DNA Repair 28 (2015) 73–82

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

BLM protein mitigates formaldehyde-induced genomic instability

Anuradha Kumari[a,b], Nichole Owen[b], Eleonora Juarez[b], Amanda K. McCullough[a,b,*]

[a] Oregon Institute of Occupational Health Sciences, Oregon Health & Science University, Portland, OR 97239 USA
[b] Department of Molecular and Medical Genetics, Oregon Health & Science University, Portland, OR 97239 USA

ARTICLE INFO

Article history:
Received 17 October 2014
Received in revised form 30 January 2015
Accepted 10 February 2015
Available online 19 February 2015

Keywords:
ATM
BLM
53BP1
Double-strand breaks
Formaldehyde

ABSTRACT

Formaldehyde is a reactive aldehyde that has been classified as a class I human carcinogen by the International Agency for Cancer Research. There are growing concerns over the possible adverse health effects related to the occupational and environmental human exposures to formaldehyde. Although formaldehyde-induced DNA and protein adducts have been identified, the genomic instability mechanisms and the cellular tolerance pathways associated with formaldehyde exposure are not fully characterized. This study specifically examines the role of a genome stability protein, Bloom (BLM) in limiting formaldehyde-induced cellular and genetic abnormalities. Here, we show that in the absence of BLM protein, formaldehyde-treated cells exhibited increased cellular sensitivity, an immediate cell cycle arrest, and an accumulation of chromosome radial structures. In addition, live-cell imaging experiments demonstrated that formaldehyde-treated cells are dependent on BLM for timely segregation of daughter cells. Both wild-type and BLM-deficient formaldehyde-treated cells showed an accumulation of 53BP1 and γH2AX foci indicative of DNA double-strand breaks (DSBs); however, relative to wild-type cells, the BLM-deficient cells exhibited delayed repair of formaldehyde-induced DSBs. In response to formaldehyde exposure, we observed co-localization of 53BP1 and BLM foci at the DSB repair site, where ATM-dependent accumulation of formaldehyde-induced BLM foci occurred after the recruitment of 53BP1. Together, these findings highlight the significance of functional interactions among ATM, 53BP1, and BLM proteins as responders associated with the repair and tolerance mechanisms induced by formaldehyde.

1. Introduction

Formaldehyde (HCHO) is a ubiquitous environmental and occupational pollutant. Both chronic and acute exposures to formaldehyde have been associated with adverse effects on human health including eye, nose, throat, and skin irritation, allergic contact dermatitis, altered lung functions and immune responses, occupational asthma, and cancer [1,2]. Based on epidemiological evidence associating formaldehyde exposure with nasopharyngeal cancer and myeloid leukemia [3–5], formaldehyde was classified as a class I human carcinogen by the International Agency on Cancer Research (IARC) in 2006. However, there exists some controversy regarding the nature, magnitude, and persistence of the adverse health effects related to formaldehyde exposure.

The primary DNA lesions resulting from formaldehyde exposure that contribute to its genotoxic and mutagenic potential are considered to be DNA-protein crosslinks (DPCs) [6–10], though the molecular mechanisms that lead to formaldehyde-induced carcinogenesis remain elusive. Formation and persistence of DPCs may pose a formidable challenge to genome stability by interfering with biological processes such as replication, recombination, and transcription. Thus, elucidating the roles of specific DNA repair and tolerance factors involved in DPC processing is important for understanding the mechanisms of formaldehyde-induced carcinogenesis. In this regard, several studies have shown that loss of specific DNA repair factors can promote formaldehyde-induced cellular and cytogenetic abnormalities [11–14].

In order to identify pathways involved in limiting formaldehyde-induced cell death, a formaldehyde cytotoxicity screen of the *Saccharomyces cerevisiae* gene deletion library was previously performed in our lab and multiple pathways were identified that are important for cell survival following formaldehyde exposure. Under low dose, chronic exposure conditions,

Abbreviations: 53BP1, p53 binding protein; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related; CGRP, calcitonin gene-related peptide; BLM, bloom protein; CHO, Chinese Hamster Ovary; DSB, double-strand break; DPC, DNA-protein crosslink.

* Corresponding author at: Department of Molecular and Medical Genetics, Oregon Health & Science University, Portland, OR 97239, USA. Fax: +1 503 494 6831.
E-mail address: mcculloa@ohsu.edu (A.K. McCullough).

http://dx.doi.org/10.1016/j.dnarep.2015.02.010
1568-7864/

homologous recombination was the primary pathway that conferred resistance to formaldehyde-induced lesions; while following acute, high dose exposure, the nucleotide excision repair (NER) pathway was critical for cell survival [15]. Interestingly, this investigation showed that a *sgs1* (a member of the RecQ superfamily) deletion mutant exhibited increased cellular sensitivity to both chronic and acute formaldehyde exposures [15]. Consistent with the yeast study, an *E. coli* RecQ mutant was sensitive to formaldehyde treatment [12]. However, both *E. coli* and yeast studies were limited to measurements of cell viability and did not further investigate the molecular mechanisms mediated by RecQ helicases in limiting formaldehyde-induced cytotoxicity.

Members of the RecQ superfamily are important for maintaining genomic integrity and thus, are referred to as guardians of the genome. Although, there exists only one RecQ family member in bacteria and yeast, in humans, the RecQ superfamily of helicases is comprised of 5 known family members: BLM, WRN, RecQL1, RecQL4, and RecQ5. Among these, *BLM* was the first to be linked to a hereditary disease known as Bloom syndrome (BS) [16]. BS is a rare autosomal recessive disorder characterized by multiple abnormalities, including immunodeficiency, pre- and post-natal growth retardation, and a high incidence of cancer [17]. Biochemical and cellular studies have demonstrated that the BLM protein is a 3′–5′ helicase that participates in critical steps associated with replication, recombination and repair [18]. BLM protein has also been shown to be required for faithful chromosome segregation during mitotic cell division [19,20]. BS cells exhibit chromosomal instability and hypersensitivity in response to several genotoxic agents, including replication stressors, topoisomerase inhibitors, and DNA crosslinking agents [21]; however, the role of BLM following exposure to DNA-protein crosslinking agents remains to be elucidated.

Germane to our interest in formaldehyde-induced genomic instability, this study investigated the potential importance of human BLM in both the DNA damage response and maintenance of genomic integrity following formaldehyde exposure. Herein, we show that BLM rescues formaldehyde-treated cells from G2/M arrest by facilitating the repair of DSBs and regulating normal mitotic progression. Additionally, our studies demonstrate a co-localization of 53BP1 and BLM proteins at sites of formaldehyde-induced DNA damage where the recruitment of BLM protein to the damage sites was found to be ATM-dependent. Overall, our findings suggest an interplay between ATM, 53BP1, and BLM proteins that is critical for mitigating formaldehyde-induced genotoxic and cytotoxic effects.

2. Materials and methods

2.1. Cells and culture conditions

Patient derived BLM-deficient (GM08505) and ATM-deficient (GM05849) SV40-transformed fibroblast cells used in this study were purchased from Coriell Cell Repositories. Wild-type (GM639, also known as GM00639) SV40-transformed cells were a kind gift from Dr. Robb E. Moses (OHSU). Cells were grown in DMEM supplemented with 10% fetal bovine serum and antibiotics (ampicillin and streptomycin, Gibco) at 37 °C in a 5% CO_2 incubator. For all experiments, sub-confluent cultures were treated for 4 h with various concentrations of formaldehyde (Fisher Scientific) and harvested at the indicated recovery times. For Click-iT EdU (5-ethynyl-2′-deoxyuridine) assays, cells were labeled with EdU (10 μM for 1 h) and processed for imaging following the manufacturer's protocol (Invitrogen, C10377).

2.2. Colony forming assays

For colony forming assays, 300–1800 cells were seeded in 100 mm or 6-well plates and incubated overnight at 37 °C prior to acute formaldehyde treatment for 4 h at the indicated concentrations. Following a 4 h treatment, formaldehyde was removed, cells were washed with PBS, and fresh media was added. After 10–15 days, colonies (>30 cells) were fixed, stained with methylene blue diluted in methanol (4 g/L), and counted.

2.3. siRNA transfection

Wild-type cells were transfected with 100 nM scramble (Dharmacon, D001810-01-05) or BLM siRNA cocktail (Dharmacon, M-007287-02-0005) according to the manufacturer's instructions using HiPerFect transfection reagent (QIAGEN). Briefly, HiPerFect (25 μL) was diluted into siRNA- or scramble-containing Opti-MEM (GIBCO) and incubated at room temperature for 10 min to allow the formation of lipid-siRNA complexes. A suspension of GM639 cells (0.2×10^6 cells in 600 μL DMEM) was added to the preformed lipid-siRNA complexes (400 μL) and incubated at room temperature for an additional 10 min. Transfected cells were diluted in DMEM and then seeded in a 12-well plate (0.1×10^6 cells per well). After a 24 h incubation, transfected cells were subjected to acute formaldehyde treatment (0–400 μM) for 4 h. Formaldehyde-treated cells were washed with PBS twice and re-transfected with scramble or BLM siRNA. After 24 h of second transfection, cells were trypsinized and seeded in 6-well plates for colony forming assays. Following 10 days of growth, plates were stained with methylene blue and colonies were counted. In parallel, siRNA- or scramble-treated cells were plated in 60 mm plates and harvested at indicated times for Western blot analyses.

2.4. Cell cycle analyses

Cells were arrested at the G1/S phase boundary by treatment with a replication elongation inhibitor, aphidicolin (1 μg/mL) for 24 h. Prior to formaldehyde treatment, aphidicolin-synchronized cells were allowed a 2 h recovery to facilitate the progression of cells into S phase. Cells were harvested at the indicated times, fixed in ice-cold 70% ethanol, and stained with propidium iodide (PI) (Invitrogen). DNA content was measured using a FACSCalibur instrument (Becton Dickinson) (Flow Cytometry Core, OHSU). Aggregated cells were excluded from the PI-stained cell suspensions by passing them through strainer-capped tubes (BD Falcon) prior to analyses. The results were analyzed using FlowJo software (Tree Star V.7.5).

2.5. Live-cell imaging

For live-cell imaging, wild-type and BLM-deficient cells were plated ($1–3 \times 10^4$ per plate), grown on a glass bottom microwell petridish (P35G-1.5-14-C, Mattek), and treated with formaldehyde (100 μM) for 4 h. Cells were then washed with PBS, and released into fresh media prior to imaging using a VivaView™ FL incubator microscope. For synchronization in S phase, plated cells were treated with aphidicolin (1 μg/mL) overnight, released into fresh media for 2 h prior to formaldehyde treatment. Four random fields were selected per sample and images were captured every 30 min for 6 days. Image brightness and contrast enhancement, and conversion to QuickTime movies were performed with ImageJ software.

2.6. Immunofluorescence studies

Cells were fixed in 4% paraformaldehyde at room temperature for 10 min, washed with PBS buffer, permeabilized with PBS-T