Nonlethal Chromosome Rearrangements in the Progeny of Dermal Fibroblasts Exposed *In Vitro* and *In Situ* to Low-Fluence HZE Particles and Gamma Rays.

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We have continued to investigate structural damage to chromosomes in the long-term progeny of single hTERT-immortalized fibroblasts exposed to ^{137}Cs gamma rays and 1 GeV/amu Iron ions. With this approach, it was reasoned that nonlethal damage would be passed on to each cell in a surviving expanded clone (macro colony). Transmission of such damage (e.g., translocations) was monitored by 24-color combinatorial chromosome painting (mFISH) for each of the clones analyzed. We isolated, and characterized 94 clones that survived 1-4 Gy exposures of 662 keV gamma rays, and 84 clones surviving 1.0 Gy of 1 GeV/amu ^{56}Fe ions. The clones have been cryogenically preserved, for further analysis.

A major impetus for this approach stems from the fact that any chromosomal damage found in the progeny of singly irradiated cells should be homogeneous within all cells of a particular clone, and thus would be amenable to submicroscopic analysis by high density CGH DNA microarrays. In particular, we are interested in determining the dose response for submicroscopic interstitial deletions, which, because of their proposed dose and frequency distributions, should allow us to make direct measurements of damage (such as the shape of the dose response) over much lower doses (e.g., $\leq$ 10 cGy). We hope to present preliminary data on this aspect of the project.

In the mean time, cytogenetic results of note to date include the following. The background frequency of translocations in 8 unirradiated clones, for which a total of 1039 cells were scored, showed a sporadic background frequency of 0.004 translocations per cell. For the majority of irradiated clones, all ten karyotypes analyzed were normal by mFISH. However, following 4 Gy of gamma rays, 21/49 clones (43%) were homogeneous for a unique stable rearrangement that was present in 10/10 karyotypes. Most of the recovered rearrangements were simple reciprocal translocations, but of the 21 clones harboring stable rearrangements, 4/21 (19%) contained complex exchanges. Similar results were obtained following exposure to ^{56}Fe particles. Of the 84 clones examined, 30 (36%) contained transmissible exchange aberrations present in all cells sampled; of these 8 (27%) were complex, mostly involving small insertions. Interestingly, the frequency of clones containing (homogeneous) stable translocations was equal to the frequency of translocations per cell in an irradiated nonclonal population that was sampled at the first postirradiation mitosis. This is irrespective of the fact that cells at the first mitosis frequently contained lethal aberrations, such a dicentrics.

Some clones irradiated with either gamma rays or ^{56}Fe ions were found to contain, in addition to stable rearrangements, additional aberrations involving one cell out of the ten that were karyotyped. Often these took the form of truncated chromosomes (not apparently involved in the parental exchange configuration) which were either terminal deletions or “one-way” exchanges. Sometimes these “sporadic” aberrations were reciprocal translocations, again, not associated with the parental exchange. We initially hypothesized that these may represent instances of delayed chromosomal instability. However, after more careful quantitative analysis, we have come to the conclusion that both types of these sporadic events may be explained on the basis of background frequencies of aberrations present in the unirradiated controls. Thus,

while both gamma rays and HZE heavy ions produce transmissible translocations in a large percentage (36% to 43%) of recovered clones, such exposures are apparently incapable of inducing genomic instability in the form of large-scale chromosomal rearrangements in these cells.

For the purpose of comparing the cellular damage response observed *in vitro* with that resulting from the irradiation of intact tissue, we are presently investigating transmissible IR-induced structural changes to chromosomes in a 3D artificial skin construct (FT200 MatTek Corp.). The constructs mimic the principle biological properties of human skin, consisting of basal, spinous, granular, and cornified epidermal layers. The cells in this construct are both metabolically and mitotically active, and express virtually all markers of differentiation specific to epidermis. The dermal compartment, which is separated from the epidermis by a well-developed basement membrane, is composed of a collagen matrix containing viable dermal fibroblasts. It is the dermal fibroblasts that constitute the initial focus of our investigation. So far, we have developed a method of physically separating the fibroblast-containing dermal layer from the epidermal layer, and have successfully produced single-cell suspensions of the fibroblasts contained within. Results regarding basic radiobiological properties of dermal fibroblasts derived from this artificial skin model will be presented that compare *in vitro* versus *in situ* exposure.