c-ABL tyrosine kinase stabilizes RAD51 chromatin association

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To The Editor:

I would like to submit a paper entitled " c-ABL TYROSINE KINASE STABILIZES RAD51 CHROMATIN ASSOCIATION " by Drs. Shimizu et al for publication in Biochemical and Biophysical Research Communications. Our manuscript describes a new role for c-ABL in regulating RAD51 functions. I have received approval for this manuscript to be submitted.

In this paper, by using self-association defective RAD51 mutants, we show that the phosphorylation of RAD51 by c-ABL stabilizes RAD51 chromatin association. This activation is abolished by replacement of Tyr-315 with Phe, indicating that the phosphorylation of Tyr-315 is the origin of this activation. However, c-ABL cannot restore the defect of the self-association defective mutants in IR-induced nuclear focus formation, suggesting that c-ABL functions during the early phase of RAD51 chromatin assembly, before RAD51 nucleo-protein filament formation. Our findings thus suggest a new model for the regulation of early steps of homologous recombination repair.

We are very happy if you kindly consider our manuscript for possible publication in Biochemical and Biophysical Research Communications.

Sincerely yours,

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c-ABL tyrosine kinase stabilizes RAD51 chromatin association

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Abstract

The assembly of RAD51 recombinase on DNA substrates at sites of breakage is essential for their repair by homologous recombination repair (HRR). The signaling pathway that triggers RAD51 assembly at damage sites to form subnuclear foci is unclear. Here, we provide evidence that c-ABL, a tyrosine kinase activated by DNA damage which phosphorylates RAD51 on Tyr-315, works at a previously unrecognized, proximal step to initiate RAD51 assembly. We first show that c-ABL associates with chromatin after DNA damage in a manner dependent on its kinase activity. Using RAD51 mutants that are unable to oligomerize to form a nucleoprotein filament, we separate RAD51 assembly on DNA to form foci into two steps: stable chromatin association followed by oligomerization. We show that phosphorylation on Tyr-315 by c-ABL is required for chromatin association of oligomerization-defective RAD51 mutants, but is insufficient to restore oligomerization. Our findings suggest a new model for the regulation of early steps of HRR.

Keywords: homologous recombination repair, RAD51, BRCA2, ATM, c-ABL, tyrosine phosphorylation.

Introduction

HRR is a major pathway for the resolution of DNA double-strand breaks (DSBs) in the somatic cells of higher eukaryotes, and is mediated by RAD51, the eukaryal orthologue of bacterial RecA. A key step in HRR involves the assembly of RAD51 onto DNA substrates at the site of DNA breakage to form an ordered, helical nucleoprotein filament, which catalyzes homologous pairing and the strand exchange reaction [1]. This RAD51 assembly is marked in cells by the formation of nuclear foci containing RAD51 in vertebrate cells of avian, rodent or human origin. Genetic studies in these organisms demonstrate that a number of ancillary molecules are necessary for RAD51 focus formation after DNA damage. These include the breast cancer suppressor, BRCA2, as well as the RAD51 paralogues XRCC2, XRCC3 and RAD51B-D. Available evidence suggests that these ancillary molecules assist RAD51 focus formation either by working as 'recombination mediators', which displace proteins like replication protein A from DNA to enable RAD51 nucleation, or by directly promoting RAD51 loading [2, 3]. An important unresolved question concerns the nature of the signaling process that triggers RAD51 assembly at sites of DNA breakage.

A c-ABL, ubiquitously expressed non-receptor-type tyrosine kinase, is activated by ionizing radiation (IR) in an ATM-dependent manner [4, 5] and plays important roles in growth arrest and cell death [6]. The results of previous studies indicate that c-ABL is involved in HRR through the phosphorylation of RAD51. c-ABL was shown to inhibit binding of RAD51 to DNA in vitro by phosphorylating it on Tyr-54 in one study [7]; the other study showed that c-ABL phosphorylates RAD51 on Tyr-315 and this phosphorylation enhances the association of RAD51 with RAD52, one of recombination mediator proteins [8]. In addition, the oncogenic form of c-ABL fusion kinase (BCR/ABL) has been shown to enhance RAD51 expression and to phosphorylate it on Tyr-315, resulting in resistance to drugs such as cisplatin and mitomycin C [9]. On the other hand, we have shown that *arg*$^{-/-}$ (ARG is the only other known member of the c-ABL family) and *atm*$^{-/-}$ DT40 chicken B cells show a mild impairment in RAD51 focus formation and HRR capacity [10-12]. More recently, a reduction in IR-induced RAD51 focus formation has also been observed in *atm*$^{-/-}$ and *abl*$^{-/-}$ mouse embryonic fibroblasts and ATM-deficient human cells [13]. These findings imply that, in higher vertebrates, c-ABL family plays some regulatory roles in RAD51 functions in an ATM-dependent manner, though they are not apparently essential components of the HRR machinery. However, how these ABL tyrosine kinase members mechanistically regulate in vivo RAD51 functions remains to be established.

In the present study, we show that c-ABL is recruited and activated in the chromatin in its kinase activity dependent manners. By using self-association defective RAD51 mutants, we further show that the phosphorylation of RAD51 by c-ABL stabilizes RAD51 chromatin association. This activation is abolished by replacement of Tyr-315 with Phe, indicating that the phosphorylation of Tyr-315 is the origin of this activation. However, c-ABL cannot restore the defect of the self-association defective mutants in IR-induced nuclear focus formation, suggesting that c-ABL functions during the early phase of RAD51 chromatin assembly, before RAD51 nucleo-protein filament formation.

MATERIALS AND METHODS

Expression plasmids. Tyr-54 and Tyr-315 of human RAD51 were mutated to Phe and Arg-167 of human RAD51 was mutated to Gly, using a PCR strategy. These RAD51 mutant cDNAs were then inserted into pEF-BOS expression vector containing HA-tag. (HA-RAD51-Y54F, HA-RAD51-Y315F and HA-RAD51-R167G). RAD51wt and R167G cDNAs were also inserted into the pFLAG-CMV-2 expression vector (Sigma) (Flag-RAD51-wt and Flag-RAD51-R167G). Using HA-RAD51-R167G as a template, we constructed HA-RAD51-R167G/Y54F and HA-RAD51-R167G/Y315F expression plasmids by PCR strategy.

Cell culture, transfection, and subcellular fractionation. 293T human embryonic kidney cells were cultured in DMEM supplemented with 10% FBS and antibiotics, in a 5% CO2 incubator at 37ºC. Transient transfection were performed by using FuGENE 6 Transfection Reagent (Roche). Subcellular fractionation was carried out essentially as described [14].

Antibodies. Antibodies recognizing specifically the phosphorylated human RAD51 (either at Tyr-54 or Tyr-315) were generated by immunization of rabbit with the ovalbumin (OVA)-conjugated peptides (NeoMPS, France): the phosphopeptide TVEAVAY(PO_3H_2)APKKELINIKGIC was used to generate the antibody recognizing RAD51 phosphorylated at Tyr-54 and the phosphopeptide KIY(PO_3H_2)DSPCLPEAEAMFY for antibody recognizing RAD51 phosphorylated at Tyr-315. The antibodies were purified by using affinity chromatography with on Sulfolink gels [9] conjugated with the corresponding phosphorylated and unphosphorylated peptides, as described by the manufacture. Rabbit anti-human RAD51 antibody (a gift of Dr. Akira Shinohara) was used for RAD51 focus detection

and immunoblot analysis as a primary antibody. Other primary antibodies for immunoblot analysis were: mouse monoclonal antibodies; anti-Flag M5, anti-β-actin AC15, anti-α-tubulin B-5-1-22 (these were purchased from Sigma.), anti-human BRCA2 Ab-1 (CALBIOCHEM), anti-Histone H3 6-6-2 (Upstate); rabbit anti-GFP antibody (abcam). Secondary antibodies for immunoblot analysis were: goat anti-rabbit IgG horseradish peroxidase conjugated (CHEMICON) and peroxidase labeled goat anti-mouse IgG (FUNAKOSHI). Protein G Sepharose beads (GE Healthcare) was used in immunoprecipitation.

Results and Discussion

The RAD51 protein is known to form helical filament structures, which is considered to be the active form for homologous pairing and strand exchange. Structural based mutagenesis revealed that the Phe-86 residue is directly involved in the monomer-monomer interaction of the human RAD51 filament [15, 16]. The human RAD51 filament model constructed with the reported crystal structure of the archaeal RadA RAD51 filament [17] suggests that the Arg-167 residue is also located in the monomer-monomer interface of the active filament of RAD51 (Supplementary Fig. 1). We therefore replaced Arg-167 of human RAD51 by Gly, which does not have side chain moiety, and studied whether this mutation (R167G) influences monomer-monomer interactions involved in RAD51 self-association, like the substitution of Phe-86 with Glu (F86E) does. Flag-tagged wild-type (WT) or R167G mutant RAD51 proteins were, thus, transiently expressed in 293T cells, and examined for their ability to bind endogenous RAD51 and BRCA2 with or without IR stimulation, by using co-immunoprecipitation method. We found that RAD51-WT and RAD51-R167G proteins displayed comparable abilities to bind endogenous BRCA2. However, while the RAD51-WT protein showed the IR-inducible ability to bind endogenous RAD51, RAD51-R167G completely lost the ability to interact with endogenous RAD51 (Supplementary Fig. 2A). The inability of RAD51-R167G to interact with other RAD51 molecules in vivo was also confirmed by the results of co-immunoprecipitation experiments with co-expressed HA-tagged RAD51-R167G and Flag-tagged RAD51-WT (data not shown).

To establish the validity of the results of experiments with the RAD51-R167G mutant, we

analyzed the ability of GFP-tagged RAD51-F86E or RAD51-SA208-209ED (Ser-208 and Ala-209 were replaced with Glu and Asp, respectively) mutants to interact with endogenous RAD51 or BRCA2. In agreement with the results of the previous studies [15, 16], GFP-RAD51-SA208-209ED retained the ability to bind endogenous RAD51, but was unable to bind endogenous BRCA2. On the other hand, GFP-RAD51-F86E retained the ability to bind endogenous BRCA2, but showed the reduced ability to interact with endogenous RAD51 (Supplementary Fig. 2B).

Our previous in vitro studies showed that mutant RAD51 proteins, in which Tyr-315 is replaced with Asp or Glu to mimic phosphorylation, were proficient in double-strand DNA (dsDNA) binding, but were defective in dsDNA unwinding, probably due to defect in filament formation on dsDNA [18]. Consistent with these findings, fluorescence spectroscopic analysis of the RAD51-Y315W mutant suggested that Tyr-315 is closed to the subunit-subunit interacting site [19] (see also Supplementary Fig1). Thus, it is of interest to study whether c-ABL has any effects on in vivo behaviors of self-association-defective RAD51 mutants. Since RAD51filament formation in vivo is a complex cellular process, especially in higher vertebrate cells, requiring transport to and assembly in chromatin, before RAD51 nucleoprotein filament formation on damaged DNA [14, 20], we examined effects of c-ABL on nuclear transport or chromatin association of RAD51. HA-tagged RAD51-WT or RAD51-R167G was transiently co-expressed with Flag-tagged active (wt) or kinase-dead (kd) c-ABL in 293T cells, and cells were fractionated into cytoplasmic, nuclear and chromatin-associated fractions. Anti-human RAD51 antibodies were used to detect endogenous RAD51 as well as transiently expressed HA-tagged RAD51, and a-tubulin and Histone H3 were used as specific makers for the soluble cytoplasmic and insoluble chromatin-associated fractions, respectively. As overall distributions of RAD51-WT, RAD51-R167G, endogenous RAD51 and c-ABL were comparable in the cytoplasmic and nuclear fractions (data not shown), data for protein levels in the nuclear fraction was not shown for simplicity.

Fig. 1A reveals several interesting findings. Firstly, while we found extensive chromatin association of c-ABL-wt (first row, lanes 8 and 11), the chromatin association of c-ABL-kd was minimal (first row, lanes 9 and 12), indicating that the efficient chromatin association of c-ABL requires intact tyrosine kinase activity. This was further confirmed by the results of experiment with imatinib, a specific inhibitor of c-ABL [21], that imatinib effectively inhibits

c-ABL chromatin association (Supplementary Fig. 3). Interestingly, the electrophoretic mobility of c-ABL-wt in the chromatin fraction (lanes 8 and 11) was significantly retarded as compared with those of c-ABL-kd in the chromatin fraction (lanes 9 and 12) or c-ABL-wt in the soluble cytoplasmic fraction (lanes 2 and 5). This suggests that c-ABL is auto-phosphorylated in the chromatin fraction, and is consistent with the fact that c-ABL-wt but not c-ABL-kd in the chromatin fraction is tyrosine-phosphorylated (unpublished data). Secondly, we found extensive chromatin association of not only transfected RAD51-wt but also endogenous RAD51 (second row, lane 7). Since the chromatin association of endogenous RAD51 was not detectable in the absence of transfected RAD51 (unpublished data), the extensive chromatin association of transfected RAD51-wt as well as endogenous RAD51 appeared to be stimulated by transfection procedures. However, when cells were transfected with RAD51-R167G, the chromatin association of not only transfected RAD51-R167G but also endogenous RAD51 was minimal (second row, lane10). This is consistent with the finding that RAD51-R167 failed to associate with endogenous RAD51 (Supplementary Fig. 2A), and further suggest that the observed defect of RAD51-R167G in chromatin association is primarily due to its inability to self-associate. Finally, we found that co-transfection of c-ABL-wt but not c-ABL-kd with RAD51-R167G greatly enhanced the chromatin association of not only RAD51-R167G but also endogenous RAD51 (second row, lanes 10-12); slight enhancement of the chromatin association of transfected RAD51-wt and endogenous RAD51 by c-ABL-wt but not by c-ABL-kd expression was also observed (second row, lanes 7-9). We also found that imatinib effectively inhibited c-ABL-mediated enhancement of RAD51-R167G chromatin association (Supplementary Fig. 3).

To study whether c-ABL actually phosphorylates Tyr-54 or Tyr-315 on RAD51 under the conditions studied, HA-tagged wild-type RAD51 or various mutants, RAD51-R167G, RAD51-Y54F or RAD51-Y315F (Tyr-54 and Tyr-315 were substituted with Phe in Y54F and Y315F, respectively) were transiently co-expressed with Flag-tagged c-ABL-wt in 293T cells, and the cytoplasmic and chromatin fractions were subjected to immunoblot analysis using antibodies specific for phosphorylated Tyr-54 or Tyr-315. As shown in Fig. 1B, c-ABL phosphorylated both of Tyr-54 and Tyr-315 on RAD51-WT (third and fourth rows, lane 10) and RAD51-R167G (third and fourth rows, lane 16), but not Tyr-54 on RAD51-Y54F (third row, lane 12), or Tyr-315 on RAD51-Y315F (fourth row, lane 14). Interestingly, we detected

the phosphorylation of Tyr-54 and Tyr-315 by c-ABL only in the chromatin fraction. This is consistent with the findings that c-ABL auto-phosphorylation, which is required for c-ABL activation [22, 23], is detected only in the chromatin fraction, as described above. To test the generality of this finding that c-ABL restore the defective chromatin association of self-association-defective RAD51-R167G, GFP-tagged RAD51-WT or RAD51-F86E were transiently co-expressed with Flag-tagged c-ABL-wt or c-ABL-kd in 293T cells and the cytoplasmic and chromatin fractions were subjected to immunoblot analysis. As shown in Fig. 1C, when cells were transfected with GFP-RAD51-F86E, the chromatin association of not only transfected GFP-RAD51-F86E but also endogenous RAD51 was minimal (second and third rows, lane 10), and cotransfection of c-ABL-wt but not of c-ABL-kd enhanced the chromatin association of not only transfected GF-RAD51-F86E but also endogenous RAD51 (second and third rows, lanes10-12). We also observed the phosphorylation of GFP-RAD51-WT and GFP-RAD51-F86E on Tyr-54 only in the chromatin fraction (fourth row, lanes 8 and 11), though the phosphorylation of Tyr-315 was detected both the cytoplasmic and chromatin fractions (fifth row, lanes 2, 5, 8 and 11). The reason for this difference is not clear at present time. It is possible that the large GFP-tag but not the HA-tag influences the in vivo phosphorylation by c-ABL.

The self-association defective GFP-RAD51-F86E mutant was previously shown to be defective in IR-induced nuclear focus formation [15]. In agreement with this finding, and consistent with the results shown in the Fig 1 that GFP-RAD51-F86E cannot stably associate with the chromatin, GFP-RAD51-F86E did not form typical RAD51 nuclear foci following IR stimulation, and a significant chromatin staining was not observed (Fig 2A, right panel). The RAD51-R167G mutant also showed defective IR-induced nuclear focus formation and could not stably associates with chromatin (Fig 2A, left panel), as expected. Since c-ABL can restore the chromatin association of these self-association-defective RAD51 mutants as shown in Fig 1, we were interested to examine whether c-ABL can restore the defect of these RAD51 mutant in IR-induced nuclear focus formation. We therefore transiently co-expressed these RAD51 mutants with c-ABL in 293T cells, with or without IR stimulation. As shown in Fig. 2B, c-ABL co-expression restored the chromatin association of these mutants, but not typical IR-induced nuclear focus formation. Thus, c-ABL can restore the stable chromatin association of self-association-defective RAD51 mutants, but cannot restore the defective IR-induced

nuclear focus formation, which depends on the ability of RAD51 to self-associate. Since c-ABL can phosphorylate both of Tyr-54 and Tyr-315 on these self-association-defective RAD51 mutants in the chromatin fraction (Fig. 1), it was of interest to study which tyrosine residue is involved in the restoration of the stable chromatin association of self-association-defective RAD51 mutants. We therefore substituted Tyr-54 or Tyr-315 with Phe in RAD51-R167G to create RAD51-R167G/Y54F or RAD51-R167G/Y315F. These HA-tagged RAD51 mutants (RAD51-R167G, RAD51-R167G/Y54F and RAD51-R167G/Y315F) were then transiently co-expressed with Flag-c-ABL in 293T cells. As shown in Fig. 2C, while the mutation of Tyr-54 did not influenced the ability of c-ABL to restore the chromatin-association defect of RAD51-R167G, the mutation of Tyr-315 abrogated the ability of c-ABL to restore the chromatin-association defect of this mutant, indicating that the phosphorylation of Tyr-315 plays a critical role in the restoration of the stable chromatin association of self-association-defective RAD51 mutants.

It is now well established that BRCA2 plays an essential role in the chromatin assembly of RAD51 during HRR [3]. Since we have not been so far able to detect a stable interaction between BRCA2 and c-ABL in vivo and c-ABL can bind to the chromatin in BRCA2-deficient Capan cells (unpublished data), it seems likely that c-ABL is recruited to chromatin independently of BRCA2, where it phosphorylates RAD51 transported to sites of DNA damage by BRCA2. This phosphorylation, mainly on Tyr-315, enhances/stabilizes the chromatin association of RAD51 proteins, assisting their assembly into an active nucleoprotein intermediate, a step which is again dependent on BRCA2 [3]. This hypothesis is consistent with the location of Tyr-315 in the possible 3-D RAD51 filament structure, which is located at the edge of the subunit-subunit interface and exposed on the inner surface of filament structure (see Supplementary Fig. 1). c-ABL cannot restore the ability of the self-association defective mutants to form IR-induced nuclear foci. Therefore, c-ABL may conceivably function at an early step in the recruitment of RAD51 to chromatin, which precedes nucleoprotein filament formation via RAD51-RAD51 self-association. We have not so far been successful in detecting in vivo Tyr-54/Tyr-315 phosphorylation of endogenous RAD51 following IR stimulation (unpublished data), raising the possibility that RAD51 modification by c-ABL may only be transient. Overall, this hypothetical proposal for c-ABL-mediated regulation of RAD51 in vivo is consistent with the results of our previous in vitro

study showing that the replacement of Tyr-315 with acidic Asp or Glu residues, mimicking constitutive Tyr-315 phosphorylation, impairs dsDNA binding [18].

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References

1. West, S.C., Molecular views of recombination proteins and their control. Mol. Cell. 4 (2003) 435-445.
2. Sung, P. and H. Klein, Mechanism of homologous recombination: mediators and helicases take on regulatory functions. Proc. Natl. Acad. Sci. U.S.A. 486 (2006) 217-247.
3. Shivji, M.K.K. and A.R. Venkitaraman, DNA recombination, chromosomal stability and carcinogenesis: insights into the role of BRCA2. DNA Repair. 3 (2004) 835-843.
4. Baskaran, R., Wood, L.D., Whitaker, L.L., et al., Ataxia telangiectasia mutant protein activates c-Abl tyrosine kinase in response to ionizing radiation. Nature 387 (1997) 516-519.
5. Shafman, T., Khanna, K.K., Kedar, P., et al., Interaction between ATM protein and c-Abl in response to DNA damage. Nature 387 (1997) 520-523.
6. Wang, J.Y.J., Regulation of cell death by the Abl tyrosine kinase. Oncogene 19 (2000) 5643-5650.
7. Yuan, Z.-M., Huang, Y., Ishiko, T., et al., Regulation of Rad51 function by c-Abl in response to DNA damage. J. Biol. Chem. 273 (1998) 3799-3802.
8. Chen, G., Yuan, S.S., Liu, W., et al., Radiation-induced assembly of Rad51 and Rad52 recombination complex requires ATM and c-Abl. J Biol Chem. 274 (1999) 12748-12752.
9. Slupianek, A., Schmutte, C., Tombline, G., et al., BCR/ABL regulates mammalian RecA homologs, resulting in drug resistance. Mol. Cell. 8 (2001) 795-806.
10. Takao, N., Kato, H., Mori, R., et al., Disruption of ATM in p53-null cells causes multiple functional abnormalities in cellular response to ionizing radiation. Oncogene 18 (1999) 7002-7009.
11. Takao, N., Mori, R., Kato, H., et al., c-Abl tyrosine kinase is not essential for ataxia telangiectasia mutated functions in chromosomal maintenance. J. Biol. Chem. 275 (2000) 725-728.
12. Li, Y., Shimizu, H., Xiang, S., et al., Arg tyrosine kinase is involved in homologous recombinational DNA repair. Biochem. Biophys. Res. Comm. 299 (2002) 697-702.

13. Yuan, S.S., H. Chang, and E.Y. Lee, Ionizing Radiation-induced Rad51 nuclear forcus formation is cell cycle-regulated and defective in both $ATM^{-/-}$ and $c\text{-}Abl^{-/-}$ cells. Mutation Res. 525 (2003) 85-92.
14. Tarsounas, M., D. Davies, and S.C. West, BRCA2-dependent and independent formation of Rad51 nuclear foci. Oncogene 22 (2003) 1115-1123.
15. Pellegrini, L., Yu, D.S., Lo, T., et al., Insights into DNA recombination from the structure of a Rad51-BRCA2 complex. Nature 420 (2002) 287-293.
16. Yu, D.S., Sonoda, E., Takeda, S., et al., Dynamic control of Rad51 recombinase by self-association and interaction with BRCA2. Mol. Cell 12 (2003) 1029-1041.
17. Wu, Y., Qian, X., He, Y., et al., Crystal structure of an ATPase-active form of Rad51 homolog from Methanococcus voltae. J Biol Chem. 280 (2005) 722-728.
18. Takizawa, Y., kinebuchi, T., Kagawa, W., et al., Mutational analyses of the human Rad51-Tyr315 residu, a site for phosphorylation in leukaemia cells. Genes to Cells 9 (2004) 781-790.
19. Conilleau, S., Takizawa, Y., Tachuwana, H., et al., Localization of tyrosine 315, a target for phosphorylation by cAbl tyrosine kinase, at the edge of the subunit-subunit interface of the human Rad51 filament. J. Mol. Biol. 339 (2004) 797-804.
20. Davies, A.A., Masson, J., Mcllwraith, M.J., et al., Role of BRCA2 in control of the Rad51recombination and DNA repair protein. Mol. Cell 7 (2001) 273-282.
21. Noble, M.E.M., J.A. Endicott, and L.N. Johnson, Protein kinase inhibitors: insights into drug design from structure. Science,303 (2004) 1800-1805.
22. Brasher, B.B. and A.V. Etten, c-Abl has high intrinsic tyrosine kinase activity that is stimulated by mutation of the Src homology 3 domain and by autophosphorylation at two distinct regulatory tyrosines. J. Biol. Chem. 275 (2001) 35631-35637.
23. Dorey, K., Engen, J.R., Kretzschmar, J., et al., Phosphorylation and structure-based functional studies reveal a positive and a negative role for the activation loop of the c-Abl tyrosine kinase. Oncogene 20 (2001) 8075-8084.

Figure Legends

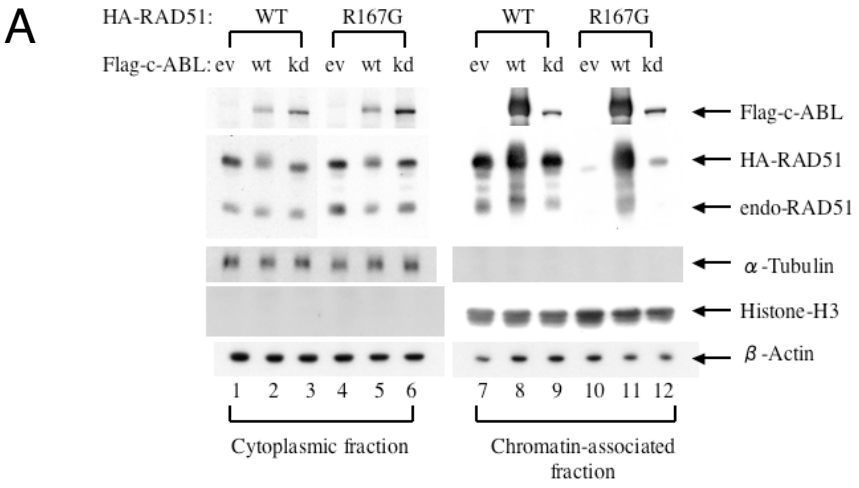
Figure 1. c-ABL restores the chromatin association of self-association-defective RAD51 mutants. (A) Flag-tagged wild-type (wt: lanes 2, 5, 8, 11) or kinase-dead (kd: lanes 3, 6, 9, 12) c-ABL and HA-tagged wild-type (WT: lanes 1-3 and 7-9) or R167G mutant (lanes 4-6 and 10-12) RAD51 expression vectors were transiently co-transfected into 293T cells in various combinations as indicated: in lanes 1, 4, 7 and 10, empty vectors (ev) were transfected. Forty-eight hour after transfection, cells were fractionated into the cytoplasmic (lanes 1-6) and chromatin-associated (lanes 7-12) fractions, and these fractions were subjected to immunoblot analysis with anti-Flag (c-ABL, first row), anti-human RAD51 (HA-RAD51 and endo-RAD51, second row), anti-a-Tubulin, anti-Histone-H3 and anti-b-Actin antibodies; HA-RAD51 and endo-RAD51 denotes transfected HA-tagged RAD51 and endogeneous RAD51,

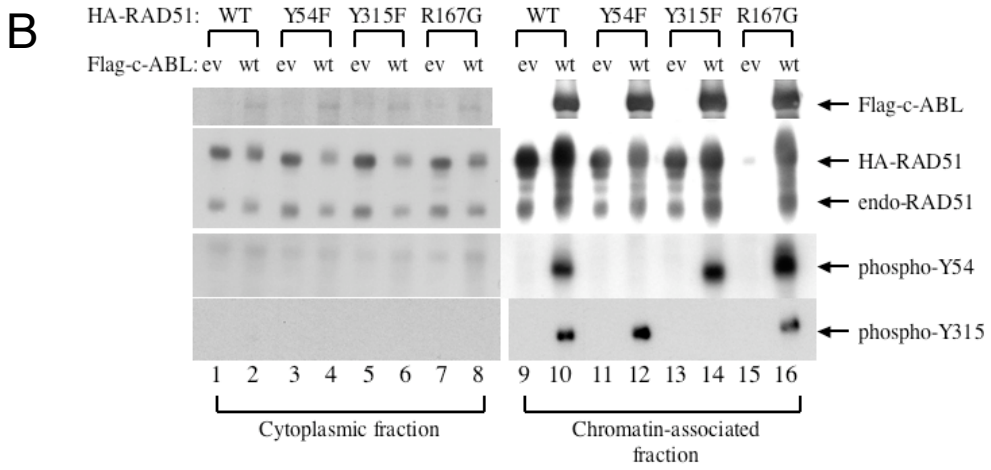
respectively. **(B)** Flag-tagged wild-type c-ABL (wt: lanes 2, 4, 6, 8, 10, 12, 14, 16) and HA-tagged wild-type (WT: lanes 1-2 and 9-10) or various mutants (Y54F, lanes 3-4 and 11-12; Y315F, lanes 5-6 and 13-14; R167G, lanes 7-8 and 15-16) RAD51 expression vectors were transiently co-transfected into 293T cells in various combinations as indicated: empty vectors (ev) were transfected in lanes 1, 3, 5, 7, 9, 11, 13, 15. Forty-eight hour after transfection, cells were fractionated into the cytoplasmic (lanes 1-8) and chromatin-associated (lane 9-16) fractions. These fractions were subjected to immunoblot analysis with anti-Flag (c-ABL, first row), anti-human RAD51 (HA-RAD51 and endo-RAD51, second row), anti-phospho-Y54 (third row), or anti-phospho-Y315 (fourth row) antibodies. **(C)** Flag-tagged empty (ev: lanes 1, 4, 7 and 10), wild-type (wt: lanes 2, 5, 8 and 11) or kinase-dead (kd: lanes 3, 6, 9 and 12) c-ABL and GFP-tagged wild-type (WT: lanes 1-3 and 7-9) or mutant (F86E: lanes 4-6 and 10-12) RAD51 expression vectors were transiently co-transfected into 293T cells in various combinations as indicated. Forty-eight hour after transfection, cells were fractionated into the cytoplasmic (lanes 1-6) and chromatin-associated (lane 7-12) fractions, and these fractions were subjected to immunoblot analysis with anti-Flag (c-ABL, first row), anti-GFP (GFP-RAD51, second row), anti-RAD51 (endo-RAD51, third row), anti-phospho-Y54 (fourth row) or anti-phospho-Y315 (fifth row) antibodies.

Figure 2. The restoration of defective chromatin association of RAD51-R167G by c-ABL is dependent on Tyr-315.

(A) Forty-eight hour after transfection with the HA-tagged or GFP-tagged wild-type (WT), or mutant (HA-R167G or GFP-F86E) RAD51 expression vectors into 293T cells, cells were exposed to IR (10 Gy) or untreated (Control). Three hour after IR treatment, RAD51 foci were visualized with anti-RAD51 antibodies. **(B)** HA-tagged RAD51-R167G or GFP-tagged RAD51-F86E and Flag-tagged empty (vector) or c-ABL expression vectors were transiently co-transfected into 293T cells as indicated. **(C)** HA-tagged RAD51 mutant (R167G, R167G/Y54F and R167G/Y315F) and Flag-tagged empty (vector) or c-ABL expression vectors were transiently co-transfected into 293T cells in as indicated.

Figure





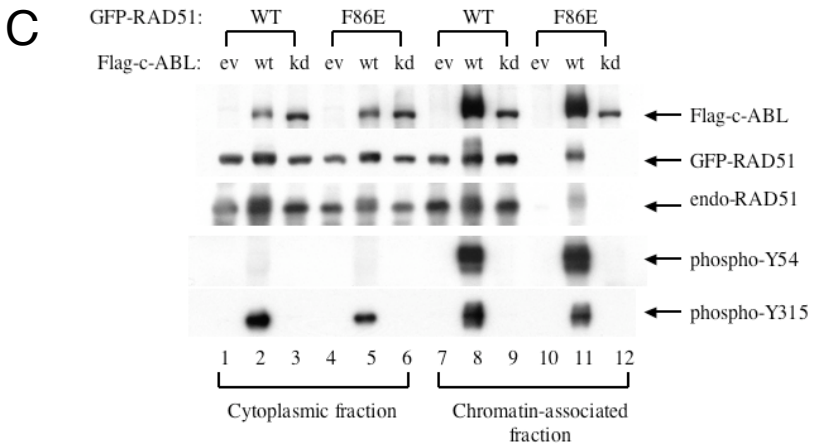


Figure 1

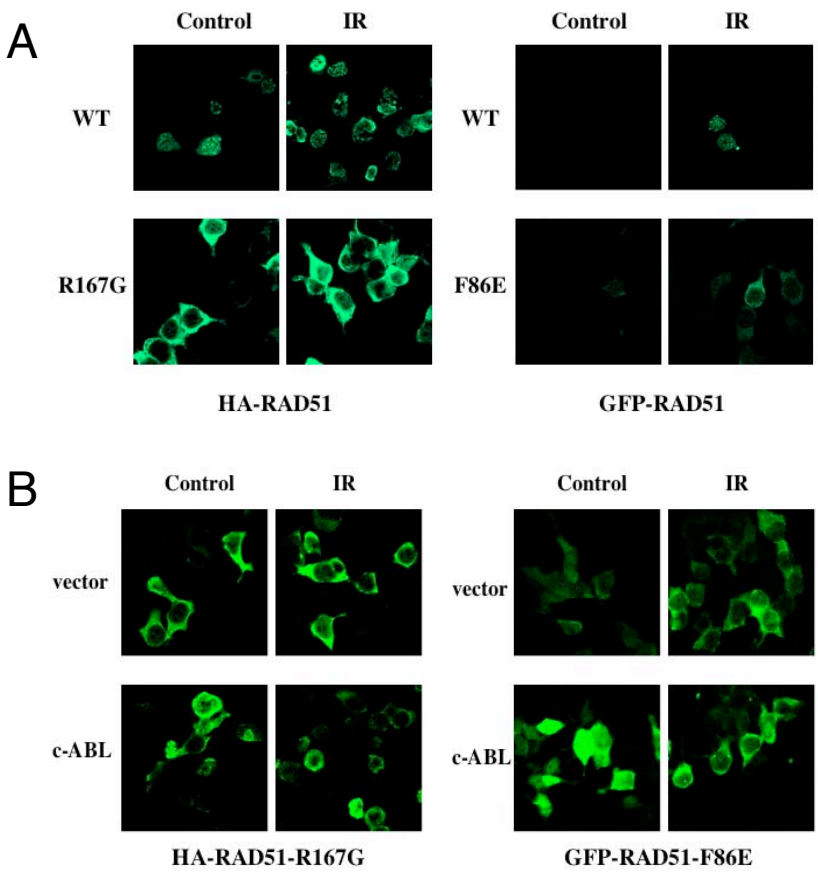

A
Control
IR
Control
IR
WT
WT
R167G
F86E
HA-RAD51
GFP-RAD51
B
Control
IR
Control
IR
vector
vector
c-ABL
c-ABL
HA-RAD51-R167G
GFP-RAD51-F86E
C
HA-RAD51:
R167G
Y54F/R167G
R167G/Y315F
vector
c-ABL

Figure 2

Electronic Supplementary Material (online publication only)

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