Biochemical and Biophysical Research Communications 554 (2021) 76–82

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

FBX4 mediates rapid cyclin D1 proteolysis upon DNA damage in immortalized esophageal epithelial cells

Jia Liu [a, b], Hui Yang [a], Pak Yan Cheung [b], Sai Wah Tsao [b], Liang Lv [c, **], Annie L.M Cheung [b, *]

[a] Center of Medical Research, The Second Xiangya Hospital of Central South University, Changsha, Hunan, PR China
[b] School of Biomedical Sciences, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong, China
[c] Department of Gastroenterology, The Second Xiangya Hospital of Central South University, Changsha, Hunan, PR China

ARTICLE INFO

Article history:
Received 4 March 2021
Accepted 16 March 2021
Available online 27 March 2021

Keywords:
Cyclin D1
F-box protein 4
ATM
DNA damage Response
Immortalized esophageal epithelial cells

ABSTRACT

It has been implied that deregulation of cyclin D1 turnover under stresses can facilitate genomic instability and trigger tumorigenesis. Much focus has been placed on identifying the E3 ligases responsible for mediating cyclin D1 degradation. However, the findings were quite controversial and cell type-dependent. Little is known about how cyclin D1 is regulated in precancerous cells upon DNA damage and which E3 ligases mediate the effects. Here we found cyclin D1 reduction is an early response to DNA damage in immortalized esophageal epithelial cells, with expression dropping to a low level within 1 h after γ-irradiation. Comparison of temporal expression of cyclin D1 upon DNA damage between immortalized NE083-hTERT and NE083-E6E7, the latter being p53/p21-defective, showed that DNA damage-induced rapid cyclin D1 reduction was p53-independent and occurred before p21 accumulation. Overexpression of cyclin D1 in NE083-E6E7 cells could attenuate G0/G1 cell cycle arrest at 1 h after irradiation. Furthermore, rapid reduction of cyclin D1 upon DNA damage was attributed to proteasomal degradation, as evidenced by data showing that proteasomal inhibition by MG132 blocked cyclin D1 reduction while cycloheximide facilitated it. Inhibition of ATM activation and knockdown of E3 ligase adaptor FBX4 reversed cyclin D1 turnover in immortalized NE083-hTERT cells. Further study showed that knockdown of FBX4 facilitated DNA breaks, as indicated by an increase in γ-H2AX foci in esophageal cancer cells. Taken together, the results substantiated a pivotal role of ATM and FBX4 in cyclin D1 proteolysis upon DNA damage in precancerous esophageal epithelial cells, implying that deregulation of the process may contribute to carcinogenesis of esophageal squamous cell carcinoma.

1. Introduction

The esophagus is exposed directly to a variety of substances that can cause injuries and irritations, and trigger inflammatory responses, resulting in release of reactive oxygen species/reactive nitrogen species (ROS/RNS). Overexpression of ROS/RNS can cause DNA damage. Cyclin D1, belonging to the family of D-type cyclins, regulates cell cycle progression and tumorigenesis [1–4]. Degradation of cyclin D1 at the early stage of DNA damage response (DDR) is crucial for maintaining genomic stability, as interference of cyclin D1 degradation compromises the intra-S-phase checkpoint and increases the frequency of chromatid breaks following DNA damage [5,6]. In the early stage of carcinogenesis, failure to degrade cyclin D1 upon DNA damage may allow DNA damages to accumulate and facilitate cancer development [7,8]. Studying events and mechanisms involved in regulation of cyclin D1 upon DNA damage in precancerous cells may provide a better understanding of the carcinogenesis process of esophageal squamous cell carcinoma (ESCC).

Immortality has been deemed as a prerequisite condition for malignant transformation of cells [9]. Immortalized cells may be

Abbreviations: APC, anaphase-promoting complex; ATM, mutated in ataxia telangiectasia; CHX, cycloheximide; DDR, DNA damage response; ESCC, esophageal squamous cell carcinoma; FBX4, F-Box protein 4; HPV, human papillomavirus; hTERT, human telomerase reverse transcriptase; IR, ionizing radiation; PRMT5, protein arginine methyltransferase 5; SCF, Skp1-Cul1-F box; reactive oxygen species, ROS; reactive nitrogen species, RNS.

* Corresponding author.
** Corresponding author.
E-mail addresses: lianglyu@csu.edu.cn (L. Lv), lmcheung@hku.hk (A.L.M Cheung).

https://doi.org/10.1016/j.bbrc.2021.03.089

considered as precancerous cells and are good models for studying the early events of the cancer development. Little is known about how cyclin D1 is regulated in precancerous cells upon DNA damage and which E3 ligases mediate the effects. A variety of E3 ligases such as anaphase-promoting complex (APC)/cdc27 [10], SCF^{FBXO31} [11], $SCF^{FBX4/alphaB\text{-}crystallin}$ [12] and SCF^{FBX8} [13], have been reported to mediate cyclin D1 degradation in different cell types. Among these E3 ligases, $SCF^{FBX4/alphaB\text{-}crystallin}$ may have an important role in cyclin D1 degradation in ESCC since mutations of FBX4 were found in 14% primary esophageal carcinoma [14]. Another F-box protein, FBXO31, was reported to specifically respond to DNA damage [11]. In this paper, we showed that rapid and p53-p21-independent cyclin D1 degradation occurs within 1 h upon DNA damage in immortalized esophageal epithelial cells, and that the E3 ligase adaptor FBX4 is involved in mediating this process.

2. Methods and materials

2.1. Cell lines and drugs

Three immortalized human esophageal epithelial cell lines were used: NE2-hTERT, established previously in our laboratory [15], and NE083-hTERT, kindly provided by Professor YL Kwong (Department of Medicine, The University of Hong Kong) [16], were immortalized by overexpression of human telomerase reverse transcriptase (hTERT); NE083-E6E7, also provided by Professor Kwong, was immortalized by expression of human papillomavirus (HPV) E6 and E7 [16]. The immortalized cells were cultured in a 1:1 mixture of defined keratinocyte serum-free medium (GIBCO, Carlsbad, CA, USA) and EpiLife (Cascade Biologics, Portland, Oregon, USA) with the supplements provided. Esophageal squamous cell carcinoma cell lines KYSE30 and KYSE150 [17], obtained from DSMZ (Braunschweig, Germany), were cultured in RPMI 1640 (Sigma, St. Louis, MO, USA) containing 10% fetal bovine serum (Invitrogen, Gaithersburg, MD, USA). KU55933 (an ATM inhibitor) and cycloheximide (CHX) were purchased from Sigma-Aldrich. MG132 was obtained from Merck Bioscience, Darmstadt, Germany.

2.2. Generation of NE083-E6E7-pBabe and NE083-E6E7-cyclin D1 stable cell lines

The retroviral vectors pBabe-puro and the cyclin D1-expressing pBabe-puro-cyclin D1-HA (kindly provided by Dr. William Hahn, Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, USA), and the VSV-G plasmid (kindly provided by Dr. H. L. Chen, Department of Microbiology, The University of Hong Kong), were transfected into the Phoenix 293 packaging cells (also from Dr. H. L. Chen). The NE083-E6E7 cells were infected at PD (population doubling) 80 with viral supernatant for 8 h. The pBabe and cyclin D1 transfectants were selected with 1 μg/ml puromycin for two weeks.

2.3. Ionizing radiation (IR)

Cells were cultured in 35 mm dish for 24 h then subjected to γ-irradiation from a 137Cs source using a Gammacell 3000 Elan machine (MDS Nordion, Ottawa, ON, Canada).

2.4. Pulse-chase analysis with cycloheximide

NE083-hTERT cells with or without exposure to 10 Gy γ-irradiation were treated with 50 μg/ml CHX for different durations up to 60 min. Cell lysates were prepared and subjected to western blot.

2.5. SiRNA interference

Selective small interfering RNAs (siRNAs) for the FBX4 (ID: s25343) and negative control siRNA were purchased from Thermo Fisher Scientific, Wilmington, Delaware, USA. Transfections with Lipofectamine RNAiMAX (Invitrogen) were performed according to the manufacturer's protocol. For the knockdown of FBX4, 3×10^4 cells were plated in 35 mm dishes, to which siRNA-Lipofectamine complexes were added, resulting in a final RNA concentration of 20 nmol/L. After 48 h, cells were collected for analysis. Details of FBXO31-knockdown and the two shRNA sequences were reported previously [18].

2.6. Immunoblotting and antibodies

Preparation of whole-cell lysates and immunoblotting were performed as described previously [18,19]. Since H2AX protein tightly binds with DNA, the following method was used to prepare samples for γ-H2AX detection. After washing with cold PBS twice to remove residual medium, adherent cells in 35 mm dish were scraped into 100 μl PBS and centrifuged at 700 rpm for 5 min at 4 °C. The supernatant was discarded and the cell pellet was then suspended in 25 μl 2X Protein Sample Buffer and sonicated briefly. The samples were denatured by boiling at 95 °C for 10 min.

Cyclin D1 antibody was purchased from BD Biosciences (San Diego, CA, USA). Anti-FBX4 antibody was purchased from Rockland (Gilbertsville, PA, USA). Antibodies against ATM, p-ATM, γ-H2AX, p-p53, p-Chk2 were obtained from Cell signaling Technology (Beverly, MA, USA). p53 and p21 antibodies were purchased from Dako (Glostrup, Denmark). Actin, cyclin E and CDK4 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). β-tubulin antibody was purchased from Sigma.

2.7. Flow cytometry

About 1–2 x 10^6 single cells were harvested and washed in cold PBS twice, then fixed in 70% ethanol overnight. Cells were washed on the next day in cold PBS once and then incubated in propidium iodide (PI) buffer (PBS containing 40 μg/ml PI and 100 μg/ml RNAase) at 37 °C for 30 min prior to analysis by flow cytometry (BD FACSCanto II Analyzer, BD Biosciences). Cell cycle was analyzed with FlowJo using Dean-Jett-Fox methods.

2.8. Immunofluorescence

Cells were fixed in 4% paraformaldehyde for 15 min at room temperature, and then permeabilized with 0.25% Triton X-100 for 5 min. The cells were incubated with 1:500 anti-γ-H2AX (Cell Signaling) for 1 h, followed by three washes in PBS and then incubated with 1:2000 secondary antibody (Alexa Fluor 488 donkey anti-rabbit IgG Invitrogen) for 1 h. Cells were then counterstained with 4′,6-diamidino-2-phenylindole (DAPI). Immunostaining of cells was visualized using confocal microscopy (Carl Zeiss LSM 700, NY, USA) with a 63X objective.

2.9. Statistical analysis

The results were analyzed using SPSS (Aspire Software International, Leesburg, VA). The data from each experiment (expressed as the mean ± SE) were compared by ANOVA. All statistical tests were two-sided, and P values < 0.05 were deemed statistically significant.

3. Results and discussion

3.1. Cyclin D1 was downregulated in immortalized esophageal epithelial cells upon DNA damage

To study the regulation of cyclin D1 under genotoxic stress in precancerous esophageal cells, the changes in cyclin D1 expression upon DNA damage were first investigated using NE2-hTERT and NE083-hTERT cell lines. Since DNA damage was reported to induce G1 arrest [20], the expression of cyclin D1 and other cell cycle molecules related to G1 phase, such as cyclin E and CDK4 were compared. Western blot results showed that cyclin D1 expression was reduced 1 h after 10 Gy of γ−irradiation, while CDK4 expression was unchanged and cyclin E slightly upregulated. γ-H2AX and p-ATM, serving as DNA damage indicators, were increased as expected (Fig. 1A). Moreover, NE083-hTERT and NE2-hTERT responded to irradiation-induced DNA damage in a dose-dependent manner, with increased activation of DNA damage response proteins, p-ATM, p-Chk2, p-p53, γ-H2AX and corresponding cyclin D1 reduction (Supplementary Fig. S1).

Cyclin D1 and cyclin E are two important cyclins that bind and activate CDK4/CDK6 and CDK2, respectively, to facilitate G1-S transition. Cyclin D1, but not cyclin E, was found to undergo rapid degradation upon DNA damage in immortalized esophageal epithelial cells. Degradation of cyclin D1 upon DNA damage may be associated with the requirement of cells to elicit a rapid G1 arrest to

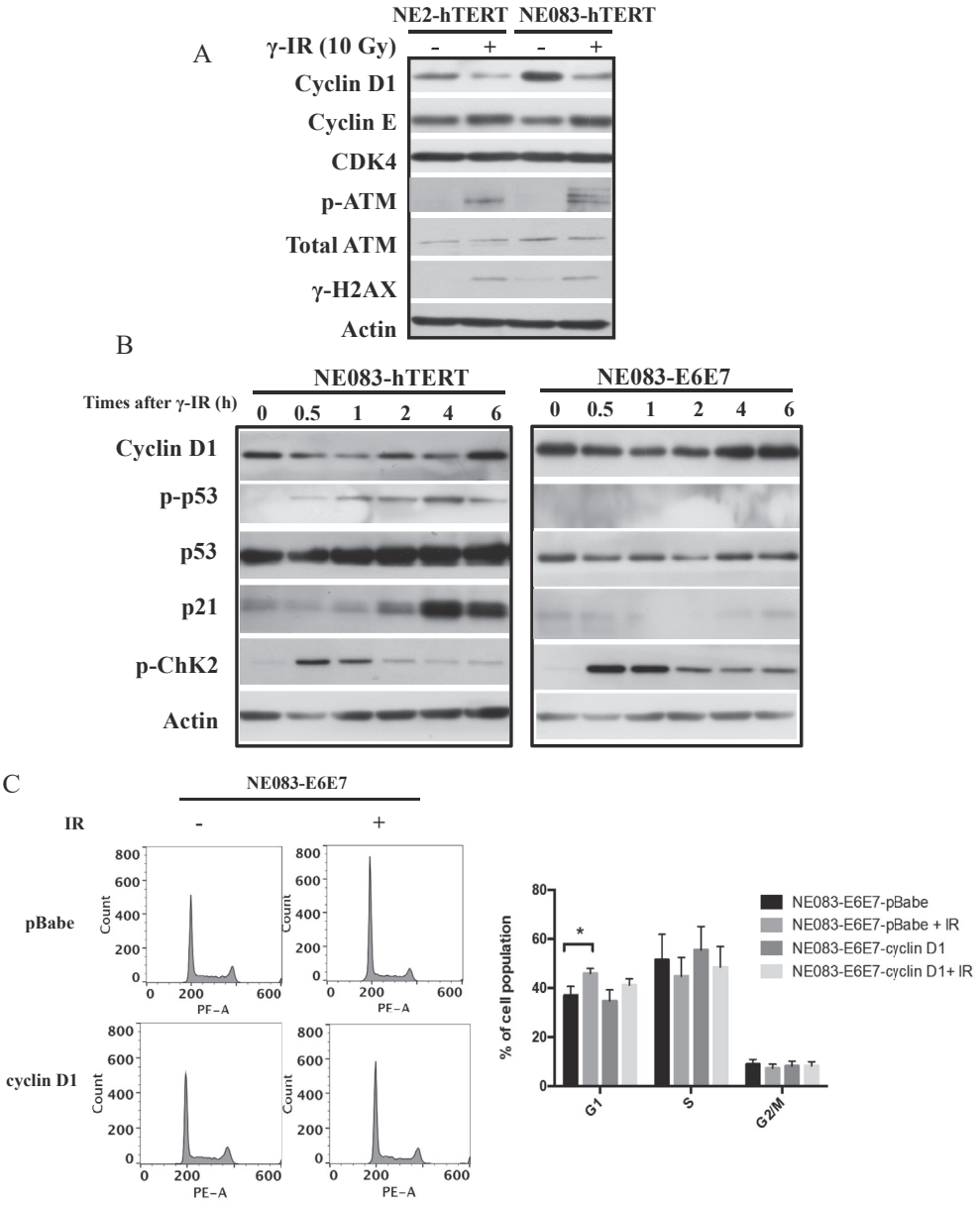

Fig. 1. Cyclin D1 reduction is an early response that occurs before p21 accumulation in immortalized esophageal epithelial cells responding to DNA damage. (A) Immortalized esophageal epithelial cells responded to DNA damage with cyclin D1 reduction. Cell lysates from NE2-hTERT and NE083-hTERT were harvested at 1 h after 10 Gy of γ −irradiation and then analyzed by western blot for cyclin D1, cyclin E, CDK4, ATM and γ-H2AX expressions. (B) DNA damage-induced cyclin D1 reduction occurred before p21 accumulation. The protein expression pattern of immortalized cells was analyzed at the indicated time intervals (h) after 10 Gy γ-irradiation. (C) Overexpression of cyclin D1 in NE083-E6E7 cells attenuated G0/G1 cell cycle arrest induced by γ −irradiation at 1 h. Stable cell lines NE083-E6E7-pBabe and NE083-E6E7-cyclin D1 were subjected to 10 Gy of γ −irradiation; cells were harvested 1 h later and stained with PI, then analyzed by FACS. Representative cell cycle analysis results are shown in the left panel. The mean percentages of cell populations at G0/G1, S, G2/M phase from three independent experiments are shown in the right panel. *, $P < 0.05$.

prevent further accumulation of DNA damage [10,21]. Its preferential degradation over cyclin E1 may be attributed to its novel functions beside cell cycle regulation, such as epigenetic regulation of gene transcription through protein arginine methyltransferase 5 (PRMT5) [22], and regulation of DNA repair [23].

3.2. DNA damage-induced rapid cyclin D1 reduction was p53-independent

Since DNA damage led to rapid downregulation of cyclin D1, further investigation was carried out to determine the temporal correlations of cyclin D1 reduction and p21 induction, both of which are important for eliciting G1 cell cycle arrest upon DNA damage. Cellular responses in two immortalized cell lines NE083-hTERT and NE083-E6E7, established from esophageal epithelial cells of the same individual, were assessed after 10 Gy γ-irradiation (Fig. 1B). In NE083-hTERT cells, DNA damage elicited a rapid cyclin D1 reduction, which was evident at 0.5 h, before p53 stabilization and p21 upregulation which occurred at around 1 h and 4 h respectively. In the NE083-E6E7 cell line, which was immortalized by overexpression of HPV E6/E7 genes, and therefore defective in p53 activation and p21 induction [16], cyclin D1 was also rapidly downregulated, indicating that this effect is p53-independent. Next, we studied the effects of cyclin D1 overexpression in NE083-E6E7 immortalized cells. The results showed that there was G0/G1 arrest 1 h after irradiation in the NE083-E6E7-pBabe control cells, but this effect was attenuated in NE083-E6E7-cyclin D1 cells which had a lower cell populations at G0/G1 phase compared with control cells (Fig. 1C). Therefore, these results suggest that cyclin D1 degradation may play a pivotal role in eliciting cell cycle arrest before p21 induction, which may be especially important for p53-deficient cells.

3.3. Reduction in cyclin D1 in response to DNA damage was due to proteasome-mediated degradation

The rapid cyclin D1 reduction upon DNA damage suggests that a post-translational modification may be involved in this process. Whether the reduction of cyclin D1 in immortalized esophageal epithelial cells was mediated by proteasomal degradation was investigated. The results showed that treatment with proteasome inhibitor MG132 could abolish the γ-irradiation-induced cyclin D1 reduction in both NE2-hTERT and NE083-hTERT cells (Fig. 2A). Then pulse-chase analysis with CHX was used to compare the degradation profile of cyclin D1 with and without γ-irradiation. The results showed that γ-irradiation facilitated the turnover of cyclin D1 (Fig. 2B). Therefore, the results supported that cyclin D1 reduction in response to DNA damage is due to proteasome-mediated degradation.

3.4. ATM and FBX4 mediated cyclin D1 degradation in immortalized esophageal epithelial cells responding to IR

Since cyclin D1 reduction under DNA stress was due to proteasomal degradation (Fig. 2), the expression levels of E3 ligase adaptors FBX4 and FBXO31, which are reported to be involved in cyclin D1 degradation in 3T3 fibroblasts [12] and SK-MEL-28 melanoma cells [11] respectively, were investigated. Western blot results studying the temporal changes of cellular response in NE083-hTERT cells showed marked reduction in cyclin D1 at the very early stage of DNA damage response, and gradual recovery at late stage (Fig. 3A), which was consistent with the results in Fig. 1. There was also gradual accumulation of FBXO31 (Fig. 3A and Supplementary Fig. S2). Yet, knockdown of FBXO31 failed to reverse IR or UV-induced cyclin D1 degradation in ESCC cells [18], indicating that

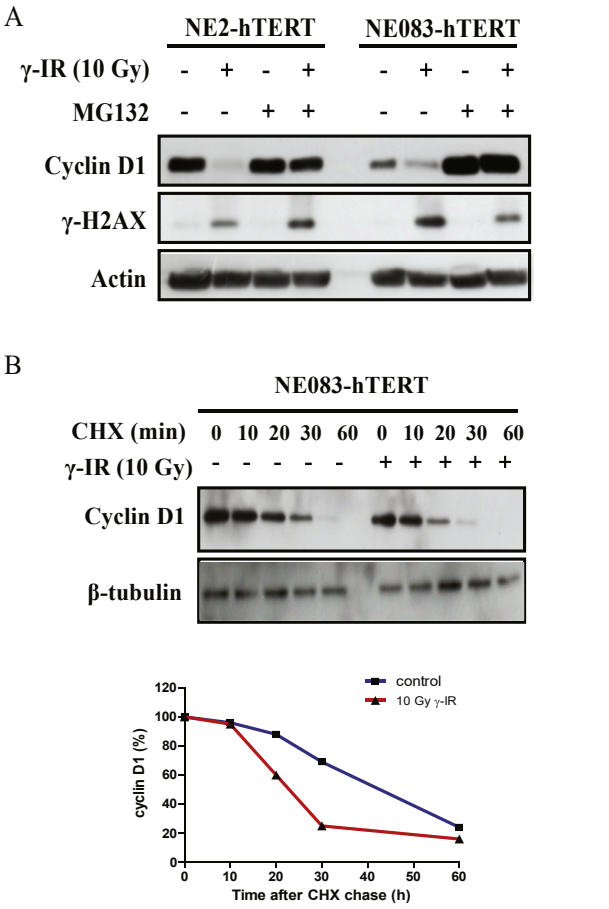

Fig. 2. Cyclin D1 reduction in response to DNA damage is due to proteasome-mediated degradation. (A) Cell lysates from indicated immortalized cells treated with 10 μM MG132 or dimethyl sulfoxide (DMSO) for 4 h followed by exposure to 10 Gy γ-irradiation were harvested for western blot. (B) Turnover of cyclin D1 in NE083-hTERT cells with or without 10 Gy γ-irradiation. The levels of cyclin D1 were analyzed using ImageJ (National Institutes of Health, Bethesda, MD) and normalized to the expression levels of β-tubulin.

FBXO31 may not be a major mediator of cyclin D1 degradation in cells of esophageal epithelial origin.

Although the expression level of FBX4 remained unchanged (Fig. 3A), its possible involvement in mediating cyclin D1 degradation cannot be ruled out. Barbash et al. found that GSK3β can mediate FBX4 phosphorylation on Ser12, thereby regulates FBX4 dimerization, and stimulating FBX4-driven E3 ligase activity [14]. It is possible that this mechanism may induce cyclin D1 degradation without apparent change in FBX4 protein expression. Therefore, we examined the effects of knockdown of FBX4 on cyclin D1 degradation and found that FBX4-knockdown could reverse γ-irradiation-induced cyclin D1 degradation (Fig. 3B), indicating that FBX4 mediates cyclin D1 turnover upon DNA damage in immortalized esophageal epithelial cells.

Interestingly, the expression level of p-ATM showed opposite trends to cyclin D1, with increased p-ATM occurring from 0.5 to 2 h when cyclin D1 expression decreased (Fig. 3A). NE083-hTERT and two ESCC cell lines, namely KYSE30 and KYSE150, were treated with KU55933, an ATM inhibitor, to study the effect of ATM inhibition on cyclin D1 degradation. The results showed that KU55933 prevented IR-induced p-ATM expression, and also blocked cyclin D1 degradation in immortalized epithelial cells (Fig. 3C) as well as ESCC cells (Supplementary Fig. S3), indicating rapid proteolytic degradation of cyclin D1 upon IR is ATM-dependent.

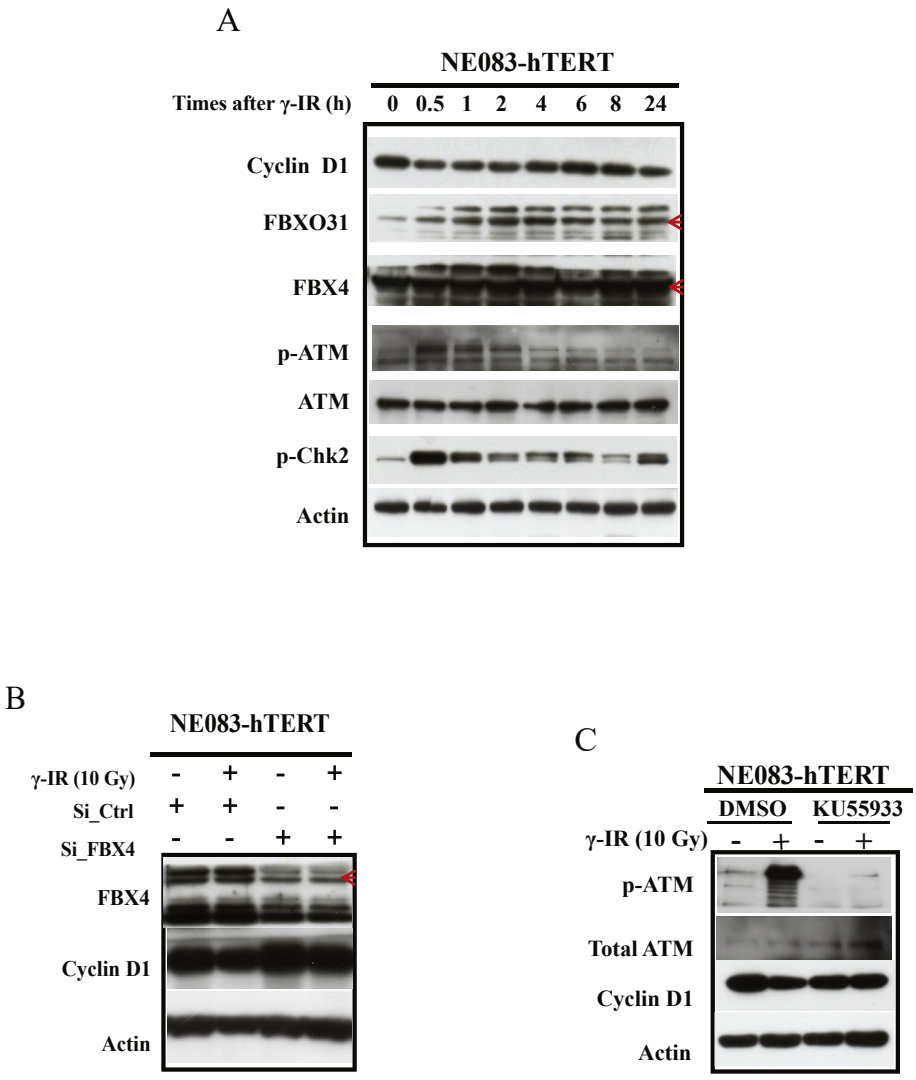

Fig. 3. Rapid cyclin D1 degradation in immortalized esophageal epithelial cells in response to IR is mediated by ATM and E3 ligase adaptor FBX4. (A) Temporal changes in expression of cyclin D1, E3 ligase adaptor proteins FBX4 and FBXO31, as well as DDR proteins p-ATM and p-Chk2 in NE083-hTERT were analyzed by western-blot. (B) Knockdown of FBX4 abolished cyclin D1 reduction upon γ-irradiation. NE083-hTERT was transfected with si-ctrl or si-FBX4 siRNA for 48 h, and cell lysates were collected after 1 h post 10 Gy γ-irradiation. (C) ATM inhibition prevented cyclin D1 degradation upon IR. Immortalized esophageal epithelial cells NE083-hTERT were treated with KU55933 for 1 h and then subjected to 10 Gy γ-irradiation. Red arrows indicate target bands.

DNA damage-induced proteolysis of cyclin D1 requires phosphorylation of cyclin D1 at Thr-286. The kinases reported to directly phosphorylate cyclin D1 at T286 include GSK3β [24], ERK1/2 [13] and p38 SAPK2 [25]. Although how DNA damage signals trigger kinases to phosphorylate cyclin D1 is unknown, the use of inhibitors and knockout experiment in previous studies proved that ATM is required for cyclin D1 phosphorylation and degradation following DNA damage [5,11]. Here, ATM inhibition was found to abolish cyclin D1 rapid degradation upon DNA damage in precancerous cells (Fig. 3C), indicating cyclin D1 is a downstream effector of ATM pathway. Further studies are needed to identify unique DDR proteins downstream of ATM that directly mediate kinases to phosphorylate and degrade cyclin D1.

3.5. Knockdown of FBX4 facilitated DNA damage-induced double strand breaks

As mutations of FBX4 were found in 14% primary esophageal carcinoma [26], but whether impaired FBX4 facilitates cancer development upon DNA damage is not well addressed, we proceeded to study the effects of FBX4-knockdown on genomic stability in ESCC cells. The results showed that knockdown of FBX4 facilitated DNA damage as indicated by an increase in γ-H2AX foci in KYSE150 compared with the siRNA control cells (Fig. 4).

FBX4 and its coactivator αB crystalline, which are components of Skp1-Cul1-F box (SCF) E3 ligase, specifically recognize Thr286-phosphorylated cyclin D1 and mediate cyclin D1 polyubiquitination and proteasomal degradation [12]. Disruption of cyclin D1 phosphorylation, nuclear export or deregulation of cyclin D1 E3 ligase adaptor FBX4-αB crystalline can contribute to cancer development. Overexpression of a cyclin D1 mutant with impaired Thr286 phosphorylation, which could not be effectively exported from the nucleus for degradation in the cytoplasm, was found to transform cells and trigger mantle cell lymphoma in a mouse model. Disruption of phosphorylation of cyclin D1 at Thr286 and genomic mutations at FBX4 were both found in esophageal cancers [26]. Here we found that FBX4 mediates cyclin D1 proteolysis in immortalized esophageal epithelial cells and knockdown of FBX4

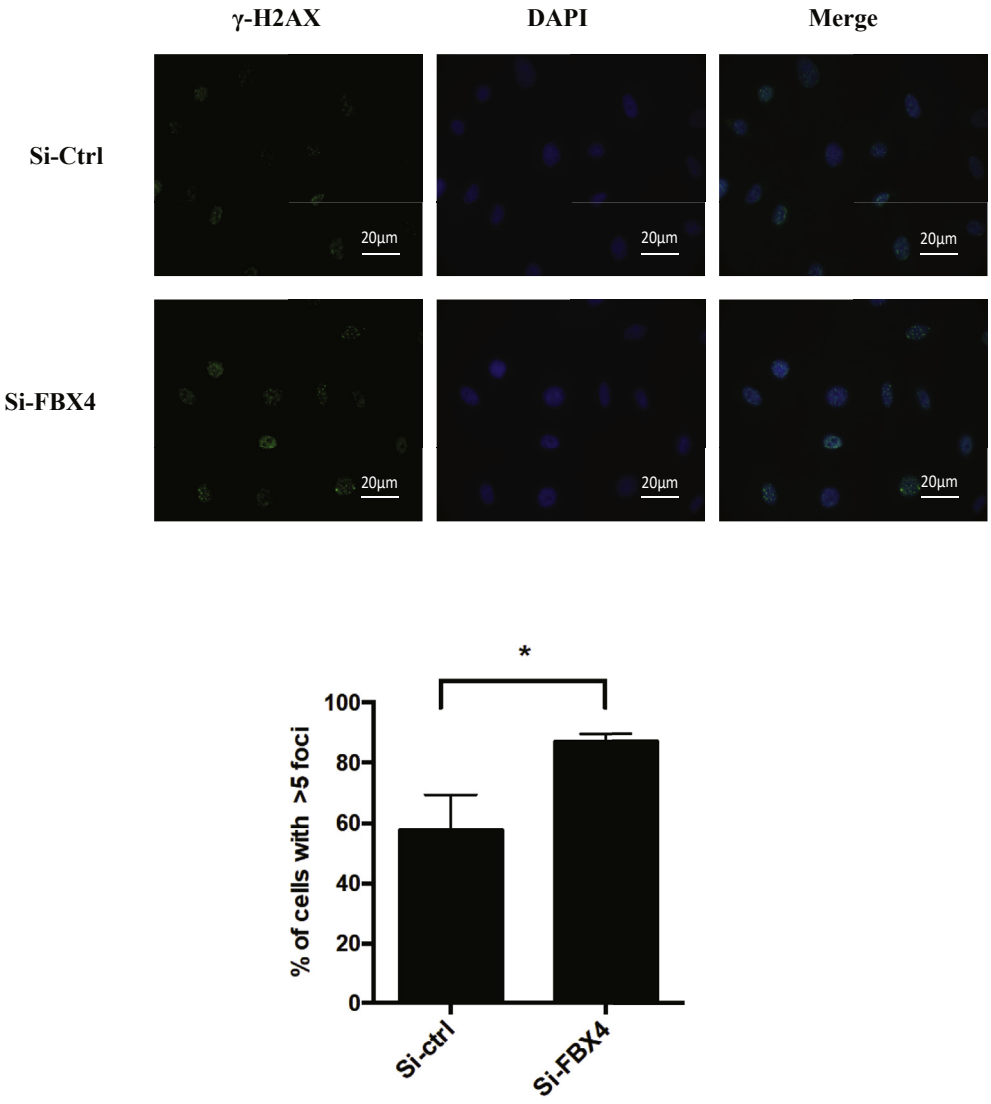

Fig. 4. Knockdown of FBX4 facilitated DNA damage-induced double-stranded breaks. KYSE150 cells expressing si-ctrl and si-FBX4 RNA were exposed to 10 Gy γ-irradiation. One h later, the cells were fixed and subjected to immunofluorescence staining for γ-H2AX. Representative results visualized using confocal microscopy with a 63X objective were shown in the upper panel. *, $P < 0.05$ compared the percentages of cells with above 5 γ-H2AX foci between si-ctrl and si-FBX4 cells from three independent experiments.

could facilitate DNA damage, suggesting a key role of FBX4-cyclin D1 in ESCC development.

In summary, cyclin D1 degradation is an early DNA damage response, and ATM and FBX4 are involved in rapid proteolysis of cyclin D1 in immortalized cells. This process is crucial for the initiation of G1 arrest before p53-induced p21accumulation; disturbance of this process may contribute to cell cycle checkpoint defects and imperfect repair of DNA lesions, thereby leading to genomic instability and carcinogenesis.

Funding

This study was supported by a grant from the University of Hong Kong (Project No. 201309176073) and funding from National Natural Science Foundation of China (Grant No. 81502630).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Dr. William Hahn, Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, USA for pBabe-puro-Cyclin D1-HA plasmid, and Dr. H. L. Chen, Department of Microbiology, The University of Hong Kong for Phoenix 293 packaging cells and VSV-G plasmid. We acknowledge the assistance of the University of Hong Kong Li Ka Shing Faculty of Medicine Faculty Core Facility for flow cytometry services.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2021.03.089.

References

[1] T. Hunter, J. Pines, Cyclins and cancer. II: cyclin D and CDK inhibitors come of

age, Cell 79 (1994) 573–582.
[2] F.I. Montalto, F. De Amicis, Cyclin D1 in cancer: a molecular connection for cell cycle control, adhesion and invasion in tumor and stroma, Cells 9 (2020).
[3] G. Tchakarska, B. Sola, The double dealing of cyclin D1, Cell Cycle 19 (2020) 163–178.
[4] S. Qie, J.A. Diehl, Cyclin D1, cancer progression, and opportunities in cancer treatment, J. Mol. Med. (Berl.) 94 (2016) 1313–1326.
[5] L.L. Pontano, P. Aggarwal, O. Barbash, E.J. Brown, C.H. Bassing, J.A. Diehl, Genotoxic stress-induced cyclin D1 phosphorylation and proteolysis are required for genomic stability, Mol. Cell Biol. 28 (2008) 7245–7258.
[6] T. Shimura, N. Kunugita, Mitochondrial reactive oxygen species-mediated genomic instability in low-dose irradiated human cells through nuclear retention of cyclin D1, Cell Cycle 15 (2016) 1410–1414.
[7] G. Di Sante, A. Di Rocco, C. Pupo, M.C. Casimiro, R.G. Pestell, Hormone-induced DNA damage response and repair mediated by cyclin D1 in breast and prostate cancer, Oncotarget 8 (2017) 81803–81812.
[8] S.Y. Wu, S.H. Lan, S.R. Wu, Y.C. Chiu, X.Z. Lin, I.J. Su, T.F. Tsai, C.J. Yen, T.H. Lu, F.W. Liang, C.Y. Li, H.J. Su, C.L. Su, H.S. Liu, Hepatocellular carcinoma-related cyclin D1 is selectively regulated by autophagy degradation system, Hepatology 68 (2018) 141–154.
[9] R.F. Newbold, R.W. Overell, J.R. Connell, Induction of immortality is an early event in malignant transformation of mammalian cells by carcinogens, Nature 299 (1982) 633–635.
[10] R. Agami, R. Bernards, Distinct initiation and maintenance mechanisms cooperate to induce G1 cell cycle arrest in response to DNA damage, Cell 102 (2000) 55–66.
[11] M.K. Santra, N. Wajapeyee, M.R. Green, F-box protein FBXO31 mediates cyclin D1 degradation to induce G1 arrest after DNA damage, Nature 459 (2009) 722–725.
[12] D.I. Lin, O. Barbash, K.G. Kumar, J.D. Weber, J.W. Harper, A.J. Klein-Szanto, A. Rustgi, S.Y. Fuchs, J.A. Diehl, Phosphorylation-dependent ubiquitination of cyclin D1 by the SCF(FBX4-alphaB crystallin) complex, Mol Cell 24 (2006) 355–366.
[13] H. Okabe, S.H. Lee, J. Phuchareon, D.G. Albertson, F. McCormick, O. Tetsu, A critical role for FBXW8 and MAPK in cyclin D1 degradation and cancer cell proliferation, PloS One 1 (2006) e128.
[14] O. Barbash, E.K. Lee, J.A. Diehl, Phosphorylation-dependent regulation of SCF(Fbx4) dimerization and activity involves a novel component, 14-3-3ϵ, Oncogene 30 (2011) 1995–2002.
[15] P.Y. Cheung, W. Deng, C. Man, W.W. Tse, G. Srivastava, S. Law, S.W. Tsao, A.L. Cheung, Genetic alterations in a telomerase-immortalized human esophageal epithelial cell line: implications for carcinogenesis, Canc. Lett. 293 (2010) 41–51.
[16] H. Zhang, Y. Jin, X. Chen, C. Jin, S. Law, S.W. Tsao, Y.L. Kwong, Papillomavirus type 16 E6/E7 and human telomerase reverse transcriptase in esophageal cell immortalization and early transformation, Canc. Lett. 245 (2007) 184–194.
[17] Y. Shimada, M. Imamura, T. Wagata, N. Yamaguchi, T. Tobe, Characterization of 21 newly established esophageal cancer cell lines, Cancer 69 (1992) 277–284.
[18] J. Liu, L. Han, B. Li, J. Yang, M.S. Huen, X. Pan, S.W. Tsao, A.L. Cheung, F-box only protein 31 (FBXO31) negatively regulates p38 mitogen-activated protein kinase (MAPK) signaling by mediating lysine 48-linked ubiquitination and degradation of mitogen-activated protein kinase kinase 6 (MKK6), J. Biol. Chem. 289 (2014) 21508–21518.
[19] J. Liu, L. Lv, J. Gong, Y. Tan, Y. Zhu, Y. Dai, X. Pan, M.S.Y. Huen, B. Li, S.W. Tsao, J. Huo, A.L.M. Cheung, Overexpression of F-box only protein 31 predicts poor prognosis and deregulates p38α- and JNK-mediated apoptosis in esophageal squamous cell carcinoma, Int. J. Canc. 142 (2018) 145–155.
[20] A. Di Leonardo, S.P. Linke, K. Clarkin, G.M. Wahl, DNA damage triggers a prolonged p53-dependent G1 arrest and long-term induction of Cip1 in normal human fibroblasts, Genes Dev. 8 (1994) 2540–2551.
[21] S. Qie, A. Yoshida, S. Parnham, N. Oleinik, G.C. Beeson, C.C. Beeson, B. Ogretmen, A.J. Bass, K.K. Wong, A.K. Rustgi, J.A. Diehl, Targeting glutamine-addiction and overcoming CDK4/6 inhibitor resistance in human esophageal squamous cell carcinoma, Nat. Commun. 10 (2019) 1296.
[22] P. Aggarwal, L.P. Vaites, J.K. Kim, H. Mellert, B. Gurung, H. Nakagawa, M. Herlyn, X. Hua, A.K. Rustgi, S.B. McMahon, J.A. Diehl, Nuclear cyclin D1/CDK4 kinase regulates CUL4 expression and triggers neoplastic growth via activation of the PRMT5 methyltransferase, Canc. Cell 18 (2010) 329–340.
[23] Z. Li, X. Jiao, C. Wang, L.A. Shirley, H. Elsaleh, O. Dahl, M. Wang, E. Soutoglou, E.S. Knudsen, R.G. Pestell, Alternative cyclin D1 splice forms differentially regulate the DNA damage response, Canc. Res. 70 (2010) 8802–8811.
[24] J.A. Diehl, M. Cheng, M.F. Roussel, C.J. Sherr, Glycogen synthase kinase-3beta regulates cyclin D1 proteolysis and subcellular localization, Genes Dev. 12 (1998) 3499–3511.
[25] O. Casanovas, F. Miró, J.M. Estanyol, E. Itarte, N. Agell, O. Bachs, Osmotic stress regulates the stability of cyclin D1 in a p38SAPK2-dependent manner, J. Biol. Chem. 275 (2000) 35091–35097.
[26] O. Barbash, J.A. Diehl, SCF(Fbx4/alphaB-crystallin) E3 ligase: when one is not enough, Cell Cycle 7 (2008) 2983–2986.