F. Tumor Diagnosis

Study of Subcellular Distribution and Binding Substances of ^{67}Ga, ^{111}In and ^{196}Yb in Tumor Tissue

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Subcellular distribution of ^{67}Ga, ^{111}In and ^{169}Yb was quantitatively determined to evaluate the role of lysosome in accumulation of ^{67}Ga, ^{111}In and ^{169}Yb in malignant tumor tissue and liver. The following animals and transplanted tumors were used: rats implanted with Yoshida sarcoma and hepatoma AH109A; mice implanted with Ehrilich tumor. ^{67}Ga, ^{111}In and ^{169}Yb-citrate were injected to the rats intravenously and to the mice intraperitoneally. Ten minutes to 48 hours after the administration of these radioactive substances, the animals were sacrificed, and the tumor tissues and liver were excised. Subcellular fractionation of tumor tissues and liver were carried out according to the method of Hogeboom and Schneider. ^{67}Ga and ^{111}In and ^{169}Yb of each fraction was counted by a well type scintillation counter, and protein of each fraction was measured according to Lowry's method.

In Yoshida sarcoma and Ehrlich tumor, most of the radioactivity was localized in the supernatant fraction, and small amount of radioactivity was accumulated in the mitochondrial fraction (lysosome contains in this fraction). But in the liver, most of the radioactivity was concentrated in the mitochondial fraction and the radioactivity of this fraction was increased with the passage of time after administration. Twenty-four hours later, about 50% of total radioactivity was accumulated in this fraction. In the case of hepatoma AH109A, radioactivity of mitochondrial fraction was increased with time after administration, and about 30% of total radioactivity was concentrated in this fraction 24 hours after administration.

From these results it is concluded that lysosome does not play an important role in the tumor concentration of ^{69}Ga, ^{111}In and ^{169}Yb and lysosome plays am important role in the liver concentration of ^{67}Ga, ^{111}In and ^{169}Yb. In the case of hepatoma AH109A it is presumed that lysosome plays considerably important role in the tumor concentration of ^{67}Ga, ^{111}In and ^{169}Yb, as hepatoma AH109A remains some nature of liver.

Study of Distribution of ^{67}Ga, ^{111}In and ^{169}Yb in Tumor Tissue by Macroautoradiography and Histological Method

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The localization of ^{67}Ga, ^{111}In and ^{169}Yb in tumor tissues was determined macroautoradiographically. ^{67}Ga-citrate, ^{111}In-citrate and ^{169}Yb-citrate were injected intravenously to the rats subcutaneously transplanted Yoshida sarcoma and were injected intraperitoneally to the mice subcutaneously transplanted Ehrlich tumor. These animals were sacrificed 3, 24 and 48 hours after injection. These tumor tissues were frozen in *n*-hexane (−70°C) cooled with dry ice-acetone. After this, these frozen tumor tissues were cut into serial thin sections (10 μm) in the crystat (−20°C). One of the slice of these sections was then placed on X-ray film and this film was developed after

exposure of several days. On the other hand, next slice of these sections were then stained using the hexatoxylin and eosin. From the observations of these autoradiogram and H·E stained slice, the following results were obtained.

It was concluded that concentration of ^{67}Ga, ^{111}In and ^{169}Yb was predominant in viable tumor tissue rather than in necrotic tumor tissue and concentration of these elements was predominant in connective tissue (which contains inflammatory tissues) rather than in viable tumor tissue, regardless of time after the administration.

Considering the above-described facts, it is presumed that binding substance of these elements is acidic mucopolysaccharide, as there are large amount of acidic mucopolysaccharide in inflammatory tissues, which has many carboxy radical, sulfonic group in its structure.

Biological and Biochemical Studies on the Tumor Affinity of Gallium Element

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^{67}Ga tumor scanning is useful in the diagnosis of cancer localization and rough estimation of therapeutic effectiveness and so on. Our interest was concentrated on the gallium affinity to tumors of different cancer biology of histopathology, negative membrane charge, cell growth and lysosomes. Various experimental rat and murine tumors as well as human malignancies were employed.

1) Histopathological difference of ^{67}Ga uptake: In vivo uptake of ^{67}Ga was greatest in undifferentiated cancer (Sato's lung cancer); adenocarcinomas (the ascitic hepatomas of AH 7974 and AH7974F), the next; melanoma and squamous cell carcinoma (WHT/Ht), and mucin-producing, slow-growing adenozarcinoma (R-1), the least.

2) ^{67}Ga in vitro uptake vs parameters of cancer biology

	Cellular membrane charge	Cellular doubling time	^{67}Ga in vitro uptake
FM3A (murine breast cancer)	μV^{-1} sec^{-1} cm -1.52 ± 0.24	hr 12	$\mu Ci/10^6$ cells 0.26
C6 (human lymphosarcoma)	-0.64 ± 0.10	16.8	0.19
P3H (human Burkitt)	-0.60 ± 0.10	19.2	0.09

3) Extracellular-to-intracellular migration of ^{67}Ga: Fractionation studies at 3 and 48 hours of labeling revealed that ^{67}Ga localizes on the cell membrane during the initial hours and then gradually moves into the cell and localizes intracellularly.

4) Lysosomal fixation of ^{67}Ga: Ultracentrifugation studies and electron microscopic analysis confirmed that ^{67}Ga fixes in the lysosomes.

5) Gallium modification of cancer biology: When it was added to FM3A cell culture, cold, nonradioactive gallium produced (1) a temporary cessation of cell increase, (2) later reduction of cell growth, (3) marked reduction of saturation density, and (4) morphological acquisition of functioning cells such as macrophages, indicating "de-cataplasia" and "re-differentiation".

Gallium adheres to the cell membrane, then enters the cell rather gradually, and fixes specifically in the lysosomes. The element seems to take hold of cancer cells in such a way so as to modify their parameters of cancer biology.