INVESTIGATIVE NUCLEAR MEDICINE

The Relationships Between the Ga-67 Uptake and Nuclear DNA Feulgen Content in Thyroid Tumors: Concise Communication

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It has been reported that Ga-67 uptake by malignant tumors differs somewhat according to the histologic type. Previously, we reported that uptake of Ga-67 is predictably low in well-differentiated adenocarcinoma of the thyroid gland but high in anaplastic carcinoma and malignant lymphoma. We studied the relationship between Ga-67 uptake and nuclear DNA content in four papillary adenocarcinomas, three follicular adenocarcinomas, three anaplastic carcinomas, and five malignant lymphomas of the thyroid gland. In anaplastic carcinoma and malignant lymphoma, the nuclear DNA content and proliferative index were significantly higher than in well-differentiated adenocarcinoma. These results suggest that there is close correlation between Ga-67 uptake and degree of malignancy of thyroid tumor cells.

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Gallium-67 is widely recognized as useful in the detection and location of malignant tumors and inflammatory lesions (*1–10*). Many theories as to the factors responsible for accumulation of Ga-67 in such lesions have been proposed, including increased acidity of tumors, greater metabolic rate and activity, increased membrane permeability, and the greater vascularity of tumor. Nonetheless, the exact mechanism of Ga-67 uptake by the malignant cell is still unknown.

Hayes et al. (*11*) and Ito et al. (*12*) have reported that uptake of Ga-67 in transplanted animal tumors is associated with viable rather than necrotic tissue. In 1981, we reported that uptake of Ga-67 is predictably low in well-differentiated adenocarcinoma of the thyroid gland but high in anaplastic carcinoma and malignant lymphoma (*13*). In general the anaplastic carcinoma and malignant lymphoma grew more rapidly and had a poorer prognosis than well-differentiated adenocarcinoma of the thyroid gland. From these results we suggested that increased Ga-67 uptake in thyroid tumors indicates a more highly malignant tumor. It is therefore of interest to assess the relationship between Ga-67 uptake and the degree of malignancy of thyroid tumors. In the present study we attempted to examine the relationship between Ga-67 uptake and Feulgen nuclear DNA content, which is related to the rate of cellular proliferation in various malignant tumor cells of the thyroid gland such as well-differentiated adenocarcinoma, anaplastic carcinoma, and malignant lymphoma.

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METHODS AND MATERIALS

Gallium-67 was administered intravenously in a dose of 3 mCi (111 MBq), and 48 hr later patients were scanned anteriorly in a supine position with the neck hyperextended. A rectilinear scanner (crystal 7.6 cm; collimator 30 holes, 10-cm focal length) was used, with a 20% window covering the 93-keV peak.

Scans were interpreted as negative if the activity equaled the background level; equivocal if it was slightly greater; and positive if it was definitely greater. This study was carried out on three anaplastic carcinomas, five malignant lymphomas, four papillary adenocarcinomas, and three follicular adenocarcinomas all being proven either by open biopsy or at surgery. All of the

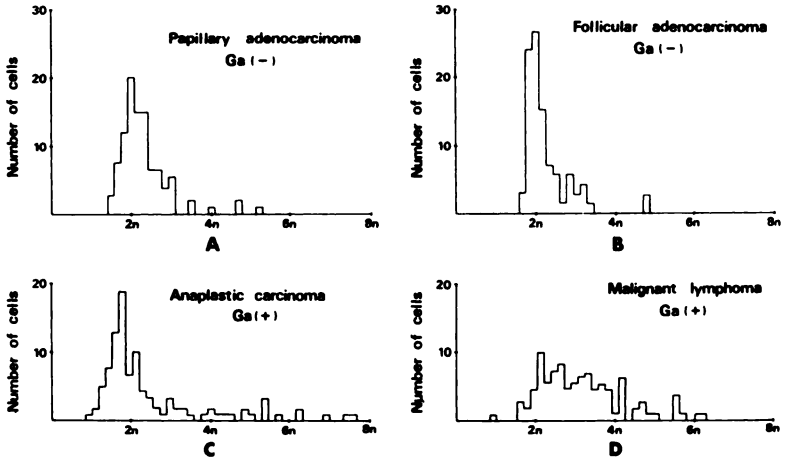

FIG. 1. Histograms of DNA distribution in malignant thyroid tumors. A: papillary adenocarcinoma, B: follicular adenocarcinoma, C: anaplastic carcinoma, D: malignant lymphoma.

malignant lymphomas were considered in the thyroid gland.

The tissue was fixed in a 10% formalin followed by dehydration and paraffin embedding according to an automatic standard procedure. The tissue was examined histologically first by hematoxylin-eosin staining. For the microspectrophotometric examination, 8- to 10-μm sections were cut and Feulgen staining was performed (12 min of hydrolysis in 1 *N* HCl at 60 °C, then Schiff's reagent at pH 1.7 for 45 min). Nuclear Feulgen DNA content was measured with an automatically registering fluorescence cytophotometer.*

For each tumor, 100 nuclei were measured at random. For every value, three measurements were performed inside and three outside the nucleus. The average of these each three measurements was accepted as representative Feulgen DNA content and was expressed in arbitrary units (A.U.). In each measurement, overlapping and cutting artifacts were avoided. We also measured the Feulgen DNA content of 20 lymphocyte nuclei in each tumor in order to determine the diploid value (2n). The results were recorded on histograms.

RESULTS

The representative DNA histograms for papillary and follicular adenocarcinomas are almost diploid with a few tetraploid cells, and the distribution is unimodal (Fig. 1, A and B). However, the representative DNA histogram for anaplastic carcinoma is broadly scattered, with hypotetraploid, tetraploid (4n), and hypertetraploid (6n) values (Fig. 1C). The representative DNA histogram for malignant lymphoma is broad-based as in anaplastic carcinoma, with hypotetraploid, tetraploid, and hypertetraploid values (Fig. 1D).

Representative Ga-67 scintigrams for these cases are shown in Fig. 2. In well-differentiated adenocarcinoma the Ga-67 scintigram was almost negative or equivocal (Fig. 2, A and B). In anaplastic carcinoma and malignant lymphoma, however, the Ga-67 scintigrams were clearly positive (Fig. 2, C and D).

The average Feulgen DNA content of 100 nuclei in each tumor was 220.1 ± 36.3 A.U. (arbitrary units) in four papillary adenocarcinomas, 243.9 ± 23.4 A.U. in three follicular adenocarcinomas, 336.4 ± 21.8 A.U. in three anaplastic carcinomas, and 333.5 ± 52.6 A.U. in five malignant lymphomas (Table 1). In other words, the nuclear Feulgen DNA contents in anaplastic carcinoma and malignant lymphoma were significantly higher than in well-differentiated adenocarcinomas. There was a highly significant difference ($p < 0.005$ by Student's t-test) both between anaplastic carcinoma and the well-differentiated adenocarcinomas, and between malignant lymphoma and the well-differentiated adenocarcinomas.

The proliferative index (PI) from DNA histograms was easily calculated using the following formula (*14*): $PI = (S + G_2 + M)/(G_0G_1 + S + G_2M)$ The average PI in each tumor was 32.9 ± 11.1 in four papillary adenocarcinomas, 29.4 ± 8.4 in three follicular adenocarcinomas, 62.2 ± 8.1 in three anaplastic carcinomas and 66.7 ± 13.8 in five malignant lymphomas (Table 1). The PI of well-differentiated adenocarcinoma was less than

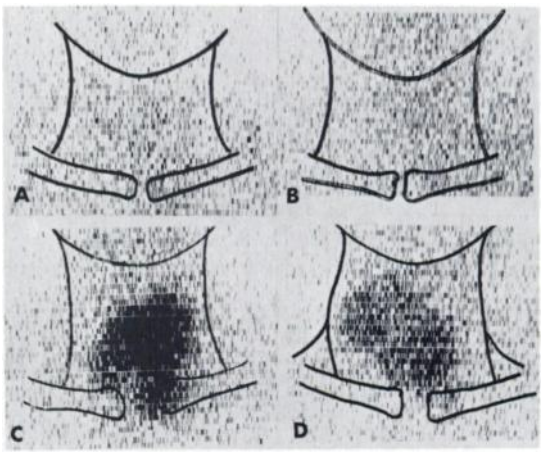

FIG. 2. Ga-67 scans of patients with papillary adenocarcinoma (A), follicular adenocarcinoma (B), anaplastic carcinoma (C), and malignant lymphoma (D).

TABLE 1. RELATIONSHIP BETWEEN PROLIFERATIVE INDEX (PI), NUCLEAR DNA CONTENT, Ga-67 UPTAKE, AND HISTOLOGIC TYPE

Histology	Case no.	PI (%)*	DNA (A.U.)*	Ga-67 scan
Papillary adenocarcinoma	1	44.3	269.0	(−)
(4 cases)	2	17.3	170.6	(−)
	3	27.5	235.2	(−)
	4	42.6	205.7	(±)
		32.9 ± 11.1	220.1 ± 36.3	
Follicular adenocarcinoma	1	23.9	241.1	(−)
(3 cases)	2	41.2	273.9	(±)
	3	23.1	216.8	(−)
		29.4 ± 8.4	243.9 ± 23.4	
Anaplastic carcinoma	1	65.4	363.2	(+)
(3 cases)	2	70.2	309.8	(+)
	3	51.2	336.1	(+)
		62.2 ± 8.1	336.4 ± 21.8	
Malignant lymphoma	1	50.7	380.9	(+)
(5 cases)	2	74.3	372.0	(+)
	3	73.2	248.5	(+)
	4	84.9	370.9	(+)
	5	50.4	295.2	(+)
		66.7 ± 13.8	333.5 ± 52.6	

* Mean ± s.d., in arbitrary units (see text). Significant difference ($P < 0.005$) could be seen both between anaplastic carcinoma and the well-differentiated adenocarcinomas, and between malignant lymphoma and the well-differentiated adenocarcinomas.

that of anaplastic carcinoma and malignant lymphoma.

There was a highly significant difference ($p < 0.005$ by Student's t-test) both between anaplastic carcinoma and the well-differentiated carcinomas, and between malignant lymphoma and the well-differentiated adenocarcinomas.

DISCUSSION AND CONCLUSION

It has been reported that Ga-67 uptake by malignant tumors differs somewhat according to the histologic type, and that accumulation is diminished following therapy (*6,15–17*). We have also previously reported that Ga-67 uptake of malignant tumors of the neck and lung differs according to the histologic type, and we suggested that the greater the Ga-67 accumulation in the tumor, the more effective radiation therapy would be (*16*).

Furthermore, we suggested that the greater the Ga-67 accumulation in lung tumor, the higher the incidence of metastasis would be and the shorter the host's survival. Gallium-67 scintigraphy thus appears to be a valuable tool in indicating prognosis (*17*). In 1981 Bidani et al. also reported that four patients with neuroblastoma, whose primary tumor did not accumulate Ga-67, subsequently exhibited a significantly better survival time compared with the six patients with Ga-67 positive tumors (*18*). In 1979, McCready similarly reported that the level of Ga-67 uptake appears to correlate with the disease process. In a series of patients studied for high, medium, and low Ga-67 uptake, those who had a high uptake had the shortest survival whereas those with the lowest uptake lived considerably longer (*19*). In 1981, we also reported that uptake of Ga-67 is predictably low in well-differentiated adenocarcinoma of the thyroid gland but high in anaplastic carcinoma and malignant lymphoma (*13*). In general, the anaplastic carcinoma and malignant lymphoma grew more rapidly and gave a much poorer prognosis than well-differentiated adenocarcinoma of the thyroid gland. It is of interest that Ga-67 uptake in malignant tumor may be correlated with the prognosis.

In this report, therefore, we measured the content of nuclear DNA in low-grade and high-grade malignant tumor cells of the thyroid and assessed its relationship to the Ga-67 uptake. The Feulgen DNA content and proliferative index (PI) were clearly different in the various malignant thyroid tumors, as seen in Table 1. The nuclear DNA content and proliferative index of anaplastic carcinoma and malignant lymphoma were significantly higher than those of well-differentiated adenocarcinoma. The DNA histograms were unimodal in well-differentiated adenocarcinoma with low-grade malignancy, but were broad unimodal or bimodal in highly malignant anaplastic carcinoma and malignant lymphoma. In general, it is found that higher polyploidy in the tumor correlates with increased malignancy. The

uptake of Ga-67 was significantly lower in papillary adenocarcinoma and follicular adenocarcinoma, but higher in anaplastic carcinoma and malignant lymphoma (Fig. 2).

In 1978, Cuvelier et al. studied the relationship between the DNA content and the degree of malignancy. They found that the distribution of the DNA was bimodal or unimodal in low-grade chondrosarcoma, but in high-grade malignant chondrosarcomas, the DNA histograms were broad unimodal or aneuploid (*20*). Similarly, Atkin et al., studying the eight-year survival rate of 67 patients with breast cancer, reported that the lower DNA content (near-diploid) group showed a significantly better survival rate than that with higher DNA content (triploid, tetraploid) (*21*). Furthermore, Temple et al. also suggested that the proliferative index (PI) is a pertinent prognostic parameter (*14*). Like these results, our data suggest that Ga-67 uptake may provide information as to the malignancy of thyroid tumors.

The mechanism of tumor localization of Ga-67 is not known with certainty, although much information has been derived regarding the biodistribution and subcellular fate of Ga-67 in a variety of tumors and other tissues in experimental animals. It is difficult to explain completely these clinical observations by the known mechanisms of Ga-67 accumulation in tumor cells. However, it is generally accepted that the intracellular concentration of Ga-67 is greater in the cytoplasm than in the nuclei of the tumor cells, and electron-microscopic autoradiography indicates that the intracellular carriers of Ga-67 are lysosomes (*22–24*). From these results, we postulate that Ga-67 in anaplastic carcinoma and malignant lymphoma of thyroid gland is chiefly concentrated intracellularly.

Bichel et al. found that the Ga-67 concentration in malignant cells related to the rate of cellular proliferation (*25*). Hammersley and Taylor (1979), reported that in mouse tumors there is a significant positive correlation between the Ga-67 uptake and the rates of both DNA and protein synthesis, but no similar relationship is seen in a smaller series of rat tumors (*26*). In 1978, Larson et al. reported that intravenously injected Ga-67 is rapidly bound to transferrin, and the transferrin-Ga complex interacts with a specific transferrin receptor on the tumor cell (*27*). On the other hand, in 1981 Hayes et al. reported that the initial in-vivo entry of Ga-67 into tumor tissue involves mainly an unbound or loosely bound form of Ga-67 (*28*).

Recently, Larson (1981) reported that during growth of sarcoma cells in tissue culture, increase in transferrin receptors preceded cell division, and the rate of iron transport into tumor cells was directly proportional to the change in transferrin receptors (*29*). Furthermore, Rudland reported that transferrin and iron are necessary to permit DNA synthesis, and this effect is apparently related to a requirement for iron in essential enzymes (*30*). Gallium is a Group IIIb metal that resembles the ferric ion in atomic configuration, charge, and in the manner in which it combines with various inorganic molecules (*31*). This suggests that a large amount of Ga-67 is transported into the tumor cell during cellular proliferation. This seems to support the hypothesis that Ga-67 deposition may therefore be related to metabolic activity in tumor cells.

We conclude that there is correlation between Ga-67 uptake and nuclear DNA content in malignant tumor cells. Further investigation and a larger study group may verify the trends suggested by this preliminary work.

FOOTNOTE

* Olympus Type MMSP-RF-KS.

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The Society of Nuclear Medicine invites manuscripts for consideration for the Fifth Annual Berson-Yalow Award. Work will be judged on originality and contribution to the fields of basic or clinical radioassay. The manuscript will be presented at the 30th Annual Meeting of the Society of Nuclear Medicine in St. Louis, MO, June 7-10, 1983, and a suitably engraved plaque will be awarded to the authors by the Education and Research Foundation of the Society of Nuclear Medicine.

The manuscript should be approximately ten pages in length (typed, double-spaced). A letter requesting consideration for the award, including the author's full mailing address and telephone number, should accompany the manuscript. Original manuscript and eight copies must be received by January 17, 1983 at the Society of Nuclear Medicine office, 475 Park Avenue South, New York, NY 10016, Attn: Mr. Dennis L. Park.

Deadline for receipt of manuscripts: January 17, 1983.

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Nominations are invited for this award, which commemorates the contributions of Dr. Paul Clarence Aebersold to the applications of nuclear physics to nuclear medicine and radiation biology, and his contributions to the Society of Nuclear Medicine. Dr. Aebersold contributed greatly to the emergence of nuclear medicine as a discipline by his energetic leadership in the provision of cyclotron-generated and reactor-produced radionuclides, and by his numerous publications and lectures.

In giving this award, the Society thus symbolically signifies its appreciation of the warm and vital person who became our first Honorary Member and whose enthusiastic encouragement and support contributed importantly to the formation and success of the Society of Nuclear Medicine.

Nominations should be supported by the curriculum vitae of the nominee and at least two letters supporting the nomination. These letters should describe briefly the contributions in basic science for which the nominee is proposed. The nominee need not be a member of the Society of Nuclear Medicine.

Please submit nominations and supporting documents to:

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Deadline for nominations: December 31, 1982.