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The p38 pathway partially mediates caspase-3 activation induced by reactive oxygen species in Fanconi anemia C cells

Michal Pearl-Yafe[a], Drora Halperin[a], Oded Scheuerman[b], Ina Fabian[a,*]

[a]*Department of Cell Biology and Histology, Sackler Faculty of Medicine, Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel*
[b]*Schneider Children's Medical Center of Israel, Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel*

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Abstract

Because Fanconi anemia (FA) cells display hypersensitivity to oxidative stress and reactive oxygen species (ROS) that act as second messengers in cellular signaling, we investigated c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) activation in two FA-C lymphocyte cell lines (HSC536/N and PD149L) and one FA-A cell line (HSC99) exposed to interferon (IFN)-γ or H_2O_2. IFN-γ induced accumulation of ROS and activation of JNK and p38 in HSC536/N and PD149L but not in HSC99 cells. Higher concentrations of H_2O_2 were needed to induce moderate intracellular levels of ROS and phosphorylation of MAPKs in FA-A than in FA-C cells. Pre-incubation with dehydroascorbic acid resulted in reduced intracellular ROS levels and inhibition of MAPK activation induced by the above treatments. To define the functional role of JNK and p38 in IFN-γ signaling, the effects of pharmacological inhibition of the MAPKs on induction of IFN-γ and anti-Fas antibody responses were determined. Treatment of HSC536/N cells with p38-specific inhibitors partially inhibited caspase-3 activation while pre-incubation with specific inhibitors of JNK had no effect. Altogether, these results suggest that FA-C cells are hypersensitive to IFN-γ and are more sensitive to oxidative stress than FA-A cells and that IFN-γ and anti-Fas antibody mediate signals for apoptosis in FA-C cells *via* p38 but not JNK pathways.

Keywords: Fanconi anemia; MAPK; IFN-γ; ROS

1. Introduction

FA is an autosomal recessive disorder characterized by cellular hypersensitivity to cross-linking agents, bone marrow failure, diverse congenital anomalies, and a marked increase in the incidence of acute myelogenous leukemia ([1,2] and reviewed in Refs. [3–5]). FA cells are reportedly hypersensitive to oxygen [6,7], although the molecular basis of this sensitivity is yet unknown. Defective hematopoiesis and hepatic steatosis was observed in mice with combined deficiencies of FANCC and Cu/Zn superoxide dismutase, suggesting that the altered redox state present in the mice is responsible for impaired hematopoiesis [8]. Other studies have shown that FANCC and NADPH cytochrome P450 reductase interact and it is suggested that FANCC serves as an intracellular antioxidant [9]. A novel interaction between FANCG and CYP2E1 (a member of the P450 superfamily that is associated with the production of free radicals (ROS)) was identified and it is proposed that FA gene products play a role in the regulation of the cellular oxidative stress [10,11]. Recent studies performed by Kontou *et al.* [12] have shown that nuclei of FA cells contain suboptimal concentrations of thioredoxin, an intracellular antioxidant and regulator of redox-sensitive gene expression [13]. Overexpression of thioredoxin in the cells removes noxious ROS and protects the DNA from mitomycin C-induced or spontaneous chromosomal instability [14].

Hematopoietic progenitor cells from mice nullizygous at the FA group C (FANCC) locus and children with FA of the C complementation group (FA-C) are hypersensitive to the apoptotic inducing effects of IFN-γ [15–17].

Recently, we have shown that FA cells are intolerant for oxidative stress and that IFN-γ involves the induction of ROS in two FA-C B-lymphocyte cell lines and in peripheral

* Corresponding author. Tel.: +972-3-6409628; fax: +972-3-6407432.
E-mail address: inaf@post.tau.ac.il (I. Fabian).

Abbreviations: DCFH-DA, 2′,7′-dichlorofluorescein-diacetate; DHA, dehydroascorbic acid; FA, Fanconi anemia; FBS, fetal bovine serum; HBSS, Hank's balanced salt solution; IFN, interferon; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; ROS, reactive oxygen species; TNF, tumor necrosis factor.

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blood neutrophils and mononuclear cells of FA patients. The study has also shown that priming of FA-C cell lines with IFN-γ followed by treatment with anti-Fas antibody (Ab) results in caspase-3 and -8 activation and apoptosis [18].

Cellular signaling pathways are regulated by the intracellular redox state of the cell. ROS may play an important role as a second messenger in signal transduction cascades and may lead to the activation of MAPKs [19–21]. Recent studies have identified JNKs and p38, also called stress-activated protein kinases, to be involved in cellular responses to environmental stresses [22,23]. JNK and p38 kinases can be activated by inflammatory cytokines, such as TNF-α and IL-1β, and a diverse array of cellular stresses, including UV light, X-rays, heat and osmotic shock, withdrawal of growth factors, and H_2O_2, and may participate in apoptosis [22,24].

H_2O_2 has been used frequently as an oxidative stimulus to identify redox-sensitive processes. The concentrations of H_2O_2 in healthy individuals is normally quite low [25]. The elevation of H_2O_2 upon viral infection or inflammation could act as a significant signal. For cells that are already activated, or cells that are sensitive to ROS (like FA cells), this signal might be expected to amplify their response by boosting MAPK phosphorylation. In the present work, we investigated FA cell lines belonging to two complementation groups, FA-C and FA-A (that provide 80% of FA patients [4,26,27]) and their corrected counterparts. The objective was to evaluate whether JNK and p38 kinases are involved in hypersensitivity of FA of the C and A complementation groups to oxidative stress and to identify more precisely the role of ROS, produced by IFN-γ and H_2O_2, on the activation and regulation of JNK and p38 signal transduction pathways. We also sought to investigate if differences in the response to oxidants exist among the two complementation groups.

2. Materials and methods

2.1. Epstein–Barr virus (EBV)-transformed cell lines

The EBV-transformed lymphoblast cell line HSC536/N, the corrected counterparts HSC536/FANCC cells (FA-C), HSC99, and the corrected counterparts HSC99/FANCA (FA-A) (gift of Manuel Buchwald, The Hospital for Sick Children, Toronto, Ont., Canada) were previously described [17,28,29]. PD149L cells are EBV-transformed lymphoblasts derived from a child with FA-C (gift of Grover Bagby, MD, Oregon Health Science University). PD149/FANCC cells were derived by transducing the PD149L cells with a retrovirus encoding both FANCC and neomycin phosphotransferase. The lymphoblast cell lines were grown in RPMI 1640 (Biological Industries Kibutz Beit Haemek) supplemented with 15% FBS (Hyclone), 1% glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin at 37°, and 5% CO_2 in a humidified atmosphere.

2.2. Measurement of ROS

The oxidation of 2′,7′-dichlorofluorescein-diacetate (DCFH-DA) (Molecular Probes) was analyzed as previously described by us [18]. In brief, cells (2×10^6/mL) were incubated with 5 μM DCFH-DA, washed, resuspended in Hank's balanced salt solution (HBSS), and plated at a density of 3750 cells/well of a 96-well plate. To each well was added 25 or 50 μM H_2O_2 or 1 ng/mL IFN-γ (R&D Systems Inc.). Fluorescence was measured over a 180-min time period with a spectrofluorometer (FL-600 Microplate Fluorescence Reader) with excitation at 488 nm and emission at 525 nm. The fluorescence at each time point was expressed as relative fluorescence, values normalized to the initial reading. In experiments where the effect of dehydroascorbic acid (DHA) on ROS production was investigated, the cells were pre-incubated with 150 μM DHA for 10 min before incubation with 5 μM DCFH-DA [18] and the fluorescence was investigated as described above.

2.3. Western blot analysis

Cells were pre-incubated in the presence or absence of 150 μM DHA and exposed to 25 or 50 μM H_2O_2 or 0.01–1 ng/mL IFN-γ for 5–60 min. Total cell protein was extracted at the indicated times, separated by 10% SDS–polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes, and immunoblotted with anti-phospho-JNK antibody (Ab detects JNK only when activated by phosphorylation at Thr183/Tyr185) and anti-phospho-p38 Ab (Ab detects p38 MAPK only when activated by dual phosphorylation at Thr180 and Tyr182) (1:3000 dilution) and anti-JNK Ab, and anti-p38 Ab (1:1000 dilution) (from New England Biolabs). The blots were incubated with secondary antibodies coupled to peroxidase. Bands were visualized using the ECL detection system, as previously described by us [18].

2.4. Fluorogenic assay for caspase-3 activity

DJNK1 and SP600125 inhibit specifically JNK activation [30,31] while PD169316 and SB202190 inhibit p38 [32,33]. HSC536/N and HSC99 cells were pre-incubated with inhibitors of JNK and p38 (2.5–25 μM) for 30 min and cultured in medium in the presence of 1 ng/mL IFN-γ and 100 ng/mL anti-Fas Ab [17] for 120 min. Cells were collected, washed, resuspended in 50 mM Tris–HCl buffer, pH 7.4, 1 mM EDTA, 10 mM EGTA and lysed by three successive freeze–thaw cycles at dry ice/37°. Cell lysates were centrifuged at 20,000 *g* for 5 min, and the supernatants were stored at −70°. The protein concentration of each sample was estimated using the Bradford Bio-Rad protein assay.

For caspase-3 activity a total of 50 μg protein was incubated with 50 μM ac-DEVD-AMC (from BIOMOL Res. Lab. Plymouth Meeting PA) at 37°, for 30 min in the dark. The release of 7-amino-4-methylcoumarine was monitored by a spectrofluorometer using an excitation wavelength of 360 nm and an emission wavelength of 460 nm.

Statistical significance was determined by using the Student's *t* test.

3. Results

3.1. Activation of JNK and p38 by IFN-γ: effect of DHA

We sought to determine whether the JNK and p38 MAPK pathways are activated in response to IFN-γ treatment. Initially, time-dependent studies were performed. The phosphorylation of the kinases was determined after exposure of HSC536/N, PD149L, HSC99 cells, and their genetically corrected counterparts to 1 ng/mL IFN-γ. In HSC536/N cells, we observed an immediate JNK and p38 activation within 5 min with maximal induction within 15 min, which was followed by a decrease to background levels (Fig. 1A and B), while no activation of JNK nor of p38 was observed in the corrected counterparts (Fig. 1B).

In a previous study, we have shown that IFN-γ induces a marked accumulation of ROS in HSC536/N cells and a moderate increase in HSC536/FANCC cells and that pre-incubation with DHA reduces ROS levels in the former cells [18]. Thus, we examined whether the activation of JNK and p38 in HSC536/N cells is due to accumulation of high concentrations of ROS and whether pre-incubation with DHA will inhibit MAPK activation. Lysates from HSC536/N cells that were pre-incubated in the presence or absence of DHA before exposure to IFN-γ were prepared and immunoblotted with antibodies against the phosphorylated forms of JNK and p38. Pretreatment of control cells with DHA did not affect JNK and p38 phosphorylation (data not shown). As expected, in HSC536/N cells JNK and p38 MAPKs were rapidly activated (within 5 min) upon exposure to IFN-γ while no phosphorylation of the MAPKs could be observed in cells pretreated with DHA (Fig. 1C).

Dose-dependent studies performed with increasing concentrations of IFN-γ showed that treatment of HSC536/N cells with 0.5 or 1 ng/mL IFN-γ for 15 min induced strong activation of the MAPKs JNK and p38 while no phosphorylation of JNK or p38 was observed at lower concentrations (Fig. 1D).

PD149L is another mutant of FA-C cells. We sought to investigate whether IFN-γ induces the activation of JNK and p38 in an additional FA-C cell line. In a similar manner, when PD149L cells were treated with 1 ng/mL IFN-γ, the activation of JNK and of p38 could be observed within 5 min and increased with time (Fig. 2A and B) while

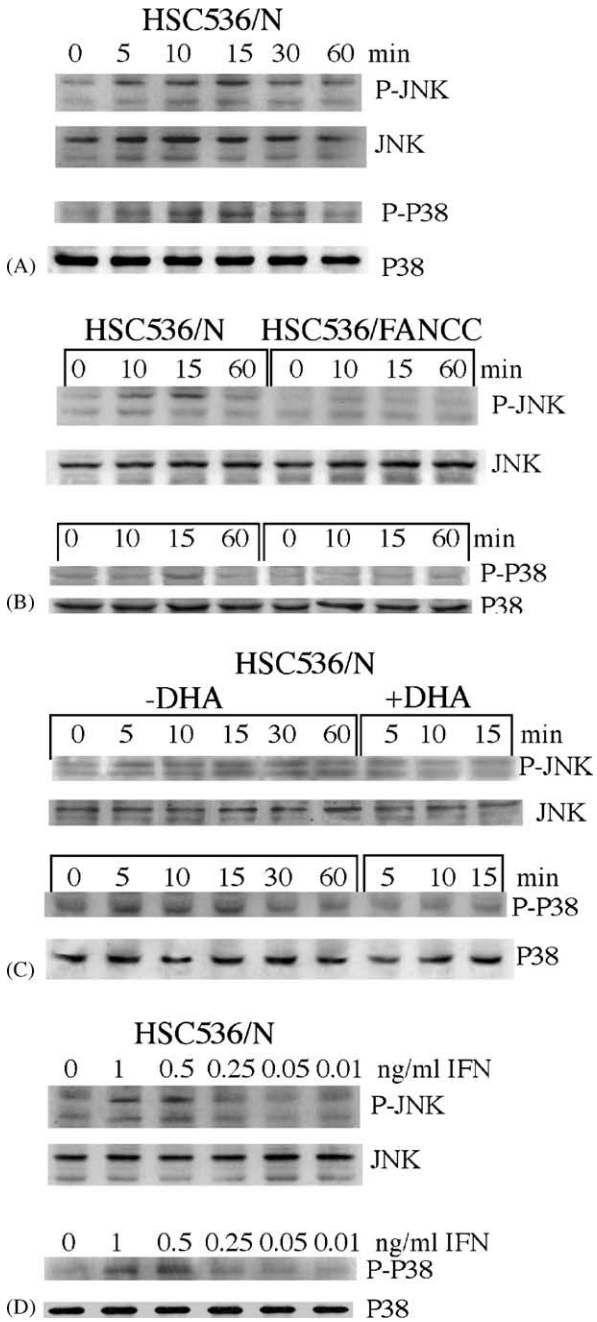

Fig. 1. Effect of IFN-γ on JNK and p38 MAPK activation in HSC536/N and HSC536/FANCC cells. (A and B) Time-dependent studies. HSC536/N and HSC536/FANCC cells were exposed to 1 ng/mL IFN-γ for the indicated times. Phosphorylation levels were measured by immunoblotting the whole cell lysates with antibodies specific for the phosphorylated forms of the enzymes. The blots were reprobed with JNK and p38 Abs to show that equivalent amounts of protein were in each lane. (C) Effect of DHA on JNK and p38 phosphorylation. HSC536/N cells were pre-incubated in the absence or presence of DHA before exposure to 1 ng/mL IFN-γ for the indicated times. Phosphorylation levels of the MAPKs were determined as above. (D) Concentration-dependent studies. HSC536/N cells were exposed for 15 min to various concentrations of IFN-γ and the phosphorylation levels of the MAPKs were determined as above. The representative blots of three experiments are shown.

no phosphorylation of the MAPKs was observed in the genetically corrected counterparts (Fig. 2B). Pretreatment of control cells with DHA did not affect JNK and p38

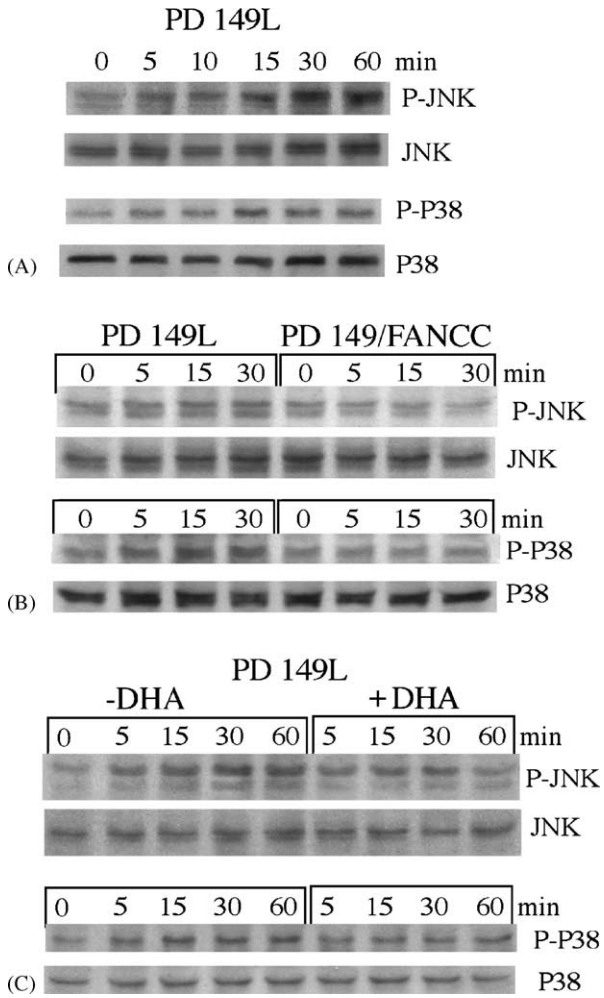

Fig. 2. Effect of IFN-γ on JNK and p38 MAPK activation in PD149L cells and PD149/FANCC cells. (A and B) Time-dependent studies. PD149L and PD149/FANCC cells were exposed to 1 ng/mL IFN-γ for the indicated times. Phosphorylation levels were measured as indicated in legends to Fig. 1. (C) Effect of DHA on JNK and p38 phosphorylation. PD149L cells were pre-incubated in the absence or presence of DHA before exposure to 1 ng/mL IFN-γ for the indicated times. The representative blots of three experiments are shown.

activation. Figure 2C indicates that pre-incubation of PD149L cells with DHA before treatment with IFN-γ decreases JNK and p38 activation.

In the next stage, we investigated whether IFN-γ induces the activation of JNK and p38 in HSC99 cells. Thus, the cells were incubated in the presence of 1 ng/mL IFN-γ for 5–60 min, lysates were prepared and immunoblotted with antibodies against the phosphorylated forms of JNK and p38, as described above. Surprisingly, no activation of JNK or p38 was observed (data not shown).

3.2. IFN-γ- and anti-Fas Ab-induced caspase-3 activation involves phosphorylation of p38 but not JNK

Our previous study has shown that IFN-γ and anti-Fas Ab induce apoptosis in HSC536/N cells through activation of caspase-3. The specificity of caspase-3 activation was examined by pre-incubation of the cells with ac-DEVD-cho (a caspase-3 inhibitor). Pre-incubation of HSC536/N cells with ac-DEVD-cho prior to exposure to IFN-γ and anti-Fas Ab inhibited caspase-3 activation by 67% [18]. In the present work, we sought to investigate whether activation of caspase-3 induced by IFN-γ and anti-Fas Ab involves JNK and p38 activation. To examine the biological significance of JNK and p38 blockade, we analyzed the effect of the MAPK inhibitors on caspase-3 activation after IFN-γ and anti-Fas Ab exposure. Using a sensitive fluorogenic assay (with DEVD-AMC serving as substrate), we observed that exposure of HSC536/N cells to IFN-γ and anti-Fas Ab enhances caspase-3 activity. Pretreatment with 2.5–25 μM SB202190 (an inhibitor of p38) resulted in $21 \pm 2\%$ to $34 \pm 4\%$ decrease, compared to cells exposed to IFN-γ and anti-Fas Ab (Fig. 3, $P < 0.05$).

Having observed that SB202190 partially inhibits caspase-3 activity, we examined the effect of the inhibitor on cell survival. Exposure of HSC536/N cells to IFN-γ and anti-Fas Ab for 4 hr resulted in $67 \pm 2.4\%$ viable cells, as determined by trypan blue exclusion. Pre-incubation with 25 μM SB202190 for 30 min, before exposure to IFN-γ and anti-Fas Ab, resulted in $77 \pm 2.3\%$ viable cells, suggesting that p38 activation contributes to cell death signaling in HSC536/N cells. Similar results were obtained upon pre-incubation of the cells with PD169316 (another inhibitor of p38) (data not shown) while pre-incubation with the JNK inhibitors SP600125 or DJNK1 did not affect caspase-3 activity (data not shown). Exposure of HSC99 cells to IFN-γ and anti-Fas Ab did not induce caspase-3 activation.

3.3. Effect of IFN-γ and H_2O_2 on the accumulation of ROS in FA-C and FA-A lymphoblast cell lines

As our data established that both JNK and p38 are activated by IFN-γ in FA-C lymphoblast cell lines but not in FA-A cells investigated by us, we determined

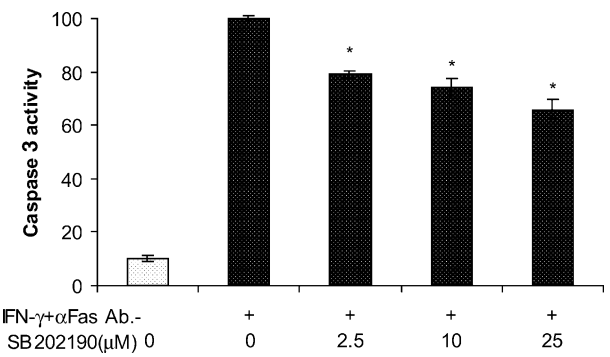

Fig. 3. Inhibition of IFN-γ- and anti-Fas Ab-induced caspase-3 activation by SB202190. HSC536/N cells were pre-incubated for 30 min with the indicated concentrations of SB202190 prior to exposure to IFN-γ and anti-Fas Ab for 120 min. Caspase-3 activity was measured using the fluorimetric assay as described in Section 2. Results expressed as mean ± SE of four experiments. $^{*}P < 0.05$.

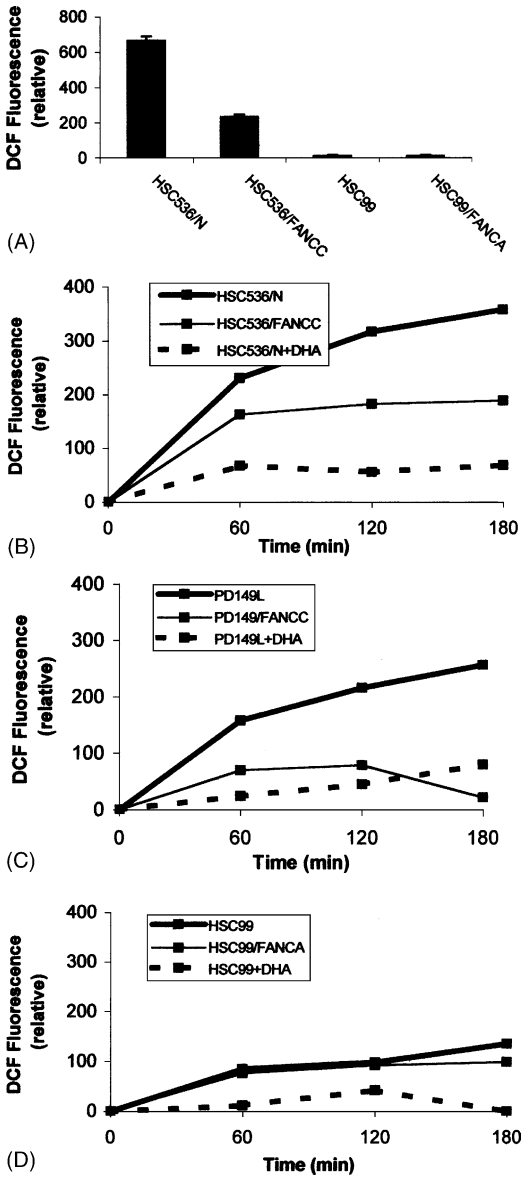

Fig. 4. Time course changes in DCF fluorescence induced by IFN-γ and H_2O_2 in FA cell lines. (A) Effect of IFN-γ on HSC536/N, HSC536/FANCC, HSC99, and HSC99/FANCA cells. The indicated FA cell lines were incubated for 15 min with DCFH-DA, washed, cultured in wells with 1 ng/mL IFN-γ for 180 min, and ROS generation was determined as described in Section 2. (B–D) Effect of H_2O_2 on HSC536/N and PD149L cells (B and C, respectively) and HSC99 cells (D). The indicated cells were pre-incubated in the absence or presence of DHA, washed and incubated for 15 min with DCFH-DA, and cultured in wells with 25 μM H_2O_2. Time course changes in DCF fluorescence were measured during 180 min. Graphic data are representative of four experiments.

intracellular ROS levels following exposure of the cells to 1 ng/mL IFN-γ. Figure 4A indicates that concomitant with our previous studies [18], treatment with 1 ng/mL IFN-γ resulted in higher ROS accumulation in HSC536/N than in HSC536/FANCC cells while no oxidation of DCFH-DA was observed in HSC99 cells nor in their corrected counterparts.

In a previous study, Zunino *et al*. investigated DNA oxidative damage in FA cells treated with H_2O_2 and found that H_2O_2-induced 8-hydroxy-2′-deoxyguanosine was higher in FA than in normal cell lines [34]. We compared accumulation of ROS in FA-C and FA-A cell lines and in their genetically corrected counterparts following their treatment with H_2O_2. Exposure of HSC536/N (Fig. 4B) and PD149L cells (Fig. 4C) to 25 μM H_2O_2 induced an immediate and marked augmentation in oxidation of DCFH-DA, which increased with time. A moderate augmentation in oxidation of DCFH-DA was induced in the genetically corrected counterparts. Contrary to these results, exposure of HSC99 and HSC99/FANCA cells to 25 μM H_2O_2 induced low augmentation in the oxidation of DCFH-DA (Fig. 4D). Figure 4B–D also shows reduced ROS accumulation in the three investigated FA cell lines that were pre-incubated with DHA and exposed to H_2O_2.

3.4. Activation of JNK and p38 by H_2O_2 in FA-C and FA-A cell lines

Having observed higher ROS levels in the two FA-C cell lines than in the FA-A cell line, following exposure to H_2O_2, we analyzed the effect of hydrogen peroxide on JNK and p38 activation in HSC536/N and HSC99 cells and in their genetically corrected counterparts. Figure 5A and B indicates that exposure of HSC536/N cells to 25 μM H_2O_2 resulted in JNK and p38 activation within 15 and 5 min, respectively, no activation of JNK nor of p38 was observed in the corrected counterparts (Fig. 5B) nor in HSC536/N cells pretreated with DHA and exposed to 25 μM H_2O_2 (Fig. 5C). No activation of JNK and p38 could be detected following exposure of HSC99 cells to 25 μM H_2O_2 (data not shown). Only exposure to higher concentrations of H_2O_2 (50 μM) resulted in the activation of the two kinases. Figure 5D and E indicates an immediate JNK and p38 activation within 5 min, while no activation of JNK and a slight activation of p38 were observed in the corrected counterparts (Fig. 5E). Pretreatment with DHA decreased MAPK activation (Fig. 5F).

4. Discussion

It is well established that increased amounts of ROS and high oxygen sensitivity are characteristic for FA cells. In a recent review, Pagano and Youssoufian hypothesize that FA proteins act directly (*via* FANCC and FANCG) and indirectly (*via* FANCA, BCRA2, and FANCD2) with the machinery of cellular defense to modulate oxidative stress [35]. In a previous study, we have shown that IFN-γ induces the accumulation of ROS in two FA-C B-lymphocyte cell lines and that pretreatment with DHA reduces the intracellular level of ROS and inhibits apoptosis [18]. In the present study, we have compared the sensitivity of EBV-immortalized FA lymphoblast cells of A and C complementation groups to IFN-γ and H_2O_2 and investigated the cellular signaling induced by these two substances.

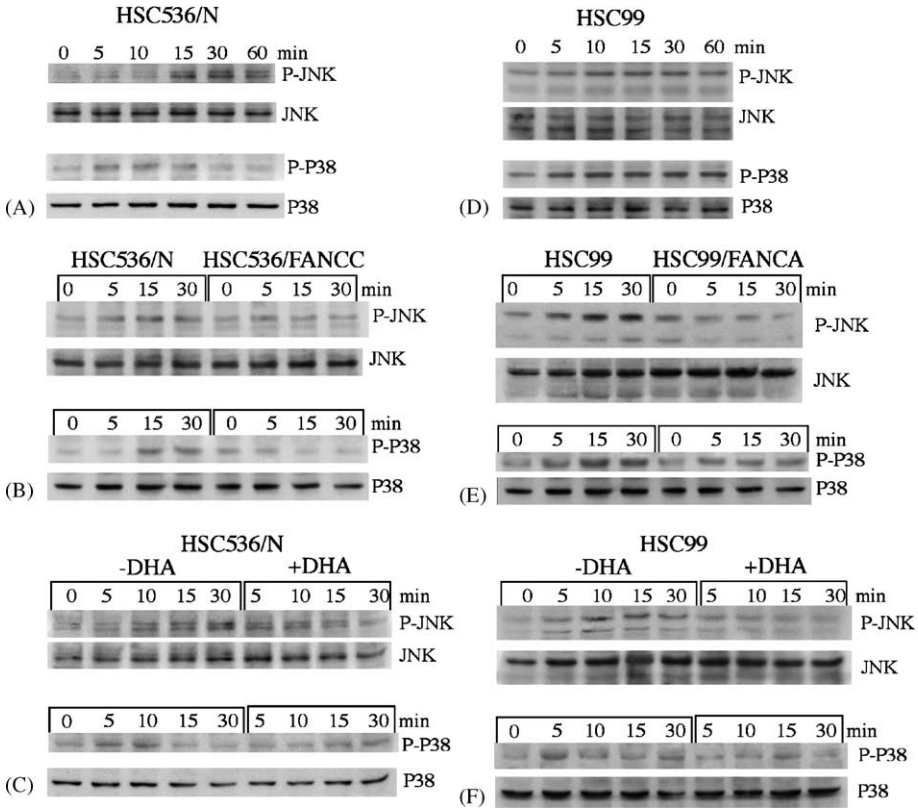

Fig. 5. Effect of H_2O_2 on JNK and p38 MAPK activation in HSC536/N, HSC99 cells, and their genetically corrected counterparts. HSC536/N (A and B) and HSC536/FANCC cells (B) were exposed to 25 μM H_2O_2 for the indicated times. Phosphorylation levels were measured as indicated in the legends to Fig. 1. (C) Effect of DHA. HSC536/N cells were pre-incubated in the absence or presence of DHA before exposure to 25 μM H_2O_2 for the indicated times. Phosphorylation levels of the MAPKs were determined as above. HSC99 (D and E) and HSC99/FANCA cells (E) were exposed to 50 μM H_2O_2 for the indicated times. (F) Effect of DHA. The cells were pre-incubated with DHA before exposure to 50 μM H_2O_2. Phosphorylation of JNK and p38 induced by H_2O_2 were measured as described above. The representative blots of three experiments are shown.

In accordance with our previously reported results, we observed that IFN-γ (1 ng/mL) induces the accumulation of ROS in HSC536/N cells, while in the present study no accumulation of ROS could be detected in HSC99 cells exposed to IFN-γ (Fig. 4A). Our study also shows that HSC536/N and PD149L cells are more sensitive and accumulate higher concentrations of ROS than HSC99 cells, upon their exposure to low concentrations of H_2O_2 (Fig. 4B and C vs. D). Thus, our findings suggest that the FANCC protein prevents the accumulation of high concentrations of ROS in the cells either by inducing enhanced detoxification or preventing over production, whereas the FANCA protein does not.

Differences have been reported in the various FA complementation groups regarding several parameters. Thus, Lackinger *et al*. [36], have compared the repair activity of oxidatively damaged DNA in lymphoblastoid cell lines of complementation groups A–E and found that the repair activity for oxidatively damaged DNA was significantly reduced in lymphoblastoid cell lines of complementation groups B–E but not in the FA-A cell line. Additional studies have shown differences in the sensitivity and response of FA-A cells to various stimuli compared to other FA complementation groups. Abnormalities of STAT1 signaling have been reported in FANCC mutant cells [37] but not in FANCA or other FA complementation groups. In contrary, some authors reported that FA-A cells undergo apoptosis more than control or FA-C or FA-E cells [34] and others have shown that fibroblasts from the complementation group A are more susceptible than fibroblasts from the complementation group D to mitochondria modifications under UVA radiation or psoralen photoactivation [38]. These findings suggest that although all FA proteins function in the FA pathway, there may be individual differences in functioning between the specific proteins.

It is well established that the stress-activated kinase cascades, which include the JNK and the p38 pathways, are activated in response to different apoptotic stimuli, including ROS. We show in the present study that JNK and p38 become activated in cells that accumulate excess intracellular concentrations of ROS, such as HSC536/N and PD149L cells exposed to IFN-γ or H_2O_2 (25 μM) or HSC99 cells exposed to higher concentrations of H_2O_2 (50 μM). The fact that pre-incubation of the cells with DHA prevented IFN-γ- or H_2O_2-induced JNK and p38 activation, and the finding that DHA reduces intracellular ROS levels, suggests that the induced phosphorylation of

the stress kinases is a result of accumulation of ROS in the cells. The observation that DHA loading abrogates increased ROS levels induced by various stimuli was described by us and by other investigators in additional cell lines and in monocytes [39–41]. Our findings are in line with Liu *et al*. who have shown that antioxidants, such as *N*-acetyl-L-cysteine, can block MAPK activation induced by oxidative stress [42].

Most importantly, our data indicate that specific inhibitors of the p38 MAPK (but not inhibitors of JNK) partially inhibit caspase-3 activation in HSC536/N cells.

Because SB202190 only partially blocked and SP600125 did not protect against caspase-3 activation, it is likely that additional pathways (like extracellular signal-regulated kinases (ERK1/2)) are operative in this model of oxidative stress induced by IFN-γ and anti-Fas Ab. Other investigators have shown that p38 is implicated in the induction of apoptosis under certain conditions, but the generation of apoptotic effects appears to be very specific to cell type and context. Thus, Xia *et al*. have shown that withdrawal of the nerve growth factor from rat PC-12 cells led to activation of JNK and p38 and induction of apoptosis in the cells [43]. Nagata and Todokoro have indicated that long exposure of SKT6 cells to osmotic or heat shock induced activation of JNK and p38 MAPK and caused apoptosis [44]. Our results are in agreement with Verma *et al*. who have shown that IFN-α and IFN-β induce the phosphorylation of p38 MAPK in CD34+ primitive human hematopoietic progenitors and that activation of this signaling cascade by type I IFN receptors is responsible for the suppressive effects of type I IFNs on normal hematopoiesis [45]. Similarly, Mayer *et al*. have shown that the function of p38 is essential for the suppressive effect of IFN-α on hematopoietic progenitors from patients with chronic myelogenous leukemia [46]. To the best of our knowledge, we provide the first evidence that IFN-γ activates the JNK and p38 MAPK pathways in FA lymphoid cell lines of the C complementation group. We propose that p38 MAPK functions as a signaling mediator of apoptosis in HSC536/N cells primed with IFN-γ.

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