Human colon cancer cells differ in their sensitivity to curcumin-induced apoptosis and heat shock protects them by inhibiting the release of apoptosis-inducing factor and caspases

R. Rashmi[1], T.R. Santhosh Kumar[1], D. Karunagaran*

Division of Cancer Biology, Rajiv Gandhi Centre for Biotechnology, Poojapura, Trivandrum 695014, India

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Abstract Mild heat treatment induced the expression of heat shock protein-70 (hsp70), hsp90 and hsp27 in two human colon cancer cell lines, one derived from primary tumor, SW480, and the other derived from the secondary lymph node tissue, SW620, of the same patient. SW620 cells appear to be more sensitive to curcumin-induced apoptosis. Heat shock protects both the human colon cancer cells from curcumin-induced apoptosis. Heat shock prevented, at least in part, the release of apoptosis inducing factor from mitochondria induced by curcumin although the release of second mitochondria derived activator of caspase and cytochrome *c* was unaffected in both the cells. Moreover, heat shock reduced curcumin-induced activation of caspases 9 and 3 but not 8.

Key words: Heat shock protein; Apoptosis; Curcumin; Apoptosis-inducing factor; Second mitochondria-derived activator of caspase; Cytochrome *c*; SW480; SW620

1. Introduction

Curcumin (diferuloyl methane), the major active ingredient of turmeric has been widely used for the treatment of inflammatory disorders for centuries in India [1]. Curcumin is also known to possess anti-proliferative, anti-mutagenic and anti-carcinogenic properties [2–4]. Several mechanisms have been proposed to explain the actions of curcumin which include blocking the production of reactive oxygen species and lipid peroxidation, and inhibiting the activities of protein kinase c, c-Jun N-terminal kinase, epidermal growth factor receptor intrinsic kinase and nitric oxide synthase [5–8]. In recent years, apoptosis has emerged as the major mechanism by which anti-cancer agents eliminate preneoplastic or cancer cells and curcumin induces apoptosis in cancer cells from colon [9], liver [10], breast [11] and leukemia [12].

Apoptosis or programmed cell death is characterized by cell shrinkage, chromatin condensation, DNA fragmentation and the activation of specific cysteine proteases known as caspases. One of the two major pathways of caspase activation involves cytokines of the tumor necrosis factor super family, which interact with their receptors and sequentially recruit receptor-associated death domains, Fas-associated death domain, caspase 8, and caspase 3; the last then cleaves various substrates leading to apoptosis [13]. In contrast, mitochondria-mediated pathway involves the release of cytochrome *c* from mitochondria, and cytochrome *c* together with apoptosis protease-activating factor-1 (Apaf-1) activates caspase 9, and the latter then activates caspase 3, resulting in apoptosis [14]. Curcumin-induced apoptosis involves both receptor- and mitochondria-mediated pathways in various cell lines [15,16]. Recently, caspase-independent apoptogenic proteins like apoptosis-inducing factor (AIF) and endonuclease G have been identified and characterized [17–19]. Tumor cells often evade apoptosis by expressing several anti-apoptotic proteins such as Bcl-2, down-regulation and mutation of pro-apoptotic genes and alterations of p53, PI3K/Akt or NF-κB pathways that give them survival advantage and thereby resist therapy-induced apoptosis [20]. Recent experimental evidence suggests that heat shock proteins like hsp70, -90 and -27 can also function as anti-apoptotic proteins by suppressing the signaling events of apoptosis [21–23]. It is also known that hsp70 and hsp27 can rescue cells not only from heat stress but also from most forms of apoptotic stimuli [21,22]. High expression of hsps especially hsp27 and hsp70, in breast, endometrial, or gastric cancer has been associated with metastasis, poor prognosis and resistance to chemo- or radio-therapy [22]. Hsp70 is abundantly and preferentially expressed in malignant human tumors and enhances the tumorigenic potential of rodent cells indicating a possible differential role for this protein between tumorigenic and non-tumorigenic cells [24].

To understand the role of hsps in curcumin-induced apoptosis, two human colon cancer cell lines, one derived from primary tumor, SW480, and the other, SW620, derived from the secondary lymph node tissue of the same patient, were induced by a sub-lethal heat exposure to express hsps. After recovery from the shock, the cells were treated with curcumin and the changes in mitochondrial membrane potential ($\Delta\psi_m$), cytochrome *c*, second mitochondria-derived activator of caspase (Smac), AIF, and the activities of caspases 9 and 3 were examined. Our results indicate that SW620 cells are more sensitive to curcumin and heat shock protects both the colon cancer cells from curcumin-induced apoptosis.

*Corresponding author. Fax: (91)-471-2348096.
E-mail address: dkarunagaran@hotmail.com (D. Karunagaran).

[1] These two authors contributed equally to this work.

Abbreviations: AIF, apoptosis-inducing factor; Smac, second mitochondria-derived activator of caspase; hsp, heat shock protein; PS, phosphatidyl serine; Apaf-1, apoptosis protease-activating factor-1; $\Delta\psi_m$, mitochondrial membrane potential; FITC, fluorescein isothiocyanate; PBS, phosphate-buffered saline; PI, propidium iodide; DAPI, 4,6-diamidino-2-phenylindole

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2. Materials and methods

2.1. Cell lines and culture conditions

Colon cancer cell lines, SW480 and SW620, were provided by Ajit Kumar, Indian Institute of Science, Bangalore, India and maintained on Dulbecco's modified Eagle's medium (Life Technologies, Inc.) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Sigma) in an atmosphere of 95% air and 5% CO_2.

2.2. Reagents and antibodies

Curcumin and 4,6-diamidino-2-phenylindole (DAPI) were procured from Sigma. Rabbit polyclonal antibodies to hsp27 (sc-9012), cytochrome *c* (sc-7159) and AIF (sc-5586) were purchased from Santa Cruz Biotechnology. Monoclonal antibodies to hsp70 (sc-24), hsp90 (sc-13119) and a goat polyclonal antibody to Smac (sc-12683) were also procured from Santa Cruz Biotechnology. A mouse monoclonal antibody to β-actin (A-5441) and alkaline phosphatase conjugated secondary antibodies were obtained from Sigma.

2.3. Induction of heat shock response and treatment with curcumin

Cells were allowed to grow up to 50% confluence and incubated at 44°C for 45 min in an incubator. Immediately after heat shock, the cells were allowed to recover for 12 h at 37°C. SW480 or SW620 cells were incubated with 50 μM curcumin (diluted from a stock of 100 mM curcumin dissolved in dimethyl sulfoxide) for 8 h after the 12 h recovery period from heat shock.

2.4. Western blotting

Cells were harvested, washed thrice in phosphate-buffered saline (PBS), and lysed in RIPA lysis buffer (50 mM Tris–Cl (pH 7.4), 1% NP 40, 40 mM NaF, 10 mM NaCl, 10 mM Na_3VO_4, 1 mM phenylmethylsulfonyl fluoride, and 10 mM dithiothreitol (DTT) and 1 μg/ml each of leupeptin and aprotinin). The cell lysates (50 μg of protein) were loaded on to SDS–PAGE and the separated proteins were transferred to nitrocellulose membrane by wet transfer method using Bio-Rad electro-transfer apparatus. After blocking with 10% non-fat milk in Tris buffered saline containing 0.2% Tween 20, the membrane was incubated with the primary antibody followed by alkaline phosphatase conjugated secondary antibody. Proteins were visualized by nitro blue tetrazolium/5-bromo-4-chloro-3-indolylphosphate reagent (Sigma). For some experiments the cells after treatment were harvested, washed twice with PBS and the cell pellet was resuspended in digitonin lysis buffer (75 mM NaCl, 1 mM NaH_2PO_4, 8 mM Na_2HPO_4, 250 mM sucrose, 190 μg/ml of digitonin) containing protease inhibitors and incubated on ice for 5 min. The releasate was centrifuged at 15 000 rpm at 4°C for 30 min and used for Western blotting as described above using antibodies to AIF or Smac or cytochrome *c* and appropriate secondary antibodies.

2.5. DAPI staining

The monolayer of cells was washed in PBS and fixed with 3% paraformaldehyde for 10 min at room temperature. The fixed cells were permeabilized with 0.2% Triton X-100 in PBS for 10 min at room temperature and incubated with 1 μg/ml of DAPI. The apoptotic nuclei (intensely stained, fragmented nuclei and condensed chromatin) were scored in percentage from 200–300 cells/sample with at least two investigators using a fluorescent microscope (Nikon TE 300).

2.6. Determination of $\Delta\psi_m$

Cells grown in 96-well plates were given heat shock and after recovery, 50 μM curcumin treatment was given for 8 h, stained with a cationic dye (ApoAlert mitochondrial membrane sensor kit, Clonetech) as per the manufacturer's protocol and analyzed by fluorescence microscopy using a band-pass filter. The cells with intact $\Delta\psi_m$ stained red while green fluorescence indicated the loss of $\Delta\psi_m$.

2.7. Fluorescein isothiocyanate (FITC)-annexin staining

The cells were grown in 48-well plates and after drug treatment for indicated period of time, washed with PBS and treated with 1× assay buffer, annexin-FITC and propidium iodide (PI) as per the protocol described in the annexin V apoptosis detection kit (sc-4252 AK) from Santa Cruz Biotechnology. After 10–20 min, the wells were washed with PBS and greenish early apoptotic cells (annexin positive) and yellowish red cells stained by PI in the later stages of apoptosis were viewed using a Nikon fluorescent microscope and photographed.

2.8. Caspase 3, caspase 9 and caspase 8 activities

The enzymatic activities of caspase 3, caspase 9 and caspase 8 were assayed spectrofluorimetrically [25]. Briefly, the whole cell lysate was incubated with 50 μM of fluorimetric substrates of caspase 3 (Ac-DEVD-AFC) or caspase 9 (Ac-LEHD-AFC) or caspase 8 (Z-IETD-AFC) in a total volume of 500 μl of reaction buffer [50 mM HEPES-KOH, pH 7.0, 10% glycerol, 0.1% 3-(cholamidopropyl)-dimethyammonio-1-propane sulfonate, 2 mM EDTA, 2 mM DTT] at 37°C for 1 h. The released AFC was quantitated using a spectrofluorometer (Perkin Elmer LS-50 B) with the excitation and emission wavelengths of 380 and 460 nm, respectively. Values of relative fluorescence units released per milligram of protein were calculated.

2.9. Immunofluorescent staining

The cells grown on glass cover slips, after appropriate treatments, were fixed and permeabilized as before and then incubated with the respective primary antibody for overnight. After extensive washing with Tris buffered saline containing 0.2% Tween 20, the cells were incubated with FITC conjugated secondary antibody at 1:50 dilution for 45 min in the dark. The cover slips were mounted with 50% glycerol-PBS, and viewed under fluorescent microscope and photographed.

3. Results

3.1. Heat shock induces the expression of hsps, 70, 90, and 27, in SW480 and SW620 cells

To study and understand the effects of heat shock on curcumin-induced apoptosis, we have used two human colon cancer cells, SW480 and SW620, established cell lines of the same patient from colon and lymph node tissues, respectively. After subjecting these cells to heat treatment at various temperatures, it was found that 44°C for 45 min is the optimal condition that consistently induces hsps without any detectable cytotoxicity (data not shown). The cells treated under this condition were then allowed to recover from the shock for 12 or 24 h and the protein expression of hsps known to be involved in apoptosis resistance (hsps 70, 90 or 27) were checked by Western blot analysis. To ensure equal loading of proteins in all the samples, β-actin controls were used. The results shown in Fig. 1 indicate that during the recovery period, the expression levels of hsps, 70, 90 and 27, increased at least up to 12 h in SW480 and SW620 cells and the basal levels of hsp27 were very low in both the colon cancer cells. The extent of induction of hsps 70, 90 and 27 from 0 to 12 h was relatively higher in SW480 cells and expression of hsps 70 and 90 did not decrease at 24 h (Fig. 1). However, the expression levels of hsp27 in SW480 and SW620, and hsp70 (but

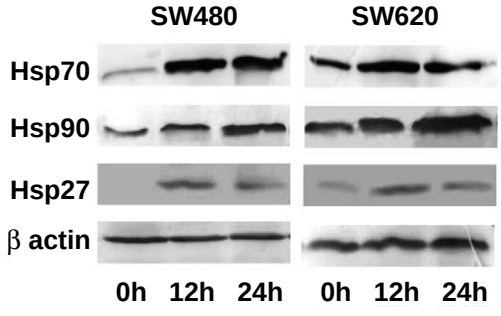


Fig. 1. Western blotting for the expression of hsps. SW480 or SW620 cells were harvested after heat shock at the indicated time intervals, and the cell lysates were loaded on to SDS–PAGE and the Western blotting for the expression hsps 70, 90, and 27, and β-actin (control) were carried out as described in Section 2. The experimental results were confirmed in another independent experiment.

not hsp90) in SW620 cells showed a slight decrease at 24 h, but the expressions of all the three hsps were still higher than the basal levels at 0 h (Fig. 1). These results suggest that the optimal conditions of heat shock chosen for the study are sufficient to induce the expression of the hsps 70, 90 and 27, in SW480 and SW620 cells and their expression was still maintained above basal levels in both the cells during the recovery period of 12–24 h.

3.2. *Heat shock inhibits curcumin-induced nuclear condensation more effectively in SW480 cells*

After confirming the expression of hsps during the recovery period, it was decided to test the effects of heat shock on subsequent treatment with curcumin. The human colon cancer cells, SW480 or SW620, were incubated with 50 μM curcumin for 8 h after the 12 h recovery period from heat shock while the untreated cells or cells exposed only to heat shock served as respective controls. DAPI staining was used to assess the percentage of apoptotic cells with condensed and fragmented chromatin over the control in SW480 and SW620 cells and the representative photographs are shown in Fig. 2. In SW480 cells treated with curcumin alone, nuclear condensation was visible in almost 86% of cells whereas all the SW620 cells treated with curcumin showed nuclear condensation (Fig. 2). If curcumin treatment was given after heat shock, 8% of SW480 and in contrast 66% of SW620 cells exhibited nuclear condensation (Fig. 2) and heat shock alone did not induce nuclear condensation in both the cells (data not shown). These results indicate that curcumin induces nuclear condensation, a typical feature of apoptosis, in SW480 and more efficiently in SW620 cells and heat shock reduces the number of cells showing nuclear condensation in both the cells and this protective effect of heat shock is relatively more in SW480 cells.

3.3. *Externalization of phosphatidyl serine (PS) and loss of membrane integrity induced by curcumin are inhibited by heat shock*

Under defined salt and calcium concentrations, annexin V is predisposed to bind PS that is externalized on to the cell surface in the very early stages of apoptosis. Hence, apoptotic cells were detected using annexin V labeled with FITC and

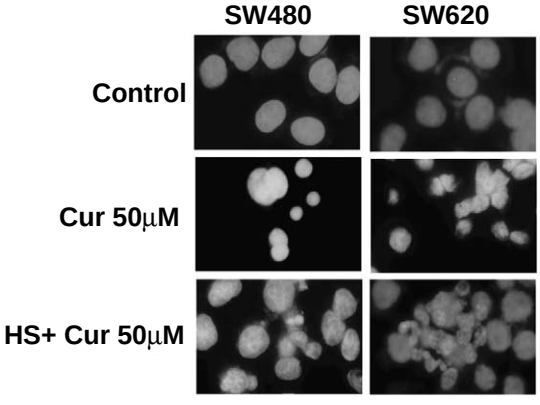


Fig. 2. Changes in nuclear condensation. SW480 or SW620 cells, treated with 50 μM curcumin for 8 h (untreated cells shown as control) or given heat shock and then treated with curcumin after the recovery period of 12 h, were fixed, permeabilized, and stained with DAPI as described in Section 2. The experiment was repeated at least three times with similar results.

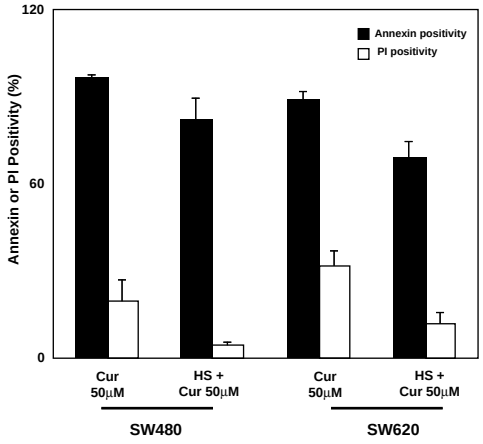


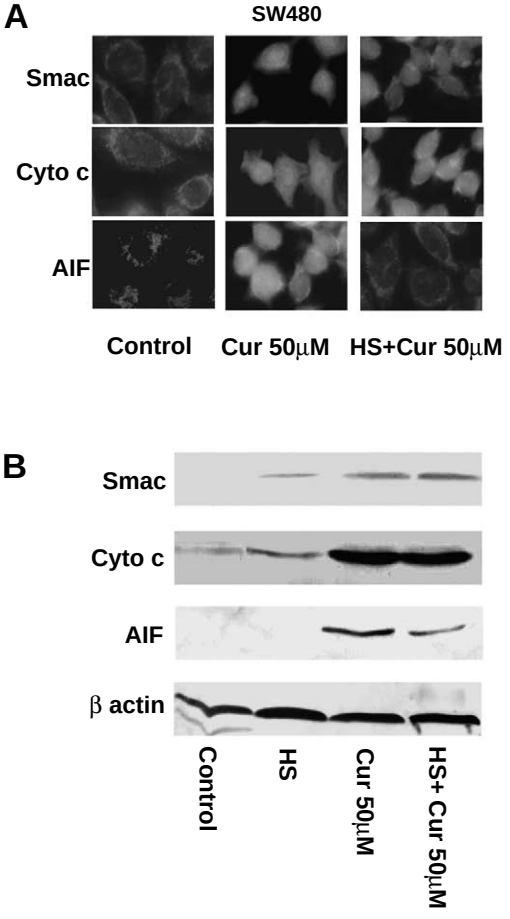
Fig. 3. Changes in annexin–PI positivity. Cells grown in 48-well plates were treated with curcumin (50 μM for 8 h) or given heat shock and then treated with curcumin after the 12 h recovery period. The cells were stained with annexin–PI mixture as per the protocol described in the annexin V apoptosis detection kit (sc-4252 AK) from Santa Cruz Biotechnology. Cells were counted under fluorescence microscope and the percentage of cells over the respective controls was calculated. The error bars indicate standard deviation and similar results were obtained in two other independent experiments.

photographed with a camera-attached fluorescent microscope. Addition of PI helps to distinguish the early apoptotic cells from late apoptotic or necrotic cells since PI cannot enter the cells in the early stages of apoptosis when the membrane integrity is intact [26,27]. SW480 and SW620 cells, which did not receive any treatment or received heat shock alone, were used as controls to calculate the percentage of annexin positivity in the treated cells. After 8 h of curcumin treatment, 96 and 89% of SW480 and SW620 cells, respectively, were positive for annexin indicating PS exposure on to the cell surface (Fig. 3). If curcumin treatment was given after heat shock, 81 and 66% of SW480 and SW620 cells, respectively, were still annexin positive (Fig. 3). For PI staining, 20% of SW480 cells were positive upon curcumin treatment and when curcumin treatment followed heat shock only 4% of them were positive (Fig. 3). Similarly, in SW620 cells, 40% of them were PI positive with curcumin treatment alone and only 11% of them were positive if curcumin treatment followed heat shock (Fig. 3). In addition, the PI positivity was more in SW620 cells treated with curcumin alone although annexin positivity was almost the same in both the cells under the same conditions (Fig. 3). Untreated cells or cells treated only with heat shock did not show PI positivity (data not shown). These results suggest that externalization of PS and loss of membrane integrity induced by curcumin are inhibited by heat shock in both the human colon cancer cells.

3.4. *Heat shock fails to prevent the curcumin-mediated release of cytochrome* c *and Smac, but partially prevents the release of AIF*

Cytochrome *c*, Smac and AIF (located within the inner mitochondrial membrane) are released into the cytosol upon apoptotic stimuli. Untreated control SW480 cells showed more granular appearance for cytochrome *c*, Smac and AIF around the nucleus (Fig. 4A). Upon curcumin treatment, both the cells showed diffuse pattern in the cytoplasm as well as nucleus for cytochrome *c*, Smac and AIF indicating their re-

lease from mitochondria (Fig. 4A). Quite interestingly, there was no difference in the patterns of staining for cytochrome *c* and Smac in SW480 cells if curcumin treatment was given after heat shock compared with the cells treated with curcumin alone (Fig. 4A). Even though the granular appearance was not very typical, AIF release was not complete in cells treated with curcumin after heat shock (Fig. 4A). Similar results were obtained in SW620 cells (data not shown). Similarly Western blot using the digitonin-lysed samples obtained from SW480 cells also showed the release of Smac, cytochrome *c* and AIF in the presence of curcumin (Fig. 4B). Interestingly, heat shock alone slightly induced the release of Smac and cytochrome *c* but not AIF and similar to the immunofluores-



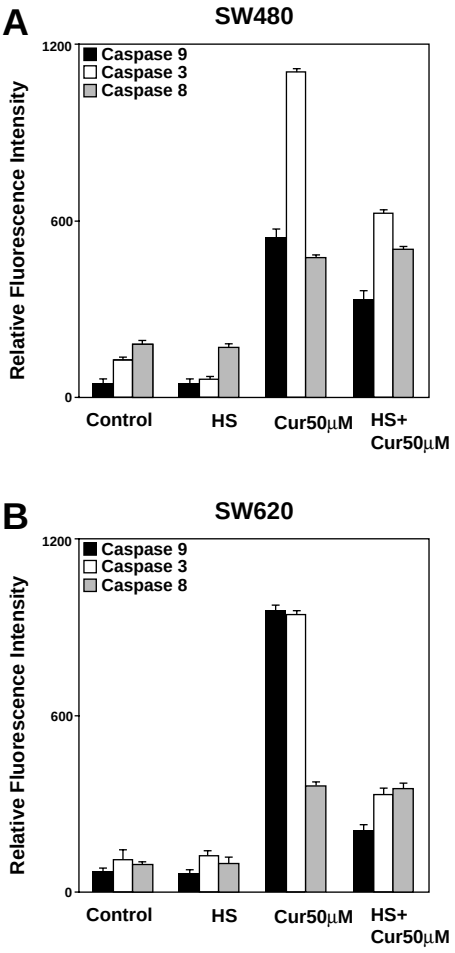


Fig. 5. Determination of the activities of caspases 9, 3 and 8. Cell lysates of untreated control or heat shock-treated or curcumin-treated or the cells that received curcumin after heat shock obtained from SW480 (A) or SW620 (B) were incubated with fluorimetric substrates of caspase 3 (Ac-DEVD-AFC) or caspase 9 (Ac-LEHD-AFC) or caspase 8 (Z-IETD-AFC) in a reaction buffer at 37°C for 1 h and the caspase activities were determined as described in Section 2. The error bars denote standard deviation and similar results were observed in another independent experiment.

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Fig. 4. Release of Smac, cytochrome *c* and AIF from mitochondria and changes in $\Delta\psi_m$. SW480 cells grown on glass cover slips from untreated control or treated with curcumin or treated with curcumin after heat shock were fixed and permeabilized and then incubated overnight with the respective primary antibody for Smac or cytochrome *c* or AIF. After extensive washing, the cells were incubated with FITC conjugated secondary antibody and mounted with glycerol and viewed under fluorescent microscope and photographed (A). The experiment was repeated at least two times with similar results. For Western blot analysis, the cells after treatment were suspended in digitonin lysis buffer containing protease inhibitors and incubated on ice for 5 min. The releasate was centrifuged and used for Western blotting using respective antibodies for AIF or Smac or cytochrome *c* or β-actin (control) and appropriate secondary antibodies (B). These results were confirmed in another independent experiment. For assessing mitochondrial potential, the cells (SW480 or SW620) grown in 96-well plates were given heat shock and after recovery, treated with curcumin and stained with a cationic dye as described in Section 2 (C). The error bars indicate standard deviation and the results were similar when the experiment was repeated two times.

cence data heat shock could partly prevent the release of AIF but not that of others (Fig. 4B). These results imply that in SW480 and SW620 cells, curcumin effectively induces the release of cytochrome *c*, AIF and Smac from mitochondria; heat treatment does not block the curcumin-mediated release of cytochrome *c* and Smac, but partially blocks the release of AIF in both the cells.

3.5. *Heat shock fails to suppress the loss of $\Delta\psi_m$ induced by curcumin*

Changes in mitochondrial potential were then determined using a cationic dye in SW480 or SW620 cells and the results show that after 8 h of curcumin treatment alone, there was almost complete loss of $\Delta\psi_m$ as all the cells showed green fluorescence and curcumin treatment after heat shock had no significant effect on this (Fig. 4C). Heat shock alone did not affect the mitochondrial potential of SW480 or SW620 cells (data not shown). These results suggest that heat shock does not prevent the loss of mitochondrial potential induced by curcumin in SW480 or SW620 cells.

3.6. *Heat shock inhibits curcumin-induced activation of caspases 3 and 9, but not 8*

The relative intensities of the cleaved free fluoroprobes of caspases 9, 3 and 8 obtained with different treatments from SW480 and SW620 cells are shown in Fig. 5A,B, respectively. Curcumin treatment significantly activated the caspases 9, 3 and 8 in both the colon cancer cells, but the extent of activation of caspase 9 was relatively more in SW620 cells (Fig. 5A,B). Similarly, heat shock significantly reduced the activation of both caspases 9 and 3, but not 8 in SW480 or SW620 cells, but the extent of inhibition of caspase 9 was relatively more in SW620 cells (Fig. 5A,B). Although heat shock alone did not change the activities of caspases 3 and 8 in SW480 and caspases 3, 9 and 8 in SW620 cells, heat shock slightly reduced the basal activity of caspase 3 in SW480 cells (Fig. 5A,B).

4. Discussion

Curcumin is recognized as a safe compound that specifically induces apoptosis in cancer cells [10]. However, cancer cells over-expressing anti-apoptotic proteins may evade curcumin-induced apoptosis and earlier results from our laboratory have shown that ectopic expression of the RelA subunit of NF-κB (known to exert anti-apoptotic effects) into murine fibrosarcoma cells protects them from curcumin-induced apoptosis [28]. In this report we have attempted to understand the relative susceptibility of primary and metastatic human colon cancer cells to apoptosis induced by curcumin and, the anti-apoptotic mechanisms by which heat shock may protect. Based on the results shown with PI, and DAPI staining and the extent of caspase 9 activation, SW620 cells appear to be more sensitive than SW480 to curcumin-induced apoptosis. In the present study, we demonstrate for the first time that heat shock can protect human colon cancer cells from curcumin-induced apoptosis by interfering with both caspase-dependent and -independent pathways supporting the anti-apoptotic role for hsps and interestingly curcumin-induced activation of caspase 8 was unaffected by prior heat treatment. Although the present results do not specify which of the hsps protects human colon cancer cells from curcumin-induced apoptosis, they do suggest the involvement of hsps 90, 70 and 27. Most of the parameters to assess apoptosis used in the present study suggest that heat shock protects both the human colon cancer cells from curcumin-induced apoptosis. However, differences were found in the extent of inhibition of curcumin-induced activation of caspase 9 by heat shock between SW480 and SW620 cells and similarly, heat shock protection is better in SW480 cells by DAPI staining. Although all the three hsps studied were up-regulated by heat shock, the quantitative differences in the levels of expression and extent of induction/sustenance of different hsps in SW480 (up-regulation of hsp70 and 27) and SW620 cells (up-regulation of hsp90) may partly account for these seemingly contradictory results. Up-regulation of hsp70 may account for heat shock-mediated effects observed after curcumin treatment in SW480 cells by DAPI staining and the differences in the extent of activation of caspases 3 and 9. Interestingly, curcumin-resistant tumor cell lines showed significantly higher expression of hsp70 [15] and hsp70 is known to interfere with the activation of caspase 9 [29]. Hsp70 also functions as a strong suppressor of apoptosis acting downstream of cytochrome *c* release and upstream of caspase-3 activation [30]. Earlier, Jaattela et al. showed that hsp70 could protect cells even after caspase 3 activation [31]. Furthermore, Hsp27 can bind to the released cytochrome *c* thereby preventing apoptosome formation that may eventually suppress activation of caspase 9 [32]. Pandey et al. demonstrated that hsp90 could also form a cytosolic complex with Apaf-1 and inhibit the formation of active caspase 3 [33]. The mechanisms by which heat shock reduces the extent of PS exposure induced by curcumin appear to be complex as PS exposure in response to curcumin is considered to be partly due to non-specific membrane disturbances induced by it even in non-nucleated cells [34]. In addition, translocation of PS to the external cell surface is not unique to apoptosis, but occurs also during cell necrosis [26]. PI staining also indicates late apoptotic or necrotic cells, but we did not visually observe necrosis in SW480 or SW620 cells treated with 50 μM curcumin.

This is the first report on the involvement of Smac that relieves the inhibitory function of inhibitor of apoptosis protein, in curcumin-induced apoptosis although other workers also reported release of cytochrome *c*, AIF and subsequent caspase activation by curcumin [21,23,35]. Since AIF release was partially blocked in human colon cancer cells by heat shock even with the loss of $\Delta\psi_m$, it raises a question whether hsp70 selectively retained AIF within the mitochondrial intermembrane space. Recently Ravagnan et al. showed hsp70 could neutralize the function of AIF by affinity binding in a cell-free system of apoptosis [36]. However, the mechanism behind the selective retention of AIF within the mitochondria in heat-treated cells is not clear. This is contrary to the notion that disruption of mitochondrial $\Delta\psi_m$ is an early event and induces the release of all apoptogenic molecules from mitochondria. Our results support the idea that the release of cytochrome *c*, Smac and AIF are separate events independent of the disruption of $\Delta\psi_m$. High level of hsp27 expression is known to prevent the translocation of cytochrome *c* to the cytosol [37] and it is possible that mild heat treatment given in the present work failed to retain cytochrome *c* within mitochondria due to insufficient increase in hsp27 in human colon cancer cells during the experimental period.

Our data support the notion that hsps can potentially block apoptosis and further work is in progress with appropriate

caspase inhibitors to understand the role of individual hsps in curcumin-induced apoptosis. The fact that cells or tissues from a wide range of tumors have been shown to express unusually high levels of one or more of hsps suggests that these proteins could play an important role not only in tumorigenesis but also in the development of drug resistance. To make conventional therapy more effective, silencing of such proteins appears to be an important approach.

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