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RAPID COMMUNICATION

Ubiquitination and degradation of SIK2 by DNA-PKcs deficiency promote radiation-induced mitotic catastrophe

Salt-inducible kinase 2 (SIK2) is a member of the AMP-activated serine/threonine kinase family. It has been reported that inhibition of SIK2 can enhance the cytotoxicity of paclitaxel,[1] promote premitotic apoptosis, and lead to cell cycle arrest in the metaphase.[2] Thus, targeting SIK2 may be a therapeutic strategy for cancers drug and radiotherapy resistance. Mitotic catastrophe is a type of abnormal mitosis leading to cell death characterized by the multipolar spindle and multinucleation, which was first discovered during an ionizing radiation (IR)-induced cell damage.[3] However, the mechanism of mitotic catastrophe is not well understood. The present study aimed to assess the effect of the knockdown of SIK2 on IR-induced mitotic catastrophe.

SIK2 protein content increased following IR stimulation, depending on the time and dose of IR (Fig. S1A, B). To further clarify the role of SIK2 in mitosis catastrophic induced by IR, we performed inhibition of SIK2 while irradiating with ^{60}Co γ-rays (Fig. S1C) and the presence of abnormal spindles such as tripole and quadrupole spindles was detected in HeLa cells. Furthermore, compared to the IR treatment alone group, the IR treatment with simultaneous knockdown of the SIK2 group showed an increased proportion of abnormal spindles from approximately 30%–50% in cells (Fig. 1A; Fig. S1D). The number of multinuclear cells also significantly increased (Fig. 1B; Fig. S1E). To summarize, these data suggest that the knockdown of SIK2 promotes IR-induced cellular mitotic catastrophe.

The yeast two-hybrid assay was performed to verify further the specific mechanism by which SIK2 regulates mitotic catastrophe, and the results showed that SIK2 might interact with DNA-PKcs (Fig. S2A). DNA-PKcs is well known for playing a key role in repairing DNA double-strand breaks (DSBs). DNA-PKcs has also been documented to regulate the mitotic process of radiation-damaged cells and is involved in maintaining the stability of the centrosome and spindle structure.[4] Consistent with previous studies, the deficiency of DNA-PKcs with IR stimulation for 48 h increased the number of multipolar spindles from 30% to 50%, and the number of multinuclear cells increased from 10% to 20% (Fig. S2B–D). These findings suggested that DNA-PKcs deficiency promotes IR-induced cellular mitotic catastrophe to a degree comparable to SIK2 knockdown. Next, the interaction between SIK2 and DNA-PKcs was assessed in the HeLa cells by co-immunoprecipitation (CO-IP) assay, and GST pulldown assay further suggested that SIK2 and DNA-PKcs can interact directly (Fig. 1C; Fig. S2E). DNA-PKcs T2609 and DNA-PKcs S2056 were colocalized with SIK2 at the centrosomes, as observed in immunofluorescence analysis (Fig. S2F). We next elucidated whether SIK2 can affect IR-induced mitotic catastrophe through its interaction with DNA-PKcs.

The knockdown or inactivation of DNA-PKcs decreased the content of the SIK2 protein (Fig. 1D; Fig. S3A). Moreover, in HeLa cell lines with stably knockdown of DNA-PKcs, compared to the control group, the number of abnormal cellular spindles decreased from 50% to 30% by over-expression of SIK2, and the proportion of multinuclear cells decreased from 17% to 8% (Fig. S3B–D). Collectively, the knockdown of DNA-PKcs can promote IR-induced mitotic catastrophe by decreasing the protein content of SIK2.

The GST pulldown assay showed that GST-SIK2 (1–926), GST-SIK2 (280–926), and GST-SIK2 (400–926) interacted with DNA-PKcs, indicating the region in which SIK2 interacts with DNA-PKcs. In contrast, GST-SIK2 (700–926) did not interact with DNA-PKcs. These results suggest the presence of some sites on SIK2 (400–700) that interact with DNA-PKcs. Therefore, we constructed the SIK2Δ400–700 plasmid for SIK2 knockout of the 400–700 amino acid residues to disrupt SIK2 interaction with DNA-PKcs (Fig. S3E, F). Our data showed that over-expression of the SIK2Δ400–700 plasmid caused a significant increase in the number of abnormal spindles and multinuclear cells induced by IR

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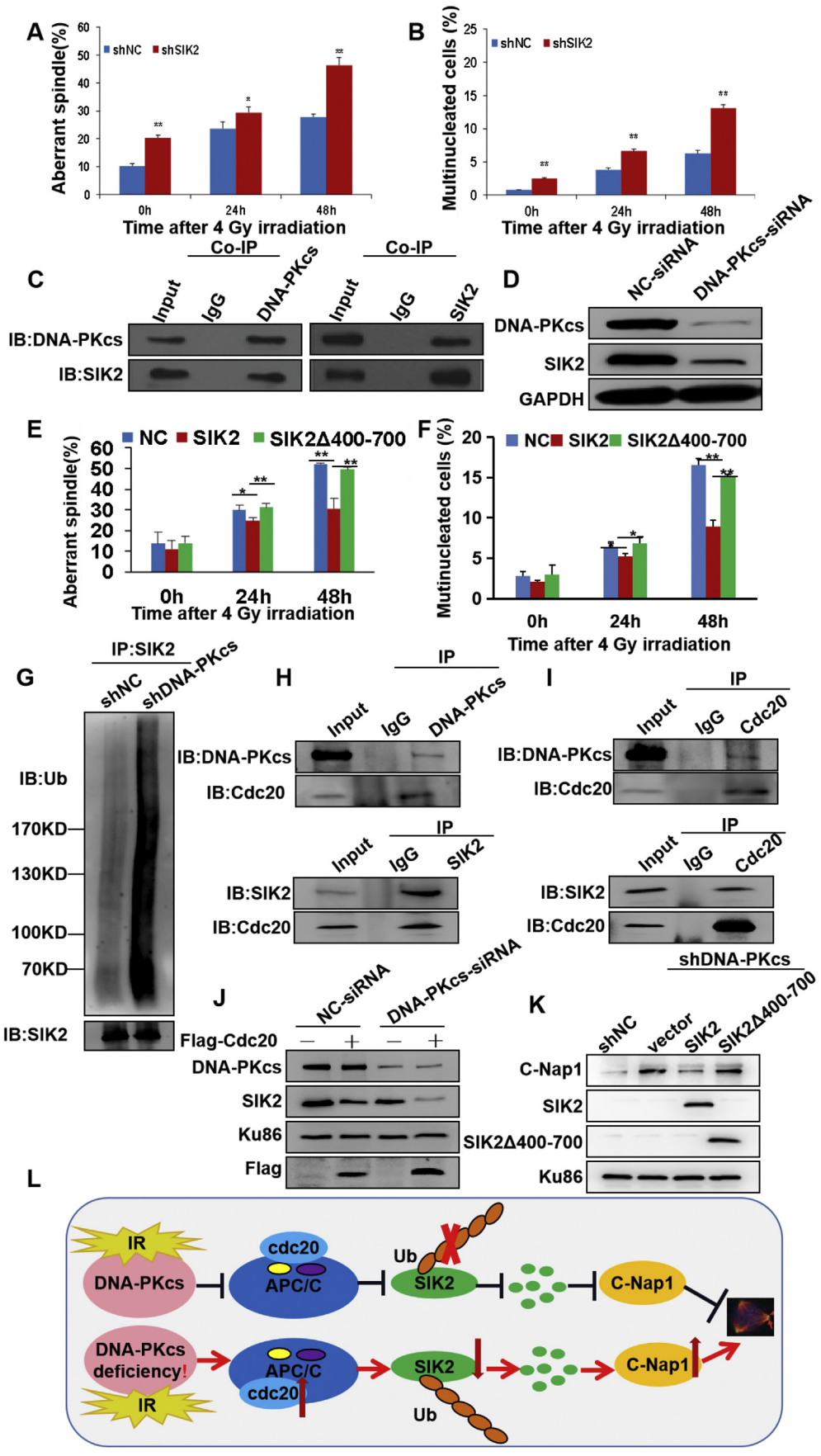
A
Aberrant spindle(%)
shNC
shSIK2
Time after 4 Gy irradiation
0h
24h
48h
B
Multinucleated cells (%)
C
Co-IP
Input
IgG
DNA-PKcs
SIK2
IB:DNA-PKcs
IB:SIK2
D
NC-siRNA
DNA-PKcs-siRNA
DNA-PKcs
SIK2
GAPDH
E
NC
SIK2
SIK2Δ400-700
F
G
IP:SIK2
shNC
shDNA-PKcs
IB:Ub
170KD
130KD
100KD
70KD
H
IP
I
Cdc20
J
Flag-Cdc20
Ku86
Flag
K
shDNA-PKcs
vector
C-Nap1
L
IR
cdc20
APC/C
Ub
DNA-PKcs deficiency!

compared with the over-expression of SIK2 (Fig. 1E, F). Therefore, SIK2 of 400—700 amino acids interacts with DNA-PKcs to reduce SIK2 protein content and participate in mitotic catastrophe caused by IR.

Next, we investigated how the knockdown of DNA-PKcs inhibits SIK2. Both the knockdown and inactivation of DNA-PKcs significantly promoted the degradation of SIK2 protein (Fig. S4A—D). The ubiquitination and degradation of SIK2 were also increased after the knockdown or inactivation of DNA-PKcs (Fig. 1G; Fig. S4E). We then identified how DNA-PKcs regulates the ubiquitination and degradation of SIK2. The ubiquitin ligase APC/Cdc20 complex is involved in split sister chromosome segregation and spindle depolymerization,[5] which may be related to DNA-PKcs-mediated degradation and ubiquitination of SIK2. The Co-IP assay results revealed that DNA-PKcs and SIK2 interact with Cdc20 (Fig. 1H, I), while the knockdown or inactivation of DNA-PKcs increased Cdc20 protein content (Fig. 5SA—D), which was achieved by inhibiting the degradation of Cdc20 (Fig. 6SA—D). This finding raised the possibility of whether Cdc20 plays a role in the knockdown of DNA-PKcs, leading to the ubiquitination and degradation of SIK2. In support of this possibility, we found that over-expression of Cdc20 decreased the protein content of SIK2, while the suppression of Cdc20 with the knockdown of DNA-PKcs increased the protein content of SIK2 (Fig. 6SE, F). In contrast, the over-expression of Cdc20 with the knockdown of DNA-PKcs inhibited the protein expression of SIK2 (Fig. 1J). These data indicated that DNA-PKcs regulates the ubiquitination and degradation of SIK2 through APC/Cdc20.

C- Nap1 is one of the core proteins that regulate centrosome condensation during mitosis, and it may thus play a role in regulating cellular mitotic catastrophe. To test this hypothesis, we performed knockdown of C-Nap1 in HeLa cells and found that both the proportion of abnormal spindles and the number of multinuclear cells were decreased (Fig. 7SA—C). We further elucidated whether C-Nap1 is involved in inhibiting SIK2 by IR-induced cellular mitotic catastrophe. The protein expression of C-Nap1 was significantly increased in cells with the knockdown of DNA-PKcs (Fig. 7SD). However, when HeLa cells with knockdown of DNA-PKcs over-expressed SIK2, the protein expression of C-Nap1 was decreased.

Moreover, the protein expression of C-Nap1 was increased in the presence of the SIK2Δ400—700 plasmid (Fig. 1K). These findings demonstrated that DNA-PKcs-mediated regulation of C-Nap1 protein expression through SIK2 may be involved in IR-induced cellular mitotic catastrophe. Furthermore, the mechanism by which SIK2 reduces C-Nap1 protein expression might be concluded as the following aspects: On the one hand, SIK2 may regulate C-Nap1 expression level at the transcription level through some key transcription factors. On the other hand, SIK2, as a serine and threonine protein kinase, may regulate C-Nap1 phosphorylation to reduce the protein expression of C-Nap1. SIK2 may also promote C-Nap1 protein degradation by modulating the activity of other proteins. The inhibition of C-Nap1 expression by SIK2 may be due to these mechanisms.

Overall, we have demonstrated for the first time that increased SIK2 ubiquitination and degradation caused by DNA-PKcs knockdown could promote IR-induced cellular mitotic catastrophe via an increase in C-Nap1 protein expression (Fig. 1L). This work provides a molecular basis for cellular mitotic catastrophe and primary experimental data for increasing sensitivity and targeting of cancer radiotherapy.

Author contributions

*__Jiaojiao Zhu__: Data curation; Writing the manuscript. *__Ying Zhang__: Performing the experiments; Data analysis. *__Ziyan Yan__: Performing the experiments; Writing-original draft. __Jianxiao Wang__: Critical regents. __Ping Wang__: Assisting with the experiment. __Xinxin Liang__: Assisting with the experiment. __Yuhao Liu__: Assisting with the experiment. __Xingkun Ao__: Assisting with the experiment. __Maoxiang Zhu__: Study concept. #__Pingkun Zhou__: Study concept; Supervision. #__Yongqing Gu__: Study concept; Critical design; Supervision; Validation.

Figure 1 Ubiquitination and degradation of SIK2 by DNA-PKcs deficiency promote radiation-induced mitotic catastrophe. **(A)** Knockdown of SIK2 in HeLa cells with 4 Gy ^{60}Co γ-ray irradiation, and immunofluorescence staining result for determining HeLa cells with aberrant spindles, which was calculated as the proportion of aberrant cells in more than 200 dividing phase cells, including cells with tripole spindles, quadrupole spindles, and multipolar spindles. **(B)** Immunofluorescence staining result for multinuclear HeLa cells, determined as the proportion of multinuclear cells in more than 200 dividing phase cells. **(C)** Determination of the interaction of DNA-PKcs and SIK2 by Co-IP. **(D)** Knockdown of DNA-PKcs, Western blot analysis of SIK2 protein content in HeLa cells. **(E)** Over-expression of SIK2Δ400—700 in HeLa cells with 4 Gy ^{60}Co γ-ray irradiation, and immunofluorescence staining result for determining HeLa cells with aberrant spindles, which was calculated as the proportion of aberrant cells in more than 200 dividing phase cells, including cells with tripole spindles, quadrupole spindles, and multipolar spindles. **(F)** Immunofluorescence staining of multinuclear HeLa cells, the cells were taken as the proportion of multinuclear cells in more than 200 dividing phase cells. **(G)** The ubiquitination level of SIK2 was determined after the knockdown of DNA-PKcs. **(H, I)** Co-IP assay for the interaction of DNA-PKcs and Cdc20, SIK2 and Cdc20 in HeLa cells. **(J)** Western blot assay of SIK2 protein content in HeLa cells following overexpression of Cdc20 and knockdown of DNA-PKcs. **(K)** Western blot analysis of C-Nap1 protein content in HeLa cells with overexpression of SIK2 or SIK2 Δ400—700 plasmid. **(L)** This schematic shows that DNA-PKcs promotes SIK2 ubiquitination and degradation through Cdc20, and SIK2 is involved in the cellular mitotic catastrophe by regulating the expression of C-Nap1. Data are expressed as mean and standard deviation ($\bar{x}(—) \pm SD$); *P*-values were determined by *t*-test in **(A, B)**; *P*-values were determined by one-way ANOVA in **(E, F)**; $^{*}P < 0.05$ and $^{**}P < 0.01$ indicate significant differences.

Conflict of interests

Authors declare no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.02.004.

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