Title: Effects of Ultralow Radiation Levels on Human Cells

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Description:

The major current US report (BEIR VII) on the effects of ionizing radiation, based to a large extent on epidemiological data, has accepted the Linear Non-threshold Hypothesis (LNT) that the dose response for producing detrimental biological effects is a linear function of radiation dose[3]. However, contrary evidence indicates that cells exposed to low levels of radiation are more resistant to subsequent radiation challenge, termed "hormesis." These contradictory views make specific predictions at the cellular and molecular levels that we can test using the unique Homestake DUSEL Facility.

If the LNT hypothesis is correct, human cells grown at ultralow radiation levels should show improved biological properties, e.g., increased growth rates, decreased spontaneous mutation rates, and decreased levels of endogenous DNA damages. The human splenocyte line TK6 is an excellent candidate as an initial line for such studies: it is a suspension line, grows well, has a well-characterized mutation system, and has a readily measured level of endogenous DNA damage clusters.

On the other hand, if hormesis occurs, ambient radiation levels could actually benefit the cell through induction of repair or toleration paths that could correct damages including those present at DNA replication or produced by radiation challenge. Thus cells grown at ultra-low radiation levels might have decreased resistance to radiation or radio-mimetic compounds, and show negative effects on growth, mutation and cluster levels.

We would carry out the experiments in two phases, according to the availability of facilities at the Homestake DUSEL Facility. Phase I would require minimal facilities (a single 37° cell culture incubator, laboratory bench, and small laboratory in which clean conditions could be maintained, a BioSafety cabinet; smaller equipment such as a microscope, etc. could be transported from BNL for the duration of the experiment). Cells would be grown for several generations (generation time ≅ 24 hr) during which their increase in numbers would be determined by counting the cells in a fixed volume. The rates of apoptosis in the cells would determined at the same time by using stains specific for different stages of apoptosis. Gene expression and proteomics of the 4850 and surface cultures could be determined using standard microarrays and protein characterization approaches. Companion cultures would be grown in a surface laboratory, and characterized by the same approaches described. Cells would also be harvested and initial processing completed (to a stage not affected by subsequent environments, e.g., return to ground level and transportation) and returned to BNL for measurement of endogenous cluster levels [1,2,4]

In Phase II, experiments requiring more extensive equipment would be carried out. This includes additional 37° incubators, dedicated microscope and other small equipment, plus, ideally a radiation source such as a ^{60}Co irradiator. These experiments

would include measurement of spontaneous mutation rates, as well as mutation rates, survival and repair of radiation-induced damage. If an irradiator could not be used, we will use a radiomimetic drug (Bleomycin) that induces many of the same DNA damages as does direct ionizing radiation exposure. The mutations rates and repair in companion cultures grown at the surface would also be characterized. Experiments will be conducted in at least two independent repeats, and statistical analysis carried out by Dr. Keith Thompson, BNL Biology Department statistics consultant.

Space requirements, specific/unusual technical issues

A cell culture clean-room laboratory is required, minimum dimensions 10 ft x 12 ft to accommodate incubators, a BioSafety cabinet plus a modest work space containing laboratory benches for minor equipment. Similar facilities at surface are also required for measurements in companion cultures.

Estimate of time required for access

Assuming the availability of facilities at DUSEL, we would anticipate carrying out pilot phase experiments in mid-2007.

Other general requirements

As biologists, we would appreciate and need support from radiation physicists in characterizing types and levels of radiation in the 4850 ft level laboratory (including that from commercially-available biological equipment) and in a surface laboratory.

References

1. **Bennett, P.V., N.S. Cintron, L. Gros, J. Laval and B.M. Sutherland.** 2004. Are endogenous clustered DNA damages induced in human cells? Free Radic Biol Med *37*:488-499.
2. **Bennett, P.V., N.L. Cuomo, S. Paul, S.T. Tafrov and B.M. Sutherland.** 2005. Endogenous DNA damage clusters in human skin, 3-D model, and cultured skin cells. Free Radic Biol Med *39*:832-839.
3. **Research, Board on Radiation Effects Research.** 2005. Health Risks from Exposure to Low Levels of Ionizing Radiation:BEIR VII Phase 2. The National Academy of Sciences Press, Washington DC.
4. **Sutherland, B.M., P.V. Bennett, O. Sidorkina and J. Laval.** 2000. Clustered damages and total lesions induced in DNA by ionizing radiation: oxidized bases and strand breaks. Biochemistry *39*:8026-8031.