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COMPREHENSIVE PROGRESS REPORT

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Project Title: The Oncogenic Action of Ionizing Radiation on Rat Skin

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1.0 Main Research Accomplishments

Progress is described in three areas corresponding to the specific aims of the proposal: 1. carcinogenesis and DNA strand breaks in rat skin following exposure by the neon ions or electrons; 2. oncogene activation in radiation-induced rat skin cancers; 3. DNA strand breaks in the epidermis as a function of radiation penetration.

Approximately 200 rats were exposed to the neon ion beam at the Bevalac in Berkeley, CA. The carcinogenicity of energetic electrons (2.0 Mev) was determined for comparison with the neon ion results. For double skin thickness irradiations electrons there was an unusually large excess of connective tissue tumors, fibromas and sarcomas. Presumably the latter tumors are occurring, because more connective tissue is exposed by deeply penetrating, i.e. energetic, beams.

Based on skin tumor yields obtained for argon ion (LET=125 kev/μ) and electron (LET=0.34 kev/μ) irradiation of rat skin, the parameters C and B in the equations:

$$Y(D) = CLD + BD^2; \qquad (1)$$

$$\text{or} \quad Y(D)/D = CL + BD \qquad (1a)$$

were evaluated. The question was asked whether the tumor yield for some unknown value of LET could be predicted. Neon ions (LET=30 kev/μ) were chosen for this test of the theory, because of the possibility that both linear and dose squared responses might be observed simultaneously. Preliminary results indicate that the neon ion response was correctly predicted and further that the initial values of C and B needed to be

adjusted for two reasons: 1) the argon ion data alone were insufficient to estimate C and B independently, 2) electrons are inherently less effective than either argon or neon ions for inducing tumors by the two track mechanism. The latter observation is the first instance, to this investigators knowledge, indicating an LET dependence of the dose-squared coefficient, B.

Experiments have established that DNA strand breaks per unit dose in the rat epidermis are reduced by about 60% if the radiation penetrates to about 0.2 mm in comparison to a penetration of 1.0 mm. These results imply that about 60% of the DNA strand breaks in the epidermis are produced by indirect radiation action, while the remaining 40% are produced by direct action. The occurrence of the penetration effect in explanted skin means it is not dependent on systemic factors. In the explanted epidermis there was a reduction in the incidence of DNA strand breaks by about 50%. The results of a 'reverse penetration' protocol indicated that DNA strand breaks are produced in even unirradiated epidermis if it is in close proximity to irradiated dermis.

The activation of oncogenes in the radiation-induced rat skin cancers followed a pattern of greater malignancy with more oncogene activation. Four highly malignant cancers exhibited activation of K-ras and c-myc oncogenes, while the remaining 8 cancers exhibited only one or the other of these two oncogenes. Of 5 squamous carcinomas, 4 showed K-ras activation and 1 showed c-myc activation. Several cancers were biopsied, a few-several times, at various stages of development. These studies showed that c-myc amplification was a relatively late event in the progression of radiation-induced squamous and basal cell carcinomas.

Highly invasive clear cell cancers (4/4) exhibited activation of both k-ras and c-myc oncogenes.

An experiment was performed to determine if the DNA in the epidermis could be broken by irradiation of underlying tissue only. The exposure was accomplished by allowing electron radiation to enter a double thickness skin fold from one direction in such a manner that the exit dose was zero. Thus the underlying tissue could be irradiated to any desired dose while the epidermis received no radiation dose. Measurement of the DNA single strand breaks in the epidermis was accomplished by previously described techniques. The unirradiated epidermis exhibited DNA strand breaks with an incidence directly related to the dose to the underlying tissue. Further studies are being performed to determine the quantitative aspects of this effect in relation to the equivalent amount of direct dose.

2.0 Plans for continuation of present objectives and new objectives

The proposed studies involve the induction of skin tumors in rats by ionizing radiation. Our objective is to obtain a better understanding of the relationship between early molecular damage to DNA and the induction of tumors by radiation. The results of these studies will provide a better rationale for extrapolating risks to low doses and between species. A simple model based on 2 events has been developed and found to fit the experimental data relating cancer yield to dose, dose rate and linear energy transfer. The model postulates a link between early molecular changes in the DNA or chromosomes of the target cells and the much later occurrence of cancer. New information on the activation of

k-ras and c-myc oncogenes in radiation-induced skin tumors offers a means for examining the genetic implications of the model.

We propose to continue developing infomation on 3 aspects of radiation carcinogenesis in rat skin, 1. DNA alterations and dose localization in depth, 2. temporal pattern of oncogene activation in irradiated skin and in radiation induced cancers. 3. validation and verification of a simple 2 event (quadratic) model of carcinogenesis. A simple quadratic model with repair has been surprisingly successful in explaining the dependence of cancer incidence on the main radiobiological parameters, such as, dose and LET. The model postulates that 2 primary events or alterations must be produced by the radiation in order to set a cell on the progression to cancer. Probably other events occur as the cells progress but these secondary events are assumed to be independent of the radiation dose and occur only incidentally as a result of the initial alterations. As an example, the primary events could be specific or non-specific chromosome breaks that lead to deletion or rearrangment of genes.

Our initial work has found 2 activated oncogenes in the radiation-induced rat skin cancers, K-ras by mutation and c-myc by amplification. Neither of these alterations seem likely to be initial in the sense as used in the model, and if they are not initial, an important question is when in cancer development do these activations occur. A major effort will be devoted to answering this question by analyzing small and large cancers by Southern blotting for presence of specific amplified oncogenes, by using in situ hybridization to localize activated cells in a cancer or in normal, irradiated tissue, and by analysis of serial

biopsies of developing cancers.

Cancer and DNA strand breaks are less effectively produced in the epidermis if the underlying dermis is not irradiated. An aim of the proposed research is to determine if the mechanism of this curious effect is mediated through movement of a protective compound or a damaging compound. This will be accomplished by freezing the tissue immediately after irradiation and then separating the epidermis mechanically and assaying for DNA strand breaks.

Specific Aims:

1. To test the expectation of the quadratic theory as it applies to carcinogenesis in rat skin by determining the magnitude of the coefficient B for heavy charged particles (neon ions) and electrons, and establishing if the initial lesion relevant to the 2 track term (dose squared) of the neon ion response is subject to biological repair.

2. To extract DNA from rat skin cancers at different stages of development and normal rat skin to test for the presence of an activated or rearranged nyc gene and for the presence of ras and other oncogenes by transfection of NIH/3T3 cell. At times after irradiation when regenerative clones are likely to be present in the epidermis in situ hybridization will be utilized to search for the presence of amplified c-myc oncogene. DNA from neon ion induced cancers will be analyzed for activation of c-myc and K-ras by the same methods applied to make similar measurements in electron induced cancers.

3. Less DNA damage and fewer cancers occur in epidermis that is in proximity to unirradiated versus irradiated underlying dermis. The hypothesis is that part of the DNA damage in the epidermis is caused indirectly either by diffusion of damaging compounds (e.g., long lived free radicals) or protective compounds (e.g., natural sulfhydryl compounds). We intend to determine by quick freezing the tissue and separating epidermis from underlying dermis at various times after irradiation whether protective or damaging compounds are the basis for this important radiobiological response that apparently occurs only in vivo.

3.0 Graduate students trained

1. Steve Hosselet, M.S., NYU, 1989.

2. Tracy Ashkenazi-Kimmel, M.S., NYU, 1988.

4.0 Bibliography, 1986-1989

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5.0 Opinion as to the present state of knowledge in this area and needed future investigations.

Knowledge about the basic mechanism of radiation carcinogenesis is advancing rapidly, but several important and interesting questions need to be answered. Ionizing radiation appears to produce its carcinogenic effect in skin by altering the DNA is 2 distinct ways. The exact nature of these alterations is unknown but could involve DNA strand breaks and rearrangements. Our recent findings of specific oncogene activations in radiation induced skin cancers provide an important approach to identifying the initial molecular lesions responsible for the progression of these cancer cells. By the use of in situ hybridization, we are able to

identify specific cells in the cancers that exhibit high levels of c-myc oncogene. We expect to be able to use this methodology to identify small clones of cells that are expressing a specific oncogene and may, therefore, be more advanced toward cancer.

The significance of this research is that new and more reliable methods to estimate radiation risks at low doses and in a variety of tissues are likely to be developed. This is particularly important at the present time because of concern about the cancer risk associated with low level exposure to radon and radon decay products. Part of the objective of these proposed experiments is to gain a better understanding of the cancer risk associated with exposure to high LET radiation, particularly as a function of the LET.

Needed future investigations include: 1. determine whether the initial lesion associated with the 2-track term of high LET radiation (particularly neon ions) is subject to radiobiological repair. Evidence exists suggesting repair may be ineffective on lesions, whether single track or 2 track, produced by radiation with LET greater than about 30 kev/micron. 2. Identify the stage in cancer progression where activation of K-ras oncogene occurs. 3. establish whether the reduced effectiveness of shallow penetrating radiation is associated with diffusion of a protective compound from unirradiated tissue or diffusion of a damaging compound from irradiated tissue. Once this is established, approaches to exploiting the potential radioprotective properties of this phenomenon could be developed.