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Perspective

Role of Mammalian Rad9 in Genomic Stability and Ionizing Radiation Response

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ABSTRACT

Eukaryotic cells have evolved DNA damage response mechanisms utilizing proficient DNA repair and cell cycle checkpoints in order to maintain genomic stability. The *Schizosaccharomyces pombe Rad9* gene was initially identified as encoding a cell cycle checkpoint protein. When the mammalian homologue of *S. pombe* Rad9 was inactivated, however, chromosomal instability was observed even in the absence of DNA damaging agents. Both an increase in chromosome end-to-end associations and telomere loss were observed in cells with inactivated mammalian Rad9. This telomere instability correlated with enhanced S- and G_2-phase specific cell killing, delayed kinetics of γ-H2AX foci appearance and disappearance, and reduced chromosomal repair after ionizing radiation (IR) exposure, suggesting that Rad9 plays a role in cell cycle phase specific DNA damage repair. Inactivation of mammalian Rad9 also resulted in decreased homologous recombinational (HR) repair, which occurs predominantly in the S- and G_2-phase of the cell cycle. These newly defined functions of mammalian Rad9 are discussed in relation to telomere stability and HR repair as a mechanism for promoting cell survival after IR exposure.

Recent studies on genomic stability have productively exploited the similarities and differences between the telomere end maintenance pathways utilized by different organisms to help identity specific components and mechanisms. One unifying concept emerging is that chromosome-end protection is a critical necessity for genome stability. Moreover, the factors involved in telomere maintenance and the potential mechanisms by which they participate in this process also seem to function in DNA repair and cell cycle checkpoints. New results now reveal that the human homologue of the yeast cell cycle checkpoint gene Rad9 functions in telomere maintenance and DNA damage repair following IR exposure.[1]

The protein products of several *rad* checkpoint genes of *Schizosaccharomyces pombe* (*rad1*$^+$, *rad3*$^+$, *rad9*$^+$, *rad17*$^+$, *rad26*$^+$ *and hus1*$^+$) play crucial roles in sensing changes in DNA structure, and several have been shown to function in the telomere maintenance. Human Rad9 (hRad9) is phosphorylated by ATM (ataxia-telangiectasia mutated gene product) in response to DNA damage[2] and together with hRad1 and hHus1, forms a nuclear complex that resembles PCNA, which is believed to sense DNA damage.[3-5] Interestingly, strains of *S. pombe* mutated in either the *rad1*$^+$, *hus1*$^+$, *rad9*$^+$ or *rad17*$^+$ genes, *S. cerevisiae* mutations in related orthologues, and nematodes altered in *mrt-2* (encoding a Rad1 homolog) all show telomere shortening. However, *S. cerevisiae* with mutant Mec3 show telomere elongation, further suggesting that these checkpoint proteins are also involved in telomere maintenance.[6-10] In human cells hRad9 along with hHus1 and hRad1 are colocalized specifically at telomeric DNA and PML (promyelocytic leukemia) bodies in ALT (alternative lengthening of telomeres) cells.[11]

The function of mammalian Rad9, a gene involved in sensing DNA damage, was examined in the context of chromosome/telomere stability and DNA repair in mammalian cells by several approaches including siRNA, expression of mutant hRad9, hRad9 overexpression and Rad9 knockout.[1] Cells with inactivated mammalian Rad9 had higher frequencies of chromosomal abnormalities, such as chromosome end-to-end associations and chromosomal breaks. The chromosomal aberrations appear to involve dysfunctional telomeres, as a significant number of cells showed telomere associations. These results support the argument that abrogating the function of mammalian Rad9, previously implicated in mediating DNA damage sensing checkpoints, increases the frequency of unrepaired chromosomal aberrations thereby also increasing the number of telomere fusions. A similar mechanism may contribute to the decreased survival of MEF cells when Mrad9 is inactivated.[12] The frequency of spontaneous as well as IR-induced chromosomal

aberrations was dramatically higher in cells with inactivated Rad9 relative to controls, suggesting that loss of telomere function occurred by breakage near telomeres, rather than by a telomerase-based mechanism.[1]

It is important to note that mammalian Rad9 is essential for genomic stability as is evident from the fact that cells with both alleles of Rad9 inactivated displayed frequent chromosome breaks with complete loss of telomeric repeats in some chromosomes, as well as chromosome end fusions, even under normal growth conditions and in the absence of exogenous genotoxic challenges.[1] The observed increases in spontaneous genomic instability and higher residual chromosome damage post irradiation cannot be attributed to the role of mammalian Rad9 to only defective cell cycle checkpoints. The fact that the G_1 checkpoint was operative in cells with reduced levels of Rad9 is supported by the observations that irradiation induced comparable frequencies of cells in G_1 and S phase compared to those cells with normal levels of hRad9.[1] Furthermore, cells with reduced levels of Rad9 displayed no defect in the S-phase specific cell cycle checkpoint as determined by radioresistant DNA synthesis.[1] In addition, mammalian Rad9 inactivation did not influence the G_2 checkpoint in the cell lines studied.[1] These studies are consistent with the observations that hRad9 knockdown had no influence on IR-induced phosphorylation of ATM Ser1981 or Chk2 Thre68,[1] further supporting the argument that mammalian Rad9 has a minimum role in cell cycle checkpoint control post-irradiation. In contrast, there was a clear and significant difference in chromosome aberration frequency of S- and G_2-phase specific aberrations/metaphase between cells with and without fully functional hRad9.[1] The role of hRad9 in cell cycle-phase specific chromosome repair was confirmed by utilizing the premature chromosome condensation technique, which revealed no difference in the kinetics of repair or residual number of chromosome breaks in G_1-phase cells with or without reduced levels of hRad9.[1] However, cells deficient in hRad9 had a higher frequency of chromosome gaps and breaks in the G_2-phase.[1] Consistent with these data, survival studies demonstrated that cells in S and G_2 phase were sensitive to irradiation if hRad9 was inactivated. Overall, these results reinforce the idea that in cells with hRad9 knockdown, the chromosomal damage that might occur during the S or G_2 phase could be due to defective chromosome repair. hRad9 is rapidly retained at DNA damage sites and colocalizes with the phosphorylated form of H2AX (γ-H2AX).[13] Interestingly, delayed appearance and disappearance of γ-H2AX foci post-irradiation was observed in cells with inactivated mammalian Rad9. These results provide strong evidence that mammalian Rad9 plays a critical role in repair of DNA damage.

Association of hRad9 with TRF2 provide further evidence that hRad9 may have a role in maintaining telomere stability. Although hRad9 knockdown has a minimal role in TRF2 association with telomeres, and inactivation of hRad9 and TRF2 did not rescue a telomere instability phenotype (Fig. 1), it is possible that TRF2 might be mediating DNA damage signaling through Rad9. This notion is further supported by the data indicating that inactivation of hRad9 influences HR but not NHEJ.[1]

Although, the recent studies demonstrate that mammalian Rad9 can influence telomere stability and HR repair,[1] it is not yet clear exactly how the protein functions in these processes. It is possible that mammalian Rad9 modulates chromatin structure, an early essential step for HR repair. Such speculation is supported by our demonstration that hRad9 inactivation delays the appearance of IR-induced γ-H2AX foci. These observations can support a role for

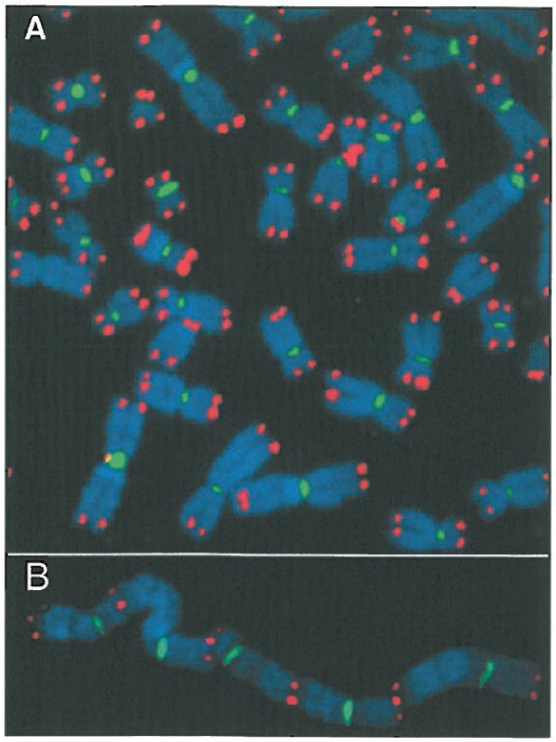

Figure 1. Telomere FISH analysis showing human metaphase chromosomal spreads. Telomere signals are red and centromere signals are green. (A) represents metaphase section of control 293 cells (B) metaphase of 293 cells with expression of mutant TRF2-$^{\Delta B \Delta M}$ and knockdown of hRad9 (note almost all chromosomes undergo telomere fusions).

Rad9 in chromatin remodeling especially at telomeres, however, it needs to be demonstrated whether the protein can influence chromatin structure beyond this limited region. Such a general chromatin alteration should influence repair in all phases of the cell cycle as has been observed in ATM null cells.[14] However, hRad9 inactivation resulted in an enhanced defect in chromosome repair confined only to the S and G_2 phases of the cell cycle, supporting its role in HR repair. Alternatively, Rad9 may interact with and influence the activity of proteins that have a role in HR repair, which occurs predominantly in S and G_2 phase cells. This is supported by our demonstration of a physical interaction between hRad9 and hRad51.[1] In fact, Shinohara and coworkers[15] demonstrated that *S. cerevisiae* orthologues of some of the hRad9 checkpoint pathway-related proteins interact with Rad51 for repair of DSBs in meiosis via recombination. Furthermore, Grushcow and coworkers[16] as well as Aylon and Kupiec[17] showed that the *S. cerevisiae* equivalents of hRad9, hRad1, hHus1 and hRad17 pathway members are involved in recombination partner choice. The high frequency of chromatid gaps and breaks in hRad9 inactivated cells support a role for the protein in HR repair, which is consistent with the observations that knockdown of hRad9 resulted decrease in HR repair.[1] Inactivation of mammalian Rad9 could thus contribute at least in part to the radiosensitivity, genomic instability and loss of telomeres observed by mediating the abrogation of HR repair.

Clearly, mammalian Rad9 is crucial for maintaining overall genomic stability and cell viability. Altered Rad9 levels dramatically affect cellular genome stability as a result of the ensuing telomere dysfunction, reduced HR repair efficiency and enhanced IR sensitivity.

The observation of Rad9 interactions with TRF2 and Rad51 suggests the protein functions in both the telomere maintenance and HR repair pathways, respectively, though the specific details of the mechanism(s) remains to be determined. Clearly, our understanding of how DNA damage sensing proteins are involved in telomere maintenance is still emerging. The combination of biochemical, structural and genetic experimental approaches that have identified the link between Rad9, DNA damage, and telomere stability will now need to be applied to develop a mechanistic understanding of how mammalian Rad9 is involved in maintaining genomic stability.

References

1. Pandita RK, Sharma GG, Laszlo A, Hopkins KM, Davey S, Chakhparonian M, Gupta A, Wellinger RJ, Zhang J, Powell SN, Roti Roti JL, Lieberman HB, Pandita TK. Mammalian rad9 plays a role in telomere stability, S- and G_2-phase-specific cell survival, and homologous recombinational repair. Mol Cell Biol 2006; 26:1850-64.
2. Chen MJ, Lin YT, Lieberman HB, Chen G, Lee EY. ATM-dependent phosphorylation of human Rad9 is required for ionizing radiation-induced checkpoint activation. J Biol Chem 2001; 276:16580-6.
3. St Onge RP, Udell CM, Casselman R, Davey S. The human G_2 checkpoint control protein hRAD9 is a nuclear phosphoprotein that forms complexes with hRAD1 and hHUS1. Mol Biol Cell 1999; 10:1985-95.
4. Volkmer E, Karnitz LM. Human homologs of *Schizosaccharomyces pombe* rad1, hus1, and rad9 form a DNA damage-responsive protein complex. J Biol Chem 1999; 274:567-70.
5. Venclovas C, Thelen MP. Structure-based predictions of Rad1, Rad9, Hus1 and Rad17 participation in sliding clamp and clamp-loading complexes. Nucleic Acids Res 2000; 28:2481-93.
6. Ahmed S, Hodgkin J. MRT-2 checkpoint protein is required for germline immortality and telomere replication in *C. elegans*. Nature 2000; 403:159-64.
7. Dahlen M, Olsson T, Kanter-Smoler G, Ramne A, Sunnerhagen P. Regulation of telomere length by checkpoint genes in *Schizosaccharomyces pombe*. Mol Biol Cell 1998; 9:611-21.
8. Nakamura TM, Moser BA, Russell P. Telomere binding of checkpoint sensor and DNA repair proteins contributes to maintenance of functional fission yeast telomeres. Genetics 2002; 161:1437-52.
9. Longhese MP, Paciotti V, Neecke H, Lucchini G. Checkpoint proteins influence telomeric silencing and length maintenance in budding yeast. Genetics 2000; 155:1577-91.
10. Corda Y, Schramke V, Longhese MP, Smokvina T, Paciotti V, Brevet V, Gilson E, Geli V. Interaction between Set1p and checkpoint protein Mec3p in DNA repair and telomere functions. Nat Genet 1999; 21:204-8.
11. Nabetani A, Yokoyama O, Ishikawa F. Localization of hRad9, hHus1, hRad1, and hRad17 and caffeine-sensitive DNA replication at the alternative lengthening of telomeres-associated promyelocytic leukemia body. J Biol Chem 2004; 279:25849-57.
12. Hopkins KM, Auerbach W, Wang XY, Hande MP, Hang H, Wolgemuth DJ, Joyner AL, Lieberman HB. Deletion of mouse *rad9* causes abnormal cellular responses to DNA damage, genomic instability, and embryonic lethality. Mol Cell Biol 2004; 24:7235-48.
13. Greer DA, Besley BD, Kennedy KB, Davey S. hRad9 rapidly binds DNA containing double-strand breaks and is required for damage-dependent topoisomerase II beta binding protein 1 focus formation. Cancer Res 2003; 63:4829-35.
14. Pandita TK, Hittelman WN. The contribution of DNA and chromosome repair deficiencies to the radiosensitivity of ataxia-telangiectasia. Radiat Res 1992; 131:214-23.
15. Shinohara M, Sakai K, Ogawa T, Shinohara A. The mitotic DNA damage checkpoint proteins Rad17 and Rad24 are required for repair of double-strand breaks during meiosis in yeast. Genetics 2003; 164:855-65.
16. Grushcow JM, Holzen TM, Park KJ, Weinert T, Lichten M, Bishop DK. *Saccharomyces cerevisiae* checkpoint genes *MEC1, RAD17* and *RAD24* are required for normal meiotic recombination partner choice. Genetics 1999; 153:607-20.
17. Aylon Y, Kupiec M. Cell cycle-dependent regulation of double-strand break repair: A role for the CDK. Cell Cycle 2005; 4:259-61.