Biomedicine & Pharmacotherapy 97 (2018) 115–119

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Original article

Curcumin sensitizes lymphoma cells to DNA damage agents through regulating Rad51-dependent homologous recombination

Qian Zhao[a,1], Jiawei Guan[a,1], Yuanhua Qin[b,1], Peng Ren[c,*], Zhiwei Zhang[a], Jian Lv[a], Shijie Sun[d], Cuili Zhang[a], Weifeng Mao[a,*]

[a] Department of Biotechnology, College of Basic Medical Sciences, Dalian Medical University, Dalian 116044, China
[b] Department of Parasitology, College of Basic Medical Sciences, Dalian Medical University, Dalian 116044, China
[c] Qianbaiji Aromatherapy, Co. Ltd, Dalian 116044, China
[d] Department of Immunology, College of Basic Medical Sciences, Dalian Medical University, Dalian 116044, China

ARTICLE INFO

Keywords:
Curcumin
DNA damage
Rad51
Homologous recombination
Apoptosis

ABSTRACT

Curcumin is a natural compound isolated from the rhizome of *Curcuma longa*. It possesses anti-tumor activity through arresting cell cycles and promoting cell apoptosis. However, the effect of curcumin on DNA damage is not well defined. In this study, we investigated the effect of curcumin on inducing DNA damage and on sensitizing lymphoma cells to anti-tumoral DNA damage drugs. Western blot showed curcumin induced γ-H2AX foci in CH12F3 lymphoma cells, which suggests curcumin induces DNA breaks. In addition, curcumin decreased the expression of Rad51, which suggests curcumin induces DNA damage through regulating Rad51-dependant homologous recombination. Rad51-dependant homologous recombination is a vital DNA repair pathway for cancer cells to resist anti-tumoral DNA damage drugs, therefore, we studied the effect of curcumin on the sensitizing lymphoma cells to various chemotherapeutic drugs. We found low level of curcumin (5 μM) sensitized lymphoma cells to anti-tumoral DNA damage agents including cisplatin, methyl methanesulfonate, hydroxyurea and camptothecin. We also found curcumin sensitized CH12F3 lymphoma cells to DNA-PK and PARP inhibitors. Flow cytometry analysis showed curcumin promoted apoptosis and western blot analysis confirmed curcumin activated caspase3-dependent apoptosis. Taken together, these results demonstrate that curcumin induces DNA damage through regulating Rad51-dependant homologous recombination and triggers caspase3-dependent apoptosis, more importantly, curcumin sensitizes lymphoma cells to various DNA damage drugs. Consequently, curcumin would be a potent agent for sensitizing lymphoma cells to anti-tumoral chemotherapeutic agents.

1. Introduction

Curcumin is a natural compound isolated from the rhizome of *Curcuma longa* [1]. Curcumin possesses anti-tumor activity in different types of cancers including lung cancer, colon cancer, breast cancer, leukemia, ovarian cancer and liver cancer [2–5]. Curcumin reportedly suppresses tumor cell proliferation and invasion, causes cell cycle arrest, and induces cell apoptosis [4,6–8]. Curcumin also has other biological effects including anti-oxidant and anti-inflammatory activities and acts as a dietary condiment [9–12]. However, the roles of curcumin in DNA damage were not well defined, and the effects of curcumin in sensitizing tumor cells to anti-tumoral drugs were not well known. Thus it is important to study the actions of curcumin in DNA damage and its effects to suppress tumor cells in combination with anti-tumoral agents.

During tumorigenesis, the mutations often occur in genes related to DNA repair, which include TP53, BRCA1, BRCA2, XRCC1, PTEN and so on [13–17]. Due to the deficient DNA repair system in tumor cells, DNA damage agents are generally used as anti-tumoral drugs to suppress tumor cell growth. Hydroxyurea is used to disrupt progression of the replication fork, which involves in the formation of DSBs at newly replicated DNA [18]. Camptothecin is an inhibitor of topoisomerase I, which can generate DSBs at the sites of DNA replication [19–21]. Cisplatin is widely used as an anti-tumoral drug which reacts with DNA to form DNA interstrand crosslinks that leads to genomic instability

Abbreviations: DSB, double-strand breaks; HR, homologous recombination; NHEJ, non-homologous end-joining; PARP, poly(ADP-ribose) polymerase; PAR, poly(ADP-ribose); MTT, methyl thiazolyl; PI, propidium iodide; SD, standard deviations; SSB, single strand break; γH2AX, phosphorylated histone H2AX; HU, hydroxyurea; CAMP, camptothecin

* Corresponding authors.
E-mail addresses: rp999@188.com (P. Ren), maoweifeng@dlmedu.edu.cn (W. Mao).

[1] Zhao, Guan and Qin equally contribute to this work.

http://dx.doi.org/10.1016/j.biopha.2017.09.078
Received 15 August 2017; Received in revised form 13 September 2017; Accepted 18 September 2017

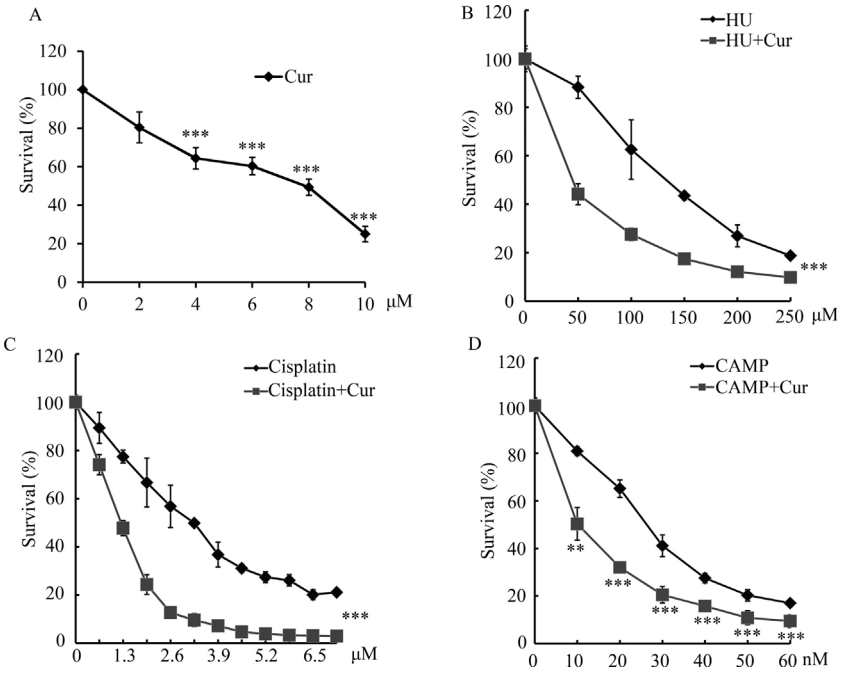

Fig. 1. Curcumin (Cur) sensitizes B lymphoma cells to DNA damage agents including hydroxyurea (HU), camptothecin (CAMP) and cisplatin. (A) The growth curves of CH12F3 cells after curcumin treatment for 36 h were measured through MTT assay. MTT assay was used to test the growth curves of CH12F3 cells treated by 5 μM curcumin in combination with different concentrations of DNA damage drugs for 24 h, (B) HU, (C) cisplatin, (D) CAMP. *P < 0.05 versus control group. **P < 0.01 versus control group. ***P < 0.001 versus control group.

[22,23]. There are two main pathways to repair double-strand breaks (DSB) including homologous recombination (HR) and non-homologous end-joining (NHEJ), in which HR reportedly contributes tumor cells to resist the DNA damage agents [24,25]. Rad51 is a vital factor to search homologous donor sequence in HR pathway. In this study, we analyzed the role of curcumin in regulating Rad51-dependent HR pathway and the effects of curcumin on sensitizing lymphoma cells to various anti-tumoral DNA damage agents.

Poly(ADP-ribose) polymerase (PARP) is a protein family that transfers mono(ADP-ribose) or poly(ADP-ribose) (PAR) group onto their target proteins, in which PARP1 is an important family member [26]. The zinc-figure domain of PARP1 protein recognizes DNA breaks and initiates DNA repair [27]. Inhibition of PARP activity can cause DNA damage, which can efficiently treat HR-deficient tumor cells, while the BRCA1/2 wildtype cells resist to PARP inhibitors [28,29]. DNA-PK is a serine/threonine kinase that plays essential roles to catalyze the downstream factors in NHEJ pathway [24]. We also analyzed the effect of curcumin on growth of lymphoma cells in combination with PARP and DNA-PK inhibitors.

2. Materials and methods

2.1. Cell lines and culture

Murine B Lymphoma CH12F3 was from T. Honjo (Kyoto University, Kyoto, Japan). The cells were cultured in RPMI-1640 medium with 10% fetal bovine serum (Hyclone, Massachusetts, USA), 100 U/ml penicillin and streptocymin, and 50 nM beta-mercaptoethanol. Cells were incubated with 5% CO_2 at 37 ˚C.

2.2. Antibodies and reagents

Curcumin, cisplatin, hydroxyurea, camptothecin, DNA-PK inhibitors (Ku-0060648, Nu-7441), PARP inhibitors (ME0328, Olaparib) were from Selleck Chemicals (Houston, USA). Antibodies against p-H2AX (Ser139) were from Cell Signaling Technologies (USA). Antibodies against Caspase3, Caspase9, Rad51, PCNA, PARP1, β-Actin were obtained from Proteintech (Chicago, USA).

2.3. Cell viability assay

Briefly, 5000 cells were seeded on each well of 96-well plate. After incubation with drugs for appropriate times, MTT were added to each well in a final concentration of 0.5 mg/ml. Subsequently, cells were incubated for 4 h. Then, culture mediums were removed. 200 μl DMSO was added to each well and plates were incubated for 15 min at 37 ˚C. Finally, the absorbance values were detected at 589 nm using microplate reader (Thermo, 354-90230, USA).

2.4. Apoptosis assay

Annexin V and propidium iodide (PI) were used to strain cells treated with curcumin. After incubation for 15 min at room temperature, the apoptotic cells were detected using flow cytometry and the results were analyzed by Flow Plus software.

2.5. Western blot

Nuclear proteins were extracted by nuclear protein extract kit (Shenggong, Shanghai, China). Whole cell proteins were extracted by RIPA buffer. Protein concentrations were quantified by Qubit 2.0 (Invitrogen, USA). Subsequently, 50 μg sample was separated by SDS-PAGE (10% for normal samples, 15% for γH2AX). After transferred to PVDF membranes, samples were detected using different antibodies.

2.6. Statistical analysis

Statistical analysis was detected by Student's *t*-tests using SPSS software. Data are presented as the mean ± standard deviations.

3. Results

3.1. Curcumin suppresses growth of lymphoma cells and sensitizes tumor cells to DNA damage agents including hydroxyurea, camptothecin and cisplatin

We analyzed the growth of CH12F3 cells under the stimulation of curcumin. MTT assay showed curcumin suppressed CH2F3 cell growth (Fig. 1A). We next analyzed the effect of curcumin on sensitizing

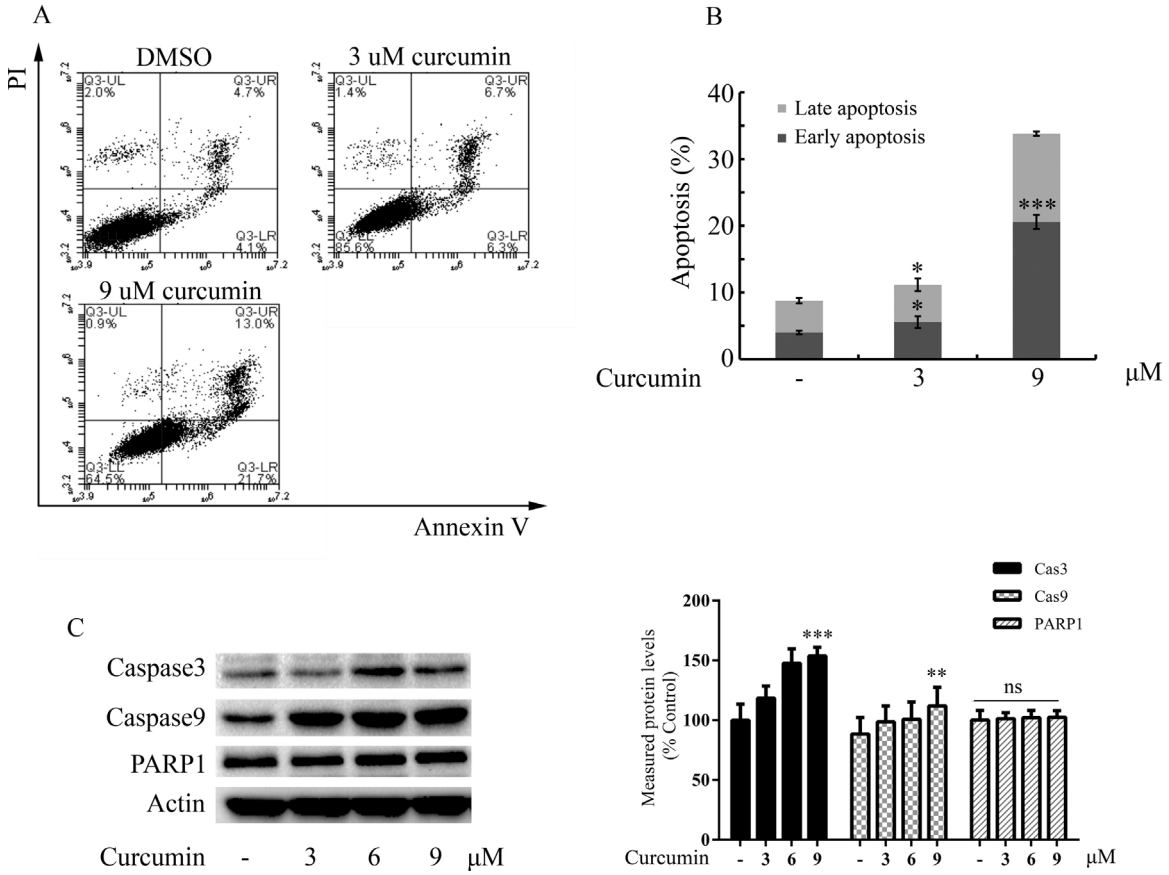

Fig. 2. Curcumin activates caspase3-dependent cell apoptosis. (A) CH12F3 cells were exposed to 3 and 9 μM curcumin for 24 h. PI and AnnexinV double-staining was used to test the apoptosis of cells. (B) Apoptosis rates were quantified in the diagrams. Data are presented as the mean ± SD from three independent experiments. *P < 0.05 versus control group. **P < 0.01 versus control group. ***P < 0.001 versus control group. (C) CH12F3 cells were treated with 3, 6, and 9 μM curcumin for 24 h. Caspase3, caspase9 and PARP1 expressions were detected through western blot. ***P < 0.001 versus control. **P < 0.01 versus control. ns, not significantly different.

CH12F3 cells to anti-tumoral DNA damage agents. Using low dose of curcumin (5 μM), we analyzed the growth curve of CH12F3 cells to curcumin in combination with anti-tumoral drugs, including hydroxyurea (HU), camptothecin (CAMP) and cisplatin. The viability of CH12F3 cells treated with 50 μM HU was 85%, while in combination with 5 μM curcumin, the same dose of HU suppressed cell viability to 43% (Fig. 1B). 5 μM curcumin also sensitized CH12F3 cells to cisplatin and CAMP (Fig. 1C, D). Therefore, these results demonstrate that curcumin sensitizes lymphoma cells to anti-tumoral chemotherapeutic drugs.

3.2. Curcumin induces caspase3-dependent apoptosis

We next analyzed the effects of curcumin on cell apoptosis. Annexin/PI staining assay showed that curcumin induced celllular apoptosis in a dose-dependent manner (Fig. 2A, B). Western blot analysis showed that caspase3, and caspase9 levels in CH12F3 cells were increased after the treatment of curcumin, revealing curcumin induces apoptosis through casepase3 and casepase9-dependent apoptosis pathway (Fig. 2C).

3.3. Curcumin induces DNA breaks and down-regulates Rad51 expression

We next investigated the DNA breaks induced by curcumin in CH12F3 cells. After treating with curcumin (0, 10, 20, 30, 40, 50 μM) for 4 h, the nuclear proteins of CH12F3 cells were extracted. The levels of γH2AX, PARP1, PCNA, and Rad51 were detected by western blot (Fig. 3A). The expression of nuclear γH2AX was up-regulated in a dose-dependent manner, which suggests high dose of curcumin induces DSB. Nuclear PARP1 and PCNA were up-regulated by curcumin (Fig. 3A). PARP1 is an important PARP family member to PARylate the target proteins, which is essential for SSB repair. PCNA is a DNA sliding clamp functioning in DNA replication. We also found curcumin down-regulated Rad51 expression, which demonstrates curcumin impairs Rad51-dependent HR.

3.4. Curcumin sensitizes lymphoma cells to DNA-PK and PARP inhibitors

We found curcumin impaired Rad51-dependent HR repair, thus the combination of curcumin and DNA-PK inhibitors could attenuate both HR and NHEJ repair, subsequently caused more unrepaired DSB and cell lethality. Therefore, we analyzed the sensitization of CH12F3 cells to curcumin in combination with DNA-PK and PARP inhibitors. MTT assay showed curcumin (5 μM) sensitized lymphoma cells to DNA-PK inhibitors (NU7441 and KU0060648) (Fig. 4A, B). PARP inhibitors have recently been clinically used to treat HR-deficient ovarian and breast cancer cells, however, the efficacy of PARP inhibitors in treating HR-proficient cancer cells is not promising. We found curcumin down-regulates Rad51-dependant HR without apparent toxic to cells. Thus, we analyzed the effect of curcumin on sensitizing lymphoma cells to PARP inhibitors. We found curcumin (5 μM) significantly enhanced the sensitivity of CH12F3 cells to PARP1 inhibitors (ME0328 and olaparib) (Fig. 4C, D), which suggests curcumin could function as a potent adjuvant for sensitizing cancer cells to PARP inhibitors.

4. Discussion

Curcumin reportedly has anti-inflammatory, anti-oxidant, anti-

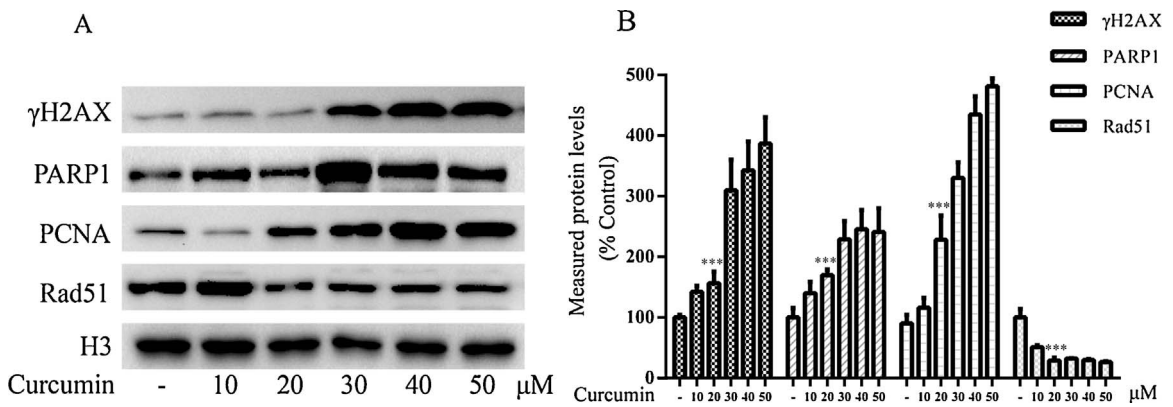

Fig. 3. Curcumin induces DNA breaks and decreases Rad51 expression. (A) Western blot was used to test the expressions of nuclear γH2AX, PARP1, PCNA, Rad51 and histone 3 (H3) in CH12F3 cells treated with different doses of curcumin. (B) The quantification of western blot was analyzed. ***P < 0.001 versus control.

infectious biological activities, thus it has been used to treat various diseases such as asthma, diabetes, hepatic and heart diseases [9,11,30]. Curcumin also has anti-cancer activity through the regulation of inflammatory cytokines, reactive oxygen species and induction of apoptosis [2,3,7]. However, the effect of curcumin on DNA damage is not well defined. Because cancer cells often possess DNA repair deficiency, DNA damage agents have been widely used to treat different types of cancer cells, in which cisplatin is the most widely used anti-tumoral genotoxic drug, however, the recovered cellular DNA repair system often helps cancer cells to resist these DNA damage drugs [22,23]. The resistance of cancer cells to chemotherapeutic drugs severely counteracts the employment of these DNA damage drugs in clinic. We found curcumin regulated Rad51-dependent HR, thus we analyzed the effect of curcumin in combination with different DNA damage drugs on the growth of lymphoma cells. Curcumin effectively sensitized lymphoma cells to cisplain, HU, and CAMP. Consequently, curcumin could be a potent drug to sensitize cancer cells to anti-tumoral genotoxic drugs. In addition, curcumin is a dietary additive that is frequently absorbed by people with food, thus it would be a relative safe phytochemical agent in combination with the DNA damage drugs to treat lymphoma cells.

The PARP inhibitors are recently promising anti-tumoral drugs, which can effectively treat BRCA1 and BRCA2 deficient cancer cells [28]. However, the resistance of cancer cells to PARP inhibitors still appears, in which the HR pathway was indicated to help cells to repair the DNA damage induced by PARP inhibitors. We analyzed the effect of curcumin on cellular DNA repair and found curcumin decreased Rad51-dependent HR pathway. Further studies showed curcumin sensitized lymphoma cells to PARP inhibitors, suggesting curcumin increases the effect of PARP inhibitors through regulating Rad51-dependent HR pathway. We also found curcumin sensitizes lymphoma cells to DNA-PK inhibitors. DNA-PK is a vital factor in NHEJ pathway. As curcumin decreases Rad51-dependent HR pathway, attenuation of NHEJ pathway through DNA-PK inhibitors could cause synthetic lethality to tumor cells.

In summary, we analyzed the DNA damage induced by curcumin and the effect of curcumin to the cellular DNA repair system. Curcumin regulates Rad51-dependent HR pathway, induces DSB and activates caspase3-dependent apoptosis. More importantly, curcumin sensitizes lymphoma cells to cisplatin, HU, CAMP, DNA-PK inhibitors and PARP1 inhibitors. Consequently, curcumin could be a potent anti-tumoral agent that can be combined with different chemotherapeutic drugs to treat lymphoma cells.

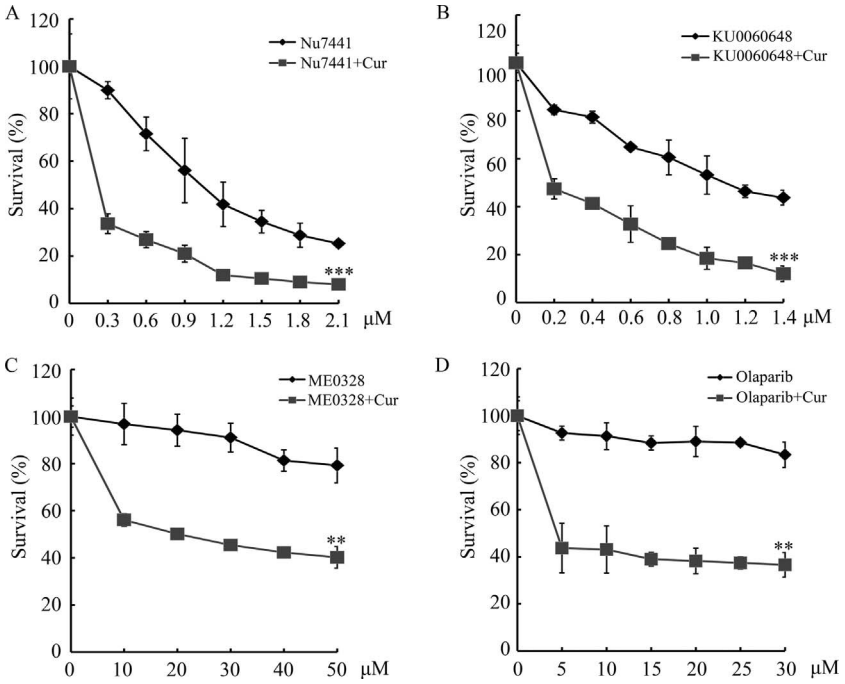

Fig. 4. Curcumin (Cur) sensitizes CH12F3 cells to DNA-PK and PARP inhibitors. MTT assay was used to test the growth curves of CH12F3 cells treated by 5 μM curcumin in combination with different concentrations of DNA-PK inhibitors including Nu7441 (A) and KU0060648 (B), PARP1 inhibitors including ME0328 (C) and olaparib (D) for 24 h.

Conflict of interest

Authors have declared that no competing interest exists.

Acknowledgements

This work was supported by Chinese National Scientific Foundation to Weifeng Mao (Nos. 81201563, 31371254).

References

[1] H. Hatcher, R. Planalp, J. Cho, F.M. Torti, S.V. Torti, Curcumin: from ancient medicine to current clinical trials, Cell. Mol. Life Sci. 65 (11) (2008) 1631–1652.
[2] A. Chen, J. Xu, A.C. Johnson, Curcumin inhibits human colon cancer cell growth by suppressing gene expression of epidermal growth factor receptor through reducing the activity of the transcription factor Egr-1, Oncogene 25 (2) (2006) 278–287.
[3] H.W. Chen, J.Y. Lee, J.Y. Huang, C.C. Wang, W.J. Chen, S.F. Su, C.W. Huang, C.C. Ho, J.J. Chen, M.F. Tsai, S.L. Yu, P.C. Yang, Curcumin inhibits lung cancer cell invasion and metastasis through the tumor suppressor HLJ1, Cancer Res. 68 (18) (2008) 7428–7438.
[4] T. Jia, L. Zhang, Y. Duan, M. Zhang, G. Wang, J. Zhang, Z. Zhao, The differential susceptibilities of MCF-7 and MDA-MB-231 cells to the cytotoxic effects of curcumin are associated with the PI3 K/Akt-SKP2-Cip/Kips pathway, Cancer Cell Int. 14 (1) (2014) 126.
[5] U. Banik, S. Parasuraman, A.K. Adhikary, N.H. Othman, Curcumin: the spicy modulator of breast carcinogenesis, J. Exp. Clin. Cancer Res.: CR 36 (1) (2017) 98.
[6] A.B. Shakor, M. Atia, I.A. Ismail, A. Alshehri, H. El-Refaey, K. Kwiatkowska, A. Sobota, Curcumin induces apoptosis of multidrug-resistant human leukemia HL60 cells by complex pathways leading to ceramide accumulation, Biochim. Biophys. Acta 1841 (12) (2014) 1672–1682.
[7] J.A. Seo, B. Kim, D.N. Dhanasekaran, B.K. Tsang, Y.S. Song, Curcumin induces apoptosis by inhibiting sarco/endoplasmic reticulum Ca ATPase activity in ovarian cancer cells, Cancer Lett. 371 (1) (2016) 30–37.
[8] N. Bortel, S. Armeanu-Ebinger, E. Schmid, B. Kirchner, J. Frank, A. Kocher, C. Schiborr, S. Warmann, J. Fuchs, V. Ellerkamp, Effects of curcumin in pediatric epithelial liver tumors: inhibition of tumor growth and alpha-fetoprotein in vitro and in vivo involving the NFkappaB- and the beta-catenin pathways, Oncotarget. 6 (38) (2015) 40680–40691.
[9] B. Joe, M. Vijaykumar, B.R. Lokesh, Biological properties of curcumin-cellular and molecular mechanisms of action, Crit. Rev. Food Sci. Nutr. 44 (2) (2004) 97–111.
[10] M.K. Bae, S.H. Kim, J.W. Jeong, Y.M. Lee, H.S. Kim, S.R. Kim, I. Yun, S.K. Bae, K.W. Kim, Curcumin inhibits hypoxia-induced angiogenesis via down-regulation of HIF-1, Oncol. Rep. 15 (6) (2006) 1557–1562.
[11] G.M. Calaf, C. Echiburu-Chau, D. Roy, Y. Chai, G. Wen, A.S. Balajee, Protective role of curcumin in oxidative stress of breast cells, Oncol. Rep. 26 (4) (2011) 1029–1035.
[12] A. Eser, D. Hizli, H. Haltas, M. Namuslu, A. Kosus, N. Kosus, H. Kafali, Effects of curcumin on ovarian ischemia-reperfusion injury in a rat model, Biomed. Rep. 3 (6) (2015) 807–813.
[13] A.N. Bullock, A.R. Fersht, Rescuing the function of mutant p53, Nat. Rev. Cancer 1 (1) (2001) 68–76.
[14] A. Juvekar, L.N. Burga, H. Hu, E.P. Lunsford, Y.H. Ibrahim, J. Balmana, A. Rajendran, A. Papa, K. Spencer, C.A. Lyssiotis, C. Nardella, P.P. Pandolfi, J. Baselga, R. Scully, J.M. Asara, L.C. Cantley, G.M. Wulf, Combining a PI3 K inhibitor with a PARP inhibitor provides an effective therapy for BRCA1-related breast cancer, Cancer Discov. 2 (11) (2012) 1048–1063.
[15] R.B. Jensen, A. Carreira, S.C. Kowalczykowski, Purified human BRCA2 stimulates RAD51-mediated recombination, Nature 467 (7316) (2010) 678–683.
[16] R. Sultana, T. Abdel-Fatah, R. Abbotts, C. Hawkes, N. Albarakati, C. Seedhouse, G. Ball, S. Chan, E.A. Rakha, I.O. Ellis, S. Madhusudan, Targeting XRCC1 deficiency in breast cancer for personalized therapy, Cancer Res. 73 (5) (2013) 1621–1634.
[17] C. Bassi, J. Ho, T. Srikumar, R.J. Dowling, C. Gorrini, S.J. Miller, T.W. Mak, B.G. Neel, B. Raught, V. Stambolic, Nuclear PTEN controls DNA repair and sensitivity to genotoxic stress, Science 341 (6144) (2013) 395–399.
[18] C. Lundin, K. Erixon, C. Arnaudeau, N. Schultz, D. Jenssen, M. Meuth, T. Helleday, Different roles for nonhomologous end joining and homologous recombination following replication arrest in mammalian cells, Mol. Cell. Biol. 22 (16) (2002) 5869–5878.
[19] S. Squires, A.J. Ryan, H.L. Strutt, R.T. Johnson, Hypersensitivity of Cockayne's syndrome cells to camptothecin is associated with the generation of abnormally high levels of double strand breaks in nascent DNA, Cancer Res. 53 (9) (1993) 2012–2019.
[20] A.F. Stewart, G. Schutz, Camptothecin-induced in vivo topoisomerase I cleavages in the transcriptionally active tyrosine aminotransferase gene, Cell 50 (7) (1987) 1109–1117.
[21] L. Ferrara, E.B. Kmiec, Camptothecin enhances the frequency of oligonucleotide-directed gene repair in mammalian cells by inducing DNA damage and activating homologous recombination, Nucleic Acids Res. 32 (17) (2004) 5239–5248.
[22] H. Huang, L. Zhu, B.R. Reid, G.P. Drobny, P.B. Hopkins, Solution structure of a cisplatin-induced DNA interstrand cross-link, Science 270 (5243) (1995) 1842–1845.
[23] M. Enoiu, J. Jiricny, O.D. Scharer, Repair of cisplatin-induced DNA interstrand crosslinks by a replication-independent pathway involving transcription-coupled repair and translesion synthesis, Nucleic Acids Res. 40 (18) (2012) 8953–8964.
[24] M.R. Lieber, The mechanism of double-strand DNA break repair by the non-homologous DNA end-joining pathway, Annu. Rev. Biochem. 79 (2010) 181–211.
[25] L. Han, W. Mao, K. Yu, X-ray repair cross-complementing protein 1 (XRCC1) deficiency enhances class switch recombination and is permissive for alternative end joining, Proc. Natl. Acad. Sci. U. S. A. 109 (12) (2012) 4604–4608.
[26] B.A. Gibson, W.L. Kraus, New insights into the molecular and cellular functions of poly(ADP-ribose) and PARPs, Nat. Rev. Mol. Cell Biol. 13 (7) (2012) 411–424.
[27] A.A. Ali, G. Timinszky, R. Arribas-Bosacoma, M. Kozlowski, P.O. Hassa, M. Hassler, A.G. Ladurner, L.H. Pearl, A.W. Oliver, The zinc-finger domains of PARP1 cooperate to recognize DNA strand breaks, Nat. Struct. Mol. Biol. 19 (7) (2012) 685–692.
[28] L. Hutchinson, Targeted therapies: PARP inhibitor olaparib is safe and effective in patients with BRCA1 and BRCA2 mutations, Nat. Rev. Clin. Oncol. 7 (10) (2010) 549.
[29] J. Balmana, S.M. Domchek, A. Tutt, J.E. Garber, Stumbling blocks on the path to personalized medicine in breast cancer: the case of PARP inhibitors for BRCA1/2-associated cancers, Cancer Discov. 1 (1) (2011) 29–34.
[30] G.M. Calaf, C. Echiburu-Chau, G. Wen, A.S. Balajee, D. Roy, Effect of curcumin on irradiated and estrogen-transformed human breast cell lines, Int. J. Oncol. 40 (2) (2012) 436–442.