Reciprocal Regulation between DNA-PKcs and Snail1 Conferring Genomic Instability

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1. Introduction

Although the roles of DNA-dependent protein kinase catalytic subunit (DNA-PKcs) involving non-homologous end joining (NHEJ) of DNA repair are well recognized, the biological mechanisms and regulators by which DNA-PKcs regulate genomic instability are not clearly defined. We show herein that DNA-PKcs activity resulting from DNA damage caused by ionizing radiation (IR) phosphorylates Snail1 at serine 100, which results in increased Snail1 expression and its function by inhibition of GSK-3-mediated phosphorylation. Furthermore, Snail1 phosphorylated at serine 100 can reciprocally inhibit kinase activity of DNA-PKcs, resulting in an inhibition to recruit DNA-PKcs or Ku70/80 to a DNA double-strand break site, and ultimately inhibition of DNA repair activity. The impairment of repair activity by a direct interaction between Snail1 and DNA-PKcs increases the resistance to DNA damaging agents, such as IR, and genomic instability. Our findings provide a novel cellular mechanism for induction of genomic instability by reciprocal regulation of DNA-PKcs and Snail1.

2. Materials and Methods

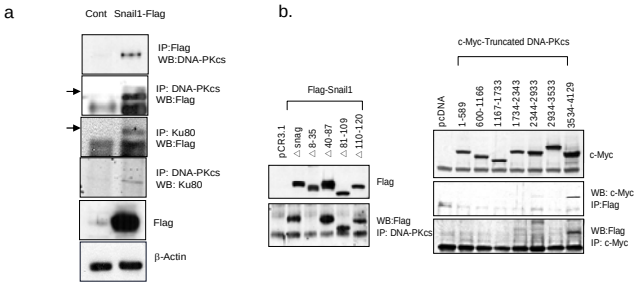
We performed Immunoprecipitation and Western blotting to study interaction between snail1 and DNA-PKcs. In addition, an kinase assay was performed in vitro to measure DNA-PKcs activity, and a trypan blue dye exclusion assay, colony forming assay and PI staining were conducted to evaluate the radioresistance of Snail1. Linear dsDNA-associated protein pull-down assay and comet assay were performed for detect of DNA-damage.

3. Results

Snail1 interacts with DNA-PKcs

When tetracycline (Tet) was added to MCF7 cells with an inducible Snail1-Flag-Tet construct, the Flag tagged Snail1 protein level was increased. Interaction of Flag with DNA-PKcs was also observed in this system, as well as the binding activity of DNA-PKcs and Ku80, indicating that induction of Snail1 affected the binding activity between DNA-PKcs and Ku80, as well as the binding activity between Snail1 and DNA-PKcs (Fig. 1a). The binding sites of Snail1 that interacted with DNA-PKcs were amino acid sequences 8–35. The binding sequences of DNA-PKcs that interacted with Snail1 were amino acid sequences 3534-4129 of the kinase domain (Fig. 1b)

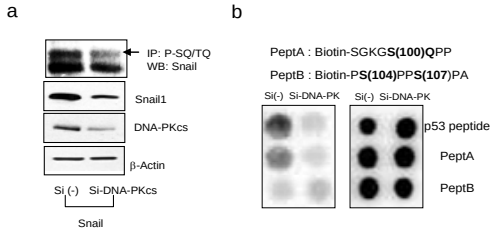
Fig.1 Snail1 interacts with DNA-PKcs.

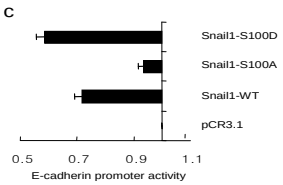


DNA-PKcs phosphorylates Snail1 at serine 100 and the interaction between the two molecules is essential for Snail phosphorylation.

we examined the Snail1 sequences in detail and found that Snail1 has one SQ/TQ site at serine 100. Phosphorylation of Snail1 at the SQ/TQ site was observed in cells that overexpressed Snail1 and treatment of cells with Si-DNA-PKcs blocked this phosphorylation (Fig. 2a). We also determined DNA-PKcs kinase activity using two types of biotin-labeled peptides as substrates; one peptide contained amino acid sequences 96–103 of Snail1, which has the SQ/TQ site (PeptA) and the other peptide contained amino acid sequences 103–109 of Snail1, which does not have the SQ/TQ site (PeptB). A higher level of DNA-PKcs kinase activity was shown when PeptA was used as a substrate, which was similar for the DNA-PKcs kinase activity when a p53 peptide was used as a substrate. However, when PeptB was used as a substrate, the DNA-PKcs kinase activity was inhibited, which was similar to Si-DNA-PKcs-treated cells. Inhibition of E-cadherin promoter activity by wild-type (WT)-Snail1 was restored by S100A. In the case of S100D, inhibition of E-cadherin activity was still present, as occurred for WT-Snail1 (Fig. 2c).

Fig. 2.DNA-PKcs phosphorylates Snail1 at serine 100 and the interaction between the two molecules is essential for Snail phosphorylation

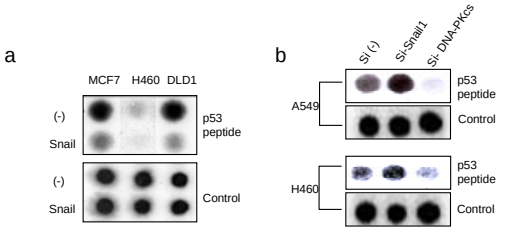




The radiation-induced kinase activity of DNA-PKcs is inhibited by Snail1

As the kinase activity of DNA-PKcs is important for DNA repair activity, we examined whether binding of Snail1 with DNA-PKcs affected the kinase activity of DNA-PKcs. Snail1 overexpression to NCI-H460, DLD2, and MCF7 cells that showed endogenously low expression of Snail1 inhibited DNA-PKcs kinase activity when a p53 peptide was used as a substrate substrate. In addition, transfection of Si-Snail1 into NCI-H460 and A549 cells showed an adverse effect. We treated cells with Si-DNA-PKcs as a positive control (Fig. 3a). Radiation-induced DNA-PKcs kinase activity was also inhibited by Snail1 overexpression (Fig. 3b).

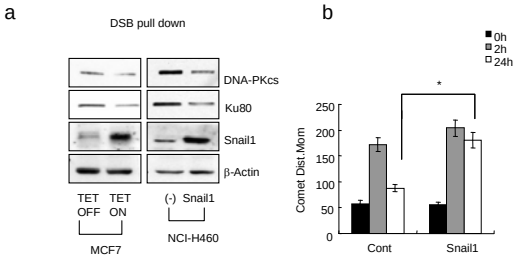
Fig. 3. Snail1 inhibits the radiation-induced kinase activity of DNA-PKcs.



Recruitment of DNA-PKcs to DNA double-strand breaks is inhibited by Snail1

A DSB pull-down assay using double-strand DNA demonstrated that Snail1 overexpression inhibited recruitment of DNA-PKcs or Ku80 to DNA DSB sites using several cell systems (Fig.4a). To elucidate the physiologic relevance, we examined DSB repair activity. As has been suggested previously, Snail1 overexpression potentiates IR-induced comet tail formation. At 24 hours after exposure to a dose of 10 Gy IR, control cells showed recovery of DSB damage; however, cells that overexpressed Snail1 showed sustained DSB damage (Fig. 4b)

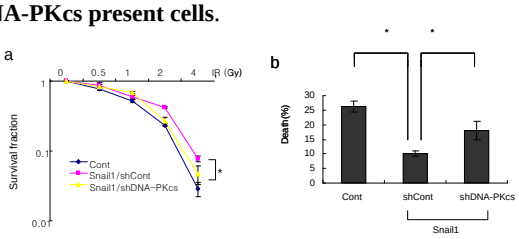
Fig. 4. Snail1 inhibits the recruitment of DNA-PKcs to DNA double strand breaks.



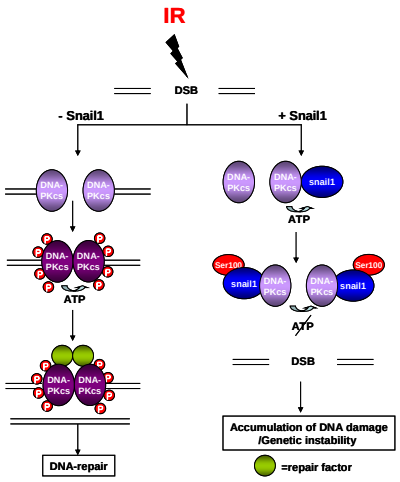
Snail does not show the resistance to DNA damage of IR in DNA-PKcs-depleted cells

Clonogenic survival studies have shown that Snail1 overexpression showed resistance to DNA damaging agents, such as 5 Gy IR for DLD cells. This effect did not occur when cells were stably transfected with Sh-RNA of DNA-PKcs (Sh-DNA-PKcs; Fig 5a). Cell death data also suggested that Snail1 overexpression increased survival after IR, while Sh-DNA-PKcs transfection restored these effects (Fig. 5b)

Fig. 5. Snail inhibits radiation-induced cell death only in DNA-PKcs present cells.



4. Conclusion



Our findings have demonstrated that the interaction of Snail1 with DNA-PKcs phosphorylated Snail1 at serine 100 by DNA-PKcs. Phosphorylated Snail1 increased repression of Snail1 promoter activity and invasiveness, which may contribute to the development of various malignancies. Phosphorylated Snail1 showed feedback inhibition of DNA-PKcs kinase activity, which resulted in the inhibition of recruitment of DNA-PKcs and Ku to a DSB site results in impaired DNA repair activity and finally induction of genomic instability.

References

[1] Lees-Miller, S.P. and Meek, K. (2003) Repair of DNA double strand breaks by non-homologous end joining. *Biochimie*, **85**, 1161-1173.

[2] Christmann, M., Tomicic, M.T., Roos, W.P. and Kaina, B. (2003) Mechanisms of human DNA repair: an update. *Toxicology*, **193**, 3-34.