FEBS Letters 581 (2007) 5275–5281

Mrg15 null and heterozygous mouse embryonic fibroblasts exhibit DNA-repair defects post exposure to gamma ionizing radiation

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Received 30 July 2007; revised 8 October 2007; accepted 11 October 2007

Available online 18 October 2007

Edited by Angel Nebreda

Abstract MORF4-related gene on chromosome 15 (MRG15) is a core component of the NuA4/Tip60 histone acetyltransferase complex that modifies chromatin structure. We here demonstrate that *Mrg15* null and heterozygous mouse embryonic fibroblasts exhibit an impaired DNA-damage response post gamma irradiation, when compared to wild-type cells. Defects in DNA-repair and cell growth, and delayed recruitment of repair proteins to sites of damage were observed. Formation of phosphorylated H2AX and 53BP1 foci was delayed in *Mrg15* mutant versus wild-type cells following irradiation. These data implicate a novel role for MRG15 in DNA-damage repair in mammalian cells.

Keywords: MORF4; NuA4; Sin3-HDAC; ATM; 53BP1

1. Introduction

Normal cells have a finite ability to divide in culture, a phenomenon known as replicative senescence. Cell fusion of normal with immortal tumor cells demonstrated that senescence is a dominant phenotype and provided the first evidence that senescence is a mechanism of tumor suppression [1]. These studies resulted in the isolation of mortality factor on chromosome 4 (MORF4) as a senescence inducing gene. MORF4 is a member of a family of transcription factors including the MORF4-related gene on human chromosome 15 (MRG15) [2,3].

MRG15 has a 96% similarity to MORF4 in amino acid sequence but fails to induce senescence upon introduction into immortal cells. The most striking structural difference between the two proteins is the presence of an N-terminal extension in MRG15, which includes a chromodomain. Proteins containing a chromodomain, characterized to date, have been found to be chromatin remodeling factors involved in causing conformational changes in chromatin by ATP-dependent movement of nucleosomes and modification of histones [4–6]. Histone modifying enzymes, the histone acetyltransferases and deacetylases (HATs, HDACs), are present in complexes involved in transcription and, recently, HAT complexes have been implicated in DNA-damage detection and repair [7]. MRG15 is present in both the NuA4/Tip60-HAT and Sin3-HDAC chromatin modifying complexes [8].

We have shown that MRG15 is important for cell proliferation in primary mouse embryonic fibroblasts (MEFs), and that deletion of the gene in mice results in gross developmental defects leading to embryonic lethality [9]. We here demonstrate that MRG15 is required for effective DNA-damage repair post exposure to ionizing radiation (IR) in MEFs and is important for efficient recruitment of DNA-repair proteins at sites of damage and acetylation of H2A and H2AX. Loss of a single copy of MRG15 in MEFs delays repair of DNA-damage post irradiation, indicating that even a modest decrease in MRG15 levels affects the function of associated complexes. This suggests a novel and critical role for MRG15 in DNA-repair in mammalian cells.

2. Methods

2.1. Cell culture and gamma irradiation conditions

Generation of *Mrg15* null and heterozygous (het) MEFs and conditions for cell culture have been described previously [9]. To determine the optimal dose of IR, MEFs were exposed to 0, 2, 3, 5 and 10 Gy from a ^{137}Cs source and seeded at 2500 cells per 60-mm tissue culture dish in triplicate. Cells were incubated for 10 days, fixed and stained and total colony number and cell numbers per colony scored [9]. Cloning efficiency was equivalent in cells exposed to 3–5 Gy and doses in this range were used in all experiments, except for detection of H2AX and 53BP1.

2.2. Colony formation and growth assays

Long-term (10 days) colony formation assays were performed as described above, following 3 Gy exposure. For cell attachment/short-term cloning efficiency and cell growth assays, γ-irradiated (3 Gy) or untreated MEFs were seeded at 100 cells per 35-mm tissue culture dish in triplicate or 3×10^4 cells per well in 24 well plates, respectively. Mass cell growth was measured by the MTT assay [10] and cell number determined from 1 to 5 days after irradiation, at 24 h intervals.

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Abbreviations: HAT, histone acetyltransferase; HDAC, histone deacetylase; IR, ionizing radiation; MEFs, mouse embryonic fibroblasts; MORF4, mortality factor on chromosome 4; MRG15, MORF4-related gene on chromosome 15; TSA, trichostatin A; het, heterozygous

0014-5793/$32.00
doi:10.1016/j.febslet.2007.10.017

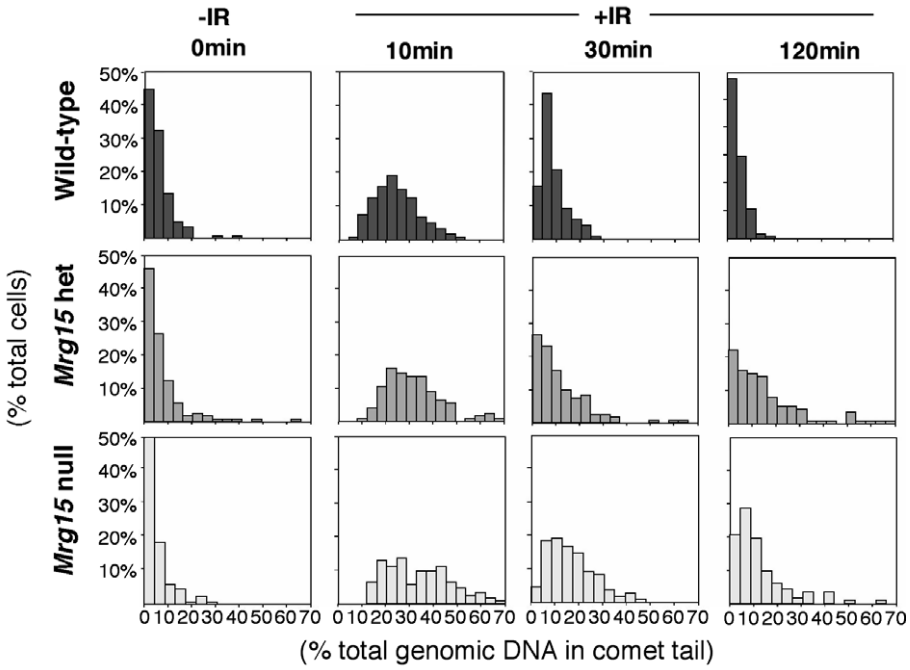

Fig. 1. MRG15 is important for DNA-repair. Wild-type, *Mrg15* het and null MEFs were untreated (−IR) or γ-irradiated at 4 Gy (+IR) and harvested at various times post exposure for comet analysis. Two clones of each genotype were tested. Distributions of percent cells with damaged DNA in tails are shown.

2.3. Strand break detection using the alkaline comet assay

Strand break repair was analyzed by single-cell agarose gel electrophoresis under alkaline conditions as described previously [11]. Cells were irradiated (4 Gy), and harvested immediately or at 30 and 120 min post exposure to IR.

2.4. Immunoblot analysis for detection of histone acetyl-K5-H2A (Ac-H2A) and phosphorylated H2AX (γ-H2AX)

Histone proteins were acid extracted from trichostatin A (TSA)-treated cells (0.4 μM, 16 h) or irradiated cells (10 Gy) at 0, 30, 45, 60, 90 and 180 min post exposure according to manufacturer's instructions (Upstate Biotechnology, Charlottesville, VA). Acid extracted histones from the same number of cells were loaded onto 15% SDS–polyacrylamide gels and Western blotted using anti-acetyl-K5-H2A (abcam, ab1764), anti-phosphorylated H2AX (Ser139) (Upstate, #05-636), or anti-histone H2A (Santa Cruz, sc-10807) antibodies, as described previously [12].

2.5. Indirect immunofluorescence to detect γ-H2AX and 53BP1 foci

Cells were fixed at 0, 30 and 60 min post irradiation (10 Gy) with cold 70% ethanol for 30 min at 4 °C. Nonspecific binding was saturated for 5 min at room temperature in block solution (1% bovine serum albumin and 10% horse serum in PBS). After incubation with anti-γ-H2AX or 53BP1 antibodies, Fluorescein and Texas Red-conjugated secondary antibodies were added. Staining with 0.5 μg/ml DAPI was done for 5 min. A Zeiss AxioVert 200M optical sectioning microscope equipped with a Zeiss AxioCam B&W CCD camera was used to collect digital images and three-dimensional deconvolution performed with the Zeiss software to resolve foci.

3. Results

3.1. Comet assays demonstrate Mrg15 null and heterozygous (het) MEFs are defective in repair of IR induced DNA-damage

MRG15 is an essential component of the NuA4/Tip60-HAT complex that has been shown to promote accessibility to chromatin and, thereby, facilitate recruitment of DNA-repair machinery to sites of DNA-damage in *Drosophila* and mammalian cells [13]. Post DNA-repair, other complexes, such as the Sin3-HDAC complex, in which MRG15 is also a component, have been postulated to restore condensed chromatin at sites of damage to maintain genome integrity. In this study, we analyzed *Mrg15* null and het MEFs to determine if they were defective in DNA-repair in response to IR. We initially quantified DNA-damage using alkaline single-cell agarose gel electrophoresis (comet assay).

MEFs derived from E13.5 wild-type, *Mrg15* null and het embryos [9], were either mock treated (−IR) or exposed to 4 Gy IR (+IR) and harvested at various times post treatment. DNA-damage in −IR was low and no major differences were observed between wild-type, *Mrg15* null and het cells. At 10 min following exposure to IR, wild-type MEFs exhibited a high percentage of DNA in the comet tail, representing damaged DNA. However, by 120 min post exposure the cells had efficiently repaired damaged DNA to levels comparable to −IR controls (Fig. 1). In contrast, the *Mrg15* null and het MEFs had un-repaired DNA in the tail at 120 min. At least two independent clones of MEF cell lines were analyzed for each genetic background and decreased DNA-repair at 120 min was observed in the *Mrg15* null and het MEF clones tested. These results demonstrate that loss of even one copy of MRG15 is sufficient to affect efficient repair of DNA-damage post IR.

3.2. Long- and short-term clonal and growth assays confirm that Mrg15 null and het MEFs have impaired growth, not increased apoptosis, following IR

Based on the results of the comet assay, we determined whether cell growth of *Mrg15* null and het cells was affected