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2'-BENZOYLOXYCINNAMALDEHYDE REGULATES PTEN INDEPENDENT OF DJ-1 IN MDA-MB-435 BREAST CANCER CELLS

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ABSTRACT

2'-Benzoyloxycinnamaldehyde (BCA) is a promising anticancer candidate against several types of cancers, including breast cancer. DJ-1 has been found to act as an oncogene, protecting cells against oxidative stress. A previous study showed that BCA-inducing antiproliferation was associated with continuous decrease in DJ-1 expression in MDA-MB-435 breast cancer cells. In this study, we found that this DJ-1 decrease after BCA treatment is associated with increased reactive oxygen species (ROS) release, and depletion of glutathione (GSH), and γ-glutamylcysteine synthetase (γ-GCS). The tumor suppressor Pten protein is known to be a downstream target for DJ-1. Therefore, Pten expression was investigated after BCA treatment and DJ-1 siRNA transfection. DJ-1 knockdown with DJ-1 siRNA transfection markedly increased Pten expression. However, unexpectedly BCA-inducing DJ-1 downregulation is associated with decreased Pten protein expression. Altogether, these data suggest that BCA downregulates Pten protein expression in MDA-MB-435 cells independent of DJ-1.

KEYWORDS

2'-Benzoyloxycinnamaldehyde (BCA), Breast cancer cells, MDA-MB-435, DJ-1, Pten.

Abbreviations: *BCA, 2'-benzoyloxycinnamaldehyde; GSH, glutathione; γ-GCS, γ-glutamylcysteine synthetase; HCA, 2'-Hydroxycinnamaldehyde; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoli-um bromide; Nrf2, NF-E2 p45-related factor 2; ROS, reactive oxygen species.*

INTRODUCTION

Cassia or Chinese cinnamon is a widely used spice made from the inner bark of the cinnamon tree. 2'-Hydroxycinnamaeldehyde (HCA) is the main effective component naturally extracted from the bark of *Cinnamomum cassia* Blume [1]. 2'-Benzoyloxycinnamaldehyde (BCA) is chemically synthesized from HCA [2]. Both HCA and BCA have been reported to have cytotoxic effects, inhibit cell proliferation and induce apoptosis *in vitro,* and inhibit tumor growth *in vivo* [3, 4]. The effect of cinnamaldehyde and its derivatives on breast cancer has not been well-understood. Recent studies indicated that BCA inhibits cell proliferation in breast cancer MCF7 and MDA-MB-435 cells [5]. The BCA-induced antiproliferation was more effective in MDA-MB-435 when compared with MCF-7 cells because of differential DJ-1 upregulation only in MDA-MB-435 cells [5]. Another study showed that cinnamon extract is able to inhibit angiogenesis in MDA-MB-231-indeced tumor in mice via vascular endothelial growth factor (VEGF) inhibition [6]. Given the review of the studies, a lot of investigations are required to elucidate the molecular mechanism(s) of cinnamadehyde and its derivatives on breast cancer. It was reported that HCA and its derivatives act as antitumor agents in diverse cancer cell lines, possibly inducing apoptosis via ROS generation and caspase-3 activation [7]. Furthermore, BCA/HCA-induced apoptosis has also been associated with the inhibition of proteasome activity [8]. BCA shows therapeutic

selectivity in K-ras-transformed cells through downregulation of thiol antioxidants [9], is a promising result for antitumor drug development. It was stated that BCA-inducing cell proliferation inhibition takes place at least in part through downregulation of DJ-1 expression. Recently, DJ-1 known as an oncogene [10], belongs to the peptidase C56 family of proteins. DJ-1 protects cells against oxidative stress in inseveral cancer models [11-16]. It was stated that DJ-1 induces glutathione (GSH) synthesis and scavenges H_2O_2 by cysteine oxidation during oxidative stress [17]. DJ-1 is known to stabilize the antioxidant transcriptional master NF-E2-related factor 2 (Nrf2), leading to the coordinated upregulation of antioxidant genes such as metallothionein and γ-GCS [18, 19]. Van der Brug and his co-workers [20] reported that, DJ-1 promotes ras signaling and suppress tumor suppressor Pten. Also, DJ-1 was reported to act as a negative regulator for the tumor suppressor protein Pten [21-23]. In a previous study, it was shown that BCA-inducing cell proliferation inhibition is associated with DJ-1 downregulation in MDA-MB-435 breast cancer cells [5]. In the current study we evaluated the effect and molecular mechanism (s) of BCA on MDA-MB-435 cells via investigating its effect on cell viability, ROS release, and the DJ-1 downstream antioxidant enzyme targets such as GSH and γ-GCS as well as the tumor suppressor Pten protein expression.

MATERIALS and METHODS

Chemicals and Reagents

MTT (3-[4, 5-dimethyl-2-thiazolyl]-2, 5-diphenyl-2H-tetrazolium bromide), 2', 7'-dichlorodihydrofluorescein diacetate (DCFH2-DA), DMSO (Dimethylesulphoxide), were purchased from Sigma (St. Louis, MO, USA). RPMI culture media, FBS and penicillin/streptomycin antibiotics were from Gibco (Invitrogen Corporation, CA, USA). Qiazol was from Qiagen (Valencia, CA, USA) and 2 X SYBR green PCR master mix from Applied Biosystems (Foster, CA, USA). Mouse monoclonal anti-DJ-1 antibody was purchased from Abcam (Cambridge, UK). Rabbit monoclonal anti-Pten antibody was from Cell Signaling. Rabbit polyclonal anti-γ-GCS (catalytic heavy chain subunit) antibody and HRP-conjugated mouse monoclonal IgG anti-β-actin antibody were from Santa Cruz (Santa Cruz, CA. USA). HRP conjugated goat anti-rabbit and anti-mouse secondary antibodies were from Pierce (Rockford, IL, USA). ECL-plus Western Blotting Detection Reagent was from Amersham Biosciences (St. Giles, Buckinghamshire, UK).

Cell culture and cell viability

Human breast cancer MDA-MB-435 cells were maintained in RPMI culture media, containing 10% FBS, 100 units/ml penicillin and 1 µg/ml streptomycin. Cells were incubated at 37 ^{o}C in a 5% CO_2 humidified atmosphere. MDA-MB-435 cells (5,000 cells/ well) were seeded into 96-well plates. On the following day, cells were treated with different concentrations of BCA as indicated and incubated for another 24 hrs. Cell viability was then estimated using MTT cell viability assay by reading the absorbance at 570 nm as previously described [5].

Intracellular ROS analysis

The effect of BCA on the intracellular ROS level was observed using the H_2O_2-sensitive fluorescent dye 2', 7'-dichlorodihydrofluorescein diacetate ($DCFH_2$-DA) as previously described [8]. MDA-MB-435 cells were treated with BCA (15, 30 and 60 µM) or 0.1% DMSO as a control and incubated for 24 hrs. Cells were treated with $DCFH_2$-DA at a final concentration 5 µg/ml and then incubated for 30 minutes. Cells were then washed twice with PBS and intracellular levels of ROS were analyzed at excitation 470 nm and emission 529

nm using Fluorescence micriplate reader (Infinite M200, TECAN, Switzerland).

Estimation of reduced glutathione

The effect of BCA on GSH was investigated as previously described [9]. Briefly, MDA-MB-435 cells were treated with different BCA concentrations for 24 hrs or with 30 µM in time intervals (0, 3, 6, 12, 24 hrs). Cells then washed with PBS, trypsinized cells were resuspended in 5 ml of PBS. 100 µl of the resuspended cells were used to determine the protein quantity. Cells were centrifuged at 600 g to obtain a packed cell pellet and added 3 volumes of 5% 5-Sulfosalicylic acid (SSA). After lyses by two cycles of freezing and thawing with liquid nitrogen, the lysates were centrifuged at 10,000 g for 10 min to collect supernatant. GSH concentration was determined by Glutathione assay kit (Sigma-Aldrich) according to manufacturer's instruments. Briefly, 10 µl of samples were reacted in 150 µl of working solution (95 mM potassium phosphate buffer, pH 7.0, 0,95 mM EDTA, 0.038 mg/ml NADPH, 0.031 mg/ml 5,5'-dithiobis(2-nitrobenzoic acid) [DTNB], 0.115 units/ml glutathione reductase, and 0.24% SSA) for 5 min and checked the changes of the optical density at 412 nm every 30 sec with VERSA max ELISA reader (Molecular device, PA, USA). The concentrations of sample glutathione were calculated from standard curve of serial diluted standard glutathione.

DJ-1 siRNA transfection and cell viability assay

MDA-MB-435 cells were transfected with either control siRNA or DJ-1 siRNA (siDJ-1) sense: 5´-CGACGAUCACUUAGAGAAATT-3´, antisense: 5´-UUUCUCUAAGUGAUCGUCGCA-3´) (Qiagen, Valencia, CA, USA) as previously described [5]. Briefly, 1 x 10^5 cells were seeded in 60-mm plates and the media replaced on the following day with serum-free media just prior to transfection. Cells were then transfected with DJ-1 siRNA at a final concentration of 10 nM using Lipofectamine^{tm} 2000 (Invitrogen, Carlsbad, CA. USA) as a transfecting agent in Opti-MEM (Invitrogen). After 12 hours, serum-free media containing the transfection mixture were replaced with fresh serum-containing media. The transfection efficiency was evaluated via downregulation of DJ-1 mRNA. Also, the effect of DJ-1 knockdown on Pten expression was investigated using real time PCR. To investigate the effect of DJ-1 siRNA on cell viability, a proper number of cells were transfected in 96-well plates for two days and MTT assay was performed.

Quantitative real time PCR

To investigate the effect of BCA and siDJ-1 on DJ-1 and Pten mRNA expression levels, real time quantitative RT-PCR analysis was performed. In brief, 1 X 10^5 cells were plated in 60-mm dishes and after 24 hrs, the cells were treated with BCA for 24 hrs more. Another set of plates was transfected with control siRNA for three days. Total RNA was extracted with Qiazol (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Five µg of extracted RNA was reverse transcribed into cDNA using a first strand cDNA synthesis kit (Applied Biosystems, Fosters city, CA, USA), and the resulting cDNA was diluted 10-fold and kept at -20^{o}C until use. The real time RT-PCR primers were designed using Primer Express 1.5 software (Applied Biosystems) as follows: DJ-1 forward, 5´-GTCATTTGTCCTGATGCCAGC-3´, and DJ-1 reverse, 5´-TCAGATAAATTCTGTGCGCCC-3´; Pten forward, 5´-TCAGTGGCGGAACTTGCAA-3´ and Pten reverse 5´-CATGAACTTGTCTTCCCGTCG-3´; GAPDH forward, 5´-AGATCATCAGCAATG-CCTCCTG-3´ and GAPDH reverse, 5´-ATGGCATGGACTGTGGTCATG-3´. DJ-1 expression was normalized using GAPDH as a housekeeping gene. Real time PCR was carried out using the ABI Prism 7500 sequence

detection system (Applied Biosystems, USA). 10 µl of SYBR Green PCR master mix, 4 µl of diluted cDNA, and 200 nM primer set were used for amplification in 20 µl reaction mixture. All samples were amplified in triplicate in a 96-well plate and the cycling conditions being as follows: 2 min at 50°C, 10 min at 95°C, and 40 cycles at 95°C for 15 seconds followed by 1 minute at 60°C. The values of Δcycle threshold (ΔCt) were calculated by normalizing the average Ct value of each treatment compared to its opposite endogenous control (GAPDH) and then calculating $2^{-\Delta\Delta Ct}$ for each treatment. Statistical analysis of the data was performed as previously described [24]. These experiments were repeated three times.

Western blot analyses

The effect of BCA on DJ-1, Pten, and γ-GCS protein expression levels was analyzed by western blot analysis using specific antibodies as previously described [5]. Briefly, cells were washed twice with cold PBS, after which 200 µL of PRO-PREP protein extraction solution (Intron, Daejon, South Korea) was added. Cell lysates were centrifuged and protein concentrations were estimated using the Coomassie protein assay reagent (Thermo Scientific, Rockford, IL. USA). 40 µg of protein samples were electrophoresed on 10-15% SDS-PAGE gels. Proteins were transferred to nitrocellulose membranes, which were blocked in 5% skim milk in TBS (25 mM Tris base and 150 mM NaCl) for 2 hrs at room temperature, and then incubated with primary antibody overnight at 4°C. Horseradish peroxidase-conjugated secondary antibody was used at 1:5000 dilutions for 1 h at room temperature and then washed three times in TBST (TBS and 0.1% Tween 20). The target proteins were detected with ECL detection.

Statistical analysis: The quantitative results were calculated and expressed as the means ± SD. Averages were graphed and the statistical differences in mean values were carried out by student t-test using Microsoft Excel-2007 software.

RESULTS and DISCUSSION

Consistently with a previous study, MDA-MB-435 cell viability was inhibited after BCA treatment in a dose-dependent manner, with an IC50 about 25 µM [5]. HCA and its derivatives, including BCA inhibits cell proliferation of several types of cancer cells via inducing apoptosis and cell cycle arrest [5, 7-9]. In the current cellular model we found that BCA-inducing antiproliferation effect is associated with ROS release (**Fig. 1**). In other cellular models it was stated also that, BCA inducing cell proliferation inhibition, apoptosis and cell cycle takes place via ROS release [8]. Joselin et al. [25] found that DJ-1 function, and localization is regulated by ROS during oxidative stress. To further study the mechanism of action of BCA in MDA-MB-435 cells, we investigated the expression alteration pattern in DJ-1 and its downstream targets GSH and γ-GCS after BCA treatment. We found in consistent with our previously published data [5] that BCA decreased DJ-1 mRNA and protein expression level in a dose and time dependent manner (**Fig. 3 & 4**). These data are consistent with the fact that DJ-1 acts as an oncogene; protecting cells against oxidative stress via several pathways; including promotion and activation of ras signaling [10, 20], stabilizing NRF2 protein by impairing Keap1 (inhibitor of NRF2) [19], increasing antioxidants expression such as GSH [17], acting as a positive regulator of the survival signaling PI3K/AKT cascade by inhibiting the phosphatase activity of PTEN [23, 26, 27], protecting cells against oxidative stress via binding with p53 transcription factor, thus

inducing inactivation of p53 and decreasing pro-apoptotic Bax [28, 29]. Furthermore, it was found that DJ-1 can improve cell survival and protect cells against apoptosis through its interaction with the Daxx protein [30, 31]. In the current study, we found that BCA-mediated MDA-MB-435 cell proliferation inhibition decreased DJ-1 expression with concurrent inhibition of GSH and γ-GCS (**Fig. 2 & 3**).

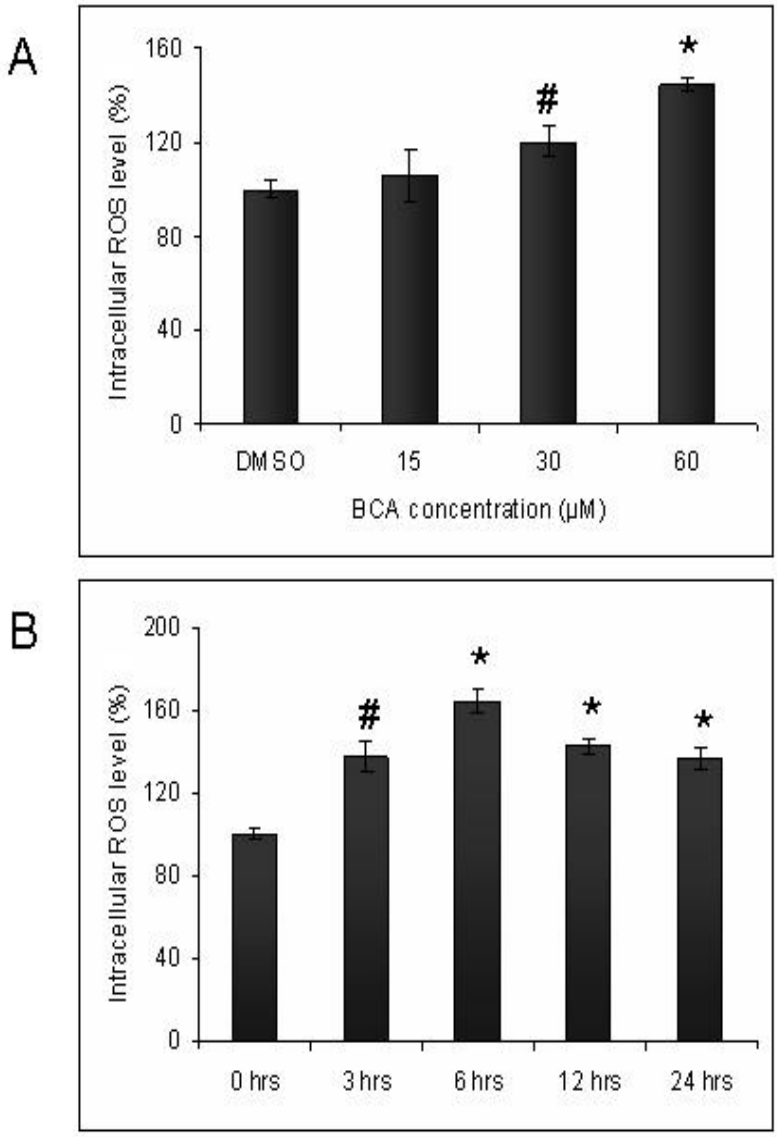

Fig.1.The effect of BCA on ROS level in MDA-MB-435 cells. Cells were treated with different doses of BCA for 24 hrs (A) and 30 µM for different intervals as indicated (B) and then the intracellular ROS was measured as shown in materials and methods.

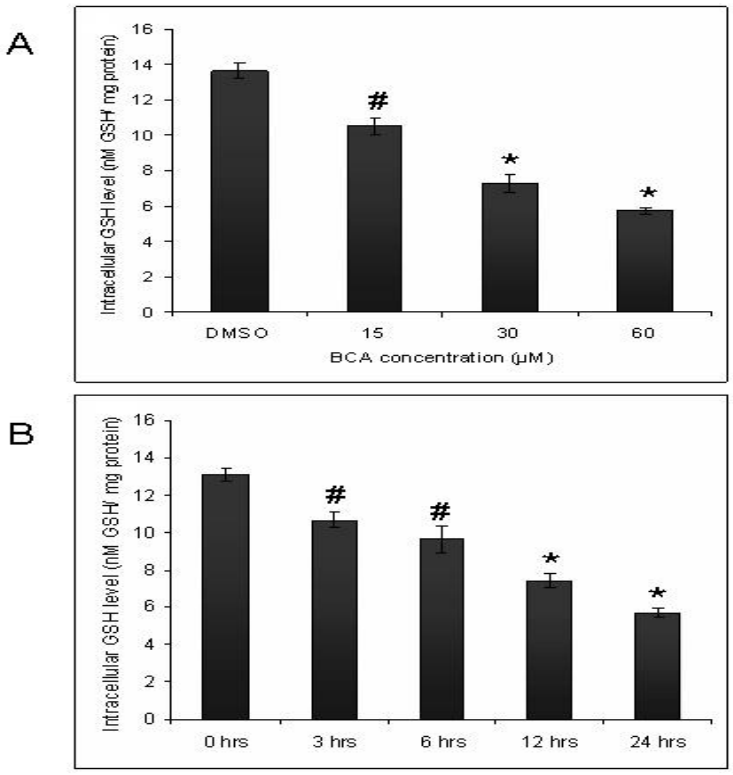

Fig.2. The effect of BCA on GSH in MDA-MB-435 cells. Cells were treated with different doses of BCA for 24 hrs (A) and 30 μM for different intervals as indicated (B) and then the GSH level was investigated as shown in materials and methods.

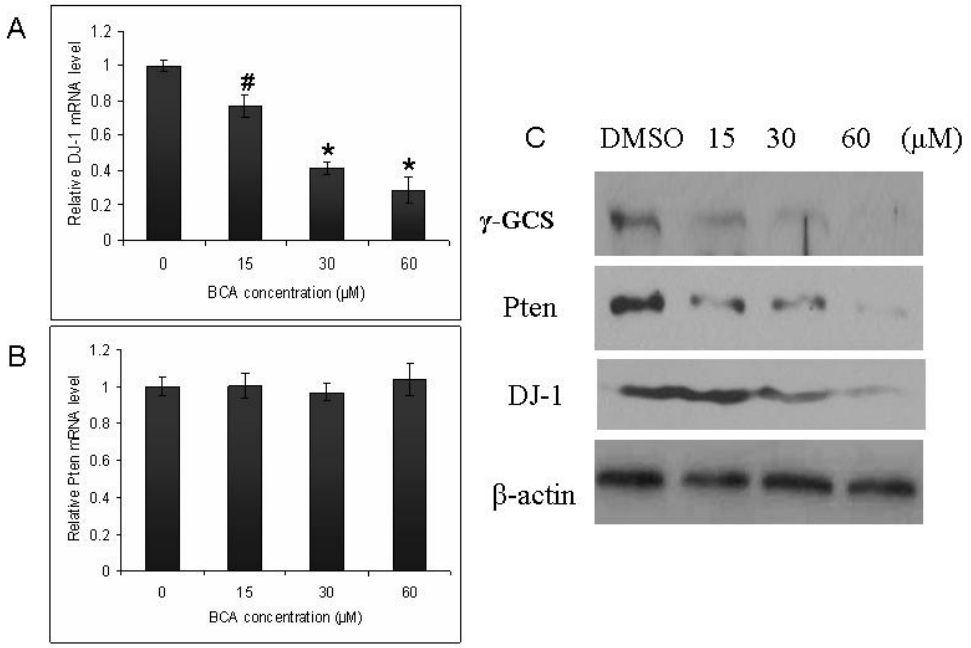

Fig.3. The effect of BCA on the DJ-1 mRNA (A), Pten mRNA (B), and protein expression of DJ-1, Pten, and γ-GCS protein expressions (C) in MDA-MB-435 cells. Cells were treated with BCA (DMSO, 15, 30, 60 μM) for 24 hrs and then mRNA and protein expression levels were investigated using real time PCR and western blot analyses; respectively as shown in materials and methods.

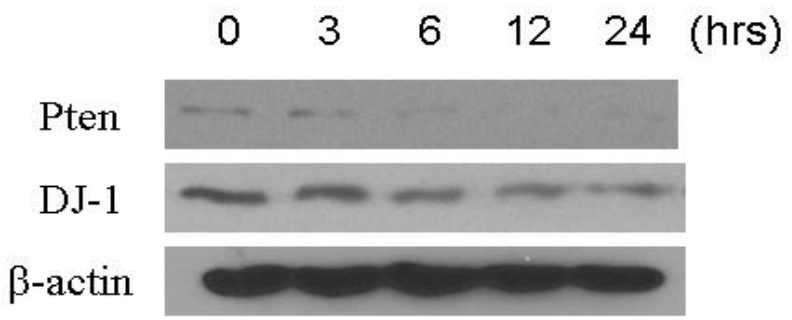

Fig.4. Time-dependent effect of BCA on the protein expression levels of DJ-1, and Pten in MDA-MB-435 cells. Cells were treated with 30 µM of BCA for 0, 3, 6, 12, and 24 hrs and then protein level was investigated using western blot analysis.

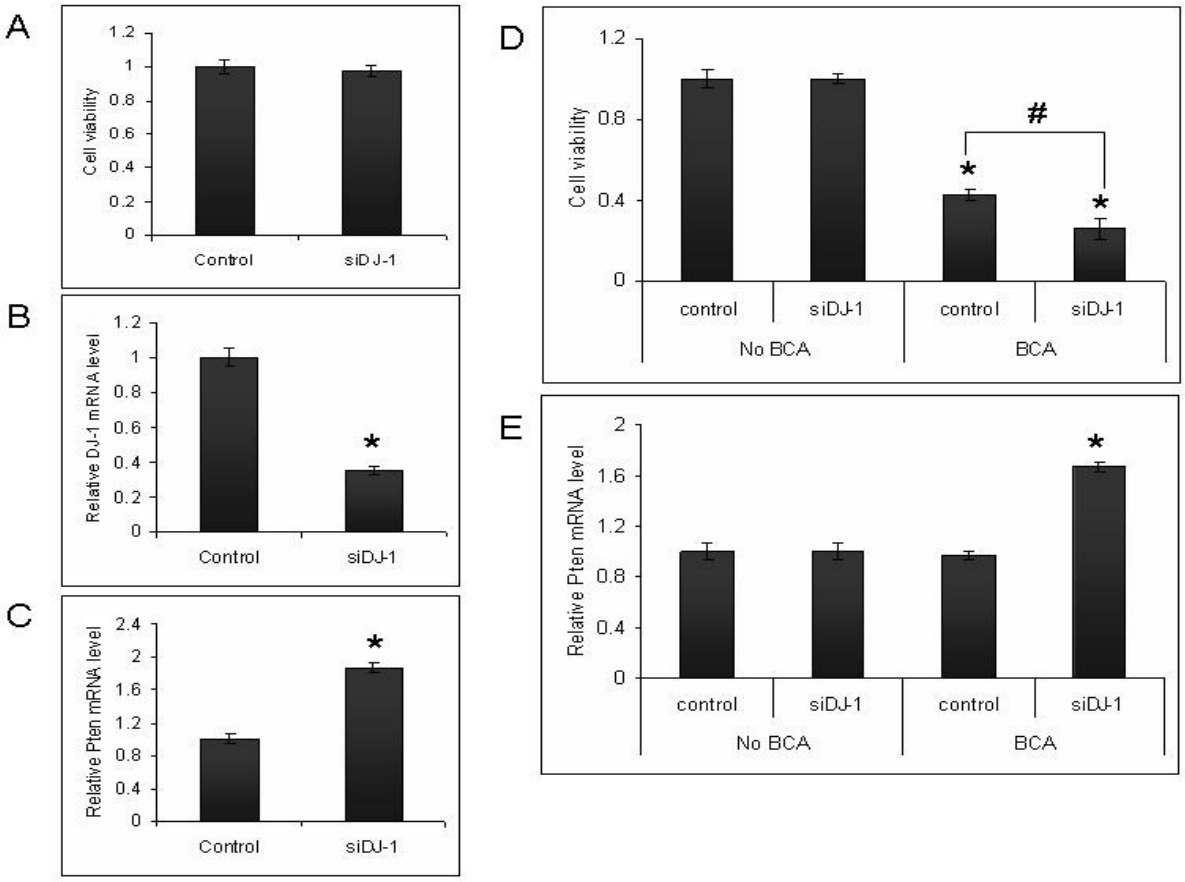

Fig.5. Pten is a downstream target for DJ-1 in MDA-MB-435 cells. Cells were transfected with DJ-1 siRNA and/or treated with BCA and then cell proliferation and Pten expression was evaluated using MTT and real time PCR analyses, respectively.

In consistent with the present data, it was stated that DJ-1 induces GSH and γ-GCS via activating NRF2 [17, 19]. Unexpectedly, we found that BCA-mediated DJ-1 downregulation in MDA-MB-435 cells showed no effect on Pten mRNA with concurrent decrease of Pten protein expression (**Fig. 3 & 4**). Based on this data, we suspected that Pten expression would be independent from DJ-1 at least in part in the current cellular model. Also, we investigated the

role of DJ-1 in cell protection and in Pten expression. We silenced DJ-1 in MDA-MB-435 cells by transfection of DJ-1 siRNA (siDJ-1) for two days. The transfection efficiency was investigated using real time qPCR (**Fig. 5B**). The effect of DJ-1 silencing on cell viability after BCA treatment was investigated using MTT assay. DJ-1 silencing showed no effect on cell viability (**Fig. 5A**). There was marked inhibition in MDA-MB-435 cell viability (**Fig. 5D**) and clear activation in Pten expression (**Fig. 5E**) due to BCA treatment after DJ-1 silencing. Several studies reported that DJ-1 functions as a negative regulator of the tumor suppressor Pten [22, 23]. In this respect, in the present study DJ-1 knockdown significantly increased Pten expression in MDA-MB-435 breast cancer cells (**Fig. 5C**). From the above data, it could be concluded that, BCA inhibited MDA-MB-435 cell proliferation in a dose dependent manner via increasing ROS release and depletion of GSH and γ-GCS. This chemosensitizing effect of BCA takes places at least in part via downregulation of DJ-1. Interestingly, despite the decrease of DJ-1 (the negative regulator of Pten), after BCA treatment the tumor suppressor protein was concurrently decreased. These data suggest that BCA regulates Pten independent of DJ-1 at least in the current cellular model, suggesting a parallel survival (via downregulation of Pten) and antiproliferative (via downregulation of DJ-1) functions of BCA in MDA-MB-435 cells. Surprisingly, DJ-1 knockdown markedly increased Pten expression. Taken together, these data refer to that Pten is regulated at least in part through DJ-1 in MDA-MB-435 in addition to other signaling pathways. However, BCA regulates Pten in a DJ-1 independent signaling pathway. Further investigations are required to clarify the regulatory effect of BCA on Pten expression in other cellular models.

CONCLUSION:

These data showed that BCA is a promising candidate for breast cancer inhibition via increasing ROS release and DJ-1 downregulation. Moreover, BCA downregulates Pten expression in MDA-MB-435 cells in a DJ-1-independent manner through other signaling pathway. Also, the above data suggest that the expression of DJ-1 is a potent marker for breast cancer progression and inhibition.

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REFERENCES

[1] B.M. Kwon, Y.K. Cho, S.H. Lee, J.Y. Nam, S.H. Bok, S.K. Chun, J.A. Kim, and I.R. Lee, 2'-Hydroxycinnamaldehyde from stem bark of Cinnamomum cassia. Planta Med 62 (1996) 183-4.

[2] L.S. Kwon BM, Cho YK, Bok SH, So SH, Youn MR, Chang SI, Synthesis and biological activity of cinnamaldehydes as anbiogenesis inhibitors. Bioorg Med Chem Lett 7 (1997) 2473-2476.

[3] B.M. Kwon, S.H. Lee, S.U. Choi, S.H. Park, C.O. Lee, Y.K. Cho, N.D. Sung, and S.H. Bok, Synthesis and in vitro cytotoxicity of cinnamaldehydes to human solid tumor cells. Arch Pharm Res 21 (1998) 147-52.

[4] C.W. Lee, D.H. Hong, S.B. Han, S.H. Park, H.K. Kim, B.M. Kwon, and H.M. Kim, Inhibition of human tumor growth by 2'-hydroxy- and 2'-benzoyloxycinnamaldehydes. Planta Med 65 (1999) 263-6.

[5] I.A. Ismail, H.S. Kang, H.J. Lee, B.M. Kwon, and S.H. Hong, 2'-Benzoyloxycinnamaldehyde-mediated DJ-1 upregulation protects MCF-7 cells from mitochondrial damage. Biol Pharm Bull 35 (2012) 895-902.

[6] J. Lu, K. Zhang, S. Nam, R.A. Anderson, R. Jove, and W. Wen, Novel angiogenesis inhibitory activity in cinnamon extract blocks VEGFR2 kinase and downstream signaling. Carcinogenesis 31 (2010) 481-8.

[7] D.C. Han, M.Y. Lee, K.D. Shin, S.B. Jeon, J.M. Kim, K.H. Son, H.C. Kim, H.M. Kim, and B.M. Kwon, 2'-benzoyloxycinnamaldehyde induces apoptosis in human carcinoma via reactive oxygen species. J Biol Chem 279 (2004) 6911-20.
[8] S.H. Hong, J. Kim, J.M. Kim, S.Y. Lee, D.S. Shin, K.H. Son, D.C. Han, Y.K. Sung, and B.M. Kwon, Apoptosis induction of 2'-hydroxycinnamaldehyde as a proteasome inhibitor is associated with ER stress and mitochondrial perturbation in cancer cells. Biochem Pharmacol 74 (2007) 557-65.
[9] J. Ock, H.A. Lee, I.A. Ismail, H.J. Lee, B.M. Kwon, K. Suk, W.H. Lee, and S.H. Hong, Differential antiproliferation effect of 2'-benzoyloxycinnamaldehyde in K-ras-transformed cells via downregulation of thiol antioxidants. Cancer Sci 102 (2011) 212-8.
[10] D. Nagakubo, T. Taira, H. Kitaura, M. Ikeda, K. Tamai, S.M. Iguchi-Ariga, and H. Ariga, DJ-1, a novel oncogene which transforms mouse NIH3T3 cells in cooperation with ras. Biochem Biophys Res Commun 231 (1997) 509-13.
[11] N. Lev, D. Ickowicz, Y. Barhum, S. Lev, E. Melamed, and D. Offen, DJ-1 protects against dopamine toxicity. J Neural Transm 116 (2009) 151-60.
[12] N. Lev, D. Ickowicz, Y. Barhum, E. Melamed, and D. Offen, DJ-1 changes in G93A-SOD1 transgenic mice: implications for oxidative stress in ALS. J Mol Neurosci 38 (2009) 94-102.
[13] M.R. Cookson, Pathways to Parkinsonism. Neuron 37 (2003) 7-10.
[14] A. Inberg, and M. Linial, Protection of pancreatic {beta}-cells from various stress conditions is mediated by DJ-1. J Biol Chem 285 (2010) 25686-98.
[15] H. Liu, M. Wang, M. Li, D. Wang, Q. Rao, Y. Wang, Z. Xu, and J. Wang, Expression and role of DJ-1 in leukemia. Biochem Biophys Res Commun 375 (2008) 477-83.
[16] J.P. MacKeigan, C.M. Clements, J.D. Lich, R.M. Pope, Y. Hod, and J.P. Ting, Proteomic profiling drug-induced apoptosis in non-small cell lung carcinoma: identification of RS/DJ-1 and RhoGDIalpha. Cancer Res 63 (2003) 6928-34.
[17] W. Zhou, and C.R. Freed, DJ-1 up-regulates glutathione synthesis during oxidative stress and inhibits A53T alpha-synuclein toxicity. J Biol Chem 280 (2005) 43150-8.
[18] C.M. Clements, R.S. McNally, B.J. Conti, T.W. Mak, and J.P. Ting, DJ-1, a cancer- and Parkinson's disease-associated protein, stabilizes the antioxidant transcriptional master regulator Nrf2. Proc Natl Acad Sci U S A 103 (2006) 15091-6.
[19] D. Malhotra, R. Thimmulappa, A. Navas-Acien, A. Sandford, M. Elliott, A. Singh, L. Chen, X. Zhuang, J. Hogg, P. Pare, R.M. Tuder, and S. Biswal, Decline in NRF2-regulated antioxidants in chronic obstructive pulmonary disease lungs due to loss of its positive regulator, DJ-1. Am J Respir Crit Care Med 178 (2008) 592-604.
[20] M.P. van der Brug, J. Blackinton, J. Chandran, L.Y. Hao, A. Lal, K. Mazan-Mamczarz, J. Martindale, C. Xie, R. Ahmad, K.J. Thomas, A. Beilina, J.R. Gibbs, J. Ding, A.J. Myers, M. Zhan, H. Cai, N.M. Bonini, M. Gorospe, and M.R. Cookson, RNA binding activity of the recessive parkinsonism protein DJ-1 supports involvement in multiple cellular pathways. Proc Natl Acad Sci U S A 105 (2008) 10244-9.
[21] R.T. Sitaram, C.J. Cairney, P. Grabowski, W.N. Keith, B. Hallberg, B. Ljungberg, and G. Roos, The PTEN regulator DJ-1 is associated with hTERT expression in clear cell renal cell carcinoma. Int J Cancer 125 (2009) 783-90.
[22] M. Fang, X.Y. Zhong, B. Du, C.L. Lin, F. Luo, L.J. Tang, and J. Chen, Role of DJ-1-induced PTEN down-regulation in migration and invasion of human glioma cells. Chin J Cancer 29 (2010) 988-94.
[23] R.H. Kim, M. Peters, Y. Jang, W. Shi, M. Pintilie, G.C. Fletcher, C. DeLuca, J. Liepa, L. Zhou, B. Snow, R.C. Binari, A.S. Manoukian, M.R. Bray, F.F. Liu, M.S. Tsao, and T.W. Mak, DJ-1, a novel regulator of the tumor suppressor PTEN. Cancer Cell 7 (2005) 263-73.
[24] K.J. Livak, and T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25 (2001) 402-8.
[25] A.P. Joselin, S.J. Hewitt, S.M. Callaghan, R.H. Kim, Y.H. Chung, T.W. Mak, J. Shen, R.S. Slack, and D.S. Park, ROS-dependent regulation of Parkin and DJ-1 localization during oxidative stress in neurons. Hum Mol Genet (2012).
[26] Y.C. Kim, H. Kitaura, T. Taira, S.M. Iguchi-Ariga, and H. Ariga, Oxidation of DJ-1-dependent cell transformation through direct binding of DJ-1 to PTEN. Int J Oncol 35 (2009) 1331-41.
[27] H. Aleyasin, M.W. Rousseaux, P.C. Marcogliese, S.J. Hewitt, I. Irrcher, A.P. Joselin, M. Parsanejad, R.H. Kim, P. Rizzu, S.M. Callaghan, R.S. Slack, T.W. Mak, and D.S. Park, DJ-1 protects the nigrostriatal axis from the neurotoxin MPTP by modulation of the AKT pathway. Proc Natl Acad Sci U S A 107 (2010) 3186-91.
[28] J. Fan, H. Ren, E. Fei, N. Jia, Z. Ying, P. Jiang, M. Wu, and G. Wang, Sumoylation is critical for DJ-1 to repress p53 transcriptional activity. FEBS Lett 582 (2008) 1151-6.
[29] J. Fan, H. Ren, N. Jia, E. Fei, T. Zhou, P. Jiang, M. Wu, and G. Wang, DJ-1 decreases Bax expression through repressing p53 transcriptional activity. J Biol Chem 283 (2008) 4022-30.
[30] E. Junn, H. Taniguchi, B.S. Jeong, X. Zhao, H. Ichijo, and M.M. Mouradian, Interaction of DJ-1 with Daxx inhibits apoptosis signal-regulating kinase 1 activity and cell death. Proc Natl Acad Sci U S A 102 (2005) 9691-6.
[31] R. Zobalova, E. Swettenham, J. Chladova, L.F. Dong, and J. Neuzil, Daxx inhibits stress-induced apoptosis in cardiac myocytes. Redox Rep 13 (2008) 263-70.

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