Oxidant Hypersensitivity of Fanconi Anemia Type C-deficient Cells Is Dependent on a Redox-regulated Apoptotic Pathway*

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Fanconi anemia is a genetic disorder characterized by bone marrow failure. Significant evidence supports enhanced apoptosis of hematopoietic stem/progenitor cells as a critical factor in the pathogenesis of bone marrow failure in Fanconi anemia. However, the molecular mechanism(s) responsible for the apoptotic phenotype are incompletely understood. Here, we tested whether alterations in the activation of a redox-dependent pathway may participate in the pro-apoptotic phenotype of primary Fancc−/− cells in response to oxidative stress. Our data indicate that Fancc−/− cells are highly sensitive to oxidant stimuli and undergo enhanced oxidant-mediated apoptosis compared with wild type controls. In addition, antioxidants preferentially enhanced the survival of Fancc−/− cells. Because oxidative stress activates the redox-dependent ASK1 pathway, we assessed whether Fancc−/− cells exhibited increased oxidant-induced ASK1 activation. Our results revealed ASK1 hyperactivation in H2O2-treated Fancc−/− cells. Furthermore, using small interfering RNAs to decrease ASK1 expression and a dominant negative ASK1 mutant to inhibit ASK1 kinase activity, we determined that H2O2-induced apoptosis was ASK1-dependent. Collectively, these data argue that the predisposition of Fancc−/− hematopoietic stem/progenitor cells to apoptosis is mediated in part through altered redox regulation and ASK1 hyperactivation.

Fanconi anemia (FA) is a heterogeneous bone marrow (BM) failure syndrome with cellular abnormalities that include chromosomal instability, increased apoptosis, and cell cycle control defects (1–5). The diversity of clinical presentation in children with FA is related, in part, to the existence of multiple complementation types, with eight FA complementation group cDNAs being identified thus far (FANCA, FANCC, FANCD1/BRCA2, FANCD2, FANCE, FANCF, FANCG, and FANCL) (6–14). Despite some clinical variability between individuals with specific FA gene mutations (15, 16), the major cause of mortality in all FA complementation types is BM failure (1, 2, 5). These studies suggest that apoptotic loss of hematopoietic stem/progenitor cells has a key pathogenic role in this disorder. Thus, understanding molecular mechanisms involved in the predisposition of FA cells to apoptosis is of critical importance to improve current treatment approaches for children with FA.

Numerous studies show that FANCA, FANCC, FANCE, FANCF, and FANCG interact in a multimeric nuclear protein complex (17–23), the formation of which is required for FANCJ to monoubiquitinate FANCD2 (14), a post-translational modification that signals relocalization of FANCD2 into nuclear foci containing BRC1A1 (24). However, the majority of Fancc resides in the cytoplasm (25, 26), and cytoplasmic localization is required for protection against genotoxin-induced apoptosis (27). Interestingly, cytoplasmic FANCC interacts with heat shock protein 70 (HSP70) to protect cells from interferon-γ TNF-α-induced apoptosis, yet FANCC-HSP70 interaction is dispensable for protection against genotoxin stress (28–30). Together these data support an additional cytoplasmic role for Fancc in suppressing apoptosis.

Participation of FANCC in redox metabolism has been proposed previously and is supported by functional interactions with cytochrome P-450 reductase (CPR) (31) and glutathione S-transferase P1 (GSTP1) (32). Evidence of oxygen sensitivity was first provided by Joenje et al. (34), who demonstrated that the chromosomal instability of primary FA cells could be reduced if grown at lowered oxygen tension (33). Several conflicting studies have been reported since this original description, although most analyses were conducted in immortalized cell lines or in cells with unidentified FA complementation type and/or mutation (34–36). Now with the availability of murine models, the issue of whether FA cells have altered redox regulation is being readdressed in primary cells. Using mice deficient in the murine Fancc homologue (Fancc), Hadjur et al. (37) showed that mice mutant at both the Fanca and superoxide dismutase 1 (SOD1) loci exhibit severe defects in hematopoiesis, including histological evidence of BM hypoplasia, an observation not detected in singly mutant mice. Although these data provide strong genetic evidence that Fancc−/− cells are hypersensitive to endogenously generated oxidants, it is unknown whether the molecular mechanism responsible for this hypersensitive response is due to altered redox signaling.

Redox signaling has a critical role in controlling multiple complex cellular processes including apoptosis, proliferation, senescence, and differentiation (38–44). This highly conserved
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regulatory process involves maintenance of the intracellular environment in an overall reduced state. Cellular oxidative stress results in the oxidation of key cysteine residues on redox-sensitive proteins, a post-translational modification that affects intracellular signaling pathways in a fashion similar to phosphorylation. A notable example of redox apoptotic signaling involves the serine-threonine kinase apoptosis signal-regulating kinase 1 (ASK1). In the normal reducing environment of a cell, ASK1 activity is inhibited via binding to thioredoxin, glutaredoxin, and glutathione S-transferases (45–51). After direct or indirect oxidative stress (i.e., H2O2, TNF-α, glucose/serum deprivation, and heat shock), these proteins are oxidized forming intramolecular disulfide bonds, which result in a conformational change and dissociation from ASK1. Unbound ASK1 is then available to autophosphorylate and subsequently phosphorylate downstream kinases, initiating an apoptotic program.

To extend our understanding of oxidative hypersensitivity in FA, we investigated whether primary Fance−/− cells exhibit alterations in the redox-dependent ASK1 apoptotic pathway. Our data demonstrate that Fance−/− cells exhibit ASK1 hyperactivation after H2O2 treatment. In addition, we show that enhanced H2O2-induced apoptosis in Fance−/− cells is ASK1-dependent and that pretreatment with antioxidants preferentially protects Fance−/− cells from apoptosis induced by H2O2 as compared with controls. Collectively, these data argue that the predisposition of primary Fance−/− cells to oxidant-induced apoptosis is mediated through hyperactivation of a redox-dependent apoptotic signaling pathway.

EXPERIMENTAL PROCEDURES

Mice—Fance+/+ mice in a C57/B16J genetic background were bred to generate Fance−/− and wild-type (WT) mice for hematopoietic progenitor assays and timed embryos for murine embryo fibroblast (MEF) cell lines as previously described (52, 53). All of the studies were approved by the Indiana University Laboratory Animal Research Center.

Hematopoietic Progenitor Assays—WT and Fance−/− BM low density mononuclear and ckit+ lin− cells were prepared as previously described (54, 55). Cells from WT and Fance−/− mice were resuspended in Iscove’s modified Dulbecco’s medium (Invitrogen) supplemented with 20% fetal calf serum (Biowhittaker, Walkersville, MD) and then exposed to H2O2 (Sigma) for 1 h. After oxidative treatment, the cells were washed and plated in clonogenic assays as described previously (55). For hypoxia exposure, low density mononuclear cells were placed in an airtight chamber before incubating with a gas mixture containing 50% O2, 5% CO2, and 45% N2 (Praxair, Indianapolis, IN). The chamber was then incubated for 4 or 16 h at 37 °C before the cells were harvested for clonogenic assays. An O2 analyzer was used to measure the O2 concentration before and after each incubation period (50 ± 3%) to ensure an airtight culture system. Control cultures were incubated at 21% O2 for 4 or 16 h.

MEF Survival Assays—MEFs were maintained as previously described (53). All of the studies were conducted in at least two or three different MEF cell lines/genotype, and only MEFs that were less than 50% confluent in the day of cloning were used for all experiments. MEFs were seeded in 24-well plates at 1 × 104 for 24 h before assessing viability by trypan blue exclusion. In some experiments, MEFs were pretreated with 20 μM selenomethionine (SeMet; Sigma) overnight or 4 mM N-acetylcysteine (NAC; Sigma) for 1 h prior to culturing with H2O2. To evaluate apoptosis, MEFs were treated with 100 μM H2O2 for 4–6 h and analyzed by terminal deoxynucleotidyltransferase-mediated nick-end labeling (TUNEL) assay as previously described (55, 56).

Retroviral Constructs and Transduction—PG13 retroviral packaging cells containing the FANCC mutants (FANCC-E251A and FANCC-del322G) in the pLXSN backbone were generously provided by Dr. Grover C. Bagby, Jr. (Oregon Health Sciences, Portland, OR) (29). Retroviral supernatants were harvested and utilized to transduce GP+E86 retroviral packaging cells as previously described (52) to pseudotype viral particles with an ecotropic envelope. The MFG-FAC retrovirus encoding the FANCC cDNA was used as a control, which previously was shown to correct mitomycin C sensitivity of Fance−/− cells to WT levels (52). The dominant negative ASK1 cDNA (ASK1−

K709M) (57) was generously provided by Dr. Hidenori Ichijo (University of Tokyo, Tokyo, Japan) in a pCMV plasmid. The ASK1-K709M DNA was subcloned into the NotI site of the bicistronic retroviral plasmid MIEG3 (58), which is 5′ to an internal bovine growth hormone gene enhancer. The packaging cell line was developed for MIEG3 and MIEG3-ASK1-K709M as previously described (52). Retroviral supernatants were collected, filtered, and stored at −80 °C until utilized for transduction of MEFs. Early passage MEFs (P0–P1) were transduced with retroviral supernatants four times over 2 consecutive days in the presence or absence of polybrene as previously described (52, 53).

ASK1 in Vitro Kinase Assay—ASK1 kinase activity was determined by depriving MEFs of serum for 4 h followed by treatment with 100 μM H2O2 for 5 min. MEFs were then washed twice with cold phosphate-buffered saline containing 1 mM sodium orthovanadate and lysed in nonionic lysis buffer. The protein concentrations were determined using the BCA assay (Pierce). The ASK1 immunoprecipitations were conducted using protein A Sepharose beads (Amersham Biosciences) and anti-ASK1 antibody (Cell Signaling, Beverly, MA). Immunobeads were subjected to an in vitro kinase reaction using either myelin basic protein (Sigma) or MKK4 (Upstate Biotechnologies, Inc.) as substrates for ASK1. The kinase mixtures contained 20 mM MgCl2, 0.1 mM sodium orthovanadate, 1 mM diithiothreitol, 30 mM β-glycerophosphate, 5 mM EGTA, 20 mM MOPS, 1 mM ATP, and 10 μg of substrate/sample before adding 2.5 μCi of [γ-32P]ATP/sample. The kinase reaction buffer was added to each sample and incubated at 30 °C for 30 min. Reactions were terminated by the addition of a sample buffer. The protein samples were separated on a 15% SDS-PAGE gel (Invitrogen), transferred to a nitrocellulose membrane, and subjected to autoradiography.

Western Blotting—Equivalent amounts of protein (200–500 μg) were separated on a 12% SDS-PAGE gel and transferred onto a nitrocellulose membrane. For immunodetection of FANCC mutants, a primary rabbit anti-FANCC antibody, previously generated by our laboratory (52), and a secondary anti-rabbit horseradish peroxidase antibody (Amersham Biosciences) were used as described (52) before visualizing with chemiluminescence (Amersham Biosciences). To document equal protein loading, the membrane was stripped and reprobed with β-actin (Sigma). For immunodetection of ASK1, rabbit anti-ASK1 antibody (Cell Signaling) was used at 1:200 dilution before incubating with the secondary antibody anti-rabbit horseradish peroxidase (1:2000 dilution).

Small Interfering RNA Transfection Protocol—Table I lists the RNA sequences used for these studies. The ASK1 siRNA sequence targeted nucleotides 570–590 of the ASK1 mRNA and was designed according to the manufacturer’s recommendations (Dharmacon, Lafayette, CO). Either sense or scrambled oligonucleotides were used as a control for every transfection experiment. WT and Fance−/− MEFs were cultured in a 6-well tissue culture dish to 30–50% confluency. The RNA oligonucleotides were diluted in Opti-MEM (Invitrogen) to obtain a 250 nM solution per Dharmacon’s recommendations. Oligofectamine transfections were conducted exactly per the manufacturer’s recommendations (Invitrogen). Following the transfection, 500 μl of Dulbecco’s modified Eagle’s medium (Invitrogen) containing 30% fetal calf serum was added without removing the transfection mixture. The cells were incubated for 72 h at 37 °C before harvesting for H2O2 cytotoxicity assays and ASK1 Western blotting. Four independent transfections were conducted with similar results.

Statistical Analyses—For all data shown, a Student’s t test was conducted to evaluate for differences between treatment groups, and a p value ≤ 0.05 was considered significant.

RESULTS

Fance−/− Hematopoietic Progenitors and MEFs Are Hypersensitive to Oxidants—Because FA patients have severe defects in hematopoietic stem/progenitor cell function, we initially tested whether Fance−/− progenitors were hypersensitive to oxidative stress. To accomplish this aim, WT and Fance−/− BM low density mononuclear cells were cultured either with H2O2 or in hyperoxic conditions (50% O2) before plating in clonogenic

| Table I siRNA sequences | RNA type | Sequence |
|-------------------------|----------|----------|
| ASK1 target             | 5′-CCG CCG UCG UCC GUC GUU CAG TTT G | |
| Sense                   | 5′-AAC CGG UGC ACG ACG CAC GAC G | |
| Scrambled               | 5′-GCG CCG UUC GCA GUA UCU CAG G | |

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progenitor assays. For studies utilizing H₂O₂, Fance −/− progenitors were significantly more sensitive to multiple H₂O₂ doses as compared with controls (Fig. 1A). In addition, Fance −/− progenitors exposed to 50% O₂ for 4 or 16 h exhibited a marked reduction in colony formation as compared with WT control cultures (Fig. 1B). BM low density mononuclear cells are a heterogeneous cell population that includes a significant proportion of differentiated cells compared with the relatively low frequency of clonogenic progenitor cells (0.01–0.5%). Given our previous observations that Fance −/− progenitors are exquisitely sensitive to multiple inhibitory cytokines such as interferon-γ and TNF-α (55), together with the knowledge that inflammatory cells such as lymphocytes and granulocytes are major sources of secreted inhibitory cytokines, it was crucial to eliminate these differentiated cells from our culture system. To test whether the observed oxidant hypersensitivity was due to an intrinsic abnormality in Fance −/− progenitor cells and not secondary to accessory cells present in BM low density mononuclear cell populations, WT and Fance −/− ckit+lin− cells were purified by fluorescence cytometry, treated with 100 μM H₂O₂, and plated in colony assays. This phenotypically defined cell population enriches for immature hematopoietic stem and progenitor cells and excludes differentiated progeny cells. Similar to prior studies with low density mononuclear cells, Fance −/− ckit+lin− cells were hypersensitive to H₂O₂ (Fig. 1C), supporting an intrinsic hematopoietic progenitor cell defect.

Because of the difficulty in obtaining sufficient numbers of primary hematopoietic progenitor cells, we established WT and Fance −/− MEFs to utilize as a cellular model system for evaluation of alterations in oxidant responsiveness in Fance −/− cells. Initial studies determined that Fance −/− MEFs exhibited an enhanced sensitivity to H₂O₂ (Fig. 2A), comparable with that observed in Fance −/− progenitors. TUNEL assays confirmed that the H₂O₂ hypersensitivity in Fance −/− MEFs was due to enhanced apoptosis (Fig. 2B). In addition, we observed that Fance −/− MEFs exhibit a slight increase in apoptosis when grown in basal conditions, similar to previous data in cultured Fance −/− hematopoietic cells (52). To test whether antioxidants protect Fance −/− MEFs from oxidant exposure, MEFs were pretreated with either SeMet or NAC before culturing with H₂O₂. Consistent with an altered redox state, Fance −/− MEFs pretreated with SeMet or NAC were protected from H₂O₂ treatment compared with control Fance −/− MEFs (Fig. 2C). Importantly, survival of Fance −/− MEFs was restored to WT levels after SeMet and NAC pretreatment. Furthermore, SeMet pretreatment of Fance −/− MEFs reduced apoptosis in basal and H₂O₂-treated conditions (Fig. 2D).
least two independent functions, separable by FANCC mutant cDNAs (29). One recognized function is to maintain normal alkylating agent sensitivity, which is associated with restoration of nuclear FA protein complex formation (corrected by FANCC-E251A mutant). The second identified function is to sustain normal PKR-mediated apoptotic signaling via an interaction with HSP70 (corrected by FANCC-del322G mutant, an FA patient-derived mutation resulting in deletion of amino acids 1–54). To determine whether oxidant hypersensitivity in Fancc−/− MEFs segregates with a known FANCC function, we transduced Fancc−/− MEFs with a retrovirus encoding either FANCC, FANCC-E251A, or FANCC-del322G. Mock-infected Fancc−/− MEFs were utilized as a control. FANCC expression in transduced MEFs was confirmed by Western blotting (Fig. 3A) before evaluating H$_2$O$_2$ sensitivity (Fig. 3B). The H$_2$O$_2$ dose-response curves for MEFs transduced with a retrovirus containing either FANCC or FANCC-E251A were indistinguishable, suggesting that the role of FANCC in regulating HSP70/PKR signaling is not required for protection against H$_2$O$_2$. In marked contrast, MEFs transduced with the FANCC-del322G mutant exhibited no protection against H$_2$O$_2$ as compared with the mock-infected control. These data show that the N-terminal domain of FANCC is required for protection against H$_2$O$_2$ in Fancc−/− cells.

**Enhanced H$_2$O$_2$-induced Apoptosis in Fancc−/− MEFs Is ASK1-dependent—**Because H$_2$O$_2$-induced apoptosis in MEFs is ASK1-dependent (59) and Fancc−/− MEFs exhibit enhanced H$_2$O$_2$-induced apoptosis (Fig. 2A), we reasoned that Fancc−/− MEFs may demonstrate increased ASK1 activity after H$_2$O$_2$ treatment. To test this hypothesis, we evaluated H$_2$O$_2$-induced ASK1 activation in WT and Fancc−/− MEFs. In vitro kinase assays revealed that Fancc−/− MEFs treated with H$_2$O$_2$ exhibited a significant increase in ASK1 activity compared with WT controls (Fig. 4, a experiment representative of five experiments with mean fold induction 2.8 ± 0.5 versus 1.5 ± 0.3 p < 0.05). To determine whether the predisposition of Fancc−/− MEFs to H$_2$O$_2$-induced apoptosis was dependent on ASK1 function, we utilized siRNA oligonucleotides to reduce ASK1 expression. Western blotting confirmed that WT and Fancc−/− MEFs transfected with the ASK1 siRNA oligomer
exhibited a significant decrease in ASK1 expression (Fig. 5A, a representative experiment). Interestingly, Fancc/H11002/H11002 MEFs transfected with the ASK1 siRNA oligomer were completely protected against H2O2 treatment compared with untreated controls (Fig. 5B), whereas WT MEFs transfected with the ASK1 siRNA oligomer did not exhibit a significant increase in survival.

To further test whether H2O2-induced apoptosis in Fancc/H11002/H11002 MEFs was dependent on ASK1 kinase activity, we constructed a bicistronic retroviral vector that encodes a catalytically inactive, dominant negative ASK1 cDNA and EGFP (MIEG3-ASK1-K709M), allowing for selection of transduced cells (EGFP+). WT and Fancc/H11002/H11002 MEFs were transduced with either MIEG3-ASK1-K709M or vector control and then evaluated for H2O2-induced apoptosis. Fancc/H11002/H11002 cells exhibited significantly less apoptosis after H2O2 treatment compared with Fancc/H11002/H11002 MEFs transfected with vector alone (Fig. 5C). Consistent with previously published data for ASK1/H11002/H11002 MEFs, inhibiting ASK1 kinase activity in WT MEFs also resulted in reduced apoptosis after H2O2 treatment, although the inhibition was more dramatic in Fancc/H11002/H11002 MEFs. Collectively, these data show that the predisposition of Fancc/H11002/H11002 MEFs to H2O2-mediated apoptosis is ASK1-dependent and identify ASK1 as a critical mediator of oxidant-induced apoptosis in Fancc/H11002/H11002 cells.

**DISCUSSION**

The first demonstration of oxygen hypersensitivity in FA cells was made over 20 years ago (33); however, since that
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Fig. 5. H$_2$O$_2$-induced apoptosis is ASK1 dependent in Fancc $-/-$ MEFs. ASK1 siRNA studies. WT and Fancc $-/-$ MEFs were transfected with either ASK1 siRNA oligonucleotides or control oligonucleotides. MEFs were incubated for 72 h at 37 °C before harvesting for ASK1 Western blot (A) and H$_2$O$_2$ cytotoxicity assays (B). The data shown are representative of four independent transfection experiments with similar results. *, $p < 0.05$; **, $p < 0.01$. C, dominant negative ASK1 studies. WT and Fancc $-/-$ MEFs were treated with a retrovirus encoding a catalytically inactive, dominant negative ASK1 (ASK1-K709M) or vector control (control). Transduced MEFs were treated with 100 μM H$_2$O$_2$ for 4 h before evaluating apoptosis by the TUNEL assay (phycoerythrin +). The percentage of apoptotic cells was calculated by dividing EGFP + PE + cells by total EGFP + cells for each condition. At least 100 cells were scored per condition per experiment. The data shown represent the means of three experiments. *, $p < 0.05$.

initial report little progress has been made to understand the molecular mechanisms involved. In fact, contradictory data have led to significant debate (60, 61). Our data in primary Fancc $-/-$ hematopoietic progenitors and MEFs clearly demonstrate hypersensitivity to oxidative agents. Furthermore, these studies are the first to establish an intrinsic defect in the oxidant responsiveness of Fancc $-/-$ progenitors, independent of differentiated hematopoietic cell populations and the BM microenvironment. Our data compliment previous studies showing that Fancc $-/-$ mice devoid of superoxide dismutase 1 expression develop a hypoplastic BM (37) linking increased endogenous oxidant stress with marrow failure in Fancc $-/-$ mice. Together these observations suggest that when Fancc $-/-$ hematopoietic cells encounter an increase in either endogenous or exogenous oxidant stress, enhanced apoptotic cell loss may occur, contributing to the pathogenesis of BM failure in FA.

Numerous reports in immortalized cell lines suggest that the loss of FA protein function may result in a pro-oxidant cellular environment (31–33, 60, 62–71). Furthermore, protein-protein interaction studies provide support for the concept that FANCC may directly participate in redox metabolism by interacting with two cytoplasmic binding proteins, GSTP1 (32) and CPR (31). These studies demonstrated that FANCC regulates the activity of both GSTP1 and CPR, with the loss of FANCC predicting increased GSTP1 oxidation (decreased activity) and increased CPR activity. In a reduced conformation GSTP1 inhibits stress-activated apoptotic signaling (50, 72, 73), suggesting that the loss of FANCC may result in altered redox-dependent stress signaling. In addition, CPR transfers electrons to cytochromes and molecular oxygen (74–77); hence increased CPR activity in Fancc deficient cells may subsequently result in a pro-oxidant cellular environment by increasing reactive oxygen species. We reasoned that if an altered redox environment was responsible for the oxidant hypersensitivity in Fancc $-/-$ MEFs, pretreatment with antioxidants that provide additional cellular reducing equivalents would preferentially protect Fancc $-/-$ cells compared with WT controls. Indeed, our data showed that SeMet or NAC pretreatment not only preferentially protected Fancc $-/-$ MEFs from H$_2$O$_2$-induced apoptosis but improved viability to WT levels, supporting a potential role for FANCC in redox metabolism.

Interestingly, we and others have previously shown that Fancc $-/-$ cells are exquisitely hypersensitive to TNF-α (55, 78), a potent activator of ASK1 (46, 59, 79). Pang et al. (28, 29) demonstrated that binding of FANCC to HSP70 acts as a negative regulator for PKR-mediated apoptotic signaling induced by costimulation with interferon-γ and TNF-α. To evaluate whether H$_2$O$_2$-induced apoptosis was dependent on HSP70 binding to FANCC, we transduced Fancc $-/-$ MEFs with a retrovirus containing a FANCC construct mutated at the HSP70-binding site (FANCC-E251A). These studies demonstrated that the FANCC-E251A mutant completely corrected the sensitivity of Fancc $-/-$ MEFs to H$_2$O$_2$, suggesting a HSP70/PKR independent mechanism. In contrast, the FANCC-del322G mutant did not protect against H$_2$O$_2$-induced apoptosis in Fancc $-/-$ MEFs. This is a particularly intriguing observation because CPR binding to FANCC is predicted to be disrupted by this mutation (31).

Given our data showing that Fancc $-/-$ cells undergo increased H$_2$O$_2$-mediated apoptosis, we investigated whether increased activation of a redox-dependent apoptotic signaling pathway was involved. Our data demonstrate that Fancc $-/-$ MEFs treated with H$_2$O$_2$ exhibit ASK1 hyperactivation. Furthermore, studies utilizing siRNA and dominant negative methodologies to decrease ASK1 activity reveal that H$_2$O$_2$-induced apoptosis in Fancc $-/-$ MEFs is ASK1-dependent. Ichijo et al. (57) identified ASK1 as an important kinase involved in oxidant- and TNF-α-induced apoptosis. Subsequent studies revealed that thioredoxin, in a reduced conformation, inhibits ASK1-dependent apoptosis by binding to ASK1 (46), which is the first demonstration that an apoptotic cascade is
directly controlled by the cellular redox environment. Since this original description, it is now recognized that the redox regulation of ASK1 is complex, involving the direct interaction of ASK1 with multiple redox-dependent binding partners including thioredoxin, glutathione S-transferases, and glutathione (45–51). The precise physiologic role that individual negative regulators have in inhibiting ASK1 activity remains unclear. However, there is evidence that these redox-dependent proteins may act as sensors for specific cellular redox stresses (46, 49, 51). Collectively, these studies suggest that ASK1 functions as a major modulator of apoptotic signaling induced by multiple types of oxidant stress including H2O2, TNF-α, glucose/serum deprivation, and heat shock (46, 48, 51). In addition, because ASK1 activity is tightly regulated by redox-dependent mechanisms (45–48), these observations support the idea that Fanc –/– cells may exhibit an altered redox environment, which predisposes to ASK1-mediated apoptosis.

An intriguing possible explanation for the enhanced propensity of Fanc –/– MEFs to ASK1-mediated apoptosis may be due to disruption of GSTP1 redox control. Previous studies showed that FANCC expression maintains GSTP1 in a reduced conformation during growth factor withdrawal and subsequently protects from apoptotic cell death (32). Importantly, reduced glutathione S-transferases are critical for inhibition of ASK1 activation and consequently oxidant-induced apoptosis (45, 50, 51). Interestingly, Gilot et al. (50) reported that GSTP1 overexpression in primary hematopoietic progenitors from ASK1-dependent mice will be important to understand the role that FANCC has in preserving survival after oxidant stress. Regardless, our data in primary Fanc –/– cells identify ASK1 as a potentially important molecular target to defend against an apoptotic fate induced by oxidant stress.

In summary, our data indicate that Fanc –/– progenitors exhibit an intrinsic defect in oxidant responsiveness. In addition, we show that the hypersensitivity of Fanc –/– cells to oxidative stress is ASK1-dependent. Furthermore, our data showing that antioxidants protect Fanc –/– cells from enhanced oxidant-induced apoptosis suggest a potential translational role for antioxidants in the prevention and/or delay of BM failure in FA. Future preclinical studies to test the potential of such an approach will be important.

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