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The role of telomere dysfunction in driving genomic instability

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Abstract. Radiation-induced genomic instability as an initiating event in radiation carcinogenesis is an attractive hypothesis that remains to be rigorously tested. Our studies have focused on the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) in this process. We have shown that effective telomeric end capping of mammalian chromosomes requires proteins more commonly associated with double-strand break (DSB) repair. Impaired end capping in DNA-PKcs-deficient genetic backgrounds not only allows dysfunctional telomeres to fuse to each other (telomere–telomere fusion), but also to broken chromosome ends created by radiation-induced DSBs (telomere–DSB fusion). Interstitial telomere sequences have been shown to be an inherent source of instability. It is also noteworthy that telomere–DSB fusions remove just one of the two ends created by a DSB, thereby rendering the remaining broken end capable of driving on-going chromosomal instability. We have used mouse Spectral Karyotyping and telomere chromosome orientation fluorescence in situ hybridization (CO-FISH) to reveal a clonal translocation possessing a telomere–DSB signal at the translocation breakpoint. Another approach has been to analyze radiation-altered cells using BAC-CGH array technology. DNA-PKcs deficient BALB/c mouse mammary vs. mammary tumor DNA revealed an amplification on chromosome 11 that has synteny to human 17q 25.1, a region frequently amplified in breast carcinoma. These studies continue to support our hypothesis that impaired telomeric function is a significant source of radiation-induced chromosomal instability that has the potential of contributing to the cancer-prone phenotype associated with even partial DSB repair deficiency.

Keywords: Telomere; DNA-repair; Radiation carcinogenesis

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1. Introduction

The mechanistic role of radiation-induced genomic instability in radiation carcinogenesis is an attractive hypothesis that remains to be rigorously tested. There are few *in vivo* studies on which to base judgments, but work in our laboratory with mouse models of radiogenic mammary neoplasia provided the first indications that certain inductive forms of genetically predisposed genomic instability may contribute to tumor development [1]. The central goal of our current research is to more firmly establish the mechanistic basis of this radiation-associated genomic instability and, from this, to assess whether such induced instability might play a major role in tumorigenesis. In the case of mouse mammary tumors, susceptibility to induced instability is expressed as an autosomal recessive trait in mammary epithelial cells and is manifest largely as excess chromatid damage. Recently published studies associate this form of instability with DNA repair deficiency, polymorphic variation in the gene encoding DNA-PKcs, and mammary associated susceptibility [2]. The underlying hypothesis being tested in our studies is that tumor-associated genomic instability is preferentially expressed in certain recombinogenic genomic domains and that these may be cell lineage-specific.

Our studies to date have focused on the induction of telomere dysfunction following exposure to ionizing radiation and the role of DNA-PKcs in this process. Telomeres consist of tandem arrays of short, repetitive G-rich sequence bound by a variety of telomere-associated proteins that together form a dynamic terminal structure that "caps" the ends of linear chromosomes, providing protection from illegitimate recombination, exonucleolytic attack and degradation. The cellular importance of functional telomeres is evidenced by the fact that they are essential for continuous cellular proliferation, an observation that has profound implications in our understanding of aging and cancer.

In striking contrast to natural chromosomal termini, broken chromosome ends produced by DNA DSBs are highly recombinogenic, and represent a major threat to the integrity of the cell's genome. As potent inducers of mutations and cell death, DSBs are arguably the most dangerous form of DNA damage. The correct repair of DSBs is essential for maintaining the genetic integrity of the cell, as erroneous repair can lead to chromosomal rearrangements such as translocations, which produce novel juxtapositions of DNA sequences at the exchange breakpoints. Cancer is frequently associated with such chromosomal abnormalities.

2. Subjects and methods

We have demonstrated that effective telomeric end capping of mammalian chromosomes unexpectedly requires proteins more commonly associated with DNA DSB repair [3]. Ku70, Ku86, and DNA-PKcs all participate in DSB repair through non-homologous end joining (NHEJ). Mutations in any of these genes cause spontaneous chromosomal end-to-end fusions that maintain large blocks of telomeric sequence at the points of fusion. The fusions, which contribute significantly to the background level of chromosomal aberrations, are not a consequence of telomere shortening, nor are they telomere associations (telomeres within $\leq 1/4$ width of chromatid of each other). We have also demonstrated that nascent telomeres produced via leading-strand DNA synthesis are especially susceptible to