Biometals (2009) 22:855–862
DOI 10.1007/s10534-009-9214-7

Modulation of intracellular iron levels by oxidative stress implicates a novel role for iron in signal transduction

Suman Deb · Erin E. Johnson · Raquel L. Robalinho-Teixeira · Marianne Wessling-Resnick

Received: 6 January 2009 / Accepted: 22 January 2009 / Published online: 4 February 2009

Abstract Reactive oxygen species (ROS) display cytotoxicity that can be exacerbated by iron. Paradoxically, HeLa cells treated with the ROS-generators menadione and 2,3-dimethoxy-1,4-naphthoquinone display increased free labile iron. HeLa cells exposed to ROS undergo apoptosis but iron chelation limits the extent of cell death suggesting the rise in intracellular iron plays a signaling role in this pathway. This idea is supported by the fact that iron chelation also alters the pattern of ROS-induced phosphorylation of stress-activated protein kinases SAPK/JNK and p38 MAPK. Thus, ROS-induced increases in cellular free iron contribute to signaling events triggered during oxidative stress response.

Keywords Iron · Reactive oxygen species · Signal transduction · Apoptosis

Introduction

Subtoxic concentrations of reactive oxygen species (ROS) are generated during normal aerobic metabolism and are considered to be intracellular signaling molecules. However, generation of ROS resulting from hyperoxia (Kazzaz et al. 1999; Lee and Choi 2003), growth factor deprivation (Kirkland et al. 2002), ultraviolet radiation (Chen et al. 2003), and chemotherapeutic drugs (Kalivendi et al. 2001) can also initiate cell death. Because iron can react with H_2O_2 by Fenton chemistry to generate reactive free radicals, including the highly toxic hydroxyl radical (•OH), it seems reasonable that cellular iron levels would be limited under these conditions to limit iron-catalyzed oxidative damage. However, cellular responses to oxidative stress can paradoxically promote increased intracellular iron levels. For example, cells treated with H_2O_2 display reduced ferritin synthesis and increased transferrin (Tf) receptor expression and uptake (Martins et al. 1995; Pantopoulos and Hentze 1995; Andriopoulos et al. 2007). Oxidative stress is also known to upregulate heme oxygenase-1 (HO-1), which degrades heme to biliverdin, CO and iron (Keyse and Tyrrell 1987; Keyse and Tyrrell 1989). While biliverdin may act as an anti-oxidant, release of free ferrous iron by HO-1 could potentially enhance free radical damage.

Despite the recognition of these paradoxical cellular responses, our knowledge about actual changes in cellular iron induced by ROS and the biological consequences of the modulation of cellular free iron levels under oxidative stress conditions is rather limited. To further elucidate the response of intracellular iron homeostasis to oxidative stress, we examined the effects of the ROS-generators menadione and

S. Deb · E. E. Johnson · R. L. Robalinho-Teixeira · M. Wessling-Resnick (✉)
Departments of Nutrition and Genetics and Complex Disease, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115, USA
e-mail: wessling@hsph.harvard.edu

2,3-dimethoxy-1,4-naphthoquinone (DMNQ) on cellular iron using the fluorescence-based calcein assay. Our results demonstrate that ROS increase the chelatable "free" iron pool due to an intracellular redistribution of iron and that this effect contributes to stress-activated signal transduction events promoting apoptosis.

Materials and methods

Cell culture

HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 300 g/l L-glutamine, 10% (v/v) fetal bovine serum, 50 units/ml penicillin G, and 50 μg/ml streptomycin.

Calcein assay to measure intracellular free iron

HeLa cells were grown to 80–90% confluency in 24-well plates. Cells were loaded with 0.25 μM calcein-AM (Molecular Probes) for 20 min at 37°C in supplemented DMEM. Cells were then washed with Hank's balanced salt solution (HBSS), and the initial fluorescence for each well was measured using a SpectrafluorPlus plate reader (Tecan; 485 nm excitation, 535 nm emission). Cells were then incubated with 50 μM menadione, 50 μM DMNQ, 50 μM ferrous ammonium sulfate (FAS) or DMSO (vehicle control) for 20 min in HBSS. To confirm that chelatable free iron was measured, cells were also preincubated with salicylaldehyde isonicotinyl hydrazone (SIH) for 15 min prior to each treatment. Fluorescence was then again measured as described above after a 90 min incubation period at 37°C and values for each well were normalized to the fluorescence intensity determined before treatment (percent initial value).

Analysis of apoptotic cells

For microscopic analysis, HeLa cells were fixed in 70% ethanol at 4°C for 16 h. After washing twice with PBS containing 10 μg/ml RNAse A, cells were stained with 2 μg/ml Hoechst 33258 for 10 min at room temperature. A Nikon Eclipse E800 fluorescence microscope was used to count the percentage of condensed nuclei characteristic of apoptosis from 500 cells per sample.

Western blot analysis

HeLa cells grown to ~80% confluency in 6-well plates were washed with PBS containing 0.1 mM $CaCl_2$ and 1 mM $MgCl_2$. Cells were exposed to 50 μM menadione or 200 μM SIH or both in the same buffer for 20 min. Cells were then washed twice with supplemented DMEM and incubated in the latter medium for 0–180 min. Exposure to SIH was continued during this time period. Whole cell extracts were prepared by scraping HeLa cells into Laemmli sample buffer (100 μl per well). Samples were sonicated for 10 s, boiled and protein content was determined using the DC assay (Biorad). Fifty micrograms were electrophoresed on 10% SDS–polyacrylamide gels and then transferred to nitrocellulose membranes. After blocking the membrane in Tris-buffered saline (TBS) with 0.1% (w/v) Tween-20 containing 5% non-fat dry milk for 1 h at room temperature, the membrane was exposed to primary antibody (1:1000) overnight at 4°C. The membrane was subsequently washed with TBS containing 0.1% Tween-20 and incubated with horse radish peroxidase (HRP)-conjugated secondary antibody (1:2000) for 1 h at room temperature. Immunolabeled bands were detected with Super Signal West Pico reagent (Pierce). In addition, immunoblots were stripped using Re-Blot^{TM} Recycling reagent (Chemicon International) for 20 min at room temperature and reprobed with appropriate antisera.

Materials

SIH was a very generous gift from Dr. Prem Ponka (Lady Davis Institute, McGill University, Montreal, Quebec, Canada). Polyclonal antibodies raised against ERK1/2 (p44/42 MAPKs), phospho-ERK1/2 (Thr202/Tyr204), p38 MAPK (Thr180/Tyr182), SAPK/JNK, phospho-SAPK/JNK (Thr183/Tyr185) were obtained from Cell Signaling Technology. Sheep anti-human ferritin was obtained from The Binding Site. Rabbit anti-sheep-HRP was obtained from Pierce and donkey anti-rabbit-HRP from Amersham.

Results

ROS increase cellular free iron levels

To characterize the influence of ROS on cellular iron homeostasis, the oxygen free radical generator

menadione was employed in this investigation. Quinones like menadione undergo redox cycling inside cells to generate ROS including H_2O_2 and the superoxide anion O_2. To directly examine whether the level of free iron actually increases in menadione-treated cells, the metal-sensitive fluorophore calcein was employed (Cabantchik et al. 1996). Briefly, HeLa cells were loaded with the acetomethyl ester of calcein (calcein-AM), which is rapidly hydrolyzed in the cytosol to release free calcein. Calcein fluorescence is quenched when iron or other metals are bound. Because calcein fails to bind calcium or magnesium ions at physiological pH and the intracellular concentration of other metals is relatively low, a decrease in cell-associated calcein fluorescence provides a relative measure of increased free intracellular iron content. As shown in Fig. 1, menadione treatment was found to quench cell-associated calcein fluorescence and this effect was blocked by the iron chelator SIH. The latter control confirms that intracellular free iron levels are increased upon treatment with the drug. These data demonstrate that a redistribution of iron stores occurs and is responsible for the quenching effect (Breuer et al. 1995). For comparison, cells incubated with ferrous ammonium sulfate (FAS) also display a similar SIH-sensitive quenching effect consistent with the import of non-Tf bound iron (NTBI) as previously described (Breuer et al. 1995; Zanninelli et al. 1997; Staubli and Boelsterli 1998). Because menadione is known to rapidly deplete cellular thiols (Toxopeus et al. 1993), the effects of DMNQ were also studied. DMNQ stimulates the formation of ROS without thiol interaction (Toxopeus et al. 1993). Similar to menadione, DMNQ promoted an increase in intracellular free iron that was blocked by SIH (Fig. 1), thus indicating that thiol depletion is not involved in this process.

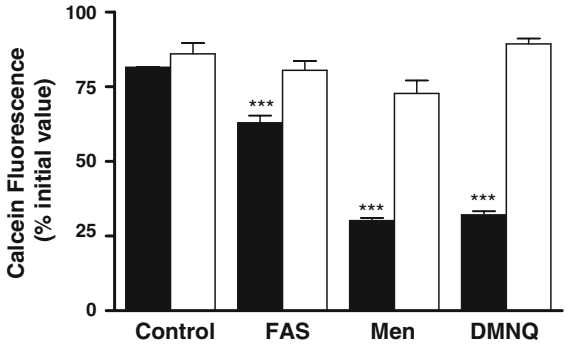

Fig. 1 ROS increase the intracellular calcein-sensitive iron pool. HeLa cells were preloaded with the iron-binding fluorophore calcein (0.25 μM) for 20 min to examine the chelatable intracellular iron pool as described in "Materials and methods". After washing to remove residual extracellular calcein, cells were incubated with either 50 μM menadione or 50 μM DMNQ, 50 μM ferrous ammonium sulfate (FAS) or both in HBSS (*closed bars*). Replicate samples were incubated under the same conditions except that these cells were preincubated with 200 μM SIH to confirm specificity of the fluorescent probe for iron (*open bars*). Intracellular fluorescence measured after 90 min incubation was normalized to the starting fluorescence and results are shown as % initial value. The mean ± SEM from a single experiment ($n = 3$) are shown. (***) denotes $P \leq 0.001$ using the Students *t* test. Similar results were obtained on at least three separate occasions

Menadione induces apoptosis of HeLa cells and this effect is blocked by iron chelation

Oxidative stress is known to elicit either proliferative or apoptotic cellular responses. One rationalization for mobilization of intracellular iron in response to ROS is that the metal is requisite for cell growth as an essential cofactor of ribonucleotide reductase, a rate-limiting enzyme for DNA synthesis. However, thymidine incorporation did not increase upon treatment of HeLa cells with menadione (data not shown). Rather, menadione was found to induce apoptosis with ~75% of the treated cells containing condensed nuclei 24 h after exposure to the quinone (Table 1). These results are consistent with several previous reports that this ROS-generator can promote apoptosis in a variety of different cell lines (McConkey et al. 1988; Juan and Wu 1993; Wu et al. 1993; Osada et al. 2001; Criddle et al. 2006).

To assess the role of cellular iron level in the apoptotic response, the influence of the iron chelator SIH was examined. As shown in Fig. 1, SIH

Table 1 Menadione induces apoptosis

Treatment	% Apoptotic nuclei
Control (DMSO)	1.9 ± 1.0
SIH	1.8 ± 0.34
Menadione	75.9 ± 8.05
SIH + Menadione	40.0 ± 5.33

HeLa cells were treated with 50 μM menadione or the same volume of carrier (DMSO) for 20 min in PBS containing 0.1 mM $CaCl_2$ and 1 mM $MgCl_2$ followed by incubation in DMEM for 24 h. To chelate intracellular iron, cells incubated with or without menadione were also incubated with 200 μM SIH as indicated. The percentage of condensed nuclei was determined in a population of 500 cells by microscopy as described in "Materials and methods"

effectively chelates the intracellular free iron pool that increases upon ROS generation, thereby reversing the quenching effect on calcein. The iron chelator also mitigates the pro-apopotic effect of menadione, with only about half the number of HeLa cells undergoing apoptosis in its presence (Table 1). Combined, these data suggest that increased cellular free iron may help to sustain or propagate signal transduction pathways promoting apoptosis through stress response pathways activated by ROS.

Menadione induces phosphorylation of stress-activated protein kinases

Candidate second messenger pathways for ROS-induced apoptosis involve mitogen-activated protein kinases (MAPKs) including extracellular signal-related kinases 1 and 2 (ERK1/2), stress-activated protein kinase/c-Jun NH_2-terminal kinase (SAPK/JNK), and p38 MAPK. In response to various cellular stimuli and stress conditions, these factors become phosphorylated via MAPK kinase signal transduction cascades. Specifically, menadione has been reported to stimulate ERK phosphorylation in HR stomach cancer cells (Osada et al. 2001), to induce JNK activity in H9c2 cardiac muscle cells (Turner et al. 1998), and to promote phosphorylation of both ERK1/2 and p38 MAPK in macrophages (Ogura and Kitamura 1998). Therefore, to investigate the activation state of ERK1/2, SAPK/JNK, and p38 MAPK in menadione-treated HeLa cells, Western blot analysis was performed using antibodies specific to phosphorylated forms of these kinases; protein expression was verified using anti-MAPK-specific antisera. Exposure to 50 μM menadione resulted in the rapid phosphorylation of 44 kDa ERK1 and 42 kDa ERK2 (Fig. 2), the 46 kDa isoform of SAPK/JNK (Fig. 3) and p38 MAPK (Fig. 4). Except for ERK1/2, a steady decline in the phosphorylated forms of these kinases was observed over a 3 h time period post-treatment. Levels of protein expression of all of the MAPKs were unaffected by menadione.

Iron chelation alters the pattern of SAPK/JNK and p38 MAPK activation in response to menadione

To further study the influence of cellular iron on MAPK activation, SIH was also added during

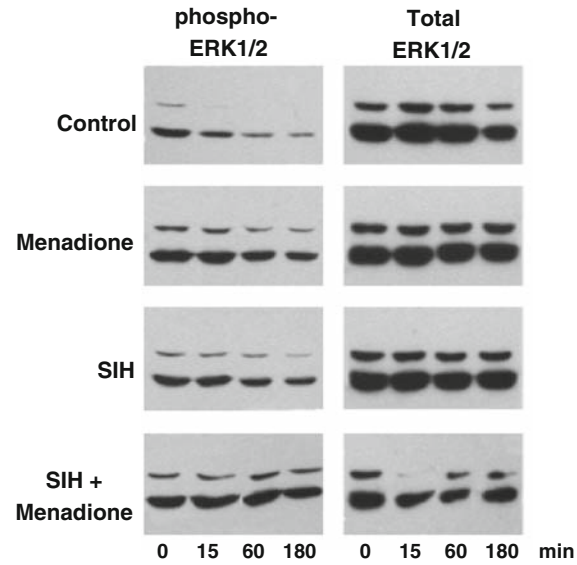

Fig. 2 Effects of menadione and iron chelation on phosphorylation of ERK1/2. HeLa cells were exposed to 50 μM menadione or to 200 μM SIH alone or in combination with menadione (SIH + Men) for 20 min. The medium was then replaced with supplemented DMEM for 0–180 min as indicated. Cells treated with SIH in the absence or presence of menadione were continuously maintained in the chelator throughout the time course. Whole cell extracts were collected as described under "Materials and methods". Phosphorylation was assessed using antibodies that specifically recognize phospho-ERK1/2 and equivalent loading was verified by reprobing the blot with the anti-ERK1/2. Shown are results of an individual experiment that reflect similar data obtained on at least three separate occasions

treatment with menadione in order to block the rise in intracellular free iron. The presence of SIH alone induced a small increase in ERK1/2 phosphorylation (Fig. 2). It did not, however, affect the onset or timecourse of dephosphorylation over the 3 h time period studied. In contrast, SIH significantly delayed the onset and extent of both SAPK/JNK (Fig. 3) and p38 MAPK (Fig. 4) phosphorylation. More pronounced effects were observed in the deactivation of these kinases. For example, menadione-induced maximal phosphorylation of p38 MAPK and SAPK/JNK within 20–60 min with a diminished signal by 180 min reflecting the transient activation and rapid dephosphorylation of these kinases. In the presence of SIH, maximal phosphorylation was not detected until 60 min and the extent of phosphorylation was significantly less compared to untreated cells. Furthermore, a prolonged state of activation was observed at 180 min relative to the

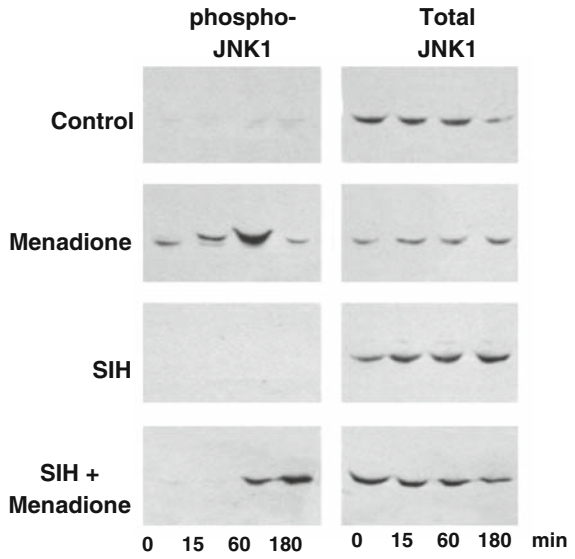

Fig. 3 Effects of menadione and iron chelation on phosphorylation of SAPK/JNK. HeLa cells were exposed to 50 μM menadione or to 200 μM SIH alone or in combination with menadione (SIH + Men) as described for Fig. 2. Phosphorylation was assessed by Western blotting cell lysates prepared in a similar fashion except that antibodies specifically recognizing phospho-SAPK/JNK were used. Equal lane loading was confirmed using anti-SAPK/JNK

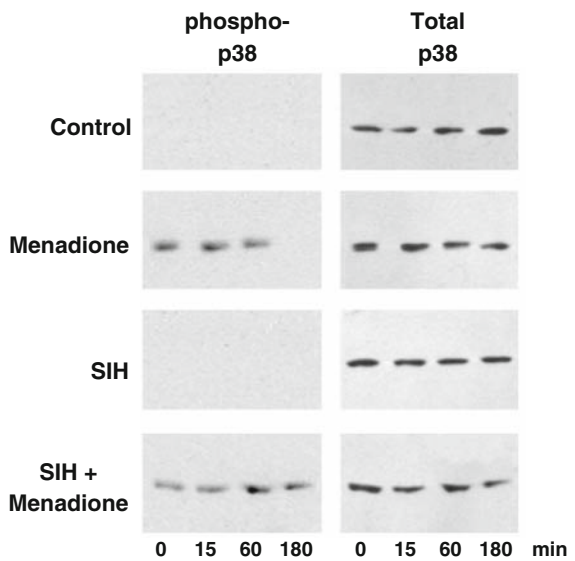

Fig. 4 Effects of menadione and iron chelation on phosphorylation of p38 MAPK. Experiments were performed identical to those in Figs. 2 and 3 except that activation of p38 MAPK was assessed using antibodies specific for phospho-p38 MAPK and that loading was verified with the anti-p38 MAPK

menadione-induced response (Figs. 3, 4). These key observations implicate a role for the paradoxical increase in cellular free iron induced by menadione in the activation/deactivation of SAPK/JNK and p38 MAPK. Moreover, because activation of SAPK/JNK and p38 MAPK can produce a pro-apoptotic response, these findings further suggest that the ability of SIH to limit menadione-induced cell death (Table 1) is due to its influence on the state of stress-activated kinase phosphorylation. These conclusions are compatible with the data of Brenneisen et al. (1998), who have reported that iron chelation reduces c-Jun phosphorylation and transcriptional activation in response to ultraviolet B irradiation, a stress that also produces ROS and that has been associated with increased iron levels (Bissett et al. 1991).

SAPK/JNK and p38 MAPK activation occurs independent of extracellular iron

Recently, it has been reported that ROS induce iron uptake by Tf (Andriopoulos et al. 2007) and that Tf receptor-dependent iron uptake contributes to ROS-induced apoptosis in endothelial cells (Kotamraju et al. 2002). To examine if extracellular iron sources play a role in stress kinase activation, experiments were performed as described above except that serum and iron salts were excluded from the cell medium during the timecourse of menadione treatment and kinase activation. As shown by the result summarized in Fig. 5, menadione-induced activation of SAPK/JNK and p38 MAPK is unaffected by the absence of extracellular iron. Likewise, SIH delays the onset and reduces the extent of phosphorylation/dephosphorylation. Although these results do not rule out a role for ROS-enhanced iron uptake in the oxidative stress response, they do indicate that the redistribution of intracellular iron stores induced by menadione is sufficient to promote activation of SAPK/JNK and p38 MAPK.

Discussion

Maintenance of iron homeostasis must be tightly regulated in response to oxidative stress. Use of the fluorometric calcein assay in our study demonstrated that ROS increase levels of intracellular free iron.

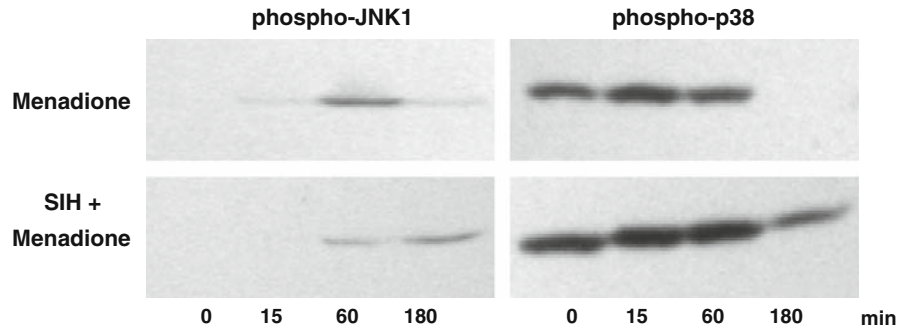

Fig. 5 Activation of SAPK/JNK and p38 MAPK does not require extracellular iron. Experiments were performed similar to Figs. 2, 3, and 4 except that after menadione treatment, HeLa cells were incubated in serum-free α-minimal essential medium which does not contain iron. Western blot analysis was carried out exactly as shown in Figs. 2, 3, and 4. Control experiments confirmed the lack of phosphorylation in untreated control cells and cells treated with SIH alone (data not shown). Shown are data from one of two experiments with similar results

A 2.5-fold increase in the chelatable iron pool was observed within 2 h of exposure to menadione. Why would the cell respond to oxidative stress by increasing intracellular free iron given that this effect would further promote metal-catalyzed ROS production? Because iron chelation mitigates menadione-induced apoptosis, we believe this paradox is explained by a role for cellular free iron in signaling pathways for oxidative stress responses (Jabs 1999). While a role for iron in apoptosis has been investigated in many different studies, these results have failed in general to provide a clear and definitive picture of how intracellular iron levels may affect this process. Chelators like SIH and desferrioxamine are often used to explore this question, but because iron deprivation can cause growth arrest due to inhibition of ribonucleotide reductase (Hoffbrand et al. 1976; Lederman et al. 1984; Nyholm et al. 1993; Chitambar and Wereley 1995), chelation of the metal itself may also induce apoptosis. In this investigation, SIH did not induce apoptosis but did protect against menadione-induced cell death. The suppression of ROS-induced apoptosis by iron chelation correlates with the observation that SIH also reduces the onset and extent of SAPK/JNK and p38 MAPK phosphorylation and delays dephosphorylation of both stress-activated kinases in response to menadione. Many studies now support a role for SAPK/JNK and p38 MAPK in apoptosis (Karin 1998; Davis 2000; Ono and Han 2000; Kyriakis and Avruch 2001). ROS are known to activate SAPK/JNK (Guyton et al. 1996; Clerk et al. 1998) and p38 MAPK (Ogura and Kitamura 1998) and redox-mediated signalling mechanisms have been characterized that are most likely responsible for this effect (Saitoh et al. 1998; Yoshizumi et al. 2000). Thus, a reasonable explanation for the observation that iron chelation protects against cell death is that SIH limits changes in redox status elicited through iron-generated ROS that mediate activation/deactivation of SAPK/JNK, p38 MAPK and possibly other elements of the apoptotic signaling pathway.

A role for iron-generated ROS in several different signaling pathways is now beginning to emerge. Along the lines of our investigation, Brenneisen et al. (1998) have also provided evidence to support the idea that the critical events in ultraviolet B activation of JNK2 rely on iron-catalyzed ROS. ROS are induced by a number of different cytokines, growth factor receptor tyrosine kinases and G protein coupled receptors, but the sources of cellular ROS are rather poorly defined and could potentially emanate from changes in the intracellular free iron level. For example, it has been directly shown that IL-1β enhances the calcein-sensitive free iron pool in astrocytoma cells (Pinero et al. 2000). Rauen et al. (2000) have shown that cold-induced apoptosis is not associated with increased cellular production of oxygen radicals but rather with an increase in the cellular calcein-sensitive iron level. These authors argue that increased cellular iron is then responsible for the generation of hydroxyl radicals that promote hypothermia injury/cold-induced apoptosis. Thus, much like cellular calcium levels that are modulated to sustain and/or propagate a number of signal transduction pathways, the intracellular free iron pool may be regulated in a similar fashion to

modulate production of cellular ROS and subsequent signaling responses.

Acknowledgments This work was supported by NIH grants DK056160, DK064750, ES014638 and the Milton Fund (Harvard University). R.L.R-T was a fellowship recipient of FAPESP (Brazil), and E.E.J. was supported by NIH grants T90 DK070078 and T32 ES007155.

References

Andriopoulos B, Hegedusch S, Mangin J, Riedel HD, Hebling U, Wang J et al (2007) Sustained hydrogen peroxide induces iron uptake by transferrin receptor-1 independent of the iron regulatory protein/iron-responsive element network. J Biol Chem 282:20301–20308. doi:10.1074/jbc.M702463200

Bissett DL, Chatterjee R, Hannon DP (1991) Chronic ultraviolet radiation-induced increase in skin iron and the photoprotective effect of topically applied iron chelators. Photochem Photobiol 54:215–223. doi:10.1111/j.1751-1097.1991.tb02009.x

Brenneisen P, Wenk J, Klotz LO, Wlaschek M, Briviba K, Krieg T et al (1998) Central role of Ferrous/Ferric iron in the ultraviolet B irradiation-mediated signaling pathway leading to increased interstitial collagenase (matrix-degrading metalloprotease (MMP)-1) and stromelysin-1 (MMP-3) mRNA levels in cultured human dermal fibroblasts. J Biol Chem 273:5279–5287. doi:10.1074/jbc.273.9.5279

Breuer W, Epsztejn S, Cabantchik ZI (1995) Iron acquired from transferrin by K562 cells is delivered into a cytoplasmic pool of chelatable iron(II). J Biol Chem 270:24209–24215. doi:10.1074/jbc.270.33.19330

Cabantchik ZI, Glickstein H, Milgram P, Breuer W (1996) A fluorescence assay for assessing chelation of intracellular iron in a membrane model system and in mammalian cells. Anal Biochem 233:221–227. doi:10.1006/abio.1996.0032

Chen Q, Chai YC, Mazumder S, Jiang C, Macklis RM, Chisolm GM et al (2003) The late increase in intracellular free radical oxygen species during apoptosis is associated with cytochrome c release, caspase activation, and mitochondrial dysfunction. Cell Death Differ 10:323–334. doi:10.1038/sj.cdd.4401148

Chitambar CR, Wereley JP (1995) Effect of hydroxyurea on cellular iron metabolism in human leukemic CCRF-CEM cells: changes in iron uptake and the regulation of transferrin receptor and ferritin gene expression following inhibition of DNA synthesis. Cancer Res 55:4361–4366

Clerk A, Michael A, Sugden PH (1998) Stimulation of multiple mitogen-activated protein kinase sub-families by oxidative stress and phosphorylation of the small heat shock protein, HSP25/27, in neonatal ventricular myocytes. Biochem J 333(Pt 3):581–589

Criddle DN, Gillies S, Baumgartner-Wilson HK, Jaffar M, Chinje EC, Passmore S et al (2006) Menadione-induced reactive oxygen species generation via redox cycling promotes apoptosis of murine pancreatic acinar cells. J Biol Chem 281:40485–40492. doi:10.1074/jbc.M607704200

Davis RJ (2000) Signal transduction by the JNK group of MAP kinases. Cell 103:239–252. doi:10.1016/S0092-8674(00)00116-1

Guyton KZ, Liu Y, Gorospe M, Xu Q, Holbrook NJ (1996) Activation of mitogen-activated protein kinase by H_2O_2: role in cell survival following oxidant injury. J Biol Chem 271:4138–4142. doi:10.1074/jbc.271.7.3604

Hoffbrand AV, Ganeshaguru K, Hooton JW, Tattersall MH (1976) Effect of iron deficiency and desferrioxamine on DNA synthesis in human cells. Br J Haematol 33:517–526. doi:10.1111/j.1365-2141.1976.tb03570.x

Jabs T (1999) Reactive oxygen intermediates as mediators of programmed cell death in plants and animals. Biochem Pharmacol 57:231–245. doi:10.1016/S0006-2952(98)00227-5

Juan CC, Wu FY (1993) Vitamin K3 inhibits growth of human hepatoma HepG2 cells by decreasing activities of both p34cdc2 kinase and phosphatase. Biochem Biophys Res Commun 190:907–913. doi:10.1006/bbrc.1993.1135

Kalivendi SV, Kotamraju S, Zhao H, Joseph J, Kalyanaraman B (2001) Doxorubicin-induced apoptosis is associated with increased transcription of endothelial nitric-oxide synthase: effect of antiapoptotic antioxidants and calcium. J Biol Chem 276:47266–47276. doi:10.1074/jbc.M106829200

Karin M (1998) Mitogen-activated protein kinase cascades as regulators of stress responses. Ann NY Acad Sci 851:139–146. doi:10.1111/j.1749-6632.1998.tb08987.x

Kazzaz JA, Horowitz S, Li Y, Mantell LL (1999) Hyperoxia in cell culture: a non-apoptotic programmed cell death. Ann N Y Acad Sci 887:164–170

Keyse SM, Tyrrell RM (1987) Both near ultraviolet radiation and the oxidizing agent hydrogen peroxide induce a 32-kDa stress protein in normal human skin fibroblasts. J Biol Chem 262:14821–14825

Keyse SM, Tyrrell RM (1989) Heme oxygenase is the major 32-kDa stress protein induced in human skin fibroblasts by UVA radiation, hydrogen peroxide, and sodium arsenite. Proc Natl Acad Sci USA 86:99–103. doi:10.1073/pnas.86.1.99

Kirkland RA, Windelborn JA, Kasprzak JM, Franklin JL (2002) A Bax-induced pro-oxidant state is critical for cytochrome c release during programmed neuronal death. J Neurosci 22:6480–6490

Kotamraju S, Chitambar CR, Kalivendi SV, Joseph J, Kalyanaraman B (2002) Transferrin receptor-dependent iron uptake is responsible for doxorubicin-mediated apoptosis in endothelial cells: role of oxidant-induced iron signaling in apoptosis. J Biol Chem 277:17179–17187. doi:10.1074/jbc.M111604200

Kyriakis JM, Avruch J (2001) Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. Physiol Rev 81:807–869

Lederman HM, Cohen A, Lee JW, Freedman MH, Gelfand EW (1984) Deferoxamine: a reversible S-phase inhibitor of human lymphocyte proliferation. Blood 64:748–753

Lee PJ, Choi AM (2003) Pathways of cell signaling in hyperoxia. Free Radic Biol Med 35:341–350. doi:10.1016/S0891-5849(03)00279-X

Martins EA, Robalinho RL, Meneghini R (1995) Oxidative stress induces activation of a cytosolic protein responsible for control of iron uptake. Arch Biochem Biophys 316:128–134. doi:10.1006/abbi.1995.1019

McConkey DJ, Hartzell P, Nicotera P, Wyllie AH, Orrenius S (1988) Stimulation of endogenous endonuclease activity in hepatocytes exposed to oxidative stress. Toxicol Lett 42:123–130. doi:10.1016/0378-4274(88)90069-0

Nyholm S, Mann GJ, Johansson AG, Bergeron RJ, Graslund A, Thelander L (1993) Role of ribonucleotide reductase in inhibition of mammalian cell growth by potent iron chelators. J Biol Chem 268:26200–26205

Ogura M, Kitamura M (1998) Oxidant stress incites spreading of macrophages via extracellular signal-regulated kinases and p38 mitogen-activated protein kinase. J Immunol 161: 3569–3574

Ono K, Han J (2000) The p38 signal transduction pathway: activation and function. Cell Signal 12:1–13. doi:10.1016/S0898-6568(99)00071-6

Osada S, Saji S, Osada K (2001) Critical role of extracellular signal-regulated kinase phosphorylation on menadione (vitamin K3) induced growth inhibition. Cancer 91:1156–1165. doi:10.1002/1097-0142(20010315)91:6<1156::AID-CNCR1112>3.0.CO;2-1

Pantopoulos K, Hentze MW (1995) Rapid responses to oxidative stress mediated by iron regulatory protein. EMBO J 14:2917–2924

Pinero DJ, Hu J, Cook BM, Scaduto RC Jr, Connor JR (2000) Interleukin-1beta increases binding of the iron regulatory protein and the synthesis of ferritin by increasing the labile iron pool. Biochim Biophys Acta 1497:279–288. doi:10.1016/S0167-4889(00)00066-5

Rauen U, Petrat F, Li T, De GH (2000) Hypothermia injury/cold-induced apoptosis—evidence of an increase in chelatable iron causing oxidative injury in spite of low O_{2-}/H_2O_2 formation. FASEB J 14:1953–1964. doi:10.1096/fj.00-0071com

Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y et al (1998) Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. EMBO J 17:2596–2606. doi:10.1093/emboj/17.9.2596

Staubli A, Boelsterli UA (1998) The labile iron pool in hepatocytes: prooxidant-induced increase in free iron precedes oxidative cell injury. Am J Physiol 274(6):G1031–G1037

Toxopeus C, van Holsteijn I, Thuring JW, Blaauboer BJ, Noordhoek J (1993) Cytotoxicity of menadione and related quinones in freshly isolated rat hepatocytes: effects on thiol homeostasis and energy charge. Arch Toxicol 67: 674–679. doi:10.1007/BF01973690

Turner NA, Xia F, Azhar G, Zhang X, Liu L, Wei JY (1998) Oxidative stress induces DNA fragmentation and caspase activation via the c-Jun NH_2-terminal kinase pathway in H9c2 cardiac muscle cells. J Mol Cell Cardiol 30:1789–1801. doi:10.1006/jmcc.1998.0743

Wu FY, Chang NT, Chen WJ, Juan CC (1993) Vitamin K3-induced cell cycle arrest and apoptotic cell death are accompanied by altered expression of c-fos and c-myc in nasopharyngeal carcinoma cells. Oncogene 8:2237–2244

Yoshizumi M, Abe J, Haendeler J, Huang Q, Berk BC (2000) Src and Cas mediate JNK activation but not ERK1/2 and p38 kinases by reactive oxygen species. J Biol Chem 275:11706–11712. doi:10.1074/jbc.275.16.11706

Zanninelli G, Glickstein H, Breuer W, Milgram P, Brissot P, Hider RC et al (1997) Chelation and mobilization of cellular iron by different classes of chelators. Mol Pharmacol 51: 842–852