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ScienceDirect
Cellular Signalling 19 (2007) 1879–1892
CELLULAR SIGNALLING
www.elsevier.com/locate/cellsig

Identification of sequences that target BRCA1 to nuclear foci following alkylative DNA damage

Wendy W.Y. Au, Beric R. Henderson *

Westmead Institute for Cancer Research, University of Sydney, Westmead Millennium Institute at Westmead Hospital, Westmead NSW 2145, Australia

Received 4 April 2007; received in revised form 24 April 2007; accepted 24 April 2007
Available online 1 May 2007

Abstract

BRCA1 is a tumor suppressor involved in the maintenance of genome integrity. BRCA1 co-localizes with DNA repair proteins at nuclear foci in response to DNA double-strand breaks caused by ionizing radiation (IR). The response of BRCA1 to agents that elicit DNA single-strand breaks (SSB) is poorly defined. In this study, we compared chemicals that induce SSB repair and observed the most striking nuclear redistribution of BRCA1 following treatment with the alkylating agent methyl methanethiosulfonate (MMTS). In MCF-7 breast cancer cells, MMTS induced movement of endogenous BRCA1 into distinctive nuclear foci that co-stained with the SSB repair protein XRCC1, but not the DSB repair protein γ-H2AX. XRCC1 did not accumulate in foci after ionizing radiation. Moreover, we showed by deletion mapping that different sequences target BRCA1 to nuclear foci induced by MMTS or by ionizing radiation. We identified two core MMTS-responsive sequences in BRCA1: the N-terminal BARD1-binding domain (aa1–304) and the C-terminal sequence aa1078–1312. These sequences individually are ineffective, but together they facilitated BRCA1 localization at MMTS-induced foci. Site-directed mutagenesis of two SQ/TQ motif serines (S1143A and S1280A) in the BRCA1 fusion protein reduced, but did not abolish, targeting to MMTS-inducible foci. This is the first report to describe co-localization of BRCA1 with XRCC1 at SSB repair foci. Our results indicate that BRCA1 requires BARD1 for targeting to different types of DNA lesion, and that distinct C-terminal sequences mediate selective recruitment to sites of double- or single-strand DNA damage.

Keywords: BRCA1; Nuclear foci; DNA repair; DNA damage; BARD1

1. Introduction

Cancer susceptibility is frequently associated with defects in DNA repair pathways [1]. Mutations in the breast cancer susceptibility gene, BRCA1, are found in up to 50% of patients with inheritable breast and ovarian cancer [2]. BRCA1 is a tumor suppressor protein which has been linked to various cellular functions including the DNA damage response, DNA repair, cell-cycle checkpoint activation and chromatin remodeling [3,4]. It displays maximum expression in S-phase cells, localizing predominantly in the nucleus in nuclear domains with proteins such as BARD1, RAD51 and BRCA2 [5–7]. BRCA1 has a zinc-binding RING motif in the amino terminus and two BRCT motifs in the carboxy terminus. The interaction of the BRCA1 and BARD1 RING domains facilitates nuclear localization of the two proteins [8,9], and results in DNA damage-associated ubiquitin ligase activity which can be abolished by the BRCA1 cancer mutation, C61G [10]. The BRCT domain of BRCA1 facilitates a transcriptional activation function [11], and interacts with many different proteins whose binding is disrupted by BRCT cancer mutations [12].

DNA damage signaling pathways are triggered by protein phosphorylation events initiated by a number of phosphoinositide kinase-related kinases [13]. BRCA1 is phosphorylated by ATM and ATR, and forms nuclear foci with ATR upon DNA damage [14]. BRCA1 is a key DNA damage-response protein and undergoes redistribution in the nucleus after DNA damage,

Abbreviations: ATM; ataxia telangiectasia mutated; ATR; ATM and Rad3 related; DMEM; Dulbecco's modified Eagle's medium; DSB; double-strand break; IR; ionizing radiation; EMS; ethyl methanesulfonate; MMTS; methyl methanethiosulfonate; MMS; methyl methanesulfonate; PBS; phosphate-buffered saline; SSB; single-strand break; YFP; yellow fluorescent protein; XRCC1; X-ray repair cross-complementing group 1.

* Corresponding author. Westmead Millennium Institute, Darcy Road (PO Box 412), Westmead NSW 2145, Australia. Tel.: +61 2 9845 9057; fax: +61 2 9845 9102.
E-mail address: beric_henderson@wmi.usyd.edu.au (B.R. Henderson).

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doi:10.1016/j.cellsig.2007.04.010

where it becomes concentrated in nuclear foci. BRCA1 nuclear foci have mostly been studied in cells treated with ionizing radiation (IR) to induce DNA double-strand breaks (DSBs), wherein BRCA1 co-localizes with a range of DNA DSB repair factors including the homologous recombination protein, RAD51 [6], and the end-joining RAD50-MRE11-NBS1 protein complex [15]. However, observations from a number of studies suggest that BRCA1 might also participate in other DNA repair pathways. BRCA1 is hyperphosphorylated [5,16] and forms distinct nuclear foci in response to UV-induced damage (e.g. base dimerization) [17]. Since DNA lesions induced by UV radiation are monitored and processed by nucleotide (NER) and base (BER) excision repair, it is possible that BRCA1 plays a role in these repair pathways. Indeed, inducible expression of BRCA1 in U2OS cells has been shown to increase the expression of the NER protein, XPC [18].

Another well known function of BRCA1 relates to the repair of DNA cross-link damage, which involves the association of BRCA1 with Fanconi anemia proteins, RAD51 and BRCA2 [19]. The lack of wild-type BRCA1 in HCC1937 cells and $BRCA1^{-/-}$ mouse ES cells confers hypersensitivity to the DNA crosslinking agents, mitomycin C [20] and cisplatin [21,22], indicating that BRCA1 is a critical component in repairing this type of damage. Mouse mammary epithelial cells with a conditional BRCA1 knockout and HCC1937 cells are also hypersensitive to treatment with the DNA alkylating chemical and single-strand break (SSB) inducer, methyl methanesulfonate (MMS) [23–26]. The normal DNA damage response can be rescued in these cells by expressing exogenous wild-type BRCA1 protein, but not by the BRCA1 C61G or A1708E mutants [15]. In addition, many BRCA1-associated proteins such as RAD50 [27], RAD51 [28], MDC1 [29], 53BP1 [30] and PCNA [31] were shown to form foci after SSB induction, which further led to speculation that BRCA1 may also participate in this DNA damage repair pathway. The first protein identified to be involved in SSB repair is XRCC1, which forms nuclear foci upon treatment of cells with DNA alkylating agents such as ethyl methanesulfonate (EMS) [32,33]. XRCC1 also forms a protein complex with PARP-1, polynucleotide kinase, DNA polymerase β and DNA ligase III at the sites of DNA breakage [34]. Like BRCA1, some of these SSB repair proteins possess BRCT motifs, which are important for cell survival upon DNA damage and repair of the DNA lesion [32].

The transfer of DNA damage signals is largely dependent on protein phosphorylation initiated by a number of PI3-kinases, including ATR which can be activated by a variety of DNA damage agents. ATR has been implicated in the response of SSB damage, in that expression of the ATR kinase-defective protein in human cells increases cellular sensitivity to MMS [35]. Moreover, ATR phosphorylates Chk1 (a BRCA1 substrate) and activates cell cycle checkpoint after MMS treatment [36]. ATR and BRCA1 were found to co-localize in nuclear foci after UV and IR treatments [14], suggesting that the two proteins may co-operate to activate different types of DNA damage response.

The aim of this study was to investigate the involvement of BRCA1 in the repair of different types of DNA damage, particularly its response to the alkylating agent, methyl methanethiosulfonate (MMTS). BRCA1 nuclear foci induced by MMTS were characterized, and the protein regions that target BRCA1 to these foci were identified. The effect of BRCA1 cancer mutations and ATR-target site mutations on the formation of MMTS-induced foci was also examined.

2. Materials and methods

2.1. Plasmid construction

The BRCA1 cancer mutants and deletion constructs used in this study are shown in Figs. 5–9. Most of the pYFP-BRCA1 plasmids and the pFlag-BARD1 plasmid have been described previously [8,9,37]. Other constructs were derived from the pFlag-BRCA1 wild-type plasmid [38] using PCR and DNA cloning, and YFP coding sequence inserted into a unique Not1 upstream of the BRCA1 sequence within the pFlag-CMV2 plasmid. For the pYFP-BRCA1(1078–1312) plasmid, DNA sequence encoding the BRCA1 amino acids (aa) 1078–1312 was PCR-amplified (by forward primer 5′-TGATGC*GTCGAC*CCAAAATT-GAATGCTATG-3′ and reverse primer 5′-TGATGC*GTCGAC*GGTGTTTG-TATTTGCAG-3′) and cloned into the SalI restriction site of the pEYFP-C1 expression vector (Clontech). The pYFP-BRCA1(N304 + 1078–1312) plasmid was produced by amplifying BRCA1 sequence aa1078–1312 by PCR and inserting it into the pYFP-BRCA1(1–304) plasmid within the EcoRI site. For the pYFP-BRCA1-(N304 + 1078–$1312^{S1143A,S1280A}$) plasmid, the S1143A and S1280A (Serine to Alanine substitutions) (bold) were introduced into the BRCA1 sequence aa 1078–1312 by PCR-based mutagenesis using the forward primer 5′-GTAGTCATGCA**GCT**CAGGTTTG-3′ and reverse primer 5′-ATGTTCCTG**AGC**TGCCTTTGCC-3′. The BRCA1($1078–1312^{S1143A,S1280A}$) fragment was then inserted into the pYFP-BRCA1(1–304) plasmid within the EcoRI site.

2.2. Cell culture and transfection

Human MCF-7 breast cancer cells and U2OS osteosarcoma cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum (FCS), Penicillin (50 U/ml) and Streptomycin (50 μg/ml), at 37 °C in a humidified atmosphere of 5% CO_2. For transfection, cells were seeded on glass coverslips and grown to approximately 50% confluency. For each sample, a maximum of 3 μg of plasmid DNA was transfected, using Lipofectamine 2000 Reagent (Invitrogen) according to the manufacturer's instruction. At 6 h post-transfection, the transfection medium was removed and replaced with the above DMEM medium.

2.3. Western blotting

Western blotting was performed as previously described [37]. Briefly, YFP-BRCA1 constructs were transfected in U2OS cells in 75 cm^2 tissue culture flasks. About 45 h post-transfection, cells were lysed in protein extraction buffer [20 mM Tris–HCl pH8.0, 100 mM NaCl, 1 mM EDTA, 0.5% NP-40 and protease inhibitor] by the freeze-thaw method. Cell lysates were spun at 13,000 rpm for 15 min at 4 °C, and the supernatant (protein extract) was boiled in sample buffer [100 mM Tris–HCl pH6.8, 20% glycerol, 0.01% bromophenol blue, 10% β-mercaptoethanol, and 5% SDS] for 5 min. Protein samples were resolved by polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes. The membranes were blocked in 5% non-fat milk-PBS for 1 h, followed by primary antibody incubation for 2 h. The membranes were incubated in horse radish peroxidase-conjugated secondary antibody for 1 h and then detected using the enhanced chemical luminescence (ECL) Western blotting detection reagent (Amersham Biosciences). YFP-BRCA1 proteins were detected using either anti-green fluorescent protein monoclonal antibody (Roche Applied Science) or the BRCA1 monoclonal antibody Ab1 (Oncogene Research).

2.4. DNA damage treatment and immunostaining

Approximately 45 h post-transfection, cells were either left untreated, or treated with the following chemicals or radiations: 2 mM Hydroxyurea for 2 h;