Chromosome Rearrangements in the Clonal Descendants of hTERT-Immortalized Human Cells Exposed to Gamma Rays

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Chromosome aberrations that are induced in response to clastogenic agents are typically studied at the first mitosis following exposure. Until the advent of fluorescence *in situ* hybridization (FISH), such analysis was largely confined to asymmetrical exchange products that are considered nontransmissible, such as dicentrics. This is in contrast to symmetrical exchanges, such as reciprocal translocations, which are presumably nonlethal, and therefore transmissible. For the purposes of future molecular analyses, our long-term objective is to isolate a series of independent cell clones, each homogeneous for a particular radiation-induced stable aberration. In the mean time, the isolation and characterization of such clones, by itself, provides some insight into the process of transmissibility and genomic stability.

Single-cell suspensions of human fibroblast cultures previously immortalized by stable transfection of the catalytic subunit of telomerase (hTERT) were irradiated with 4 Gy of 137Cs gamma rays, before being plated at low density for clonal growth. Surviving clones were randomly isolated and grown up for cytogenetic analysis using 24-color multiplex FISH (mFISH). Ten cells were karyotyped from each clone.

To date we have characterized 49 independent radiation-exposed clones, and an approximately equal number of clones not exposed to radiation. The background frequency of translocations in 7 unirradiated hTERT control clones— for which more extensive scoring was done— was remarkably low; less than 1%, a value comparable to that found *in vivo* for mitogen-stimulated human peripheral lymphocytes. For many of the irradiated clones, all ten karyotypes were normal by mFISH. However, 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. Somewhat surprisingly, however, of the 21 clones that were homogeneous for stable rearrangements, 4/21 (19%) harbored complex exchanges, one of which involved four breakpoints and three different chromosomes.

No evidence of radiation-induced chromosomal instability in the form of jumping translocations was observed. However we did find a tendency for cells within irradiated clones to occasionally exhibit chromatid-type breaks and gaps, which were not found in unirradiated control clones, suggesting some sort of latent expression of radiation damage.

We also examined the frequency of symmetrical (ostensibly nonlethal) translocations in a population of *uncloned* cells at the their first postirradiation mitosis. Interestingly, this frequency was remarkably similar to the frequency of viable clones containing these transmissible aberration types. This is irrespective of the fact that the translocation-bearing cells at the first mitoses frequently also contained dicentrics (and other asymmetrical products) that almost certainly would be lethal to the cell. In the near future we plan to analyze clones surviving exposure to 1 GeV/nucleon iron ions.

The results underscore the significance of both simple and complex aberrations to the long-term survival of human cells exposed to ionizing radiation.