Activating PTEN by COX-2 inhibitors antagonizes radiation-induced AKT activation contributing to radiosensitization

Zhen Meng [a, b], Ye-Hua Gan [a, b, *]

[a] Central Laboratory, Peking University School and Hospital of Stomatology, 22 Zhongguancun Avenue South, Haidian District, Beijing 100081, China
[b] Department of Oral & Maxillofacial Surgery, Peking University School and Hospital of Stomatology, 22 Zhongguancun Avenue South, Haidian District, Beijing 100081, China

A B S T R A C T

Radiotherapy is still one of the most effective nonsurgical treatments for many tumors. However, radioresistance remains a major impediment to radiotherapy. Although COX-2 inhibitors can induce radiosensitization, the underlying mechanism is not fully understood. In this study, we showed that COX-2 selective inhibitor celecoxib enhanced the radiation-induced inhibition of cell proliferation and apoptosis in HeLa and SACC-83 cells. Treatment with celecoxib alone dephosphorylated phosphatase and tensin homolog deleted on chromosome ten (PTEN), promoted PTEN membrane translocation or activation, and correspondingly dephosphorylated or inactivated protein kinase B (AKT). By contrast, treatment with radiation alone increased PTEN phosphorylation, inhibited PTEN membrane translocation and correspondingly activated AKT in the two cell lines. However, treatment with celecoxib or another COX-2 selective inhibitor (valdecoxib) completely blocked radiation-induced increase of PTEN phosphorylation, rescued radiation-induced decrease in PTEN membrane translocation, and correspondingly inactivated AKT. Moreover, celecoxib could also upregulate PTEN protein expression by downregulating Sp1 expression, thereby leading to the activation of PTEN transcription. Our results suggested that COX-2 inhibitors could enhance radiosensitization at least partially by activating PTEN to antagonize radiation-induced AKT activation.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Radiotherapy is still one of the most effective nonsurgical treatments for many tumors [1], although new therapeutic strategies targeting molecules critical for tumors have shown promise [2]. Radiation impedes cancer cell growth by inducing cytotoxicity mainly through DNA damage [3]. However, radioresistance remains a major impediment to radiotherapy [4,5]. Cancer cells resistant to radiotherapy can result in the local recurrence [6,7]. Exposure of cancer cells to radiation can activate several signal pathways that lead to radiation resistance, including NF-κB and signal transducer and activator of transcription 3 (STAT3) [8]. These pathways are linked to radioresistance via the upregulation of cyclin D1, vascular epithelial growth factor (VEGF), matrix metalloproteinases (MMPs),

and pro-inflammatory cytokines [9,10]. DNA-dependent protein kinase catalytic subunit (DNA-PKcs) and ataxia telangiectasia-mutated gene (ATM) induce radioresistance by triggering reparation of radiation-induced DNA damage [11,12].

The PI3K/AKT signaling pathway also plays a crucial role in the radioresistance of cancer cells. Phosphorylation of AKT at S473 or T308 is the activated form of AKT [13]. AKT promotes cell proliferation, inhibits apoptosis, and enhances cell invasion, thereby regulating various cellular functions [14]. The PI3K/AKT pathway is believed to be involved in three major radioresistance mechanisms, as follows: intrinsic radioresistance, tumor-cell proliferation, and hypoxia [15]. For example, the PI3K/AKT pathway is involved in resistance to radiotherapy by upregulating VEGF and capillary-like tubule formation in the tumor [16]. PI3K and AKT activation is involved in cell cycle regulation by degradation of cyclin D1 and cyclin-dependent kinase 4 (CDK4), thereby resulting in resistance to radiation-induced apoptosis [17–19]. Therefore, targeting PI3K/AKT pathway might be an important way to decrease radioresistance.

* Corresponding author. Central Laboratory, Peking University School of Stomatology, 22 Zhongguancun Avenue South, Haidian District, Beijing 100081, China. Fax: +86 10 62173402.
E-mail address: kqyehuagan@bjmu.edu.cn (Y.-H. Gan).

Phosphatase and tensin homolog deleted on chromosome ten (PTEN) is an important tumor-suppressor and is a key negative regulator of the PI3K/AKT pathway by dephosphorylating phosphatidylinositol-3,4,5-trisphosphate (PIP3) [20,21]. Deletions and mutations of PTEN were observed in many cancers [22]. Membrane translocation is critical for PTEN to function as a tumor suppressor because its main substrate, PIP3, is located in the plasma membrane [23]. Membrane-bound PTEN is the activated form of PTEN [23–25]. However, phosphorylation of PTEN at S380, T382, T383, and S385 decreases PTEN membrane translocation, thereby deactivating PTEN [24]. Therefore, we speculate that agents that can activate PTEN would antagonize AKT's function in radioresistance.

Cyclooxygenase-2 (COX-2) is overexpressed in several tumors, including lung cancer, colon cancer, breast cancer, and prostate cancer [26]. COX-2 is the rate limiting enzyme that converts arachidonic acid to prostaglandins. Arachidonic acid is mainly converted to prostaglandin E2 (PGE2), which acts on multiple signal pathways involved in cell proliferation, cell apoptosis, angiogenesis, invasion, and promotion of tumor progression [27]. Radiation can induce COX-2 [28]. Conversely, COX-2 inhibitors can also enhance tumor radioresponse by inhibiting nuclear epidermal growth factor receptor transport and DNA repair or phosphorylation of STAT3 [29,30]. These studies suggest that COX-2 inhibitors can be used as potential enhancers of radiosensitization. However, the mechanism underlying COX-2 inhibitors enhancement on radiosensitization is not fully understood.

In this study, we showed that COX-2 inhibitors enhanced radiosensitization by activating PTEN to antagonize radiation-induced AKT activation in HeLa and SACC-83 cells.

2. Material and methods

2.1. Cell culture and treatments

HeLa cells were incubated in Dulbecco's modified Eagle's medium (GIBCO) with 10% fetal bovine serum (FBS) at 37 °C with 5% CO_2. SACC-83 cells, which were derived from human salivary adenoid cystic cancer (SACC) [31], were incubated in RPMI medium 1640 (GIBCO) with 10% FBS at 37 °C with 5% CO_2. Cells were exposed to radiation (Cobalt-60) at different doses at dose rate of 2.544 Gy/min and then cultured for 24 h. Cells were also exposed to different doses of celecoxib or valdecoxib alone for 24 h. In another treatment, cells were exposed to a combination of radiation and COX-2 inhibitor (either celecoxib or valdecoxib); first, cells were treated with radiation and then with celecoxib or valdecoxib for 24 h.

2.2. Plasmids, reagents and antibodies

Wild type or mutant PTEN promoter-reporters were constructed in our previous study [32]. Celecoxib and valdecoxib were purchased from Selleck Chemicals. Anti-PTEN (#9552), anti-phospho-AKT (T308) (#9275), anti-phospho-AKT (S473) (#4058), and anti-phospho-PTEN (S380/T382/T383) (#9554) antibodies were purchased from Cell Signaling Technology. Anti-β-actin (I-19) antibody (sc-1616) was purchased from Santa Cruz Biotechnology.

2.3. Protein extraction and Western blot

Whole cell lysates were extracted with RIPA lysis buffer (Applygen). Membrane proteins were extracted using Mem-PER Eukaryotic Membrane Protein Extraction Reagent Kit (Thermo). Protein concentrations were determined by BCA protein assay (Thermo). Equal amounts of samples were subjected to 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE)

and transferred onto a polyvinylidene fluoride membrane (Millipore). The membrane was blocked with 5% fat-free milk in TBS-T (50 mmol/L Tris, pH 7.5; 150 mmol/L NaCl; 0.05% Tween 20) for 1 h. After incubation with primary antibodies diluted 1:1000 in TBS-T containing 1% milk overnight at 4 °C, the membrane was washed extensively with TBS-T and then incubated with a secondary antibody conjugated with fluorophore for 1 h at room temperature. After extensive washing with TBS-T, the membrane was visualized with Odyssey infrared imaging system (Odyssey LI-COR). For internal controls of equal loading, the blots were also stripped and reprobed with β-actin antibody. Protein expressions of target genes were quantitated. The target bands on Western blots were scanned, and densitometry was performed. Data were presented as mean ± standard deviation (SD) of three independent experiments.

2.4. Real-time quantitative PCR

Total RNA was extracted using TRIzol reagent (Invitrogen). Reverse transcription and real-time PCR were performed as described previously [32]. The primers for human PTEN are as follows: 5′-GACCATAACCCACCACAGC-3′ (sense) and 5′-CCAGTTCGTCCCTTTCCAG-3′ (antisense). The primers for human β-actin are as follows: 5′-CGGGAAATCGTGCGTGAC-3′ (sense) and 5′-CAGGCAGCTCGTAGCTCTT-3′ (antisense). All the primers were designed using the Primer Premier Version 5.0 software. The efficiency of all the primers was confirmed by sequencing their conventional PCR products. Real-time PCR was performed using a 7500 real-time PCR system of Applied Biosystems with FastStart Universal SYBR Green Master (Roche).

2.5. Luciferase assay

Luciferase assay was performed as described previously [32]. Briefly, PTEN reporter plasmid (1 μg) was transfected with Lipofectamine 2000 (Invitrogen) into HeLa or SACC-83 cells in a 12-well plate. The transfected cells were lysed in a cell lysis buffer 24 h after transfection. Luciferase activity was measured with a LB960 microplate luminometer (Berthold) using luciferin as the substrate, according to the manufacturer's instructions (Promega).

2.6. Cell proliferation assay

Cell proliferation assay was performed using Cell Counting Kit-8 (CCK-8, Dojindo) according to the manufacturer's instructions. Briefly, cells exposed to or not exposed to radiation treatment were seeded into 96-well plates (1.5×10^3 cells/well) and treated with 20 μM celecoxib or valdecoxib for 0, 24, 48, or 72 h. CCK-8 at 10 μL was added to each well after COX-2 inhibitor treatment. Cells were further incubated at 37 °C for 3 h and measured for absorbance at 450 nm. Data were presented as mean ± SD of at least three independent experiments.

2.7. Assessment of cell apoptosis

Cells were washed with phosphate-buffered saline (PBS) thrice, fixed with 10% formaldehyde for 5 min, and incubated with 5 mg/ml 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) in the dark for 3 min at room temperature. After washing with PBS, the cells were examined under a fluorescence microscope (Nikon). The cells presenting features of nuclear condensation and fragmentation were identified as apoptotic cells and were counted within the six randomly selected fields. The rate of apoptotic cells was presented as mean ± SD of at least three independent experiments.

2.8. Statistical analysis

Statistical analysis was performed using SPSS 11.5 for Windows. All data were presented as mean ± SD. Differences between multiple groups were analyzed by one-way analysis of variance. A value of $P < 0.05$ was considered as statistically significant.

3. Results

3.1. COX-2 inhibitor celecoxib enhanced radiation-induced inhibition of cell proliferation and apoptosis

To examine whether COX-2 inhibitor celecoxib could enhance radiosensitization, HeLa and SACC-83 cells were exposed to radiation with 8 Gy at the dose rate of 2.544 Gy/min or 20 μM celecoxib (a selective inhibitor for COX-2) alone for 24 h. A treatment involving the combination of radiation and celecoxib was also performed. Radiation treatment was initially performed followed by celecoxib for 24 h. As shown in Fig. 1A—D, treatment with radiation or celecoxib alone only resulted in a slight inhibition of cell proliferation and a slight increase in apoptosis, whereas treatment with celecoxib after radiation treatment significantly enhanced radiation-induced HeLa cell proliferation inhibition and apoptosis. Similar results were also observed in SACC-83 cells (Supplementary Fig. 1).

3.2. COX-2 inhibitor celecoxib upregulated PTEN partially by inhibiting Sp1, and activated PTEN and inactivated AKT

To understand the mechanism underlying the enhancement of radiosensitization by COX-2 inhibitor celecoxib, we treated HeLa or SACC-83 cells with increasing doses of celecoxib and examined the activated forms of PTEN and AKT. As shown in Fig. 2A and B and Supplementary Fig. 2, the protein expression of PTEN was upregulated after treatment with celecoxib, whereas the phosphorylation of PTEN (S380/T382/T383) and AKT (both T308 and S473) were inhibited in HeLa or SACC-83 cells. Since membrane-bound PTEN is the activated form of PTEN and is negatively regulated by phosphorylation at the C-terminal tail, we further detected the change of membrane-bound PTEN after treatment with celecoxib. Correspondingly, membrane-bound PTEN was also dose-dependently increased after the treatment.

To confirm whether upregulation of PTEN protein expression by celecoxib was due to the activation of PTEN transcription, we detected PTEN promoter activity and mRNA expression after treatment with celecoxib. As shown in Fig. 2C and D, both PTEN promoter activity and mRNA expression were dose-dependently upregulated by celecoxib in HeLa or SACC-83 cells. Since we previously showed that Sp1 is an important negative regulator of PTEN transcription [32], and other group also showed that COX-2 inhibitors can downregulate Sp1 expression [33], we further detected whether Sp1 was involved in celecoxib-induced PTEN overexpression. As shown in Fig. 2A and B and Supplementary Fig. 2, Sp1 was dose-dependently downregulated in HeLa or SACC-83 cells after treatment with celecoxib. Moreover, mutation of Sp1 binding site (at −918/−913, the translational start site was defined as +1), which is responsible for the negative regulation of PTEN transcription by Sp1 in our previous study [32], in the PTEN full-length promoter reporter construct partially abolished celecoxib-induced increase in PTEN promoter activity in HeLa cells (Fig. 2E). These results suggested that COX-2 inhibitor celecoxib upregulated PTEN protein expression at least partially by inhibiting Sp1 to activate PTEN transcription.

3.3. Radiation inactivated PTEN and activated AKT

To examine whether radiation could affect the activation of PTEN and AKT, we exposed HeLa and SACC-83 cells to increasing doses of radiation and examined the phosphorylation of PTEN and AKT. As shown in Fig. 3 and Supplementary Fig. 3, radiation induced phosphorylation of PTEN at S380, T382 and T383 without affecting the expression of total PTEN. Radiation also induced phosphorylation of AKT at T308 and S473 without affecting the expression of total AKT, thereby suggesting that radiation inactivated PTEN and activated AKT.

3.4. COX-2 inhibitors antagonized radiation-induced inactivation of PTEN and activation of AKT

To further detect whether COX-2 inhibitors could antagonize radiation-induced inactivation of PTEN and activation of AKT, we examined the phosphorylation of PTEN and AKT and membrane translocation of PTEN after HeLa or SACC-83 cells were exposed to radiation or celecoxib (or valdecoxib) alone, or to a combination of radiation and COX-2 inhibitor. First, the cells were radiated and then were treated with celecoxib or valdecoxib for 24 h. As shown in Fig. 4A and Supplementary Fig. 4A, celecoxib completely blocked radiation-induced increase in PTEN phosphorylation, thereby rescuing radiation-induced decrease of membrane-bound PTEN and correspondingly blocking radiation-induced increase in AKT phosphorylation. Treatment with valdecoxib showed similar results to that with celecoxib (Fig. 4B and Supplementary Fig. 4B). These results suggested that COX-2 inhibitors contributed to radiosensitization at least partially by antagonizing radiation-induced inactivation of PTEN and activation of AKT.

4. Discussion

In this study, we showed that COX-2 inhibitors could upregulate PTEN expression and promote PTEN membrane translocation or activation to contribute to radiosensitization of cancer cells. Our results provided new information, as follows: radiation could increase PTEN phosphorylation to decrease PTEN membrane translocation or activation; and COX-2 inhibitors could upregulate and activate PTEN to block radiation-induced activation of AKT.

Activation of AKT by radiation could be due to the inactivation of PTEN by radiation. Radiation induced AKT phosphorylation at T308 and S473 in the two types of cancer cells. This finding was consistent with that of a previous study, in which radiation induced AKT phosphorylation and correspondingly promoted oxidative stress [34]. Moreover, radiation induced PTEN phosphorylation and decreased PTEN membrane translocation or activation. To the best of our knowledge, the present study is the first report on the induction of PTEN phosphorylation by radiation. We further confirmed that induction of PTEN phosphorylation by radiation resulted in the reduction of PTEN membrane translocation or activation. This finding was consistent with the knowledge that PTEN phosphorylation at the C-terminal is an inactivated form of PTEN and is not localized in the membrane [24]. Given that PTEN is a key negative regulator of AKT and is inactivated by radiation, radiation-induced activation of AKT could have been caused by the inactivation of PTEN by radiation. However, other factors may also have contributed to radiation-induced activation of AKT. Certainly, the mechanism underlying PTEN inactivation or PTEN phosphorylation induction by radiation needs to be explored. PTEN inactivation by radiation contributed to radiation-induced activation of AKT, and this finding supports our speculation that agents that can activate PTEN could be used to antagonize the AKT's function in radiosensitization, as is the case for COX-2 inhibitors.
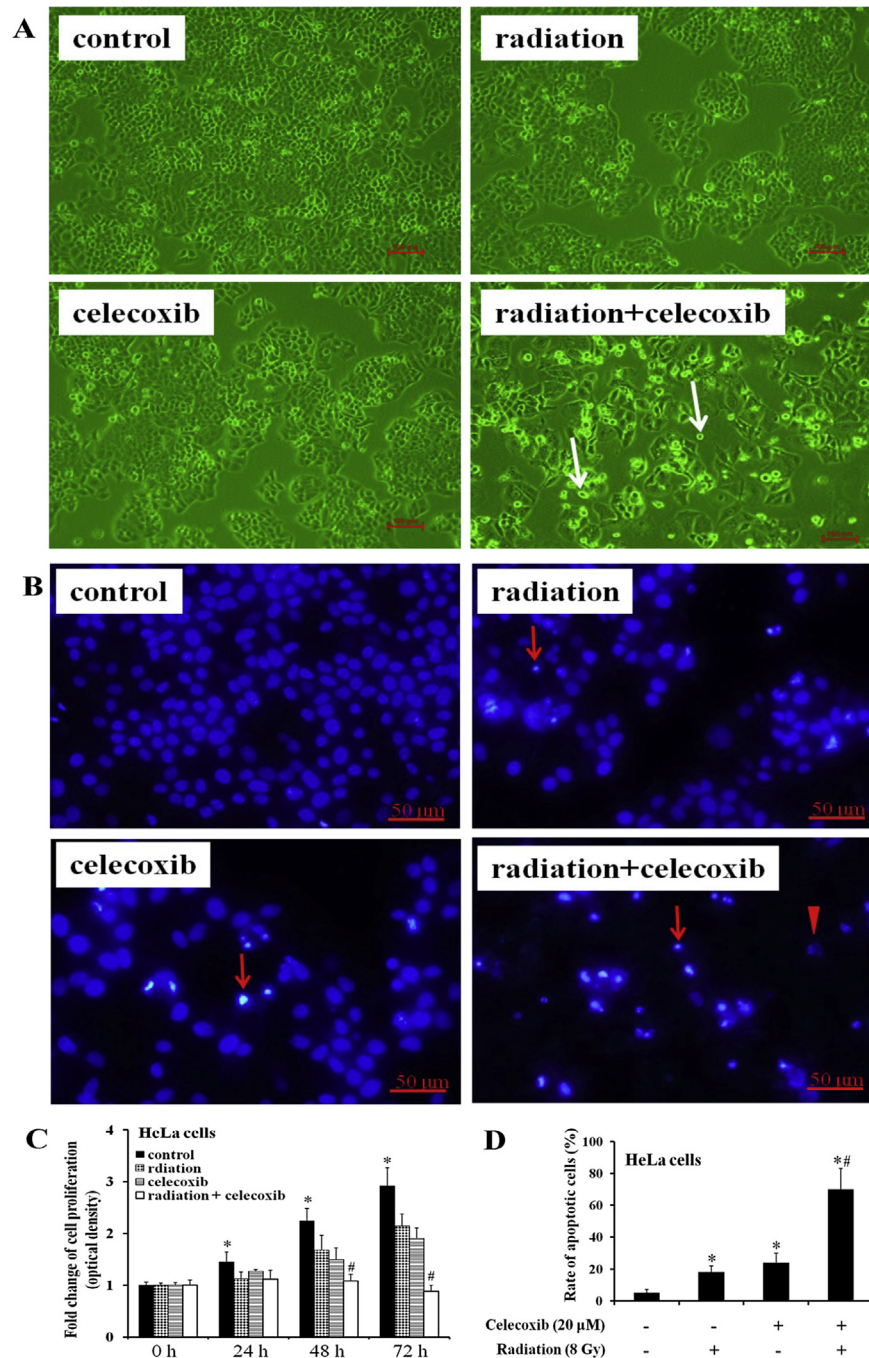
Fig. 1. COX-2 inhibitor, celecoxib, enhanced radiation-induced inhibition of cell proliferation and apoptosis. (A) Microphotographs of cells after different treatments. Apoptotic cells were shrunk and floating (arrows). (B) Fluorescent microphotographs of cells after different treatments. Cells were stained with DAPI after different treatments and those with nuclear condensation (arrows) or fragmentation (triangle) were identified as apoptotic cells. Bar = 50 μm. (C) Cell proliferation after different treatments. Data were presented as mean ± SD of at least three independent experiments. $^*P < 0.05$ vs. the radiation or celecoxib or combinational group; $^\#P < 0.05$ vs. the radiation or celecoxib group (n = 3). (D) Apoptotic rate of cells after different treatments. Data were presented as mean ± SD of at least three independent experiments. $^*P < 0.05$ vs. the control group; $^\#P < 0.05$ vs. the celecoxib or radiation group (n = 3).

COX-2 inhibitors enhanced radiosensitization at least partially by activating PTEN to antagonize radiation-induced activation of AKT. We observed that COX-2 inhibitor celecoxib could significantly enhance radiosensitization of the two types of cancer cells. We also observed that both COX-2 inhibitors, namely, celecoxib and valde-coxib, not only significantly induced PTEN expression, but also dephosphorylated PTEN, thereby promoting PTEN membrane translocation or activation. These findings indicated that COX-2 inhibitors were PTEN inducers and PTEN activators. COX-2 inhibitors also inactivated AKT. COX-2 inhibitors completely blocked radiation-induced increase of PTEN phosphorylation, thereby rescuing radiation-induced decrease of PTEN membrane translocation and correspondingly blocking radiation-induced activation of AKT. Considering that AKT activation plays a crucial role in radioresistance [17–19], our results suggested that upregulating and activating PTEN by COX-2 inhibitors contributed to the
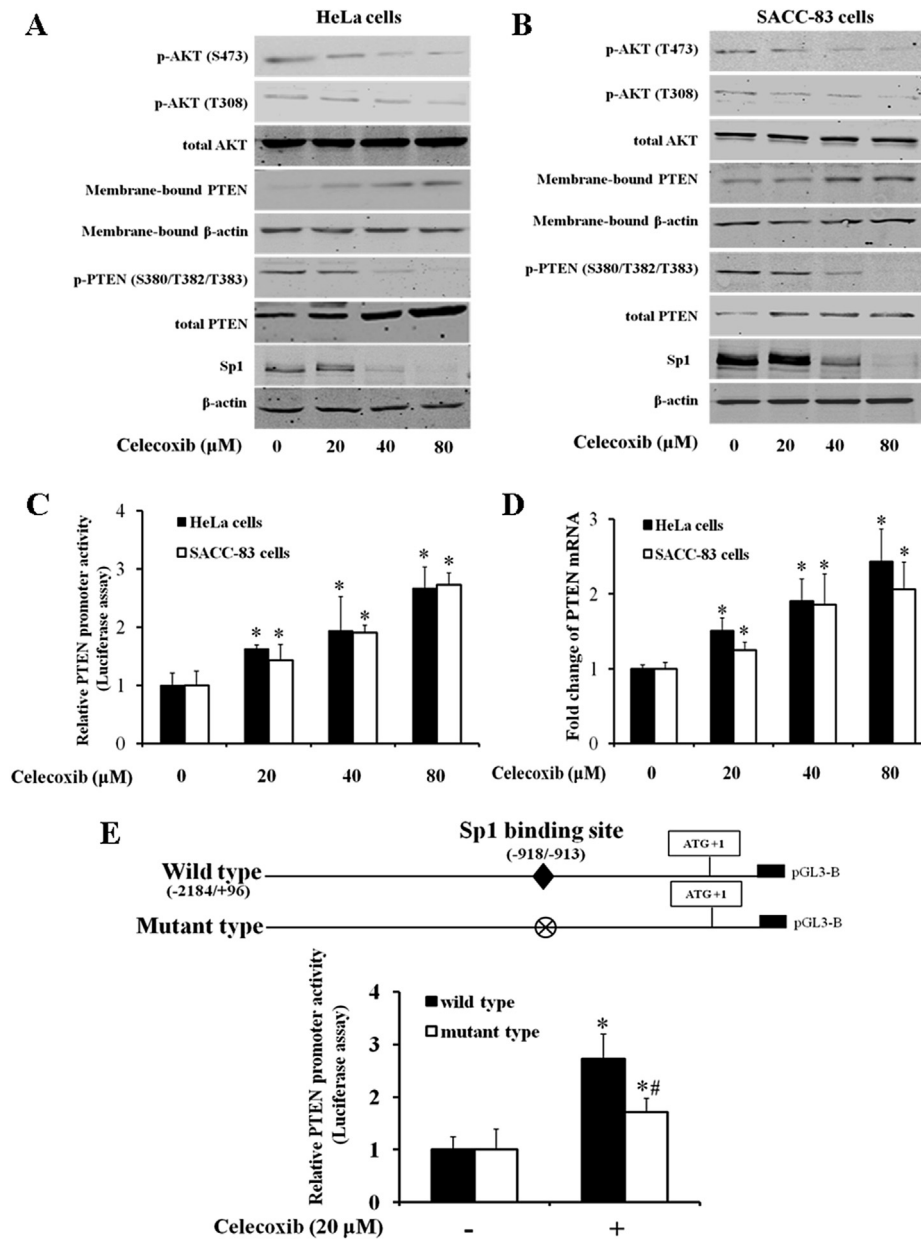
Fig. 2. COX-2 inhibitor celecoxib activated PTEN, inactivated AKT, and upregulated PTEN. (A, B) Western blot analysis of AKT, PTEN, and Sp1 after treatment with celecoxib. HeLa (A) and SACC-83 cells (B) were treated with different doses of celecoxib for 24 h. Cells were lysed or extracted for membrane proteins and subjected to Western blot assay. p-AKT, phosphorylated AKT; p-PTEN, phosphorylated PTEN. (C) Celecoxib upregulated PTEN promoter activity. HeLa or SACC-83 cells were transfected with PTEN promoter-reporter constructs and treated with celecoxib for 24 h, after which luciferase activity was measured and normalized by total protein concentration.*$P < 0.05$ vs. the control ($n = 3$). (D) Celecoxib increased PTEN mRNA expression. The mRNA expression of PTEN was quantitated by real-time PCR after HeLa cells were treated with celecoxib for 24 h *$P < 0.05$ vs. the control ($n = 3$). (E) Sp1 was involved in celecoxib-induced PTEN promoter activity. Upper panel shows the schematic map of PTEN promoter. The PTEN promoter is simulated by a straight line. The Sp1-binding site is represented by a filled diamond shape, and the mutated Sp1-binding site is represented by a crossed circle. The translational start site was defined as (+1) because multiple transcriptional start sites between −958 and −821 before the initiation codon ATG were reported. The black box represents the luciferase reporter (pGL3-B, pGL3-Basic plasmid). Lower panel shows the data of luciferase assay. Luciferase activity was measured after HeLa cells were transfected with wild type or mutated PTEN promoter-reporter constructs and treated with 20 μM celecoxib for 24 h.

antagonism of radiation-induced AKT activation. This occurrence could be another important mechanism underlying COX-2 inhibitors' enhancement of radiosensitization besides the other previously reported mechanisms [29,30]. Therefore, our results further supported the assumption that COX-2 inhibitors could be a clinically available and promising enhancer of tumor radiotherapy. A phase I clinical study already showed that celecoxib can improve radiotherapy of non-small cell lung cancer patients [35]. However, according to our results, the enhancement of radiosensitization by

COX-2 inhibitors could be compromised in tumors with aberrant PTEN. Further studies are needed to evaluate how much the enhancement on radiosensitization by COX-2 inhibitors would be compromised in PTEN-null cells. Nevertheless, our results have clinical relevance because the application of COX-2 inhibitors as an enhancer for radiotherapy may be more suitable for tumors whose PTEN was not mutated or deleted.

COX-2 inhibitors upregulated PTEN protein expression at least partially by inhibiting Sp1 expression, thereby activating PTEN
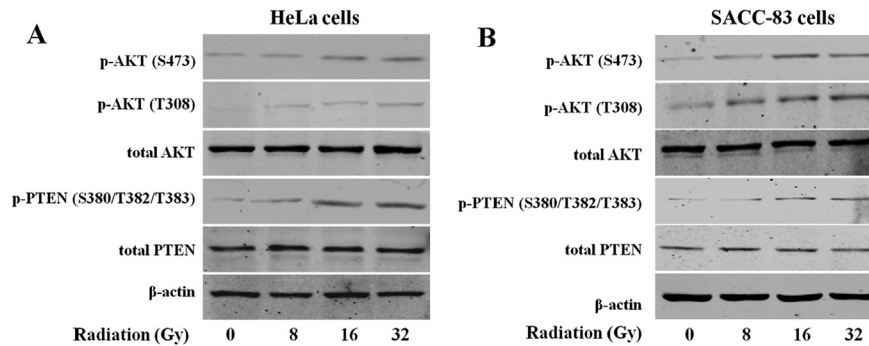
Fig. 3. Radiation inactivated PTEN and activated AKT. (A, B) Western blot analysis of AKT and PTEN after radiation. HeLa (A) and SACC-83 cells (B) were lysed and subjected to Western blot after exposure to increasing doses of radiation.
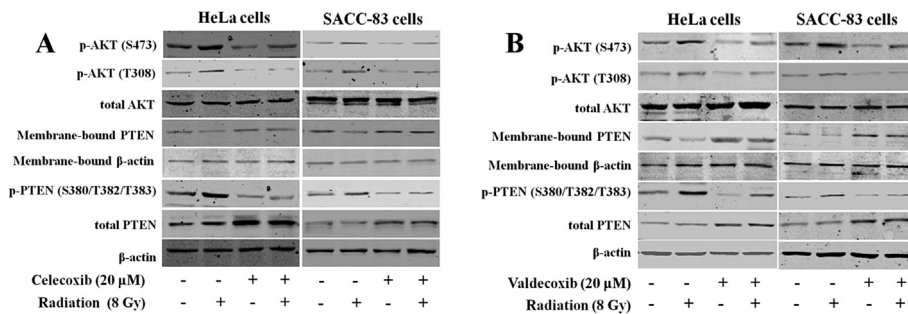

Fig. 4. COX-2 inhibitors antagonized radiation-induced inactivation of PTEN and activation of AKT. (A) Western blot analysis of AKT and PTEN after different treatments. HeLa and SACC-83 cells were treated with radiation, celecoxib, or both radiation and celecoxib. (B) HeLa and SACC-83 cells were treated with radiation, valdecoxib, or both radiation and valdecoxib. Cells were lysed or extracted for membrane proteins and then subjected to Western blot.

transcription. Several phosphorylation sites of PTEN have been identified, including S362, T366, S370, S380, T382, T383, and S385 in the C-terminal of PTEN [36–39]. Phosphorylation of the C-terminal of PTEN contributes PTEN stabilization because mutations at S380, T382, and T383 in the C-terminal of PTEN lead to low stability of the PTEN protein [36]. Our study showed that COX-2 inhibitors dephosphorylated PTEN at S380, T382, and T383 in the C-terminal. However, COX-2 inhibitors did not downregulate the total PTEN protein, but rather conversely upregulated it. This finding may explain why COX-2 inhibitors strongly activated PTEN transcription, thereby sufficiently compensating for and even exceeding the dephosphorylation-induced loss of PTEN stability. COX-2 inhibitor celecoxib could dose-dependently upregulate PTEN mRNA and activate the PTEN promoter. Moreover, we also showed that COX-2 inhibitor-induced upregulation of PTEN was at least partially due to the inhibition of Sp1 expression, since COX-2 inhibitor celecoxib dose-dependently downregulated Sp1 expression, and the mutation of the Sp1 specific binding site in the PTEN promoter partially abolished the effect of celecoxib on PTEN promoter activity.

In conclusion, we showed that COX-2 inhibitors activated PTEN to antagonize radiation-induced PTEN inactivation and AKT activation, thereby contributing to radiosensitization.

Conflict of interest

None declared.

Acknowledgments

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.03.008.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.03.008.

References

[1] L.W. Brady, S. Kramer, S.H. Levitt, R.G. Parker, W.E. Powers, Radiation oncology: contributions of the United States in the last years of the 20th century, Radiology 219 (2001) 1–5.

[2] L. Mao, Oral squamous cell carcinoma - progresses from risk assessment to treatment, Chin. J. Dent. Res. 15 (2012) 83–88.

[3] S. Kesari, S.J. Advani, J.D. Lawson, K.T. Kahle, K. Ng, B. Carter, C.C. Chen, DNA damage response and repair: insights into strategies for radiation sensitization of gliomas, Future Oncol. 7 (2011) 1335–1346.

[4] L. Milas, U. Raju, Z. Liao, J. Ajani, Targeting molecular determinants of tumor chemo-radioresistance, Semin. Oncol. 32 (2005) S78–S81.

[5] J. Bernier, Current state-of-the-art for concurrent chemoradiation, Semin. Radiat. Oncol. 19 (2009) 3–10.

[6] S. Lee, M.J. Lim, M.H. Kim, C.H. Yu, Y.S. Yun, J. Ahn, J.Y. Song, An effective strategy for increasing the radiosensitivity of human lung cancer cells by blocking Nrf2-dependent antioxidant responses, Free Radic. Biol. Med. 53 (2012) 807–816.

[7] M. Provencio, A. Sanchez, P. Garrido, F. Valcarcel, New molecular targeted therapies integrated with radiation therapy in lung cancer, Clin. Lung Cancer 11 (2010) 91–97.

[8] M.A. Brach, H.J. Gruss, T. Kaisho, Y. Asano, T. Hirano, F. Herrmann, Ionizing radiation induces expression of interleukin 6 by human fibroblasts involving activation of nuclear factor-kappa B, J. Biol. Chem. 268 (1993) 8466–8472.

[9] H. Harant, R. de Martin, P.J. Andrew, E. Foglar, C. Dittrich, I.J. Lindley, Synergistic activation of interleukin-8 gene transcription by all-trans-retinoic acid and tumor necrosis factor-alpha involves the transcription factor NF-kappaB, J. Biol. Chem. 271 (1996) 26954–26961.

[10] M. Hinz, D. Krappmann, A. Eichten, A. Heder, C. Scheidereit, M. Strauss, NF-kappaB function in growth control: regulation of cyclin D1 expression and G0/G1-to-S-phase transition, Mol. Cell. Biol. 19 (1999) 2690–2698.

[11] K. Valerie, L.F. Povirk, Regulation and mechanisms of mammalian double-strand break repair, Oncogene 22 (2003) 5792–5812.

[12] P. Dent, A. Yacoub, J. Contessa, R. Caron, G. Amorino, K. Valerie, M.P. Hagan, S. Grant, R. Schmidt-Ullrich, Stress and radiation-induced activation of multiple intracellular signaling pathways, Radiat. Res. 159 (2003) 283–300.

[13] J. Cicenas, The potential role of Akt phosphorylation in human cancers, Int. J. Biol. Markers 23 (2008) 1–9.

[14] X. Zhang, B. Jin, C. Huang, The PI3K/Akt pathway and its downstream transcriptional factors as targets for chemoprevention, Curr. Cancer Drug Targets 7 (2007) 305–316.

[15] J. Bussink, A.J. van der Kogel, J.H. Kaanders, Activation of the PI3-K/AKT pathway and implications for radioresistance mechanisms in head and neck cancer, Lancet Oncol. 9 (2008) 288–296.

[16] I. Shiojima, K. Walsh, Role of Akt signaling in vascular homeostasis and angiogenesis, Circ. Res. 90 (2002) 1243–1250.

[17] S.J. Jeong, C.A. Pise-Masison, M.F. Radonovich, H.U. Park, J.N. Brady, Activated AKT regulates NF-kappaB activation, p53 inhibition and cell survival in HTLV-1-transformed cells, Oncogene 24 (2005) 6719–6728.

[18] K. Soderlund, G. Perez-Tenorio, O. Stal, Activation of the phosphatidylinositol 3-kinase/Akt pathway prevents radiation-induced apoptosis in breast cancer cells, Int. J. Oncol. 26 (2005) 25–32.

[19] J.L. Nakamura, A. Karlsson, N.D. Arvold, A.R. Gottschalk, R.O. Pieper, D. Stokoe, D.A. Haas-Kogan, PKB/Akt mediates radiosensitization by the signaling inhibitor LY294002 in human malignant gliomas, J. Neurooncol. 71 (2005) 215–222.

[20] L.C. Cantley, B.G. Neel, New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 4240–4245.

[21] F. Vazquez, W.R. Sellers, The PTEN tumor suppressor protein: an antagonist of phosphoinositide 3-kinase signaling, Biochim. Biophys. Acta 1470 (2000) M21–M35.

[22] N. Chalhoub, S.J. Baker, PTEN and the PI3-kinase pathway in cancer, Annu. Rev. Pathol. 4 (2009) 127–150.

[23] F. Vazquez, S. Matsuoka, W.R. Sellers, T. Yanagida, M. Ueda, P.N. Devreotes, Tumor suppressor PTEN acts through dynamic interaction with the plasma membrane, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 3633–3638.

[24] G. Denning, B. Jean-Joseph, C. Prince, D.L. Durden, P.K. Vogt, A short N-terminal sequence of PTEN controls cytoplasmic localization and is required for suppression of cell growth, Oncogene 26 (2007) 3930–3940.

[25] S.M. Walker, N.R. Leslie, N.M. Perera, I.H. Batty, C.P. Downes, The tumour-suppressor function of PTEN requires an N-terminal lipid-binding motif, Biochem. J. 379 (2004) 301–307.

[26] R.E. Harris, Cyclooxygenase-2 (cox-2) blockade in the chemoprevention of cancers of the colon, breast, prostate, and lung, Inflammopharmacology 17 (2009) 55–67.

[27] D. Wang, R.N. Dubois, Prostaglandins and cancer, Gut 55 (2006) 115–122.

[28] K.K. Steinauer, I. Gibbs, S. Ning, J.N. French, J. Armstrong, S.J. Knox, Radiation induces upregulation of cyclooxygenase-2 (COX-2) protein in PC-3 cells, Int. J. Radiat. Oncol. Biol. Phys. 48 (2000) 325–328.

[29] K.H. Chen, C.C. Hsu, W.S. Song, C.S. Huang, C.C. Tsai, C.D. Kuo, H.S. Hsu, T.H. Tsai, C.Y. Tsai, L.C. Woung, S.H. Chiou, K.H. Lu, Y.W. Chen, Celecoxib enhances radiosensitivity in medulloblastoma-derived CD133-positive cells, Childs Nerv. Syst. 26 (2010) 1605–1612.

[30] M.Y. Yang, H.T. Lee, C.M. Chen, C.C. Shen, H.I. Ma, Celecoxib suppresses the phosphorylation of STAT3 protein and can enhance the radiosensitivity of medulloblastoma-derived cancer stem-like cells, Int. J. Mol. Sci. 15 (2014) 11013–11029.

[31] K. Hu, S.L. Li, Y.H. Gan, C.Y. Wang, G.Y. Yu, Epiregulin promotes migration and invasion of salivary adenoid cystic carcinoma cell line SACC-83 through activation of ERK and Akt, Oral Oncol. 45 (2009) 156–163.

[32] X.X. Kou, T. Hao, Z. Meng, Y.H. Zhou, Y.H. Gan, Acetylated Sp1 inhibits PTEN expression through binding to PTEN core promoter and recruitment of HDAC1 and promotes cancer cell migration and invasion, Carcinogenesis 34 (2013) 58–67.

[33] M. Abdelrahim, S. Safe, Cyclooxygenase-2 inhibitors decrease vascular endothelial growth factor expression in colon cancer cells by enhanced degradation of Sp1 and Sp4 proteins, Mol. Pharmacol. 68 (2005) 317–329.

[34] K. Datta, S. Suman, A.J. Fornace Jr., Radiation persistently promoted oxidative stress, activated mTOR via PI3K/Akt, and downregulated autophagy pathway in mouse intestine, Int. J. Biochem. Cell Biol. 57 (2014) 167–176.

[35] Z. Liao, R. Komaki, L. Milas, C. Yuan, M. Kies, J.Y. Chang, M. Jeter, T. Guerrero, G. Blumenschien, C.M. Smith, F. Fossella, B. Brown, J.D. Cox, A phase I clinical trial of thoracic radiotherapy and concurrent celecoxib for patients with unfavorable performance status inoperable/unresectable non-small cell lung cancer, Clin. Cancer Res. 11 (2005) 3342–3348.

[36] F. Vazquez, S. Ramaswamy, N. Nakamura, W.R. Sellers, Phosphorylation of the PTEN tail regulates protein stability and function, Mol. Cell. Biol. 20 (2000) 5010–5018.

[37] R. Fragoso, J.T. Barata, Kinases, tails and more: regulation of PTEN function by phosphorylation, Methods (2014), http://dx.doi.org/10.1016/j.ymeth.2014.10.015.

[38] S.J. Miller, D.Y. Lou, D.C. Seldin, W.S. Lane, B.G. Neel, Direct identification of PTEN phosphorylation sites, FEBS Lett. 528 (2002) 145–153.

[39] J. Torres, R. Pulido, The tumor suppressor PTEN is phosphorylated by the protein kinase CK2 at its C terminus. Implications for PTEN stability to proteasome-mediated degradation, J. Biol. Chem. 276 (2001) 993–998.