Most human cancers appear to be clonal in origin; that is, they are derived from a single cell that has apparently undergone the process of malignant transformation in vivo. Whether the progeny of this cell will eventually give rise to an invasive, malignant tumor depends upon a number of host and tissue factors. The malignant transformation of cells in vitro is also a complex, multi-step process by which normal cells acquire the various phenotypic characteristics of cancer cells. Three major steps appear to be involved: the development of morphologic transformation, immortality, and tumorigenicity. Although rodent cells will readily undergo immortalization in vitro either spontaneously or in response to treatment with chemical or physical carcinogens, immortalization appears to be the rate-limiting step in the transformation of human diploid cells (1).

In contrast to cells derived from tumors, normal human diploid fibroblasts have a limited proliferative capacity in vitro. After subcultivation for several months, the cells gradually assume a senescent morphology and proliferation ceases after about 50 mean population doublings. Although treatment of these cells with physical or chemical carcinogens can result in phenotypic changes associated with morphologic transformation, such as conversion to anchorage-independent growth, this phenomenon is rarely
associated with the development of immortalized cells (2,3). The genetic basis for escape from the commitment to senescence in human cells has not been examined systematically. Although numerous attempts to achieve complete transformation of human fibroblasts by radiation or chemical carcinogens have generally proven unsuccessful, immortalization can be achieved in cells transfected with certain viral sequences.

In this report, we describe two approaches to gaining a better understanding of factors involved in the immortalization of human diploid fibroblasts. In the first, cells were treated with single or multiple doses of x-rays and followed throughout their lifespan in vitro. Our aim was to establish a technique whereby diploid cells could be systematically transformed to immortality by x-rays, and to correlate this process with the development of specific karyotypic changes. In the second approach, cells were transfected with SV40 early region containing the large T-antigen. The appearance of changes in cell growth, chromosomal abnormalities, and frequency of spontaneous mutations were correlated with the emergence of immortalized cells in order to gain information concerning the genetic basis for this phenomenon. Finally, we examined the influence of transfection with SV40-T and immortalization on cellular radiosensitivity.

**MATERIALS AND METHODS**

Normal human diploid fibroblast cell strains were obtained from the Human Genetic Mutant Cell Repository, Camden, NJ. They were grown and maintained by standard techniques in Eagle's Minimal Essential Medium supplemented with 10% fetal bovine serum as described elsewhere (4). Early passage cultures were x-irradiated or transfected with SV40-T, then continuously passaged by subcultivation at a 1:4 dilution at approximately weekly intervals throughout their lifespan in vitro. When the cells reached senescence or "crisis", they were maintained in the incubator for several months with regular medium