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Effect of LET and microdistribution of radiation on the transformation in vitro and vivo

COMPREHENSIVE REPORT OF ACTIVITIES

Department of Energy

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DISCLAIMER

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1. Objectives and Research Accomplishments

The overall objective of this research program is to learn more about the mechanisms which determine the carcinogenic effects of ionizing radiation, particularly as they relate to high LET radiation exposure. The approach is an in vitro one, involving the study of malignant transformation and the induction of specific gene mutations in mammalian cells. The objectives outlined in the original application focused on the study of the basic characteristics of alpha radiation transformation in vitro. A particular goal was to evaluate the relative effectiveness of focal vs. diffuse irradiation in the induction of transformation. At the time of the last application (3 years ago) more emphasis was placed on the study of mechanisms of radiation carcinogenesis by studying the events evolved in the process of radiation-induced malignant transformation. This included an investigation of the effects of non-carcinogenic secondary factors and promoting agents on radiation transformation in vitro. We also propose at this time to initiate the studies of the effects of Auger electron-emitting radionuclides as another approach to the examination of the role of the distribution of radiant energy within cells and tissues.

During the early stages of the contract period, a $^{238}PuO_2$ external alpha radiation source was constructed and mounted in a specially designed chamber which allowed uniform alpha particle irradiation of monolayer cultures in 100 mm plastic Petri dishes. The source consisted of 8 mCi of $^{238}PuO_2$ electroplated on a 10 cm stainless steel dish. Alpha particles reached the cells through a honeycomb grid colimator. Detailed dosimetric measurements were carried out on the source by use of two different techniques; these both indicated an output of 24 rads/min.

At this time it became evident that the mouse 10T½ cell transformation assay which was in current use in the laboratory would not be appropriate for these studies. As the cells must be irradiated with the medium removed, they were required to remain in the "dry" state for significant periods of time; 10T½ cells appeared usually sensitive to this treatment. We therefore developed the A31-1-1 mouse BALB/3T3 cell assay in our laboratory, and adapted it to the study of alpha particle induced transformation from the $^{238}PuO_2$ irradiator. Radiation times of sufficient duration could be utilized with these cells without adverse effects on viability.

The response of the BALB/3T3 cells to 220 kV x-rays was characterized, and compared with the biological effects of alpha radiation. An RBE of 2.4 was obtained for cell killing of 3T3 cells by alpha radiation. The transformation frequency increased approximately exponentially over the range of 50-250 rads; the maximum RBE for the induction of malignant transformation by alpha radiation in growing cells was approximately 3. The RBE for alpha radiation of non-proliferating cells, however, appeared to be much higher; the transformation frequency of x-irradiated cells held in the stationary phase of growth for 6 to 144 hours after irradiation declined by nearly two orders of magnitude, while no such decline was observed in alpha-irradiated cells. This finding supports the hypothesis that carcinogenic damage induced by high LET radiation in mammalian cells is inefficiently repaired as compared with x-ray damage, and that the carcinogenic effect of exposures to high LET radiation may be simply cumulative in nature. It

further suggests that the effective RBE for alpha radiation in non-proliferating cell populations in vivo may be much higher than one would predict based on measurements in growing cells.

The induction of sister chromatid exchanges (SCE) and gross chromosomal aberrations by alpha radiation was studied in parallel experiments. The maximally effective dose for the induction of SCE by alpha particles was about 5% of that required for x-rays, though the actual frequencies induced were somewhat lower. As in the case of survival and transformation, there was no change in the level of chromosome aberrations in alpha-irradiated cells when they were held in the stationary phase of growth after exposure. Aberrations declined rapidly in x-irradiated cells held under similar conditions.

More recent studies have focused on the biological effects of radionuclides incorporated into cellular DNA, particularly the Auger electron-emitting radionuclide Iodine-125. Decay of this isotope leads to an intense deposition of energy in the DNA immediately surrounding the site of incorporation leading to a localized, high LET-like effect. Iodine-125 was extremely potent in inducing malignant transformation and 6-thioguanine resistant mutations when it was incorporated into cellular DNA as ^{125}I-iodeoxyuridine. Molecular measurements indicated that each disintegration produced at least one DNA double-strand break, and the probability for a mutation arising from a decay anywhere in the HPRT gene was approximately 1.0. The most striking finding was the marked efficiency with which ^{125}IdUrd induced both transformation and mutations at dose levels of only a few total radioactive disintegrations per cell; doses which produced little or no cell killing.

Mechanistic studies of malignant transformation in vitro suggest that this process involves at least two steps. The first is an immediate consequence of the radiation exposure. It is a frequent alteration occurring in a large fraction of the irradiated cells. Thus, though the studies described above with ^{125}IdUrd indicate that it involves DNA damage, it does not appear to reflect a mutation in a specific structural gene or group of genes. The second step, the malignant transformation of one or more or of the progeny of the irradiated cell, is a rare event occuring with the frequency of approximately 10^{-6}. This event occurs randomly during the growth of the irradiated progeny to confluence. In these characteristics the second step resembles a mutation. We suggest the hypothesis that cells respond to non-specific DNA damage by the induction of a process which enhances the spontaneous transformation frequency of their progeny during subsequent growth.

The most notable result in the studies of potential promoting agents is the observation that epidermal growth factor (EGF) can act as a potent promoter of transformation initiated by low doses of radiation. To our knowledge, this result provides the first evidence that a naturally occurring, endogenous polypeptide growth factor can enhance malignant transformation. The endogenous steroid hormone 17-Beta-estradiol induced transformation by itself, but did not appear to act as a true promoting agent.

2. Future objectives

The long term goal of this research program will continue to be as in the past to gain information concerning the mechanisms which determine the carcinogenic effects of ionizing radiation, particularly as they relate to high LET radiation exposure. Increased emphasis will be placed during the next contract period on the biological effects of low dose, low dose-rate exposures. We will continue our studies with incorporated radionuclides in an attempt to learn more about the influence of cellular localization of Auger-emitting radioisotopes and of the spectrum of energy distribution in DNA on their biological effects. A particular focus will be on studies of mutagenesis in human diploid lymphoblasts, as the kinetics of their induction by incorporated radionuclides is similar to that for malignant transformation. The logistics of these mutation experiments are much improved in comparison with transformation experiments, and allow the accumulation of a greater body of data at much reduced time and cost. Important observations with the mutation assay, however, will be tested in the BALB/3T3 transformation system.

A new direction proposed for the next contract period is an examination of the mutagenic and carcinogenic effects in vitro of fast neutron exposure. Fast neutrons are another type of high LET radiation to which human populations may potentially be exposed. Research in this area will focus on the effects of low dose, low dose-rate exposure.

3. Graduate Students and Post-Doctoral Fellows Trained in the Laboratory of Radiobiology, 1977-1983.

A. Doctoral Students Trained (Date of Doctoral Degree)

Frederick B. Oleson (1978)
Glen B. Zamansky (1978)
Gerald L. Chan (1979)
Susan Shami (1980)
Esmail K. Shubber (1981)
Robert J. Zimmerman (1981)
Peter K. LeMotte (1981)
Andrew G. Grosovsky (1982)
Joseph P. Frank (1982)

B. Doctoral Students Currently in Training (Date Doctoral Degree Expected)

Lun H. Lam (1984)
Patricia Hentosh (1984)
David W. Yandell (1985)
Harrison Weed (1986)
Amy Kronenberg (1987)
Diana M. Moeller (1987)

C. Post-Doctoral Research Fellows (Dates of Training)

Steven A. Hawley, (1976-78)
Arnold W. Malcolm, (1976-77)
Mark A. Ritter, (1976-78)
Clifford A. Selsky (1976-78)
Thomas R. Barfknecht (1977-79)
Edward P. Clark (1978-79)
Albert J. Fornace, Jr. (1978-80)
Sheila D. Long (1978-80)
Timothy J. Kinsella (1979-80)
Howard L. Liber (1980-82)
Zeda Rosenberg (1980-82)
Jane Warren (1980-81)
Gary W. West (1980-81)
Robert S. Umans (1980-83)
Nesrine Baturay (1982-)
Andrew G. Grosovsky (1982-)
Harris S. Targovnik (1982-)
Josephine Carew (1983-)
Molly R. Schwenn (1983-)

4. Bibliography of Research supported by this contract.

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2. Kennedy, A.R. and R.R. Weichselbaum. Effects of 17 β-estradiol on radiation transformation in vitro; inhibition of effects by protease inhibitors. Carcinogenesis 2: 67-69, 1981.

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7. LeMotte, P.K. and J.B. Little. A comparison of the lethal effects of intracellular radionuclides in human and rodent cells. Radiation Res. 95: 359-369, 1983.

8. Robertson, J.B., A. Koehler, J. George and J.B. Little. Oncogenic Transformation of mouse BALB/3T3 cells by plutonium-238 alpha particles. Radiation Res. (in press, 1983).

9. Liber, H.L., P.K. LeMotte and J.B. Little. Toxicity and mutagenicity of ^{125}IdUrd and ^{3}H-TdR incorporated in the DNA of human lymphoblast cells. Mutation Res. (in press, 1983).

10. LeMotte, P.K. and Little, J.B. DNA damage induced in human diploid cells by decay of incorporated radionuclides. Cancer Res. (in press, 1983).

11. Little, J.B., P.K. LeMotte, and H.L. Liber. Quantitative studies of cytotoxicity, mutagenesis and oncogenic transformation by radioisotopes incorporated into DNA. Chapter 22, In: Human Carcinogenesis (C.C. Harris and H.N. Autrup, eds.), Academic Press, New York, (in press, 1983).

12. Kennedy, A.R., J. Cairns and J.B. Little. The timing of the steps in transformation of C3H 10T½ cells by x-irradiation. Nature (submitted).

13. Kennedy, A.R. and J.B. Little. Evidence indicating that the second step in x-ray induced transformation in vitro occurs during cellular proliferation. Radiation Res. (Submitted).

5. Other Support for Overall Research and Training Program
Laboratory of Radiobiology

Aside from this contract from the Department of Energy, all of our support comes from the National Institutes of Health, primarily the National Cancer Institute. The various research and training grants from which we derive support are listed below, along with a brief description of their activities.

NCI, Research Grant ROl CA-11751 "Effects of Radiation on Stationary Cells."
P.I. John B. Little
30% of Effort (no salary)
4/1/79 - 3/31/84 (Renewal of application submitted)
Current Annual Budget $148,076

The overall objective of this research program is to gain knowledge about mechanisms which determine the radiosensitivity of mammalian cells, with particular reference to those involved in the response of stationary or very slowly proliferating cell populations to x-irradiation. A particular focus is on molecular and cellular processes associated with the repair of potentially lethal damage in cells x-irradiated during the stationary phase of growth, in order to gain a better understanding of the molecular basis for the cellular effects of radiation.

NCI, Research Grant ROI CA-34037. "Radiation Mutagenesis in Human Cells."
P.I. - John B. Little
10% of effort (no salary)
2/1/83 -1/31/86
Current Annual Budget $56,648

The overall goals of this research project are to examine the mutagenic effects of low doses and low dose rates of ionizing radiation in human cells. The specific aims are to determine the dose response relationship for the induction of mutations by low doses of x-rays, and to examine the effects of dose-rate on the induction of mutations by two techniques. These are by comparing the effects of single and multiple daily exposures to x-rays, and by varying the specific activity and duration of incubation with tritiated water.

NCI, Training Grant T32 CA-09078, "Radiobiology Training Program".
P.I. - John B. Little
10% of effort (no salary)
7/1/80 - 6/30/85
Current Annual Budget $176,900

This training program is run in collaboration with the Departments of Radiation Therapy and Nuclear Medicine at the Harvard Medical School. In offers predoctoral (6) and postdoctoral (4) training in radiobiology. There has been a 50% cut in support for research activities including staff, supplies, publication costs etc., and we have been informed that funds in future years may be limited to student and fellow stipends.

NIEHS, Center Grant P30 ES-00002, "Environmental Health Center".
P.I. - John B. Little
12/1/82 - 11/30/87
Current Annual Budget $787,914

This Center Grant offers core support for senior staff and shared facilities for environmental health related research activities throughout the School of Public Health. These include programs in the Departments of Biostatistics, Epidemiology, Cancer Biology, Toxicology, Environmental Science and Physiology, including programs in Occupational Health and Respiratory Biology. It does not support specific research projects, which must be funded by ROI grants. A significant portion of Dr. Little's salary is actually paid by this Center Grant, although the percent of effort figures appear under the respective NIH ROI grants.

NIH, Program Project POI CA-12662 "Cancer Research Center"
P.I. - Samuel Hellman (Dept. of Radiation Therapy, Harvard Medical School)
5% of effort
3/01/82 - 2/28/87
Current Annual Sub-budget: $25,200.

This sub-budget is designed primarily to support a clinical research fellow from the Harvard Joint Center for Radiation Therapy to study under Dr. Little's overall direction. The project involves the study of the response of human tumor cells to fractionated radiation.

NIH Research Grant PO1 CA-33624 "Epidemiologic and Laboratory Investigation of Cancer-prone Children".
P.I. - Warren W. Nichols, Institute for Medical Research, Camden, NJ
10% of effort
5/01/83 - 4/30/86
Current Harvard subcontract: $43,222.

The objective of this project is to obtain and study skin fibroblasts and lymphocytes from a specifically defined population of children identified as having a probably genetic predisposition to cancer by epidemiologic methods. The patients will be identified by Dr. Louise Strong at the M.D. Anderson Hospital in Houston, and blood and skin biopsy specimens sent to the Institute for Medical Research where detailed cytogenetic studies will be carried out on them. In parallel studies, we will examine the response of these cells to various DNA damaging agents in terms of their clonogenic survival.