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Tumor Suppressor p53 Can Protect Normal Cells Against Dendrosomal Curcumin-Induced Apoptosis

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ABSTRACT

Curcumin is a natural substance with anti-cancerous properties without many disadvantages of currently-used anticancer drugs. Its toxicity is significantly higher in tumor cells compared with normal cells. We hypothesized the difference of p53 function between normal and tumor cells as one of the presumable causes of this phenomenon. We knocked down the expression of p53 in normal fibroblasts using anti-p53 siRNA and subsequently explored the effects caused by dendrosomal curcumin- a novel nanoformulation of curcumin- on these cells in terms of apoptosis induction and gene expression analysis. The results of MTT assay demonstrated dendrosomal curcumin is selectively cytotoxic for melanoma cancer cells without any considerable effects on normal fibroblasts. Knocking-down of p53 in normal fibroblast cells caused increase of NF-κB1 and decrease of p21 expression level. Treating p53-suppressed normal fibroblast cells with dendrosomal curcumin led to a robust increase in apoptosis rate of the cells. Taken together, these results imply the fact that p53 can protect normal cells from dendrosomal curcumin-induced apoptosis.Therefore, dendrosomal curcumin- in addition to being a chemotherapeutic compound-represents potential capacities to be used as an effective chemopreventive agent.

Keywords: Cancer, Dendrosomal curcumin, Normal fibroblast, p53, Knockdown, Apoptosis

INTRODUCTION

According to the statistics provided by World Health Organization, cancer is one of the leading causes of death worldwide [1]. In spite of significant advances made over the last half century in our knowledge of cancer biology, neither the incidence of cancer nor the rate of cancer-asscoaited death has not changed. Most drugs currently available for cancer treatment are very toxic, highly inefficient, or greatly expensive and thus beyond the reach of majority of people [2]. Design of effective novel drugs for treatment of cancer is necessary for decreasing side effects esspecially on the function of normal cells [3].

Curcumin (diferuloylmethane) is a polyphenol extracted from the rhizome of turmeric (*curcuma longa*) with therapeutic properties such as antioxidant, anti inflammatory, anti parasitic, and anticancer activities and no side effects caused by currently used anticancer drugs [2,4]. Despite of the considerable promise of curcumin as an efficacious and safe compound, the widespread clinical application of this agent in the cure of cancer and other diseases has been limited due to poor aqueous solubility and consequently minimal bioavailability. To improve the bioavailability of curcumin, numerous approaches such as taking advantage of curcumin nanoparticles have been undertaken [5]. Here, we increased the bioavailibity of curcumin by encapsulating it in a type of polymeric nano-particle (dendrosomal curcumin:DNC) being synthetized by our laboratory. The safety and effectiveness of DNC have been demonstrated in our previous works [6-10].

The toxicity of curcumin is significantly higher in tumor cells than normal ones [11]. The cause for this phenomenon is not fully understood, but several reasons have been suggested; First, absorption and fluorescence spectroscopic methods have shown that cellular uptake and fluorescence intensity of curcumin is significantly higher in tumor cells

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than normal cells [12].

The second reason is the lower levels of glutathione in tumor compared with normal cells. This functions as an important intracellular radical scavenger and protects cells against reactive oxygen species (ROS) as well as against many toxins, mutagens, and drugs; thus the lower glutathione levels lead to the enhancemnet of sensitivity of tumor cells to curcumin [13].

The third reason is that most tumor cells, but not normal ones, express constitutively active NF-κB and mediate their survival. Curcumin can suppress the survival and proliferation of tumor cells by suppressing NF-κB-regulated gene products [2,8]. However, in addition to the suggested reasons, we hypothesized that the difference of p53 expression and function between normal and tumor cells can be one of the most significant presumable causes of the phenomenon.

p53 is a key tumour suppressor and master regulatory transcription factor that is altered in most human cancers. It is probably one of the most widely studied proteins because of its pivotal role in tumorogenesis, cell death, and survival [14]. The p53 protein has a broad range of biological functions, including regulation of cell cycle, apoptosis, senescence, DNA metabolism, angiogenesis, differentiation and the immune responses [15]. More than half of all human cancers have been reported to carry p53 mutations, and recent studies have suggested that many tumors that retain wild-type p53 are defective in either the ability to induce or the ability to respond to p53 [16,17].

Several studies have demonstrated that inactivation or alteration of p53 gives rise to enhancing the sensitivity of normal cells following exposure to stress [18-25], hence in this condition curcumin could induce cellular death. To reach this purpose, we knocked down the p53 gene in normal cells by using p53-specific siRNA. This was performed through treating cells with appropriate doses of curcumin followed by mesuerment of apoptosis rate *via* flocytometric metods.

In the present study, we found that short interfering RNA (siRNA)-mediated silencing of p53 in normal human fibroblasts, together with dendrosomal curcumin treatment, efficiently induces apoptosis. However, these phenomena are not induced by either siRNA-mediated silencing of p53 or dendrosomal curcumin treatment alone. Thus, we can propose that p53 protects normal fibroblasts against dendrosomal curcumin.

MATERIALS AND METHODS

Cells and Reagents

SK-MEL3 (a human melanoma cell line) and HFSF-PI 3 cells (normal human dermal fibroblast) were obtained from Pasteur Institute (Tehran, Iran). Cells were cultured in their adopted medium (RPMI 1640 for SK-MEL3 cells and Dulbecco's modified Eagle's medium (DMEM) for HFSF-PI3 cells) containing 10% fetal bovine serum (Gibco) and 1% Pen-Strep (Gibco). All cells were maintained at 37 °C in a humidified incubator with 5% CO_2, and were passaged every 3 days or as required using 0.25% trypsin-EDTA (Invitrogen).

Curcumin was purchased from Sigma-Aldrich Company, USA. Dendrosome nanoparticle specified Den O400, a nonionic biode-gradable denderic glycol ester (MW: 590 Da, HLB: 12.5 Mh/M, hydroxyl value: 95 mg KOH/g and acid value: mg KOH/g) was a gift from Institute of Biochemistry and Biophysics, University of Tehran,Iran.

Dendrosomal Curcumin Preparation

DNC preparation was performed using optimized protocol in our laboratory [8]. Briefly, different weight/weight ratios of dendrosome/curcumin ranging from 50:1 to 10:1 were examined before settling a suitable ratio of 25:1. Curcumin was dissolved in various amounts of dendrosome and checked for absorbance spectrum by UV spectrophotometery (TECAN, Switzerland). Then, the appropriate mixture of dendrosome and curcumin was evaluated for excitation/emission value in comparison with curcumin dissolved in PBS and 1% methanol as control samples. The loading of dendrosome nanocarriers with curcumin molecules was performed using the protocol suggested by MaLing Gou *et al.* Briefly, curcumin and dendrosome were co-dissolved in 5 ml of acetone; this solution was added into 5 ml of PBS while stirring constantly. Then, the acetone was evaporated in rotary

evaporator. The curcumin/dendrosome micelle solution was sterilized using a 0.22 µm syringe filter (Millex-LG, Millipore Co., USA). Finally, DNC was stored in 4 ºC in a light-protected condition. For *in vitro* experiments, DNC was diluted in complete culture medium [10].

Cell Viability Assay

Cell viability was determined by measuring mitochondrial succinate dehydrogenase activity using MTT (3-[4,5-dimethylthiazol-2-yl]2,5-iphenyltetrazolium bromide) assay according to the manufacturer's instructions (Sigma-Aldrich, USA). Briefly, melanoma cancer cells (SK-MEL3) and normal human dermal fibroblasts were seeded onto a 96-well plate in 200 µl of medium. After 24 h, cells were treated with various concentrations of dendrosomal curcumin (0-20 µM) for an additional 24, 48, or 72 h. At each time point, 20 µl of MTT (5 mg ml^{-1}) was added to each well and the cells were incubated for an additional 4 h at 37 °C. Culture medium was then removed and the formazan crystals were dissolved by addition of DMSO (200 µl).Absorbance was measured at 490 nm using ELISA reader (Bio-Tek).

RNA Interference

Human cellular p53 siRNA sequence pools (sc-29435) and scramble control siRNA pools (sc-37007) were purchased from Santa Cruz company. siRNA transfection was done 24 h before dendrosomal curcumin treatment. Briefly, one day before transfection, normal fibroblasts were incubated in growth medium without antibiotics such that they became 30-50% confluent at the time of transfection. The cells were transfected with 40-80 nM siRNA in Opti-MEM (Invitrogen) and Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's instructions; HFSF-PI3 cells were incubated for 6 h at 37 ºC (5% CO_2) with siRNAs in Lipofectamine and Opti-MEM (without serum or antibiotics). After 6 h, the medium was replaced by fresh complete medium containing 10% FBS. After siRNA transfection, real-time PCR analysis was carried out to confirm the efficiency of siRNA knock-down.

Apoptosis Assay; Annexin V/FITC Staining

For quantification of apoptosis after dendrosomal curcumin treatment in p53-deficient fibroblasts, Annexin V staining was performed by Annexin V-FITC kit (Roche, Germany) according to the manufacturer's manual. As mentioned above, 24 h after siRNA transfection, normal fobroblasts were incubated with 15 µM of dendrosomal curcumin for an additional 24 h. Then,these cells were harvested, and centrifuged at 200 × g for 5 min. Afterwards, the cell pellet was resuspend in 450 µl of binding buffer and 50 µl of annexin V-FITC /PI solution was then added. Finally, after 15 min incubation at room temperature and in a dark place, the samples were analyzed by flow cytometry (BD FACSCanto™ II (USA). A total of 10,000 events/sample were acquired. Flowcytometric datas were analyzed by Flowing Software 2.

RNA Extraction and Real-Time PCR

Total RNA was extracted from cells using Trizol reagent according to the manufacturer's instruction (Invitrogen). The quality and quantity of the isolated RNAs were evaluated by gel electrophoresis and UV spectrophotometry at 260 nm, respectively. Residual genomic DNA was removed using RNAase-free DNase I (fermentas) at 37 °C for 30 min followed by DNase heat inactivation at 65 °C for 10 min by addition of EDTA. Briefly, DNase-treated RNA was used in first strand cDNA synthesis reaction using PrimeScript II reverse transcriptase (Takara). cDNA synthesis reaction was performed at 37 °C for 15 min and terminated at 85 °C for 5 s. Real-time PCR using 5X HOT FIREPol®EvaGreen® HRM Mix (ROX) (Solis BioDyne) was carried out in duplicate in a 7500 real-time PCR system (Applied Biosystems) according to the manufacturer's instructions. In each sample, for compensating the variations in the amounts of input RNA and the efficacy of reverse transcriptase, the housekeeping gene GAPDH was used as internal control. Real-time PCR datas were analyzed using $\Delta\Delta C_T$ method.

Statistical Analysis

All data were expressed as the mean ± SD of at least three experiments. Statistical differences among data groups were analyzed by one-way ANOVA or analyzed by Student's t-test when appropriate, using GraphPad Prism 5.04 software (San Diego, CA, USA). A probability level of $P < 0.05$ was considered statistically significant.

RESULTS

Effect of Dendrosomal Curcumin on Cell Viability

To determine the toxicity of curcumin on SK-MEL3 cancer cells as well as human normal fibroblasts (HFSF-PI3), the cells were incubated with various concentrations of DNC (0-20 µM) for 24, 48 and 72 h and cell viability was then measured by MTT assay.

As shown in Fig. 1,dendrosomal curcumin induces cell death in melanoma cells(SK-MEL3) in a dose-dependent and time-dependent manner, as the IC_{50} values of DNC for SK-MEL3 cell line was relatively reduced within 48 and 72 h after treatment in comparison with 24 h incubation time. Interestingly, normal fibroblasts were not altered following exposure to different concentrations of dendrosomal curcumin (up to 20 µM), which were cytotoxic for sk-mel3

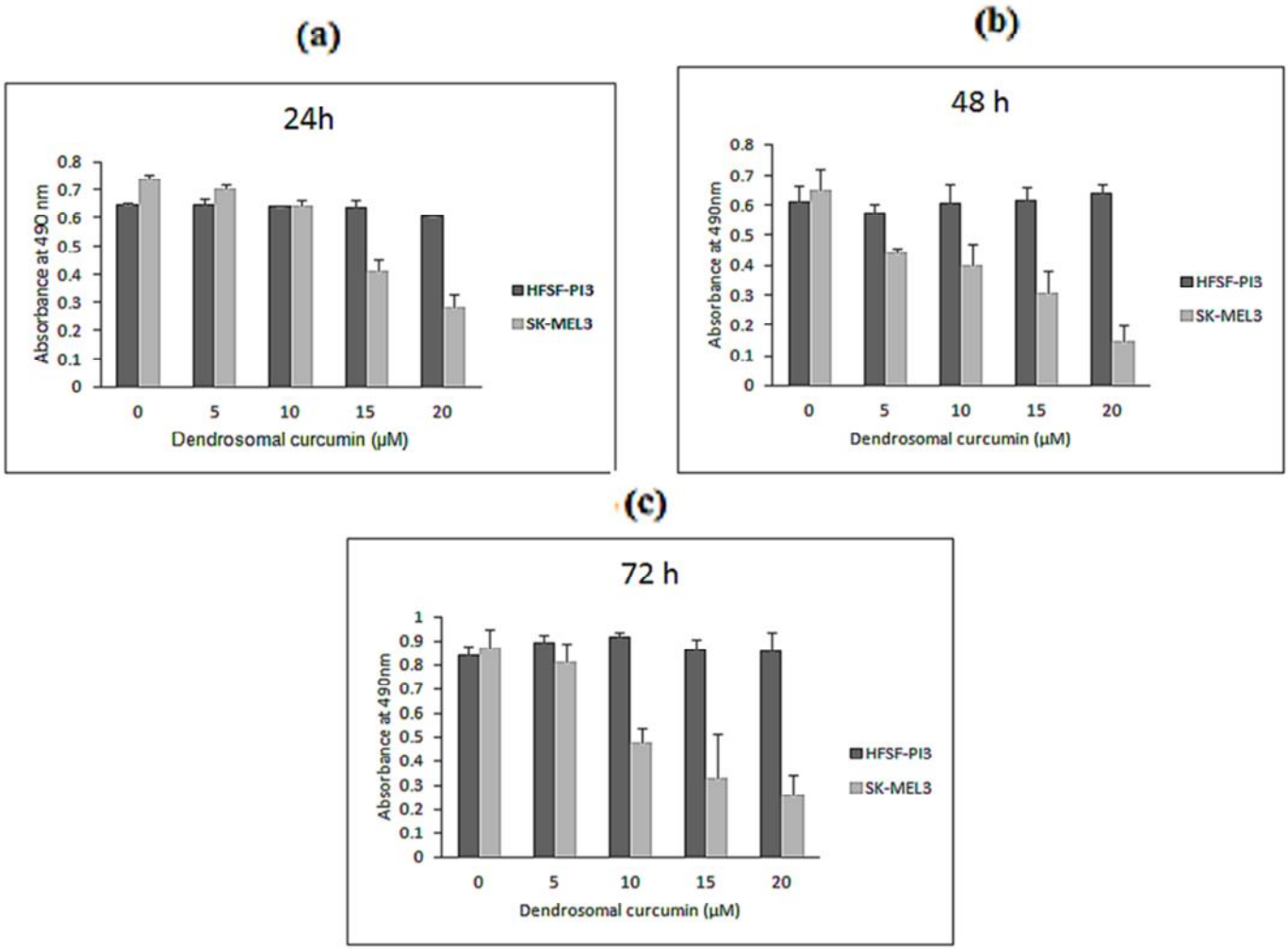

Fig. 1. Cytotoxic effect of dendrosomalcancerous cells. Normal fibroblasts (HFSF-PI3) and melanoma cancer cells (SK-MEL3) were treated with different concentrations of dendrosomal curcumin (0-20 µM) for 24, 4 8, or 72 h and cell viability at each time point was measured by MTT assay (a,b,c). Dendrosomal curcumin significantly inhibits proliferation of melanoma cells in a dose- and time-dependent manner but has not any cytotoxic effect on normal skin fibroblasts.

melanoma cells.

Dendrosomal Curcumin Uptake in Normal Fibroblasts

Dendrosomal curcumin uptake in normal fibroblasts was much lower than cancer cells. To investigate whether knock-down of the p53 gene has any effect on dendrosomal curcumin uptake in normal fibroblasts, 24 h after siRNA transfection, these cells were treated with 15 µM dendrosomal curcumin. Then, its cellular absorption was evaluated by fluorescence microscopy.We observed the increasing dendrosomal curcumin uptake in normal cells transfected by 2 different doses of p53 siRNA, compared with cells transfected with scramble siRNA (Fig. 2).

P53 Protects Normal Fibroblasts from Dendrosomal Curcumin-Induced Apoptosis

In order to study the p53 involvement in different sensitivity of normal and cancer cells to dendrosomal curcumin, the gene was knocked down in normal human dermal fibroblasts (HFSF-PI3) by transfecting 2 different concentrations of p53-specific siRNA (p53 siRNA). p53 downregulation was confirmed by real-time PCR. After successful knock-down of p53 (approximately 60 and 80%), the cells were treated with 15 µM DNC. 24h after curcumin treatment, apoptosis assay was performed by Annexin V/FITC staining. The results were compared with other control groups including; 1) normal fibroblasts without any treatment, 2) DNC-treated normal fibroblasts, 3) p53 siRNA-treated normal fibroblasts and 4) Scramble siRNA + DNC-treated normal fibroblasts.

AnnexinV/FITC-PI staining demonstrated that dendrosomal curcumin dose not induce apoptosis in normal fibroblasts treated with 15 µM concentration for 48 h. In addition, knocking down of p53 in these cells did not induce any significant increased apoptosis. But, after the reduction of p53 levels in the cells, dendrosomal curcumin treatment led to apoptosis induction in a dose dependent manner,

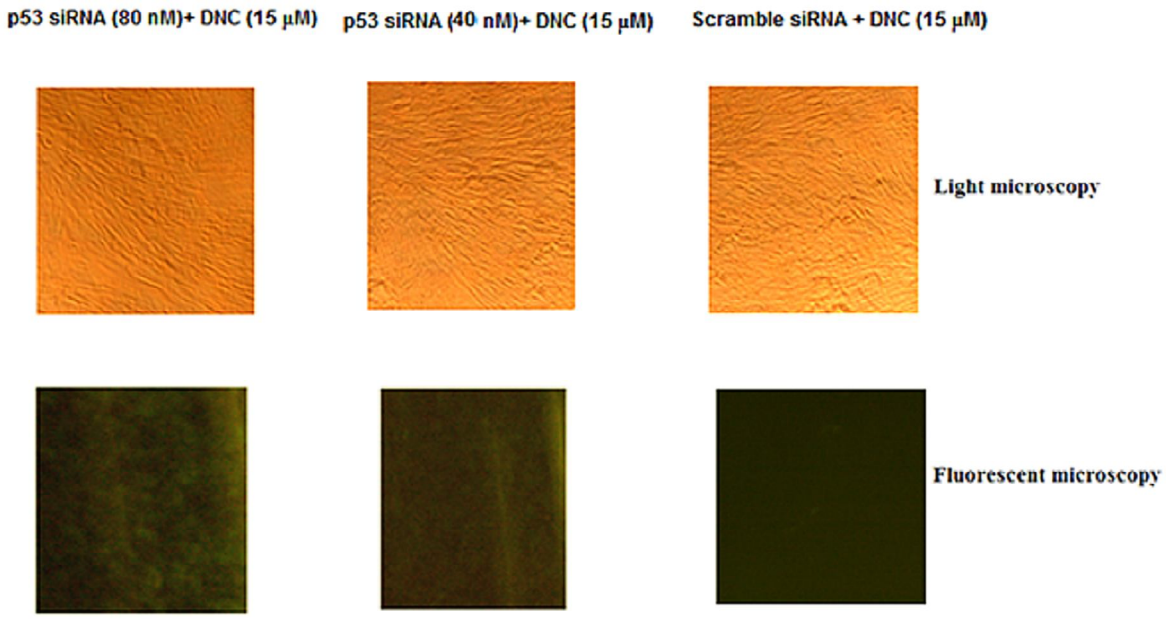

Fig. 2. Microscopic visualization of dendrosomal curcumin. 24 h after siRNA transfection to normal fibroblast, the cells were treated with 15 µM DNC and checked after 5 h by light and fluorescence microscopy. Under fluorescent microscope, fluorescence signals are observed in different intensities in test and control samples. DNC: dendrosomal curcumin (100 × magnification).

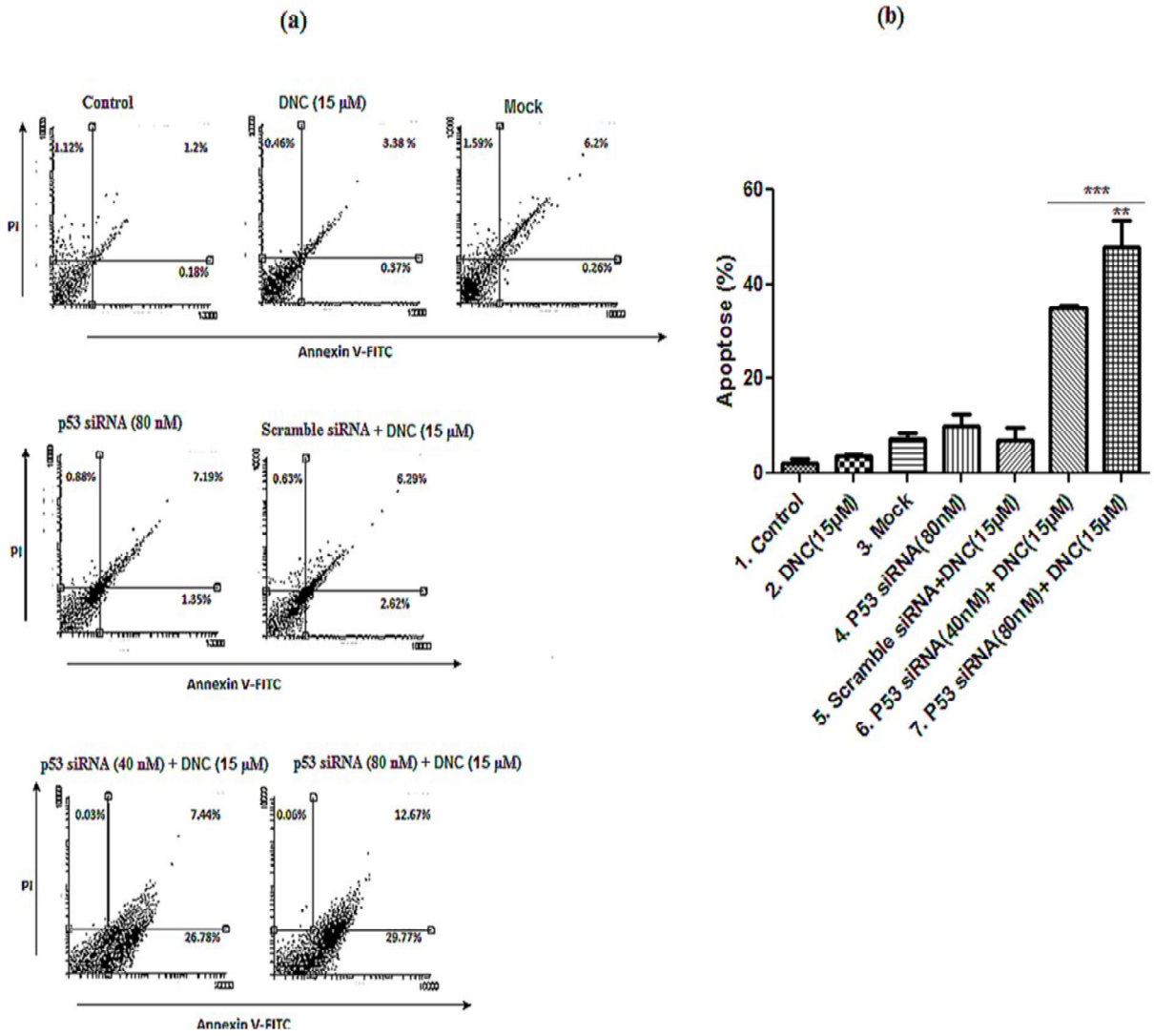

Fig. 3. p53 knockdown increased the vulnerability of normal fibroblasts to dendrosomal curcumin (a, b). After dendrosomal curcumin treatment, apoptosis rate in p53-silenced fibroblasts (by 2 different doses of p53 siRNA) was compared with several control groups. Dendrosomal curcumin caused apoptosis induction in p53-silenced normal fibroblasts and apoptosis rate was significantly dependent on p53 siRNA dose **: $p < 0.01$, ***: $p < 0.001$; cells positive for annexin V undergo apoptosis. Flow cytometric assays did not indicate any significant apoptosis in four control groups, 1) DNC-treated normal fibroblasts, 2) p53 siRNA-treated normal fibroblasts, 3) lipofectamin-treated normal fibroblasts (mock), 4) Scramble siRNA+DNC-treated normal fibroblasts. DNC: dendrosomal curcumin. PI: propidium iodide.

which was markedly enhanced compared with control siRNA-treated fibroblasts. Furthermore, in other control groups no significant apoptosis was detected (Fig. 3).

Gene Expression Analysis

P53 Knocking-down in normal fibroblasts alters expression of the p21 and NF-κB1 genes. To assess the effect of p53 knocdown on the expression levels of p21 and NF-κB1genes that play roles in regulating cell cycle, apoptosis, proliferation and survival pathways, whole-cell RNA was extracted from cells and real-time PCR was performed after exposure of normal fibroblasts to p53 siRNA for 48 h. The results revealed that exposure of normal fibroblasts to p53 siRNA can lead to the reduction of p21 level and enhancement of NF-κB1 expression (Fig. 4).

DISCUSSIONS

Despite all progress made in curcumin research, there exist a few papers addressing its differential effects on normal *vs*. tumor cells. Our results demonsrated dendrosmal curcumin (up to 20 μM) is selectively cytotoxic for cancer cells and does not cause any damage to normal fibroblasts. This clearly supports previous observations that indicate more sensitivity of tumor cells than normal ones to curcumin [11,12].

Because of high prevalence of p53 mutations in various types of human tumors and deactivation of p53 pathway in many tumors that retain wild-type p53 [18-25], we hypothesized that difference of p53 function between normal and tumor cells is one of the most significant presumable causes of the phenomenon.We evaluated the effect of p53 knockdown on the induction of cell death in normal human fibroblasts treated with dendrosmal curcumin.

Our results demonstrated that normal fibroblasts do not undergo apoptosis following dendrosomal curcumin treatment. But, targeting of p53 by RNA interference increases the sensitivity of fibroblasts to dendrosmal curcumin-induced apoptosis. A similarly protective effect provided by p53 has been reported in normal fibroblasts against UV-irradiation and multiple chemotherapeutic agents such as DNA crosslinkers cisplatin,carboplatin, doxorubicin and thiostrepton, the tubulin polymerizing agent paclitaxel, and the alkylating agents melphalan and nitrogen mustard.There are several possible explanations for these observations. The p53 protein is a central player in regulating DNA damage-induced apoptosis in many cell types. A number of previous studies, particularly those using cancer cell lines, have shown that the loss of p53 function results in increased resistance to chemotherapeutic

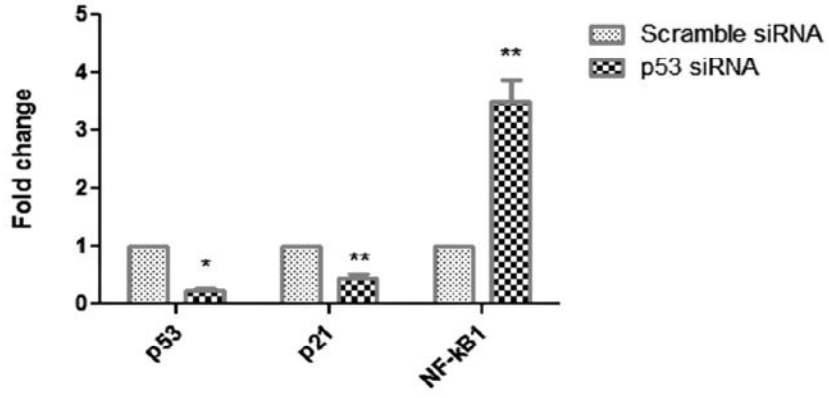

Fig. 4. The downstream effect of p53 knockdown on the expression of p21, Bcl-2, BAX and NF-κB1 in HFSF-PI3 cells. After p53 suppression, qRT PCR results exhibited the reduction of p21 and augmentation of NF-κB1 gene expression but not any significant change in Bcl-2 and BAX expression. *:$p < 0.05$, **: $p < 0.01$.

agents.In contrast, it is also well-established that p53 has anti-apoptotic activities and several studies have found increased cytotoxicity to a wide variety of drugs in primary, nontransformed cells lacking p53.These conflicting results indicate that p53 inactivation, in absence of other genetic alterations, leads to enhanced sensitivity not resistance against multiple chemotherapeutic agents.This is due to the fact that drug resistance in tumor cells may be the result of several genetic abnormalities. In addition, the role of p53 in drug sensitivity may be dependent on the type of chemotherapeutic agent [22].

Another explanation is that the antiapoptotic role of p53 may be due to the contribution of to cell survival through several mechanisms, including upregulation of p21 as a CDK inhibitor, which is a major transcriptional target of p53. Here, we found that after p53 knockdown, normal fibroblasts exhibit lower p21 expression levels than scramble siRNA transfected cells (Fig. 4). This is consistent with a previous study suggeting a direct correlation between levels of p21 expression and resistance to apoptosis in normal fibroblasts. Also, they demonstrated that p53 is mainly responsible not only for the stress-induced, but also for basal expression of p21 [18]. Thus, normal cells with wild-type p53 show higher levels of p21 expression and potentially more resistance to apoptosis.

But, with regard to differential effect of dendrosomal curcumin it might be suggested that knockdown of p53 in normal fibroblasts appears to make a model similar to cancer cells with such characteristics. This has previously been reported as reason for higher toxicity of curcumin in tumor cells compared with their normal counterparts; p53 knockdown in normal fibroblasts leads to the enhancement of dendrosomal curcumin uptake, NF-κB1 gene expression and ultimately their sensitivity to dendrosomal curcumin.

We investigated expression of the NF-κB1 (p50/p105) gene, one member of the mammalian NF-κB family, that initiates the canonical NF-κB activation pathway [26]. Our data demonstrated that p53 knockdown in normal fibroblasts causes increasing NF-κB1 gene expression. This phenomenon is one of the proposed reasons for sensitivity of cancer *vs.* normal cells to curcumin.

The transcription factor NF-κB is constitutively expressed in almost all types of cancers. It suppresses apoptosis in a wide variety of tumor cells and mediates their survival [27,28]. Several mutations directly/indirectly induce the upregulation of NF-κB that include loss/mutation p14 INK/ARF, p16 INK4a, PTEN and p53 [29]. The induction of NF-κB DNA-binding activity involves both the release of cytoplasmically stored factor from its inhibitor known as I kappa B and driving the NF-κB gene expression [30].

Curcumin can suppress the survival and proliferation of tumor cells by inhibiting the activation of NF-κB and expression of various oncogenes regulated by NF-κB that are involved in cellular proliferation, anti-apoptosis, and metastasis [11].

Several previous studies have reported antioxidant functions for p53 in normal cells. It can influence the expression of antioxidant genes such as GPX1 (glutathione peroxidase-1), ALDH4 (aldehyde dehydrogenase), and GLS2 (glutaminase 2) [31,32]. On the other hand, normal cells contain higher glutathione levels in comparison with tumor cells. Thus, it is expected that knocking down of p53 in normal fibroblasts influences the expression levels of p53 target antioxidant and pro-oxidant genes and finally results in the reduction of glutathione and increased susceptibility of these cells to oxidative stress-induced death. Several previous studies have observed oxidative stress-induced apoptosis after curcumin treatment [33].This may be another possible explanation for increased sensitivity of normal fibroblasts to dendrosomal curcumin treatment.

Taken together, we demonstrated the effect of p53 knockdown on the sensitivity of normal fibroblast to dendrosomal curmumin treatment. Our results support the notion that p53 protects normal human fibroblasts from dendrosomal curcumin-induced apoptosis. So, our findings are consistent with the hypothesis that one of the reasons behind different sensitivity of cancer versus normal cells to dendrosomal curcumin is p53 expression. This appears to be functional in an *in vitro* model of fibroblasts that removal of this “guardian of the genome” makes cells more vulnerable to curcumin-induced death. Acordingly, p53 as a tumor suppressor, is responsible for protecting cells from tumorigenic alterations and its mutations may led to

additional abnormalities that initiate cancer. The results presented here suggest that dendrosomal curcumin has potential characteristics to eliminate both cancer cells with/without p53 mutations and normal cells with p53 mutation. Thus, in addition to being a chemotherapeutic compound, dendrosomal curcumin represents potential to be used as an effective chemopreventive agent.

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REFERENCES

[1] A. Jemal, F. Bray, M.M. Center, J. Ferlay, E. Ward, D. Forman, CA: A Cancer journal for clinicians. 61 (2011) 69.

[2] S. Shishodia, M.M. Chaturvedi, B.B. Aggarwal, Curr Probl Cancer 31 (2007) 243.

[3] J.L. Watson, R. Hill, P.W. Lee, C.A. Giacomantonio, D.W. Hoskin, Exp. Mol. Pathol. 84 (2008) 230.

[4] C.L. Lin, J.K. Lin, J. Cancer Mol. 4 (2008) 11.

[5] P. Anand, A.B. Kunnumakkara, R.A. Newman, B.B. Aggarwal, Mol. Pharm. 4 (2007) 807.

[6] M.N. Sarbolouki, M. Sadeghizadeh, M.M. Yaghoobi, A. Karami, T. Lohrasbi, J. Chem. Technol. Biotechnol. 75 (2000) 919.

[7] M. Sadeghizadeh, B. Ranjbar, M. Damaghi, J. Chem. Technol. Biotechnol. 83 (2008) 912.

[8] E. Babaei, M. Sadeghizadeh, Z.M. Hassan, M.A. Feizi, F. Najafi, S.M. Hashemi, Int. Immunopharmacol. 12 (2012) 226.

[9] M. Khaniki, A.M. Alizadeh, S. Azizian, M.A. Mohaghgheghi, M. Sadeghizadeh, F. Najafi, Eur. J. Pharmacol. 689 (2012) 226.

[10] M. Tahmasebi Mirgani, B. Isacchi, M. Sadeghizadeh, F. Marra, A.R. Bilia, S.J. Mowla, F. Najafi, E. Babaei, Int. J. Nanomedicine. (2013).

[11] J. Ravindran, S. Prasad, B.B. Aggarwal, Aaps. J. 11 (2009) 495.

[12] A. Kunwar, A. Barik, B. Mishra, K. Rathinasamy, R. Pandey, K.I. Priyadarsini, Biochim. Biophys. Acta 1780 (2008) 673.

[13] C. Syng-Ai, A.L. Kumari, A. Khar, Mol. Cancer Ther. 3 (2004) 1101.

[14] A.J. Levine, M. Oren, Nat. Rev. Cancer 9 (2009) 749.

[15] K. Suzuki, H. Matsubara, J. Biomed. Biotechnol. 2011 (2011).

[16] K.M. Ryan, A.C. Phillips, K.H. Vousden, Curr. Opin. Cell Biol. 13 (2001) 332.

[17] D. Menendez, A. Inga, M.A. Resnick, Nat. Rev. Cancer 9 (2009) 724.

[18] M. Halasi, D.P. Schraufnagel, A.L. Gartel, Cell Cycle. 8 (2009) 2850.

[19] P. Lassus, M. Ferlin, J. Piette, U. Hibner, Embo. J. 15 (1996) 4566.

[20] P. Lassus, C. Bertrand, O. Zugasti, J.P. Chambon, T. Soussi, D. Mathieu-Mahul, U. Hibner, Oncogene 18 (1999) 4699.

[21] B.C. McKay, C. Becerril, M. Ljungman, Oncogene 20 (2001) 6805.

[22] D.S. Hawkins, G.W. Demers, D.A. Galloway, Cancer Res. 56 (1996) 892.

[23] L.J. Stubbert, J.M. Smith, J.D. Hamill, T.L. Arcand, B.C. McKay, Mutat. Res. 663 (2009) 69.

[24] V. Chaturvedi, L.A. Sitailo, J.Z. Qin, B. Bodner, M.F. Denning, J. Curry, W. Zhang, D. Brash, B.J. Nickoloff, Oncogene 24 (2005) 5299.

[25] J.C. O'Connor, D.M. Wallace, C.J. O'Brien, T.G. Cotter, Invest. Ophthalmol. Vis. Sci. 49 (2008) 4237.

[26] K.S. Ahn, B.B. Aggarwal, Ann. N Y Acad. Sci. 1056 (2005) 218.

[27] S. Shishodia, H.M. Amin, R. Lai, B.B. Aggarwal, Biochem. Pharmacol. 70 (2005) 700.

[28] M.M. Chaturvedi, B. Sung, V.R. Yadav, R. Kannappan, B.B. Aggarwal, Oncogene 30 (2011) 1615.

[29] Y. Ueda, A. Richmond, Pigment Cell Res. 19 (2006) 112.

[30] P.C. Cogswell, R.I. Scheinman, A.S. Baldwin, J. Immunol. 150 (1993) 2794.

[31] A.A. Sablina, A.V. Budanov, G.V. Ilyinskaya, L.S. Agapova, J.E. Kravchenko, P.M. Chumakov, Nat. Med. 11 (2005) 1306.

[32] W. Hu, C. Zhang, R. Wu, Y. Sun, A. Levine, Z. Feng, Proc. Natl. Acad. Sci. USA 107 (2010) 7455

[33] J.L. Watson, R. Hill, P.B. Yaffe, A. Greenshields, M. Walsh, P.W. Lee, C.A. Giacomantonio, D. W. Hoskin, Cancer Lett. 297 (2010) 1.