Introduction

Telomeres are specialized nucleoprotein structures that contribute to genomic stability by protecting natural chromosomal termini, an essential function inferred cytogenetically from the end-to-end fusions that result when telomeric end-capping fails. In striking contrast to telomeres, broken chromosome ends resulting from DNA double-strand breaks (DSB) are highly recombinogenic and represent a major threat to genomic integrity. These lesions have the potential for involvement in chromosomal rearrangements that contribute to genomic instability and, ultimately, tumorigenesis. Previously, we showed that effective end-capping of mammalian telomeres requires proteins more commonly regarded as being involved in DSB repair by nonhomologous end-joining (NHEJ), i.e., Ku70, Ku80, and DNA-PKcs (1). Initially, this requirement seemed paradoxical, but it is now better appreciated that a plethora of DNA repair proteins interact with telomeres (2).

DNA-dependent protein kinase (DNA-PK), composed of a catalytic subunit (DNA-PKcs) and a heterodimeric regulatory subunit (Ku70/Ku80), is a caretaker of genomic stability, regulating access to DNA ends (3). Recent studies investigating the role of DNA-PKcs in NHEJ have provided valuable insight, showing that regulation occurs through phosphorylation-mediated conformational changes (3, 4). Two clusters of autophosphorylation sites, one situated around Thr-2609 and the other around Ser-2056, are essential for DNA-PKcs in NHEJ (5–7). Phosphorylation of the Thr-2609 cluster (Thr-2609 cluster) is a critical event for proper telomere end-processing and that ligase IV (NHEJ) is required for uncapped telomere fusion. We also find uncapped telomeres in cells from the BALB/c mouse, which harbor two single-nucleotide polymorphisms that result in reduced DNA-PKcs abundance and activity, most markedly in mammary tissue, and are both radiosensitive and susceptible to radiogenic mammary cancer. Our results suggest mechanistic links between uncapped/dysfunctional telomeres in DNA-PKcs–deficient backgrounds, radiation-induced instability, and breast cancer. These studies provide the first direct evidence of genetic susceptibility and environmental insult interactions leading to a unique and ongoing form of genomic instability capable of driving carcinogenesis.

Abstract

The mechanisms by which cells accurately distinguish between DNA double-strand break (DSB) ends and telomeric DNA ends remain poorly defined. Recent investigations have revealed intriguing interactions between DNA repair and telomeres. We were the first to report a requirement for the nonhomologous end-joining (NHEJ) protein DNA-dependent protein kinase (DNA-PK) in the effective end-capping of mammalian telomeres. Here, we report our continued characterization of uncapped (as opposed to shortened) dysfunctional telomeres in cells deficient for the catalytic subunit of DNA-PK (DNA-PKcs) and shed light on their consequence. We present evidence in support of our model that uncapped telomeres in this repair-deficient background are inappropriately detected and processed as DSBs and thus participate not only in spontaneous telomere-telomere fusion but, importantly, also in ionizing radiation–induced telomere-DSB fusion events. We show that phosphorylation of DNA-PKcs itself (Thr-2609 cluster) is a critical event for proper telomere end-processing and that ligase IV (NHEJ) is required for uncapped telomere fusion. We also find uncapped telomeres in cells from the BALB/c mouse, which harbors two single-nucleotide polymorphisms that result in reduced DNA-PKcs abundance and activity, most markedly in mammary tissue, and are both radiosensitive and susceptible to radiogenic mammary cancer. Our results suggest mechanistic links between uncapped/dysfunctional telomeres in DNA-PKcs–deficient backgrounds, radiation-induced instability, and breast cancer. These studies provide the first direct evidence of genetic susceptibility and environmental insult interactions leading to a unique and ongoing form of genomic instability capable of driving carcinogenesis. [Cancer Res 2009;69(5):2100–7]
(15–17). Out-of-place interstitial (TTAGGG)n sequences are expected to have unusual properties wherein consequences for the cell are not well understood. Cytogenetic studies in mammalian cells correlating interstitial telomere sequences (ITS) with sites of spontaneous and radiation-induced chromosomal rearrangements suggest that they may destabilize chromosomes (18). ITS might accumulate telomere proteins (TRF1, TRF2) and, thus, may represent special challenges for replication (2, 19). It is also noteworthy that a telomere-DSB rejoining reaction results in an “orphaned” chromosomal fragment containing an open DSB, a situation similar to that known to promote ongoing genomic instability triggered by the loss of a single telomere (20).

Recent investigation of radiation-related human breast cancer risk in the U.S. Radiologic Technologists cohort found three different single-nucleotide polymorphisms (SNP) in the DNA-PKcs gene (Prkdc) that significantly increased breast cancer risk from occupational and medical diagnostic IR exposures (21). The BALB/c mouse, which is both radiosensitive and susceptible to radiogenic mammary cancer, provides a relevant model in which to test the hypothesis that SNPs and the resulting partial deficiencies of DNA-PKcs result in uncapped telomeres and IR-induced telomere-DSB fusions. The BALB/c phenotype has been attributed to a variant allele of the DNA-PKcs gene, PrkdcBALB, which possesses two naturally occurring SNPs that result in reduced DNA-PKcs abundance and activity, most markedly in mammary gland tissue (22–24). No individuals null for DNA-PKcs have been identified; therefore, pertinent human DNA-PKcs deficiencies likely involve haploinsufficiencies or SNPs (rather than complete loss of function) and low-penetrance genes fixed in the human population at relatively high frequencies. Reduced function resulting from such alterations might be expected to lead to subtle phenotypes, perhaps only being revealed after insult (e.g., IR exposure). Also relevant in this regard are reports of decreased DNA-PKcs expression associated with invasive carcinoma of the breast (25) and significantly lower DNA-PKcs activity in peripheral blood lymphocytes from breast cancer patients versus controls (26).

Here, we sought to further characterize telomere dysfunction in DNA-PKcs–deficient backgrounds, including the BALB/c mouse. Our results provide evidence supporting our hypothesis that uncapped telomeres in DNA-PKcs–deficient backgrounds are inappropriately recognized and processed as DSBs. The consequences of uncapped telomeres include ongoing telomere-DSB fusion events in the progeny of irradiated BALB/c cells, which precede and continue during the expression of cytogenetic instability and the emergence of preneoplastic mammary epithelial clones. By providing a link between a genetic defect (partial DNA-PKcs deficiency), the cellular dysfunction associated with this defect (telomere uncapping), and its consequence (radiation-induced genomic instability), our studies suggest a unique mechanism of individual genetic susceptibility to genomic instability resulting from environmental insult.

Materials and Methods

Cells. SD1 (DNA-PKcs deficient) mouse cells expressing vector, wild-type (WT) DNA-PKcs, autophosphorylation-mutant ABCDE (T2609A, S2612A, S2602A, T2638A, and T2638A) and autophosphorylation-mutant PQR (S2023A, S2029A, S2041A, S2053A, and S2056A) were constructed as previously reported (6). Mouse embryonic fibroblasts (MEF) deficient in p53 (p53-/−) or doubly deficient for p53 and ligase IV (p53-/−/ligIV−/−) have been described (27). The kinase activity of DNA-PKcs was inhibited via exposure to 55 μmol/L NU7026 [2-(morpholin-4-yl)-benzo[b][1]chomane-4-one; Sigma-Aldrich, Inc.] for a single cell cycle. Mammary epithelial and fibroblast cells were extracted from virgin female BALB/cByJ and C57BL/6 mice (Jackson Laboratory) and maintained as described previously (28, 29). BALB/c p53−/− mammary fibroblasts and epithelial cells have been described elsewhere (30), as were the SCID p53−/− fibroblasts (1). Radiation-induced growth variants, IRO 6-1 and IRO 6-2, were isolated during studies of IR-induced instability in primary mouse mammary epithelial cells (ref. 31; Supplementary Table 2).

Irradiations. γ-Ray exposures were delivered at a dose rate of 3.9 Gy/min in a calibrated, sealed source Mark I 137Cs γ-iradiator (J.L. Shepherd and Associates).

FISH. After irradiation, cultures were incubated for various times and processed for telomere FISH (32). Specialized applications were also used: CO-FISH (17) and spectral karyotyping (SKY) with telomere CO-FISH (SKY-CO-FISH); both were optimized and side-by-side images were evaluated (17, 33, 34).

Image analysis. Preparations were examined using a Zeiss fluorescence microscope (Axioplan 2ie MOT). 4',6-Diamidino-2-phenylindole (DAPI) and Cy3 excitor/dichroic/barrier filter sets (Carl Zeiss MicroImaging, Inc.) were used to detect counterstained chromosomes and telomere signals, respectively. Images of chromosomes were captured with a charge-coupled device camera (model CV-M4 CL, JAI PULNiX, Inc.), controlled by a Dell precision 360 workstation running Isis FISH imaging software (Metasystems).

Scoring criteria. Telomere fusion necessitates that telomeres of adjoining chromosomes fuse into a single FISH signal and the DAPI signal remain continuous (1). Telomere-DSB fusion appears as single-sided (only on one chromatid of a mitotic chromosome) interstitial blocks of CO-FISH telomere signal. Telomere association is defined as telomeres of adjacent chromosomes touching or in very close proximity (≤1/4 width of a chromatid), yet remaining as separate signals. Unless noted, at least 25 metaphases were scored for each condition.

Immuno-FISH. Cells were grown and irradiated on two-well chamber slides (Nalgene Nunc International), fixed in 4% formaldehyde, permeabilized in 0.2% Triton X-100 in PBS, and blocked in 5% milk. Mouse anti-γH2AX antibody (Millipore Corporation) was mixed in 5% milk and incubated for 1 h at room temperature. Cells were rinsed in PBS, and

Figure 1. Telomeric repeats are present at translocation breakpoints. SKY-CO-FISH distinguishes between telomere-independent (DSB-DSB) and uncapped telomere–dependent (Tel-Tel and Tel-DSB) chromosomal rearrangements. Telomere-DSB rearrangement, translocation between chromosomes 8 and 12, was a clonal event.
Results

Characterization of telomere uncapping with DNA-PKcs deficiency. We have previously reported telomere-DSB fusion frequencies in DNA-PKcs−deficient backgrounds using CO-FISH (15). To unambiguously show that telomere-DSB fusion occurs at chromosomal translocation breakpoints, we combined SKY (33) with telomere CO-FISH (17). SCID p53−/− and BALB/c p53−/− fibroblasts exposed to IR (1 and 2 Gy of 137Cs γ-rays) were collected for analyses; absence of p53 facilitated continued cycling of mouse cells despite telomere dysfunction (35). Visualization of SKY and CO-FISH telomere patterns revealed the presence of single-sided ITS (telomere-DSB fusion; ref. 15) at chromosomal translocation breakpoints (Fig. 1). Consistent with previous G-banding studies,8 SKY-CO-FISH confirmed that any chromosome can suffer telomeric end-capping failure (i.e., not chromosome-specific) and the presence of clonal rearrangements (e.g., T8;12), supporting telomere-DSB fusions as covalent linkages and, therefore, potentially transmissible. These results provide direct evidence that uncapped telomeres in DNA-PKcs−deficient backgrounds are detected and processed as DSBs and, thus, participate in chromosomal rearrangements after IR exposure.

To provide additional evidence that uncapped telomeres in DNA-PKcs−deficient backgrounds are misidentified as DSBs, we investigated the role of the NHEJ repair pathway in telomere fusion formation. MEFs deficient in p53 (p53−/−) or doubly deficient for p53 and ligase IV (p53−/− lig IV−/−; ref. 27) were exposed to NU7026, a specific chemical inhibitor of the kinase activity of DNA-PKcs (36); DNA ligase IV is essential for NHEJ, and the lack of p53 rescues ligase IV deficiency lethality without affecting NHEJ (37, 38). After a single cell cycle in the presence of NU7026, cells were collected and analyzed by telomere FISH. A dramatic induction of telomere fusion was observed in p53−/− MEFs grown in the presence of NU7026 with telomere CO-FISH (17). SCID p53−/− and BALB/c p53−/− mice (lacking DNA-PKcs protein) participate in inappropriate telomere fusion events (1), we next examined telomeric end-capping failure resulting from partial DNA-PKcs deficiency in mammary fibroblasts derived from BALB/c versus C57BL/6 mice. Previous studies have identified a unique variant of Prkdc, the gene encoding DNA-PKcs, in BALB/c mice (31). The PrkdcAB−/− variant is associated with decreased DNA-PKcs abundance and activity, increased

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8 S.D. Bouffler and S.M. Bailey, unpublished data.
radiation sensitivity, and susceptibility to IR-induced genomic instability and mammary carcinogenesis. We observed a significant increase in the frequency of telomere fusion in cells derived from BALB/c (variant Prkdc) compared with C57BL/6 (WT Prkdc; Fig. 4A). After IR exposure, the frequency of telomere-DSB fusion in BALB/c cells increased as a function of dose, indicating that, indeed, uncapped telomeres are fusing to IR-induced DSBs. TIF frequencies were also elevated in BALB/c mammary fibroblasts compared with C57BL/6, regardless of IR exposure (Fig. 4B), evidence that uncapped telomeres are triggering a damage response. Whereas an increase of TIFs in C57BL/6 after IR was observed, that seen in BALB/c was nearly twice that in C57BL/6. Interestingly, the prevalence of telomere-DSB fusion in BALB/c was higher than that of dicentric chromosomes, suggesting that telomere-DSB fusion is relatively frequent in DNA-PKcs–deficient backgrounds. We have also shown that partial deficiency of DNA-PKcs in human cells results in telomere-DSB fusion frequencies that plateau with increasing dose (42).

Consequence of telomere uncapping in the DNA-PKcs–deficient BALB/c mouse. We have previously shown that BALB/c is sensitive to radiation-induced genomic instability compared with C57BL/6 mice and others (31). This has been attributed to the variant Prkdc gene expressed in BALB/c resulting in decreased abundance and activity of DNA-PKcs (23). However, the mechanisms responsible for generating this radiation-induced genomic instability are unknown. Our experiments showing that BALB/c experiences telomeric uncapping led us to examine the contribution of this telomere dysfunction in the observed radiation-induced genomic instability. It is important to note again that the telomeric uncapping phenotype seen in BALB/c is expressed primarily as telomere-DSB fusion after insult, not as spontaneous telomere-telomere fusion, and thus is more subtle than when end-capping completely fails, as in cells expressing dominant-negative alleles of TRF2 (43), SCID cells (1), or cells in which the kinase activity of DNA-PKcs has been inhibited (ref. 40; Fig. 2B).

Figure 3. Autophosphorylation of DNA-PKcs at Thr-2609 cluster is required for telomeric end-capping function. A, representative image of an internal telomere sequence observed in cells unable to phosphorylate DNA-PKcs at the Thr-2609 cluster. B, similar increases in internal telomere signals are observed in kinase-dead mutants and Thr-2609 mutants, indicating a critical role for this site in telomeric end-capping (*, P < 0.05 versus WT by Student's t test).

Figure 4. Telomere dysfunction in BALB/c (variant Prkdc allele) compared with C57BL/6 (WT Prkdc allele) mouse mammary fibroblasts. A, telomere-DSB fusions are observed after IR exposure only in BALB/c, and increase as a function of dose; frequencies rival that of dicentrics (Dic). B, increased TIF formation, indicative of a damage response, is observed in BALB/c versus C57BL/6 (*, P < 0.05 versus C57BL/6 by Student's t test).
precedes or is concomitant with our previously reported delayed chromatid-type chromosomal instability in the same experimental system (31). The presence of telomere-DSB fusion, but not telomere-telomere fusion, led us to conclude that telomere uncapping is not causative of the delayed instability in BALB/c but rather a contributing factor in driving genomic instability forward; i.e., telomere-DSB fusion, by virtue of their nature and transmissibility, may contribute to ongoing genomic instability.

We also examined telomere dysfunction in two immortalized subclones clones (6-1 and 6-2), which were isolated from an altered growth variant (IRO) that emerged from a population of irradiated BALB/c mammary epithelial cells (Supplementary Table S2). Although immortalized, IRO remained diploid and expressed increased levels of chromatid-type aberrations compared with nonirradiated controls, a clear indication of continued chromosomal instability. At low passages, neither 6-1 nor 6-2 clones were tumorigenic, and 6-2 remained nontumorigenic through at least 27 passages. In striking contrast, injection of 6-1 at passages 21 and higher resulted in tumor formation, clearly showing the preneoplastic nature of clone 6-1 (44). Intriguingly, after IR exposure, we observed an elevated frequency of telomere-DSB fusion in 6-1 (tumorigenic) with no evidence of such fusions in 6-2 (Fig. 6). The presence of telomere-DSB fusion in the preneoplastic 6-1 clone, particularly in the unirradiated samples, is reflective of ongoing instability. This is consistent with our evaluation of chromatid-type aberrations in these clones, which revealed elevated levels in the tumorigenic clone, but not in the nontumorigenic clone (31).

**Discussion**

Maintenance of genomic stability obligates cells to correctly distinguish between and properly process two very different types of double-stranded DNA ends: telomeres and DSBs. A better understanding of this fundamental discrimination provides insight into two critical cellular pathways: the DNA damage response and telomere end-capping function. The aim of the present study was to further elucidate and characterize the role of the NHEJ repair protein DNA-PKcs in mammalian telomeric end-capping, as well as to identify the consequences of telomere dysfunction in DNA-PKcs–deficient backgrounds. The BALB/c mouse provided an especially relevant model in this regard because it possesses partial deficiency of DNA-PKcs due to two SNPs in *Prkdc* and is radiosensitive and susceptible to IR-induced mammary carcinogenesis.

We conclusively show that uncapped telomeres in DNA-PKcs–deficient backgrounds mistakenly fuse to IR-induced DSBs, supporting our hypothesis that such dysfunctional telomeres behave as DSBs. SKY-CO-FISH provided the first direct evidence of interstitial telomere-DSB fusion at translocation breakpoints between chromosomes. These characteristic telomere-DSB fusions, which maintain large blocks of displaced ITSs, can involve any chromosome; uncapping is not chromosome specific but rather depends on the presence of an uncapped telomere and a suitable substrate, whether it be a DSB or another dysfunctional telomere. SKY-CO-FISH also confirmed the presence of clonal rearrangements, supporting telomere-DSB fusion as covalent linkages and, therefore, potentially transmissible.

BALB/c mammary fibroblasts and epithelial cells exhibit telomere-DSB fusions after IR exposure, which increase in frequency in a dose-dependent manner. The presence of TIFs in BALB/c irrespective of IR exposure reveals inherent telomere instability, providing evidence that the cell improperly identifies uncapped telomeres in this background as DSBs, then misjoins them either to other uncapped telomeres (spontaneous telomere fusion) or to IR-induced DSBs (telomere-DSB fusion). DNA-PKcs–deficient cells can (and do) improperly fuse uncapped telomeres to DSBs at levels consistent with or even higher than more common chromosomal rearrangements, such as dicentrics. This scenario suggests that uncapped telomeres are being improperly identified as DSBs because they trigger a DNA damage response and participate in DSB repair pathways.

The requirement for DNA ligase IV, an essential NHEJ protein, in telomere fusion formation establishes that uncapped telomeres do indeed participate in DSB repair pathways, namely, NHEJ. Strikingly, p53+/− ligIV−/− MEFs, in which the kinase activity of DNA-PKcs was inhibited, did not display telomere fusion events. However, they did display an abundance of telomere association, intimating that uncapped telomeres are processed for ligation via NHEJ but are unable to reestablish the phosphodiester linkage without ligase IV. It must be appreciated that ample telomere sequence is present at the points of fusion in DNA-PKcs–deficient backgrounds because previous studies have shown that ligase IV is dispensable for telomere fusion of critically shortened telomeres in telomerase-deficient mouse cells (45). Distinctions between chromosome fusions arising from shortened (loss of sequence) versus uncapped (loss of structure) telomeres have been drawn previously (46). Uncapped telomere fusions resulting from depletion of the telomere protein TRF2 have also been shown to be generated by DNA ligase IV–dependent NHEJ (47). Circumstance and cause of

**Figure 5.** Ongoing telomere instability is present in BALB/c mammary epithelial cells. Elevated levels of telomere-DSB fusion, indicative of ongoing telomere dysfunction, are evident in BALB/c but not in C57BL/6. **A,** this ongoing telomere instability can be seen in unirradiated BALB/c cells as a gradual increase in telomere-DSB fusion (Telo-DSBs) compared with C57BL/6. **B,** the progeny of irradiated BALB/c cells show a striking increase in telomere-DSB fusion compared with C57BL/6 at 12 PD.
Telomeric dysfunction may govern the choice of repair pathway for end fusion, as recently proposed (48). Our demonstration that telomeres in DNA-PKcs-deficient backgrounds are identified as DSBs and that NHEJ mediates telomere fusion formation in these instances led us to hypothesize that DNA-PKcs acts in similar fashion at telomeres as it does at DSBs. Recent investigations examining the role of DNA-PKcs in NHEJ have highlighted the importance of two clusters of autophosphorylation sites (5–7). Our results provide the first evidence that phosphorylation of DNA-PKcs itself at the Thr-2609 cluster is an important target for effective telomeric end-capping function and are consistent with the concept that phosphorylation of DNA-PKcs at telomeres mimics that occurring at sites of DSBs, which then causes a conformational change that facilitates protein accessibility to the DNA end and subsequent critical end-processing (3, 4, 8).

Still, key differences must exist between DNA-PKcs presence at telomeres versus DSBs. Perhaps the most relevant, and most obvious, may be that there is only one telomeric DNA end, whereas at DSBs there are two DNA ends in close proximity. It was recently shown that the autophosphorylation of DNA-PKcs can transpire in trans- (9), an occurrence requiring juxtaposition of two DNA-PKcs molecules at the synapse. At telomeric ends, however, presumably only one DNA-PKcs molecule is present, and thus the ability to autophosphorylate in trans- would be compromised. Interestingly, ATM was recently shown to preferentially phosphorylate DNA-PKcs at the Thr-2609, but not Ser-2056, cluster, in response to IR (10). Taken together with evidence of reciprocal action of these two autophosphorylation sites, our data showing that the Thr-2609 cluster, but not the Ser-2056 cluster, is critical for telomeric end-capping function suggest that ATM, not DNA-PKcs, may be the kinase responsible for phosphorylation of this cluster at telomeres. However, it must also be appreciated that, in addition to debate over the role of ATM in Thr-2609 phosphorylation (9), the kinase activity of ATM is attenuated at telomeres through interactions with TRF2 (49).

Our data support a model in which DNA-PKcs rapidly binds to newly replicated telomeres, perhaps preferentially to those produced by leading-strand DNA synthesis, where it is absolutely required (39). The reality of strand-specific differences in telomeric end-processing is now supported by several studies (50, 51). We envision that once DNA-PKcs loads onto newly replicated, essentially blunt-ended leading-strand telomeres, the huge protein acts to sterically block the telomeric end from further processing. Subsequent autophosphorylation of the DNA-PKcs Thr-2609 cluster induces a conformational change that allows access to as yet unidentified factors that properly process the end and generate an extensive 3’ single-stranded overhang, thereby distinguishing it from a DSB. The notion of DNA-PKcs physically blocking telomeric DNA ends is also supported by our work with a specific chemical inhibitor of DNA-PKcs kinase activity. We have shown that kinase-inhibited DNA-PKcs more severely impedes telomere end-capping than does the absence of DNA-PKcs (40), an observation reminiscent of a previous report showing that cells expressing an unphosphorylatable form of DNA-PKcs are more radiosensitive than cells completely lacking DNA-PKcs (4).

When contemplating the consequence of the role of DNA-PKcs at telomeres, a critical determinant for significance is the frequency at which telomere fusions form in DNA-PKcs-deficient backgrounds, especially those relevant to the human condition, such as partial deficiencies resulting from SNPs or haploinsufficiency. Whereas there has been some discrepancy as to the reported frequencies of these events, with immortalization status and manner of DNA-PKcs depletion among the contributing factors to these inconsistencies, telomere fusions have consistently been reported in DNA-PKcs-deficient backgrounds (1, 13, 14). The lack of a dramatic telomere phenotype, of the sort seen when the critical telomere protein TRF2 is depleted for example (43), could indicate either that DNA-PKcs is not strictly required for end-capping function or, more likely, that its importance is underestimated due to the fact that NHEJ is required for the very pathway required for fusion formation.

In the present study, we expand our characterization of DNA-PKcs-deficient telomere dysfunction by examining the relationship between telomere uncapping and IR-induced genomic instability, a key intermediate process on the path of tumorigenesis. To this end, we used primary mammary epithelial cells derived from the DNA-PKcs-deficient BALB/c mouse, which experiences increased...
IR-induced genomic instability and susceptibility to mammary tumorigenesis and, as we show here, telomere uncapping. A marked increase in telomere-DSB fusion in BALB/c cells was observed after exposure. We also find evidence of delayed formation of telomere-DSB fusion, suggestive of a role for these events in ongoing IR-induced genomic instability.

It is important to appreciate that telomere-DSB fusions represent sensitive markers of delayed instability because they require an uncapped telomere (occurs with replication) and the presence of DSBs at long time points after exposure. Telomere-DSB fusion may perpetuate instability in several ways, including resulting in an open DSB end (20). ITS themselves have been shown to be a source of chromosomal instability (18), which may encounter inordinate replication stalling (2, 19), a circumstance that can also drive genomic instability. In this regard, it is intriguing that the observed IR-induced telomere instability occurred immediately preceding our previously reported IR-induced cytogenetic instability in BALB/c (31); i.e., replication fork stalling occurring on one chromatid encountering ITS may well contribute to chromatid-type breaks observed in subsequent cell cycles. Additionally, the slow rejoining kinetics of DSBs associated with DNA-PKcs deficiency after IR exposure has been shown to contribute to genomic instability, a significant source of which was identified as sister chromatid fusion (52). Delayed rejoining kinetics (more time) combined with postreplicative misjoining not only of DSBs but also of uncapped telomeres (more opportunity), as we show here, most certainly adds up to increased probability of incorrect end-joining.

Whereas telomere shortening has been associated with radiosensitivity, instability, and cancer, here we provide the first evidence that telomere uncapping associated with even partial DNA repair deficiency may be involved in driving IR-induced instability and, thus, carcinogenesis via inappropriate telomere-DSB fusion. Further evidence for this linkage comes from the observation that telomere instability was observed in a preneoplastic mammary epithelial clone, but not in a nonneoplastic clone, derived from the same growth variant that emerged from an unstable population of irradiated mammary epithelial cells.

In conclusion, we have expanded our characterization of telomere dysfunction with DNA-PKcs deficiency and established the presence of significant telomere uncapping in the BALB/c mouse. These studies begin to uncover the manner by which DNA-PKcs interacts with telomeres, and we present evidence supporting the critical role of phosphorylation of DNA-PKcs itself for its interaction with telomeric DNA ends. Analysis of consequence and time course for expression of telomere-DSB fusion and IR-induced genomic instability is suggestive of a role for telomere dysfunction in driving this instability. Importantly, subtle repair deficiencies, such as result from SNPs or haploinsufficiencies relevant to the human condition, can result in subtle telomeric uncapping, which may not present much of a problem most of the time (52). However, as we show here, after IR exposure (e.g., accidental, therapeutic), the resulting telomere-DSB fusions can fuel ongoing instability and emergence of preneoplasia, ultimately driving carcinogenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Received 7/24/2008; revised 11/10/2008; accepted 12/18/2008; published OnlineFirst 02/24/2009.

Grant support: NIH/National Cancer Institute grants CA-09236-30 (R.L. Ullrich) and CA-043322-20 (R.L. Ullrich and S.M. Bailey), DOE grant DE-FG02-01ER63239 (R.L. Ullrich and S.M. Bailey), and NASA grant NNX08DL68G (S.M. Bailey).

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We thank Drs. Penelope Jeggo and Daniel Medina for cell lines and Edwin H. Goodwin for helpful discussions and critical reading of the manuscript.

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Dysfunctional Telomeres and DNA-PKcs Deficiency


Telomere Dysfunction and DNA-PKcs Deficiency: Characterization and Consequence

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doi:10.1158/0008-5472.CAN-08-2854

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