Published in final edited form as:

Leuk Res. 2012 February ; 36(2): 241–244. doi:10.1016/j.leukres.2011.10.007.

BCR-ABL1 kinase facilitates localization of acetylated histones 3 and 4 on DNA double-strand breaks

Rafal Falinski, Margaret Nieborowska-Skorska, and Tomasz Skorski*

Department of Microbiology and Immunology, School of Medicine, Temple University, Philadelphia, PA, USA

Abstract

BCR-ABL1 kinase-positive leukemia cells accumulate high numbers of DNA double-strand breaks (DSBs) induced by the reactive oxygen species (ROS) or cytotoxic agents. To repair these lesions and prevent apoptosis BCR-ABL1 kinase stimulates the efficiency of DSB repair in leukemia cells. Histone acetylation-dependent chromatin re-modeling plays a crucial role in this process. Here we report that leukemia cells expressing BCR-ABL1 kinase displayed an enhanced histone acetylase activity (HAT) and reduced histone deacetylase activity (HDAC), which was associated with abundant expression of acetylated histone 3 and 4 (Ac-H3 and Ac-H4, respectively). Moreover, Ac-H3 and Ac-H4 readily co-localized with the spontaneous and mitomycin C-induced DSBs in BCR-ABL1 –positive leukemia cells suggesting that histone acetylation and chromatin re-modeling is important for efficient repair of numerous DSBs.

Keywords

BCR-ABL1; leukemia; histone acetylation; DNA repair

1. Introduction

BCR-ABL1 tyrosine kinase transforms hematopoietic stem cells (HSCs) to leukemia stem cells (LSCs) to induce chronic myeloid leukemia in chronic phase (CML-CP). Although ABL tyrosine kinase inhibitors (TKIs), such as imatinib, dasatinib and nilotinib frequently induce complete cytogenetic or major molecular responses (CCyR and MMR, respectively), leukemia cells may acquire additional genetic changes that confer TKI resistance and induce more aggressive blast phase (CML-BP) (1). We, and others reported that cells expressing BCR-ABL1 kinase accumulate DNA double-strand breaks (DSBs) and facilitate DSBs repair by non-homologous end-joining (NHEJ), homologous recombination (HR) and single-strand annealing (SSA). The repair is not faithful and leads to accumulation of genetic aberrations. DSB repair occurs within the context of chromatin, which structure is altered after the damage. Since chromatin structure creates a natural obstacle for the repair mechanisms, histone modifications may play a significant role by increasing the accessibility of DSB and facilitating the assembly of repair proteins. One of the first

© 2011 Elsevier Ltd. All rights reserved.

*Corresponding author: Tomasz Skorski, Department of Microbiology and Immunology, School of Medicine, Temple University, 3400 N. Broad Street, MRB 548, Philadelphia, PA 19140, USA, tel: 215-707-9157, fax: 215-707-9160, tskorski@temple.edu.

The authors declare no conflict of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

modifications is ATM, ATR and DNA-PKcs -dependent phosphorylation of histone 2AX on serine 139 (γ -H2AX) on a megabase chromatin domains involved in DSBs, which has a central function in recruiting and assembly of a specific DNA repair proteins (2). Although the presence of γ -H2AX on DSBs is widely accepted, some of these lesions may be repaired independently of γ -H2AX (3). Next, histone 3 and histone 4 are acetylated on their N-terminal tails (Ac-H3 and Ac-H4, respectively), which could promote DSB repair by destabilizing the higher-order structure of chromatin (4). We have shown previously that BCR-ABL1 kinase enhanced the number of DSBs induced by the endogenous reactive oxygen species (ROS) and by cytotoxic treatment and that this effect was accompanied by upregulation of the expression of γ -H2AX and elevation of the number of γ -H2AX foci (5, 6). Here we investigated if BCR-ABL1 kinase also regulates the expression and presence of Ac-H3 and Ac-H4 at DSBs.

2. Design and methods

32Dcl3 murine hematopoietic parental cells (P) and BCR-ABL1 –32Dcl3 counterparts (B/A) were maintained in the presence of a threshold concentration of IL-3 -rich WEHI-conditioned medium. CML-CP patient cells were obtained from the Stem Cell and Leukemia Core Facility of the University of Pennsylvania, after receiving informed consent. CD34⁺ CML cells were obtained using human CD34⁺ selection cocktail (StemCell Technologies). CD34⁺ cells from healthy volunteer were purchased from Cambrex Bio Science Walkersville. CD34⁺ cells were maintained in medium supplemented with recombinant stem cell factor and granulocyte-macrophage colony-stimulating factor (PeproTech). The research activities involving human samples were approved by the Institutional Review Board. Cells were treated or not with 1 μ M imatinib for 48 hours to inhibit BCR-ABL1 kinase and/or with 0.5 μ g/mL mitomycin C (MMC) for 1 hour to induce DSBs (5). Histone acetylase (HAT) and histone deacetylase (HDAC) activities were measured by the appropriate detection kits (Upstate and Biovision, respectively) following the manufacturers protocols. Protein expression was determined by Western analysis of the total cell lysates with the use of primary antibodies recognizing γ -H2AX, Ac-H3, Ac-H4, caspase-3 and β -actin (Upstate). Nuclear localization of the indicated histones was visualized on cytopins by immunofluorescence as described previously (6).

3. Results and discussion

Since histone acetylation has a leading role in chromatin modification during DSB repair, we tested if HAT and/or HDAC activities were de-regulated in the presence of BCR-ABL1. Using specific assay kits we found that BCR-ABL1 –32Dcl3 leukemia cells displayed enhanced HAT-H3 and HAT-H4 activities and reduced HDAC activity in comparison to parental cells (Fig. 1). Imatinib treatment inhibited HAT-H3 and HAT-H4 and enhanced HDAC activities in BCR-ABL1 –32Dcl3 cells indicating that these effects depend on BCR-ABL1 kinase.

Ac-H3 and Ac-H4 proteins were upregulated in BCR-ABL1 – 32Dcl3 leukemia cells in comparison to parental cells; the overexpression was visible in untreated leukemia cells as well as in those after MMC treatment (Fig. 2). Imatinib diminished the expression of Ac-H3 and Ac-H4 in BCR-ABL1 –32Dcl3 leukemia cells suggesting that the kinase activity is responsible for the effect. BCR-ABL1 kinase-mediated elevation of Ac-H3 and Ac-H4 was accompanied by some fluctuations in the expression of γ -H2AX protein. In concordance with previous report untreated BCR-ABL1 - 32Dcl3 leukemia cells expressed higher levels of γ -H2AX protein than their parental counterparts (Fig. 2), perhaps reflecting the difference in the number of ROS-induced DSBs (6). Three hours after MMC treatment both cell types gained more γ -H2AX expression as they probably accumulated additional DSBs, but as

expected the expression was still higher in BCR-ABL1 -32Dcl3 leukemia cells (5). Twelve hours after MMC treatment parental cells accumulated very high levels of γ -H2AX, conversely, it was barely detectable in BCR-ABL1 -32Dcl3 leukemia cells probably as a result of accelerated DSB repair (5). Incubation of BCR-ABL1-32Dcl3 cells with imatinib reduced the level of “spontaneous” γ -H2AX but its expression was readily detectable at 12 hours after MMC treatment (Fig. 2). Altogether, it appears that BCR-ABL1 kinase enhanced the expression of Ac-H3 and Ac-H4 which coincides with high levels of γ -H2AX in untreated cells as well as in those 3 hours after MMC treatment.

To determine if elevated expression of Ac-H3 and Ac-H4 might have an impact on enhanced DSB repair in leukemia cells (BCR-ABL1 -32Dcl3 and CD34+ CML-CP), we detected Ac-H3 and Ac-H4 on DSBs marked by γ -H2AX nuclear foci. We observed a partial co-localization of γ -H2AX foci only with Ac-H4 in 32Dcl3 parental and CD34+ normal cells 3 hours after MMC treatment; at 12 hours the Ac-H3 and Ac-H4 foci were barely detectable (Fig. 3 and 4). In contrast, partial co-localization of γ -H2AX foci with Ac-H3 was detected in untreated and MMC-treated (3 hours) BCR-ABL1 -32Dcl3 and CD34+ CML-CP cells; in addition, co-localization of γ -H2AX foci and Ac-H4 was readily detectable in these cells at 3 hours after the treatment. These effects were abrogated by incubation of BCR-ABL1 -32Dcl3 leukemia cells with imatinib (Fig. 3), suggesting that chromatin remodeling around DSBs is facilitated by BCR-ABL1 kinase.

Twelve hours after MMC treatment Ac-H3 and Ac-H4 foci predominated in BCR-ABL1 -32Dcl3 and CD34+ CML-CP cells; conversely numerous γ -H2AX foci persisted in parental and CD34+ normal cells (Fig. 3 and 4), and in BCR-ABL1 -32Dcl3 leukemia cells treated with imatinib (Fig. 3), in concordance with Western analysis (Fig. 2). Numerous Ac-H3 and Ac-H4 foci in the absence of γ -H2AX foci may indicate active transcription in BCR-ABL1 leukemia cells successfully recovering from DNA damage stress. The abundance of persisted γ -H2AX foci may represent irreparable DNA damage in normal cells undergoing apoptosis. This hypothesis is supported by detection of an abundant active caspase 3 in 32Dcl3 parental cells and in BCR-ABL1 -32Dcl3 cells treated with imatinib, in comparison to imatinib-naive BCR-ABL1 -32Dcl3 leukemia cells (Fig. 2).

In summary, we detected accumulation and enhanced localization of Ac-H3 and Ac-H4 on DSBs in BCR-ABL1 -positive leukemia cells. Our observation is in concordance with other report indicating “loss-of-function” of HDAC1 and hyperacetylation of H4 associated with the presence of BCR-ABL1 kinase (7). Since Ac-H3 and Ac-H4 can play an important role in efficient DSB repair, we suggest that their localization on DSBs may facilitate the repair in BCR-ABL1 -positive leukemia cells (8).

Acknowledgments

This work was supported by the grants R01CA089052 and R01CA123014 from NCI. RF and MN-S performed experiments, and TS designed the studies and wrote the manuscript.

References

1. Perrotti D, Jamieson C, Goldman J, Skorski T. Chronic myeloid leukemia: mechanisms of blastic transformation. *J Clin Invest.* 2010 Jul 1; 120(7):2254–2264. [PubMed: 20592475]
2. Kuo LJ, Yang LX. Gamma-H2AX - a novel biomarker for DNA double-strand breaks. *In Vivo.* 2008 May-Jun; 22(3):305–309. [PubMed: 18610740]
3. Yuan J, Chen J. MRE11-RAD50-NBS1 complex dictates DNA repair independent of H2AX. *J Biol Chem.* 2010 Jan 8; 285(2):1097–1104. [PubMed: 19910469]
4. Wurtele H, Verreault A. Histone post-translational modifications and the response to DNA double-strand breaks. *Curr Opin Cell Biol.* 2006 Apr; 18(2):137–144. [PubMed: 16487697]

5. Nieborowska-Skorska M, Stoklosa T, Datta M, Czechowska A, Rink L, Slupianek A, et al. ATR-Chk1 axis protects BCR/ABL leukemia cells from the lethal effect of DNA double-strand breaks. *Cell Cycle*. 2006 May; 5(9):994–1000. [PubMed: 16687921]
6. Nowicki MO, Falinski R, Koptyra M, Slupianek A, Stoklosa T, Gloc E, et al. BCR/ABL oncogenic kinase promotes unfaithful repair of the reactive oxygen species-dependent DNA double-strand breaks. *Blood*. 2004; 104(12):3746–3753. [PubMed: 15304390]
7. Brusa G, Zuffa E, Mancini M, Benvenuti M, Calonghi N, Barbieri E, et al. P210 Bcr-abl tyrosine kinase interaction with histone deacetylase 1 modifies histone H4 acetylation and chromatin structure of chronic myeloid leukaemia haematopoietic progenitors. *Br J Haematol*. 2006 Feb; 132(3):359–369. [PubMed: 16409301]
8. Lee HS, Park JH, Kim SJ, Kwon SJ, Kwon J. A cooperative activation loop among SWI/SNF, gamma-H2AX and H3 acetylation for DNA double-strand break repair. *EMBO J*. 2010 Apr 21; 29(8):1434–1445. [PubMed: 20224553]

Fig. 1. BCR-ABL1 regulates histone acetylation/deacetylation. Total cell extracts were prepared before (0), and 3 hours (3) and 12 hours (12) after MMC treatment of 32Dcl3 cells (P), BCR-ABL1 -32Dcl3 cells (B/A), and BCR-ABL1 -32Dcl3 cells pre-treated with imatinib (IM). HDAC and HAT activities for H3 and H4 were determined using specific kits.

Fig. 2. BCR-ABL kinase modulates the expression of Ac-H3, Ac-H4 and γ -H2AX. Parental cells (P), BCR-ABL1 -32Dcl3 leukemia cells (B/A) and B/A cells pre-incubated with imatinib (IM) were untreated (0) or treated with MMC for 3 hours (3) and 12 hours (12). The expressions of Ac-H3, Ac-H4, γ -H2AX and active caspase-3 were detected by Western analysis; actin served as loading control.

Fig. 3. BCR-ABL1 kinase enhances co-localization of Ac-H3 and Ac-H4 with DSBs. Parental cells (P), BCR-ABL1 -32Dcl3 leukemia cells (B/A) and B/A cells pre-incubated with imatinib (IM) were untreated (0) or treated with MMC for 3 hours (3) and 12 hours (12). Co-localization (yellow) of γ -H2AX foci (red) with Ac-H3 foci (green) or Ac-H4 foci (green) was detected by immunofluorescence on the cytopins (Ac-H3 + γ -H2AX and Ac-H4 + γ -H2AX, respectively). Nuclei were stained with DAPI (blue). Representative cells in each group are shown.

Fig. 4.

Enhanced co-localization of Ac-H3 and Ac-H4 with DSBs in CD34+ CML-CP cells. CD34+ cells from healthy donor (N) and CML-CP patient (CML-CP) were untreated (0) or treated with MMC for 3 hours (3) and 12 hours (12). Co-localization (yellow) of γ -H2AX foci (red) with Ac-H3 foci (green) or Ac-H4 foci (green) was detected by immunofluorescence on the cytopins (Ac-H3 + γ -H2AX and Ac-H4 + γ -H2AX, respectively). Nuclei were stained with DAPI (blue). Representative cells in each group are shown.