konica, Japan) for 4 min, fixed in Fuji general purpose fixer (Fuji, Japan) for 8 min and washed in gently running water for 30 min at 18.5°C . The slides were, then, dried in clean air below 20°C .

Second ARG process for [^3H]Thd

Three days after the first ARG for the complete decay of ^{18}F , the second ARG was processed using ET2F stripping film (Fuji, Japan). Under the safety light, the ET2F film was stripped from the plate, floated on the 0.05 % potassium bromide in 1 % glycerin solution at 18.5°C and picked up on the slide to cover the specimen with first autoradiogram. The film-coated slides were dried and stored in exposure boxes with silica-gel for 3 weeks at 4°C . After the exposure, the ET2F films were developed for 2 min, fixed for 4 min, washed and dried as described above. The specimens were stained with hematoxylin and eosin. Non-radioactive tumour sections were included in each group on a separate slide as a chemographic control.

The project described in this report, utilized animals maintained in the animal care facility of our institution, was fully accredited by the Laboratory Animal Care Committee of Tohoku University.

Results

Fig. 1 shows a pair of the double-tracer micro-ARG by 2-[^{18}F]FDOPA and [^3H]Thd. The grains by 2-[^{18}F]FDOPA were diffusely distributed over the area but some of them overlapped on melanin. The cells in the S-phase of mitotic cycle were labeled with [^3H]Thd.

The grains seemed to be the highest in the [^3H]Thd unlabeled melanocytes. It means that the highest concentration of 2-[^{18}F]FDOPA was observed in the non-S phase melanocytes. The [^3H]Thd unlabelled non-melanocytes showed the lowest accumulation of 2-[^{18}F]FDOPA. The uptake seems to be induced mainly by amino acid transport. The [^3H]Thd labelled cells, regardless of pigmented or not, have the slightly greater number of 2-[^{18}F]FDOPA grains than the [^3H]Thd unlabelled non-melanocytes. The amino acid demand seems to be enhanced during the cell proliferation.

Discussion

The interaction between the melanogenesis and the proliferation of melanoma cells has remained to be determined for the past 20 years⁵⁾. [³H]Thd is a DNA precursor, and the labeled cells are in the DNA synthesis phase (S-phase) of mitotic cycle. 2-[¹⁸F]FDOPA is an analog of L-DOPA, which is a substrate of melanin synthesis, and is incorporated into melanin^{1,2,3)} as an indicator of the melanogenesis. Double-tracer autoradiography allows the simultaneous investigation of two distinct metabolic processes in one system of experimental model in vivo. With this technique, the localization and differentiation of the melanogenesis from the DNA synthesis could be successfully demonstrated in the cellular level in vivo.

In the clinical application, the 2-[¹⁸F]FDOPA-PET imaging of malignant melanoma may visualize the total of melanogenetic activity and the amino acid demand via amino acid transport¹⁾. Although the distinction of melanogenesis from the basic amino acid demand of the tumour might be difficult in an image at 1 hr after injection, it may be easier with time because the incorporation of 2-[¹⁸F]FDOPA into melanin increased with time³⁾. The evaluation of pre- and post-treatment of the maturation/differentiation induction therapy of melanoma may also be useful because the increase of the active melanogenetic population by the therapy²⁾ is considered to induce higher accumulation of 2-[¹⁸F]FDOPA in the melanoma.

Acknowledgments

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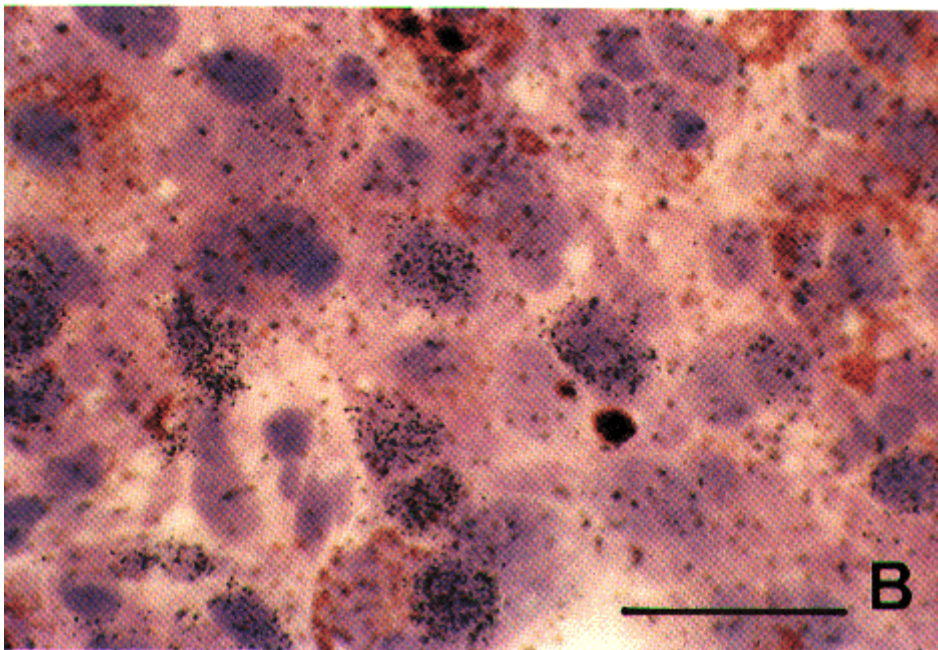
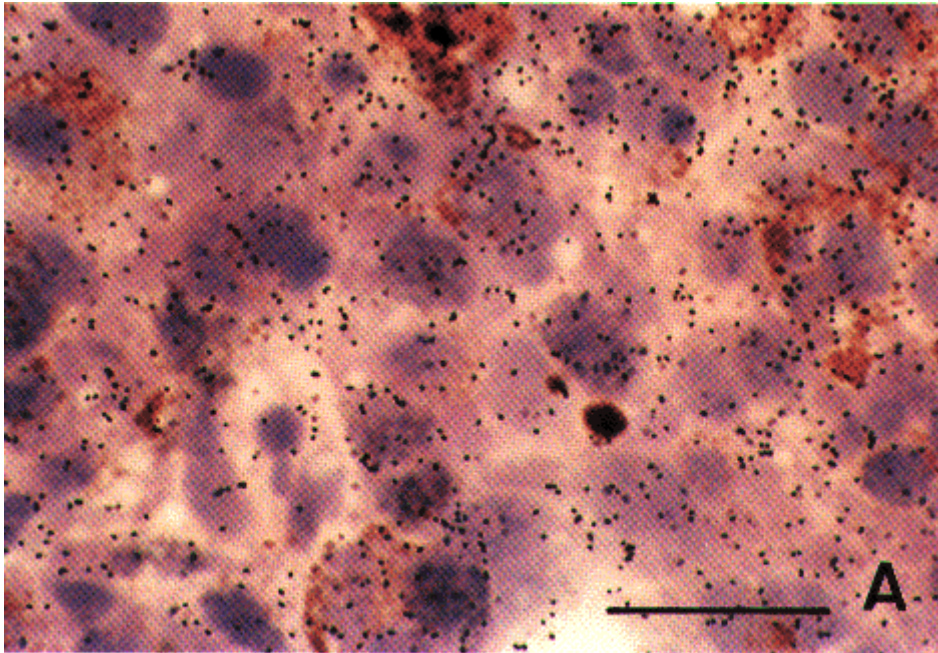


Fig. 1. A pair of the double-tracer micro-ARG by 2-[¹⁸F]FDOPA and [³H]Thd. A: focused on the 2-[¹⁸F]FDOPA microautoradiogram. B: focused on the [³H]Thd microautoradiogram. Brown pigments: melanin. Bar: 30 μ m.