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Letter to the Editor

TnT: T antigen and telomerase, an explosive route to cancer *

Sir,

Early this century when Hanahan and Weinberg enumerated the six phenotypes that they defined as the 'Hallmarks of Cancer', they segregated genomic instability as 'an enabling characteristic' rather than defining it as a hallmark. Reading more carefully, we learn that they have set genomic instability aside as a mechanism 'that enables evolving populations of premalignant cells to reach these six biological endpoints'. The notion was not new however, the hypothesis having been elegantly summarized by Peter Nowell in 1976.

Human fibroblasts do not become spontaneously immortal or tumorigenic when they are cultured *in vitro*. In the 1980s, a number of reports began to emerge that suggested that human cells could be transformed, albeit rarely, by gamma rays, carcinogens or certain viruses. The common denominator of these studies was repetitive treatments, successive for radiation and carcinogens, continuous for viruses. We began working on a model using the SV40 virus. The large T antigen was identified as the suspect carcinogen and constructs were made that allowed us to test the hypothesis that T antigen was necessary and sufficient for transformation to tumorigenicity of otherwise refractive human fibroblasts. We suspected that the protein performed this role by causing genome instability.

The results were dramatic, when a large T antigen gene was transfected into human diploid fibroblasts virtually every cell expressing the protein had some observable chromosome damage when metaphase spreads were observed using crude Giemsa staining followed by aberration scoring. Few transformed traits analogous to the hallmarks of cancer were observed. Some 30 cell populations were expanded to greater than 10 million cells per culture each and then grown, past the normal senescence stage to an apoptotic crisis stage. All but 10 cells of roughly 300 million died. These 10 cells formed expandable immortal cell lines that had become telomerase positive. These newly immortal lineages, continued to evolve in culture and to acquire the 'hallmarks' at different rates with no further treatment other than cell growth and T antigen expression. Several lines became tumorigenic, after continued cell doublings. We, therefore, hypothesized that T antigen provides the 'enabling characteristic' of genomic instability and telomerase provides an infinite number of cell divisions with which to select the various cancer phenotypes within a single lineage of cells.

In order to determine how T antigen acted as a genomic destabilizer, we made a series of mutations in the gene and repeated our studies looking at chromosome aberrations. Wild type T antigen abrogates p53 DNA damage checkpoints. Mutations in T antigen that decreased T:p53 interactions showed a clear decrease in resultant chromosome aberrations indicating how valuable checkpoint control is to dividing cells. The overall transformation model is in complete agreement with an enabling role for genomic instability, in the process of carcinogenesis.

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