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CITY AND STATE NEWARK NJ ZIP CODE 07103

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A-00891

Form Approved Through 5/2004 OMB No. 0925-0001

Department of Health and Human Services
Public Health Services

Grant Progress Report

Review Group	Type	Activity	Grant Number
RAD	5	R01	CA83838-04

Total Project Period From: 07/01/2000 Through: 06/30/2005

Requested Budget Period: From: 07/01/2003 Through: 06/30/2004

1. TITLE OF PROJECT
Effects of nonuniform distributions of radioactivity

2a. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Name and address, street, city, state, zip code)
Howell, Roger W.
Univ. of Med. & Dentistry of NJ
Department of Radiology, MSB F-451
185 S. Orange Ave.
Newark, NJ 07103

3. APPLICANT ORGANIZATION (Name and address, street, city, state, zip code)
UMDNJ - New Jersey Medical School
185 South Orange Avenue
PO Box 1709
Newark, New Jersey 07101-1709

2b. E-MAIL ADDRESS
rhowell@umdnj.edu

4. ENTITY IDENTIFICATION NUMBER
1221775306A2

2c. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
Radiology

2d. MAJOR SUBDIVISION
New Jersey Medical School

5. TITLE AND ADDRESS OF ADMINISTRATIVE OFFICIAL
Robert McLaughlin, University Controller
Liberty Plaza
335 George Street, RM #4116
New Brunswick, NJ 08900-2688
E-MAIL: grants_newark@umdnj.edu

6. HUMAN SUBJECTS
[X] No [ ] Yes
6a. Research Exempt [ ] No [ ] Yes
6b. Human Subjects Assurance No. FWA00000036
If Exempt ("Yes" in 6a): Exemption No.
6c. NIH-Defined Phase III Clinical Trial [ ] No [ ] Yes
If Not Exempt ("No" in 6a): IRB approval date
[ ] Full IRB or [ ] Expedited Review

7. VERTEBRATE ANIMALS
[X] No [ ] Yes
7a. If "Yes," IACUC approval Date
7b. Animal Welfare Assurance No. A3158-01

8. COSTS REQUESTED FOR NEXT BUDGET PERIOD
8a. DIRECT $ 157,500
8b. TOTAL $ 247,275

9. INVENTIONS AND PATENTS
[X] No [ ] Yes If "Yes," [ ] Previously Reported [ ] Not Previously Reported

10. PERFORMANCE SITE(S) *(Organizations and addresses)*
Univ. of Med & Dentistry of NJ
New Jersey Medical School
Department of Radiology
185 S. Orange Ave.
Newark, NJ 07103

11a. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR *(Item 2a)*
TEL 973 972-5067
FAX 973 972-6474

11b. ADMINISTRATIVE OFFICIAL NAME *(Item 5)*
Robert McLaughlin
TEL 732-235-9123
FAX 732-235-9231

11c. NAME AND TITLE OF OFFICIAL SIGNING FOR APPLICANT ORGANIZATION *(Item 14)*
NAME Jane Fant, MS
TITLE Assistant Dean for Research
TEL 973-972-7090 FAX 973-972-3585
E-MAIL njms-research@umdnj.edu

12. Corrections to Page 1 Face Page

13. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.
SIGNATURE OF PI/PD NAMED IN 2a. *(In ink. "Per" signature not acceptable.)* R. Howell
DATE 4/30/03

14. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.
SIGNATURE OF OFFICIAL NAMED IN 11c. *(In ink. "Per" signature not acceptable.)* Sharon McFalone "for" Jane Fant
DATE 4/30/03

A-00892

PROGRESS REPORT SUMMARY

GRANT NUMBER

PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR	PERIOD COVERED BY THIS REPORT FROM	THROUGH
Roger W. Howell, Ph.D.	07/01/2002	06/30/2003

APPLICANT ORGANIZATION

UMDNJ - New Jersey Medical School

TITLE OF PROJECT (Repeat title shown in Item 1 on first page)

Effects of nonuniform distributions of radioactivity

A. Human Subjects (Complete Item 6 on the Face Page)

Involvement of Human Subjects [X] No Change Since Previous Submission [ ] Change

B. Vertebrate Animals (Complete Item 7 on the Face Page)

Use of Vertebrate Animals [X] No Change Since Previous Submission [ ] Change

SEE PHS 2590 INSTRUCTIONS.

WOMEN AND MINORITY INCLUSION: See PHS 398 Instructions. Use Inclusion Enrollment Report Format Page and, if necessary, Targeted/Planned Enrollment Format Page.

Has there been a change in the other support of key personnel since the last reporting period? (If yes, explain the changes, if no state so.) YES.

Dr. Howell has 5% effort on a subcontract from NIH/NCI grant 2R44CA086568-02A1. The PI of the NIH/NCI grant is William Clark, Ph.D. The PI of the subcontract is Lionel Zuckier, MD. The title of the project is Phosphorylatable Monoclonal Antibodies for Tumor Therapy. The term of this subcontract is 04/01/2003 to 03/31/2004. There is no overlap.

Dr. Howell has 5% effort on DOE grant DE-FG02-02ER63447 entitled Cellular responses to low dose/very low dose rate ionizing radiation: The role of oxidative metabolism. The PI of the grant is Edouard I. Azzam. The term of this grant is 1/1/03 to 12/31/06. There is no overlap.

Will there be, in the next budget period, a significant change in the level of effort for key personnel from what was approved for this project? YES.

We anticipate that Bogdan I. Gerashchenko will receive a fellowship award from the New Jersey State Commission on Cancer Research (NJCCR) effective June 1, 2003. While we have received a letter of approval, the support of the grant is dependent on Legislative appropriations. The title of the project is Effects of radiation on unirradiated bystander cells. The period of support is 6/1/2003 to 5/31/2005. The first year of support is $28,250 and the second $29,800. The project examines the bystander effects of external gamma rays using flow cytometry techniques. Because the project focusses on gamma rays and uses newly developed techniques, there is no overlap with the present grant, however, the topics are related and concurrent work will enhance both projects. The NJCCR fellowship does not cover full support at NRSA levels and allows supplementing salary from other sources. Accordingly, Dr. Gerashchenko's effort on this NIH grant will be reduced to 38% effort. The remaining support will come from the NJCCR grant. Accordingly, his efforts will contribute to both projects in a synergistic fashion.

Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total budget. YES.

The surplus remains because it took many months to recruit the third post-doctoral fellow that was planned in our 2002 Progress Report. This is a national problem. However, we are fortunate to have recruited Massimo Pinto, Ph.D. who received his Ph.D. at the Gray Lab in the UK. We anticipate his arrival next month. His presence will greatly contribute to the project as well as using the unobligated balance. Another factor that will consume the unobligated balance is the skyrocketing salaries for post-doctoral fellows. Finally, we will continue to recruit highly skilled post-doctoral fellows and students that can substantially contribute to this project.

Progress Report Summary

a. Specific Aims.

We intend to make a small change to Specific Aim 5. In the original proposal, the comet assay was to be used to study DNA damage caused by nonuniform distributions of radioactivity. Difficulties have been encountered using this assay with the multicellular cluster model. Accordingly, we are initiating studies using induction of micronuclei as an indicator of DNA damage. Like, the comet assay, this assay will enable us to distinguish the labeled and unlabeled cells from one another. Therefore, micronuclei can be monitored separately in the two populations of cells. Preliminary studies look promising.

b. Studies and Results.

Development of flow cytometry as a strategy to study radiation-induced bystander effects. One important aspect that this project examines is the bystander effect induced by nonuniform distributions of radioactivity. Accordingly, we have been actively developing new techniques to study radiation-induced bystander effects. In view of our capacity to distinguish labeled and unlabeled cells, flow cytometry offers an excellent approach to study bystander effects in unlabeled cells. Dr. Gerashchenko has been developing such an approach to study proliferative responses of bystander cells, initially using gamma rays in the development process. His approach has been very successful and has led to a publication in Cytometry (see attached proof). We are presently preparing a second manuscript that addresses the mechanisms involved in the observed bystander response. Dr. Gerashchenko has also initiated studies with tritiated thymidine using the same co-culture system. Plans are in place to eventually use this approach with the multicellular cluster model.

I-131 iododeoxyuridine. As predicted in our 2002 Progress Report, we have completed a series of studies on nonuniform distributions of I-131 iododeoxyuridine. Chinese hamster V79 cells were labeled with I-131 iododeoxyuridine, mixed with unlabeled cells, and multicellular clusters (4,000,000 cells) were formed by gentle centrifugation. Thus, the labeled cells were randomly located in the cluster to achieve a uniform distribution of radioactivity at the macroscopic level, yet nonuniform at the multicellular level. The clusters were assembled as described and then maintained at 10.5°C for 72 h to allow I-131 decays to accumulate. The clusters were then dismantled and the cells were plated for colony formation. When 100% of the cells were labeled, the surviving fraction of cells in the cluster was exponentially dependent on the cluster activity down to 0.1% survival. In contrast, when 10% of the cells were labeled, it was observed that the survival fraction begins to saturate at about 0.5% survival. Absorbed dose estimates reveal that the mean lethal cluster dose is 4.5, 5.7,

and 6.4 Gy for 100%, 10%, and 1% labeling, respectively. These data indicate that when the distribution of I-131 is uniform at the macroscopic level, but nonuniform at the multicellular level, the mean absorbed dose to a tissue element may not be a suitable quantity for use in predicting biological effect. Rather, cellular and multicellular dosimetry approaches may be necessary to predict the biological effects of incorporated I-131. This is unexpected given that the average range of the beta particles is about 30-40 cell diameters. These results could have a substantial impact on absorbed dose calculations and prediction of biological response of incorporated beta particle emitters. A manuscript that describes these findings has been submitted to the Journal of Nuclear Medicine. A copy of the manuscript is attached to this report.

Gene expression and stress responses following incorporation of beta particle emitters. A collaboration was established between our laboratory and that of Valerie Hu at George Washington University. DNA microarray analyses were used to investigate the effect of cell-incorporated S-35 methionine on human colorectal carcinoma cells. The beta-radiation-induced gene expression profile was compared to that induced by external gamma-radiation. Studies showed that S-35 methionine at 100 μCi/ml induced a much more robust transcriptional response than gamma-radiation (2000 cGy) when evaluated 2 hr after the labeling or irradiation period. Furthermore, detailed cellular dosimetry calculations showed that the absorbed dose delivered by S-35 was well below the 2000 cGy delivered by the gamma rays. Interestingly, the cellular response to internal beta-radiation was greater not only with respect to the number of genes induced, but also with respect to the level of gene induction. Not surprisingly, the induced genes overlapped with the set of gamma-responsive genes. However, a distinct beta-gene induction profile that included a large number of cell adhesion proteins was also observed. Taken together, these studies demonstrate that metabolic incorporation of a low energy beta-emitter, such as S-35 methionine, can globally influence a diverse set of cellular activities that can, in turn, affect the outcome of many experiments by altering the cell cycle, metabolic, signaling, or redox status (setpoint) of the cell. Additional studies on the mechanism of beta-induced proliferation arrest and cell death, and on the significance of its differential gene induction/repression profile in comparison to pulsed gamma-irradiation, may lead to new insights into the ways in which ionizing radiation can interact with cells. Accordingly, these results are highly relevant to the effects of nonuniform distributions of radioactivity.

A multi-port low-fluence alpha-particle irradiator. Whenever studies are carried out with incorporated radionuclides, it is always prudent to compare results with external beams of radiation. While we had a gamma ray irradiator on hand, we did not have an alpha particle irradiator. Accordingly, in collaboration with two other members of our Department, Edouard Azzam and Sonia deToledo, we completed a new alpha particle irradiator. This irradiator will be highly useful as we continue our studies with Po-210, an alpha particle emitter. Benchmark studies can be obtained with the external beam an compared to our results for Po-210. A copy of the manuscript describing this irradiator is attached to this report.

c. Significance.

We have made major strides in developing techniques for using flow cytometry to study the effects of nonuniform distributions of radioactivity. This gives us the capacity to study the effects in both labeled and unlabeled cells in a very precise manner. This novel approach will be fully utilized in the upcoming years. Our studies with I-131 have shown that despite conventional wisdom, use of the average absorbed dose to tissue is not appropriate for predicting the biological effects of medium-energy beta emitters. Multicellular and cellular dosimetry approaches must be used for this

purpose. This has the potential to reshape conventional methodology for absorbed dose calculations. Finally, our collaborative work with Valerie Hu has demonstrated that incorporated radionuclides can induce distinctly different cellular responses than external beams of gamma rays. Furthermore, cellular dosimetry calculations indicate that these responses can be induced at low doses compared to external gamma rays. This unanticipated result may have substantial implications for use of incorporated radionuclides in metabolic labeling studies and more broadly impact future development of radiopharmaceuticals.

d. Plans.

Our studies with I-131 took substantially longer than anticipated. Therefore, we will be initiating the Po-210 studies in early May 2003 with the hope of finishing these studies by then end of the summer. While we have made substantial progress in using cell sorting technology to separately seed labeled and unlabeled cells into 6 well culture dishes, work remains to be done to perfect this technology. We will be tackling this problem alongside our Po-210 studies that do not rely on this technology. Work will continue on our web site and development of our dose-response modeling.

e. Publications.

B.I. Gerashchenko and R.W. Howell. Flow cytometry as a strategy to study radiation-induced bystander effects in co-culture systems. Cytometry, In press. Proof attached.

N.F. Marko, P.B. Dieffenbach, G. Yan, S. Ceryak, R.W. Howell, T.A. McCaffrey, and V.W. Hu. Does metabolic radiolabeling stimulate the stress response? Gene expression profiling reveals differential cellular responses to internal versus external gamma radiation. The FASEB Journal. In press. Copy attached to report.

P.V.S.V. Neti and R.W. Howell. When may a nonuniform distribution of I-131 be considered uniform? An experimental basis for multicellular level dosimetry. J. Nucl. Med., submitted. Copy attached to report.

S.M. deToledo, P.V.S.V. Neti, E.I. Azzam, and R.W. Howell. A multi-port low-fluence alpha particle irradiator: Fabrication, testing, and benchmark radiobiological studies. Radiat. Res., submitted. Copy attached to report.

A-00896

Principal Investigator/Program Director (Last, first, middle): Howell, Roger W.

PERSONNEL REPORT

Place this form at the end of the signed original copy of the application. Do not duplicate.

GRANT NUMBER
R01CA83838-04

All Key Personnel for the Current Budget Period

Name	Degree(s)	SSN	Role on Project *(e.g. PI, Res. Assoc.)*	Date of Birth *(MM/DD/YY)*	Annual % Effort
Roger W. Howell	Ph.D.	031-52-2283	PI	03/22/59	25
Dandamudi V. Rao	Ph.D.	029-44-6119	Co-investigator	04/05/44	5
Helene Z. Hill	Ph.D.	011-28-8632	Co-investigator	04/10/29	4
Bogdan I. Gerashchenko	M.D., Ph.D.	135-11-7545	Post-doc	08/13/63	100
Prasad VSV Neti	Ph.D.	143-11-3192	Post-doc	07/30/66	100
Tiffany Cooke			Summer student		17

A-00897