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EFFECT OF GROWTH RATE AND SIMIAN ADENOVIRUS-7 TRANSFORMATION ON IN VITRO ^{67}Ga BINDING TO HAMSTER EMBRYO CELLS

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The influence of growth rate and malignant transformation on ^{67}Ga uptake was studied using cultured hamster embryo fibroblasts. Normal nonconfluent cells in log phase of growth bound approximately twice as much isotope as did confluent cells in a plateau phase of growth. In contrast, cells transformed by simian adenovirus-7 (SA-7), which were also undergoing log growth, bound almost no ^{67}Ga. These results suggest that, although the rate of cellular proliferation may influence tissue affinity for ^{67}Ga, other factors must also be considered when studying the effect of malignant transformation.

Cellular characteristics influencing uptake of ^{67}Ga remain poorly understood despite the widespread use of this nuclide as a tumor-scanning agent (*1*). Since the introduction of this technique, it has been recognized that normal tissues such as liver, spleen, and bone, in addition to a broad range of solid tumors, serve as foci for accumulation of this isotope (*2*). Elucidation of the underlying cellular requirements for incorporation of ^{67}Ga may help delineate the full scope of clinical applications of this agent and reveal structural and functional differences between normal and malignant cells.

A link between rapid cell growth and the extent of ^{67}Ga localization has been suspected, but experimental evidence on this relationship is contradictory. Edwards and Hayes (*3*) first noted that this radionuclide accumulates in viable as opposed to necrotic tumor tissue. Bichel and Hansen (*4*) studied ^{67}Ga uptake by a transplantable murine ascites plasmacytoma and by normal murine bone marrow. In both systems rapid cellular proliferation was accompanied by increased binding of the isotope. These authors concluded that gallium affinity is not tumor-specific but is related to and possibly dependent on growth rate in normal and malignant tissue. Noting that stimulation of normal human lymphocytes with phytohemaglutinin is accompanied by a 60% increase in uptake of ^{67}Ga, Merz, et al (*5*) suggested that enhanced radionuclide localization results from entrance of the lymphocyte into cell division after lectin exposure.

Lack of correlation between growth rate and extent of ^{67}Ga accumulation has also been reported. Orii (*6*) studied regenerating normal rat liver and proliferating Yoshida sarcoma cells. Uptake of ^{67}Ga was independent of growth rate in each system. Otten, et al (*7*) reported that while localization of ^{67}Ga accompanies embryogenesis in BALB/c mice, uptake of the isotope does not parallel cell growth. The ability of nondividing human granulocytes to bind ^{67}Ga (*8*) further indicates that rapid growth is not a prerequisite for uptake of the isotope.

The present study of hamster embryo (HE) cells in tissue culture is directed at evaluating the influence of cellular growth rate and malignant transformation in a single cell line. Nonconfluent normal cells proliferate rapidly with a doubling time of about 18 hr; in the confluent state, cell division has markedly slowed as a result of contact inhibition; simian adenovirus-7 (SA-7) transformed cells have lost contact inhibition and proliferate at approximately the same rate as normal nonconfluent HE cells (doubling time about 19 hr). Consequently, the HE cell line provides models of rapidly growing normal cells (nonconfluent normal HE cells), essentially nonproliferating normal cells (confluent normal HE cells), and rapidly growing malignant cells (SA-7 transformed HE cells). The specific objective of this study was to compare ^{67}Ga uptake of these three types of HE cells in order to determine if rapid log phase of

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growth influences cellular incorporation of ^{67}Ga and, if it does, to determine whether rapidly proliferating normal and SA-7 transformed cells have equivalent affinities for this nuclide.

MATERIALS AND METHODS

Primary HE fibroblasts were prepared from 12–14 day embryos of Syrian golden hamsters. These cells were grown in Eagles' medium supplemented with additional amino acids and vitamins plus 10% fetal calf serum (Grand Island Biological Co.). Transformation was effected by the procedure of Casto (*9*) employing a virus concentration of 1.2 plaque-forming units of SA-7 per cell. Subcutaneous injection of 10^6 cells into adult Syrian golden hamsters resulted in palpable tumors in all hamsters within 30 days. A representative sample from each set of identical cultures was trypsinized and counted in a hemocytometer. Variation of cell concentration between duplicate samples was less than 5%.

Carrier-free ^{67}Ga (1.05 μCi) (New England Nuclear) in 1 ml of 0.9% NaCl was added to cells in the original culture medium and incubated for 24 hr. Each culture was then washed three times with fresh culture medium. To each plate was then added 1 ml of 0.25% trypsin and 1 ml of 0.02% EDTA. The cells were transferred to counting tubes and the plates rinsed with an additional 2 ml of culture medium. The radioactivity retained by the cells was then determined in a well scintillation counter.

RESULTS

Table 1 shows the relative growth rate of the nonconfluent, confluent, and SA-7 transformed HE cells. Normal nonconfluent cells have a doubling time of 18.3 hr, similar to that of SA-7 transformed cells (18.9 hr). Confluent normal cells proliferate at a much slower rate as can be seen from the fractional increase in cell number after 24 hr. Since the confluent cells no longer proliferate exponentially, calculation of their doubling time is not meaningful. This slow increase in cell number in confluent monolayers of cultured fibroblasts has consistently been observed in our laboratory.

TABLE 1. RELATIVE GROWTH RATES OF NORMAL AND SA-7 TRANSFORMED HE CELLS

	No. cells per culture (in millions)		
	Zero time	24 hr	Percent increase
Normal confluent	1.04	1.48	47
Normal nonconfluent	0.185	0.46	149
SA-7 transformed	3.0	7.22	141

TABLE 2. BINDING OF ^{67}Ga BY HE CELLS AFTER 24-HR INCUBATION IN TISSUE CULTURE MEDIUM

	μCi/10^6 cells	Experiments (No.)
Normal confluent	1.6 ± 0.09	15
Normal nonconfluent	2.9 ± 0.13	15
SA-7 transformed	0.013 ± 0.002	15

Table 2 shows the extent of binding of ^{67}Ga by each class of cells. The virally transformed cells bound almost undetectable amounts of the isotope. Normal nonconfluent cells in log phase of growth bound more isotope than the confluent population. All differences were found to be significant ($p < 0.001$) by Student's t-test. In each instance sufficient excess ^{67}Ga was present to avoid the possibility that the concentration of the isotope might be rate-limiting. Thus, normal nonconfluent cells took up a total of only 1.3% of the available isotope, normal confluent cells took up 2.3% of the available isotope, and SA-7 transformed cells took up only 0.09% of the total available isotope. Although there were 15 times more SA-7 transformed cells than nonconfluent normal cells in these experiments, the SA-7 cells took up ten times less total isotope.

DISCUSSION

In the present study the difference between confluent and nonconfluent normal HE cells is consistent with the suggestion that the rate of cellular proliferation influences ^{67}Ga uptake (*3*). Unfortunately, these cells do not grow in suspension culture and we cannot exclude the possibility that increased uptake of ^{67}Ga by nonconfluent cells may result in part from greater membrane surface exposed to the isotope, a purely mechanical difference. Nevertheless, the difference in uptake between the two populations is of the same order of magnitude as that noted by Merz, et al (*4*) in their stimulated and nonstimulated lymphocyte populations.

The influence of viral transformation on the affinity of HE cells for ^{67}Ga was most unexpected. The SA-7 transformed cells bound approximately 200 times less isotope than normal nonconfluent HE cells despite the fact that transformed cells have lost contact inhibition and undergo unrestrained rapid growth. Other known effects of tumor virus transformation of cultured mammalian cells include changes in surface glycoproteins (*10*), increased ability to bind lectins (*11*), decreased intercellular adhesiveness (*12*), and increased transport of sugars and amino acids (*13*). These are largely cell surface (membrane) phenomena. Less is known about the

internal environment of transformed cells. The markedly decreased affinity of SA-7-transformed cells for ^{67}Ga could thus result from loss of a membrane receptor, alteration in membrane transport, or loss of an intracellular macromolecular binder. These alternatives are presently under study. To the extent that tumor virus transformation of cultured HE cells is a useful model of in vivo neoplasia (*14*), these results indicate that malignant transformation is not invariably accompanied by increased uptake of ^{67}Ga. Normal cells in log phase of growth because of nonconfluence are not equivalent in gallium affinity to cells of the same origin that are in log growth because of malignant transformation. Uptake of ^{67}Ga by HE cells appears to be determined by some cellular characteristic other than growth rate that is drastically altered as a result of malignant transformation by SA-7.

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