Emerging Potential of Immediate Early Response Gene X-1 in Cardiovascular and Metabolic Diseases

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Immediate early response gene X-1 (IEX-1) or immediate early response 3 is an immediate early response gene that is highly expressed in epithelial, endothelial, and immune cells, including macrophages.[1–4] Similar to other immediate early response genes, its induction can be rapidly induced in response to several stimuli, such as UV light, inflammatory cytokines, and mechanical strain.[4–8] Under stress, many immediate early response genes, such as c-Fos, c-Jun, c-Myc, Egr-1, and other zinc-finger proteins, usually serve as transcription factors, activators, or repressors to regulate the transcriptional activity of several genes that are critical for a cell to appropriately respond to stress.[4,9–12] However, IEX-1 lacks a DNA binding domain and its putative transcription activity remains elusive.[4,13] Investigations from our laboratory show that IEX-1 controls mitochondrial superoxide production by regulating the electron transport chain and oxidative phosphorylation.[13–16] This action of IEX-1 might explain the transcriptional activity associated with IEX-1 because the reactive oxygen species (ROS) at low levels can act as a signaling molecule to alter gene transcription through reduction-oxidation–sensitive transcription factors or a signaling pathway, such as nuclear factor-κB (NF-κB) and nuclear factor erythroid 2-related factor.[17–19] Consistent with a key role of mitochondria in vascular and metabolic functions, IEX-1 deficiency impairs vascular function and increases energy expenditure in mice. A key role of IEX-1 in regulation of mitochondrial respiration and ROS production may also help to explain how IEX-1 produces both antiapoptotic and proapoptotic actions in different cell types. Macrophages, the central player in the pathogenesis of many chronic inflammatory conditions, express high levels of IEX-1. In agreement with the putative role of IEX-1 in macrophage function, we recently identified a crucial role of IEX-1 in many chronic conditions, including hypertension, sepsis, insulin resistance, obesity, and arthritis,[16,20–22] revealing previously unknown functions of IEX-1 in cardiovascular and metabolic diseases.

In this article, we reviewed the findings in IEX-1 knockout mice from our laboratory and others and primarily focused on the roles of IEX-1 in cardiovascular and metabolic disorders. We also alluded to the important findings from other laboratories that are relevant for its role in cardiovascular and metabolic diseases. In addition, an attempt is made to underline the involvement of IEX-1 in inflammation and its possible link with the lipid-mediated metabolic diseases. However, because of the limitations and scope of this article, we refrained from discussing an important role of IEX-1 in many types of cancers that has been widely reported before.

IEX-1 Regulates Mitochondrial F1F0-ATP Synthase Activity

One of the well-described functions of IEX-1 is its ability to regulate apoptosis in various cell types, which is in a good agreement with its role in the control of mitochondrial oxidative phosphorylation, given that mitochondria are key in the initiation of this process.[13,23,24] The oxidative phosphorylation takes place at the respiratory chain in the inner mitochondrial membrane, which consists of 5 enzyme complexes (I–V) (Figure 1). The first 4 complexes oxidize nicotinamide-adenine dinucleotide, reduced form, and flavin adenine dinucleotide, reduced form, produced by the Krebs cycle and the β-oxidation, to generate a proton gradient across the mitochondrial inner membrane. F1F0-ATP synthase

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(complex V) uses this electrochemical gradient to produce ATP, the major source of energy in a cell. When mitochondrial respiration is compromised (eg, during oxygen deprivation [hypoxia]), the F1F0-ATP synthase reverses its action and begins to hydrolyze ATP (ATPase) to maintain the mitochondrial membrane potential. This ATPase activity can be repressed by an inhibitory protein F1F0-ATPase inhibitor (IF1) to preserve energy.[25,26] IF1 binds to subunit of F1 complex through its inhibitory domain and inhibits ATP hydrolysis.[26] Increased IF1 activity during diminished mitochondrial respiration conserves ATP by inhibiting F1F0-ATPase at the expense of membrane potential and protects against ischemia.[26] Conceivably, increasing IF1 expression under normal mitochondrial function may inhibit oxidative phosphorylation and decrease ATP synthesis.[27] This scenario, thus, would promote a switch from oxidative phosphorylation to glycolysis. Indeed, tumor cells in which glycolysis is putatively high (Warburg effect) exhibit increased IF1 expression concomitant with reduced F1F0-ATP synthase activity.[27]

Investigation from our laboratory has provided crucial evidence that IF1 activity is tightly regulated by IEX-1 in the mitochondria.[15] By using the yeast 2-hybrid assay and coimmunoprecipitation techniques, we demonstrated that IEX-1 directly interacts with the C-terminus of IF1. This renders IF1 sensitive to degradation by a mitochondrial protease.[15] Thus, an increase in IEX-1 expression reduced IF1 level by promoting its degradation. Consequently, IEX-1– mediated IF1 degradation caused an increase in ATP hydrolysis by augmenting ATPase activity and prevented an increase in mitochondrial membrane potential induced by apoptotic stimuli.[13,15] In agreement with its stimulatory action in mitochondria, overexpression of IEX-1 inhibited both basal and apoptotic stimuli-induced ROS production in multiple cell types by facilitating electron transfer along the respiratory chain.[13,15] Furthermore, gene silencing of IF1 produced the similar effects to IEX-1 overexpression on ATP hydrolysis and ROS production, corroborating a key role of IF1 degradation in the effects produced by IEX-1.[15] These observations are in close agreement and correlate well with the IEX-1–mediated antiapoptotic effect in many cell types, revealing an important role of IEX-1 in regulation of cellular reduction-oxidation homeostasis. In contrast to the aforementioned observations, silencing of IEX-1 expectedly increased IF1 levels that, in turn, inhibited F1F0-ATP synthase activity. Reduced F1F0-ATP
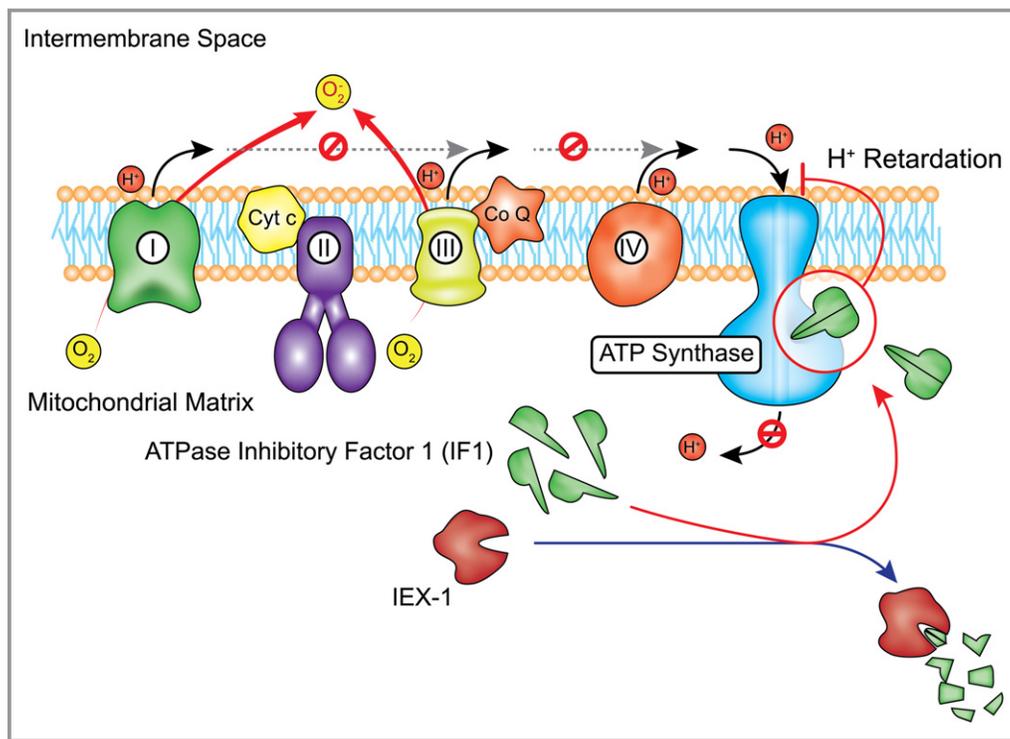
Figure 1. Proposed mechanism of immediate early response gene X-1 (IEX-1) in the regulation of reactive oxygen species (ROS) production in mitochondria. IEX-1 targets F1F0-ATPase inhibitor (IF1) for degradation and maintains a high level of F1F0-ATPase activity and more efficient electron transport along the respiratory chain, resulting in reduced ROS production. In contrast, a deficiency of IEX-1 prevents the degradation of IF1, which, in turn, reduces F1F0-ATPase activity, thereby hindering the electron transport. This phenomenon causes an increased ROS production as a result of proton (H^+) retardation along the respiratory chain. Co Q indicates coenzyme Q; Cyt c, cytochrome c; O_2, oxygen; O_2^-, superoxide radicals.

synthase activity consequently perturbed proton transport along the respiratory chain and impaired mitochondrial membrane potential, resulting in excessive mitochondrial ROS (mROS) production.[13,15,20] Moreover, because of the inhibition of F1F0-ATP synthase activity, oxidative phosphorylation was downregulated in IEX-1–deficient cells, as a result of which these cells switched their cellular metabolism from oxidative phosphorylation to glycolysis.[15,21] Accordingly, IEX-1–deficient mouse embryonic fibroblasts used more glucose to compensate energy depletion and proliferate better in complete media. However, they produced more lactate under hypoxia and were susceptible to glucose deprivation.[13,15] These findings argue that interaction of IEX-1 and IF1 may have an important role in regulation of mitochondrial respiration and ROS production in aerobic conditions in addition to their putative role during hypoxia. This notion may have greater implications than were previously thought because IEX-1 is abundantly expressed and is highly inducible in different cell types, such as small and large arteries, keratinocytes, immune cells (including macrophages and T cells), and white adipose tissue (WAT).[1,15,20,21] This pattern of IEX-1 expression provided first key evidence supporting a potential physiological importance of this novel protein in cardiovascular and chronic inflammatory conditions.

Antiapoptotic and Proapoptotic Actions of IEX-1

Apoptosis is a tightly regulated process wherein the mitochondria take a central part. IEX-1 is highly expressed in mitochondria and has been reported to produce both antiapoptotic and proapoptotic actions.[28] However, the survival action is usually observed when the basal cellular ROS level is relatively low, as observed under in vivo physiological conditions.[15,29,30] For example, overexpression of IEX-1 in T cells inhibited apoptosis and caused a lupuslike syndrome and T-cell lymphoma in mice.[29,30] Likewise, IEX-1 protected T cells from apoptosis induced by ligation of Fas and the T-cell receptor–CD3 complex.[31] Similarly, in nontumor cell lines, such as megakaryoblastic cells UT7, or in CHO-ER cells, IEX-1 prevented staurosporin-induced apoptosis.[32,33] In contrast, the lack of IEX-1 in CHO or mouse embryonic fibroblasts cells increased apoptosis and reduced their survival under a low glucose condition.[15] These observations strongly support a survival function of IEX-1 under physiological conditions.

It is well documented that the amount of ROS generated and the extent of oxidative stress in a cell determine the fate of the cell to die or survive.[34,35] At lower intracellular concentrations, ROS may induce growth and proliferation by its action as a secondary messenger to activate transcription of various survival genes that involve mitogen-activated

protein kinase and proangiogenic factors, including vascular endothelial growth factor, interleukin-8, and NF-κB.[36–38] Interestingly, the antiapoptotic activity of IEX-1 was strongly correlated with its ability to suppress intracellular ROS production.[13] Ectopic expression of IEX-1 suppressed intracellular ROS production induced by apoptosis inducers.[13] Perhaps, by maintaining intracellular ROS concentrations at a low level under apoptotic conditions, IEX-1 activates oxidant-sensitive signaling to promote cell proliferation and survival, as previously mentioned[15,21] (Figure 2A). Furthermore, an antiapoptotic activity of IEX-1 may also be contributed by its putative action on prosurvival factor myeloid cell leukemia-1 (Mcl-1).[39] Mcl-1 is an antiapoptotic member of the Bcl-2 family, which translocates into the nucleus to induce a survival effect in the presence of DNA damage or genomic instability that occurs, for example, after treatment with anticancer drug alkylating agents. In the nucleus, Mcl-1 triggers Chk1 activation, causes G2 checkpoint arrest, and repairs DNA lesions, promoting cell survival.[39] IEX-1 is essential for Mcl-1 nuclear translocation during stress.[39] Its expression rapidly increases under stress as during γ-irradiation or treatment with the cytotoxic agents[4] (Figure 2A). Thus, by promoting Mcl-1 migration into the nucleus, IEX-1 may induce a survival action in cells under stress. Given a critical role of Mcl-1 in tumor growth, understanding how IEX-1 expression is regulated under stress may provide a novel therapeutic strategy for cancer chemotherapy. Collectively, these studies support the antiapoptotic action of IEX-1 under physiological conditions.

In addition to its antiapoptotic action, IEX-1 has also been reported to promote apoptosis in various tumor cell lines. Tumor cells lines, such as HeLa, HEK-293, HaCaT, Colo320 colorectal cancer, NB4 leukemia, U87-MG glioma, T47D breast cancer, and PT45-P1 pancreatic cancer, exhibit high levels of ROS, even at the basal level.[2,28,40–48] Tumor cells also generally exhibit the Warburg effect, an increased dependency on glycolysis for ATP production attributable in part to a defective mitochondria and hypoxia.[49–51] Glycolysis is an inefficient source of ATP production, which produces far less ATP molecules than oxidative phosphorylation from a given amount of glucose. We have previously reported that overexpression of IEX-1 reduces IF1 level in mitochondria by promoting its degradation.[15] This results in an increase in F1FO-ATPase activity, causing acceleration of ATP degradation. Thus, forced overexpression of IEX-1 in tumor cells or in the cells where ATP generation is already compromised would further dramatically deplete the ATP levels (Figure 2A), thereby inducing apoptosis as a result of severe deprivation of energy.[52] Interestingly, exposure of cells to UVB, tumor necrosis factor-α, or metabolic stress by glucose deprivation has been demonstrated to target prosurvival protein Mcl-1 for proteasomal degradation, thereby reducing its cellular
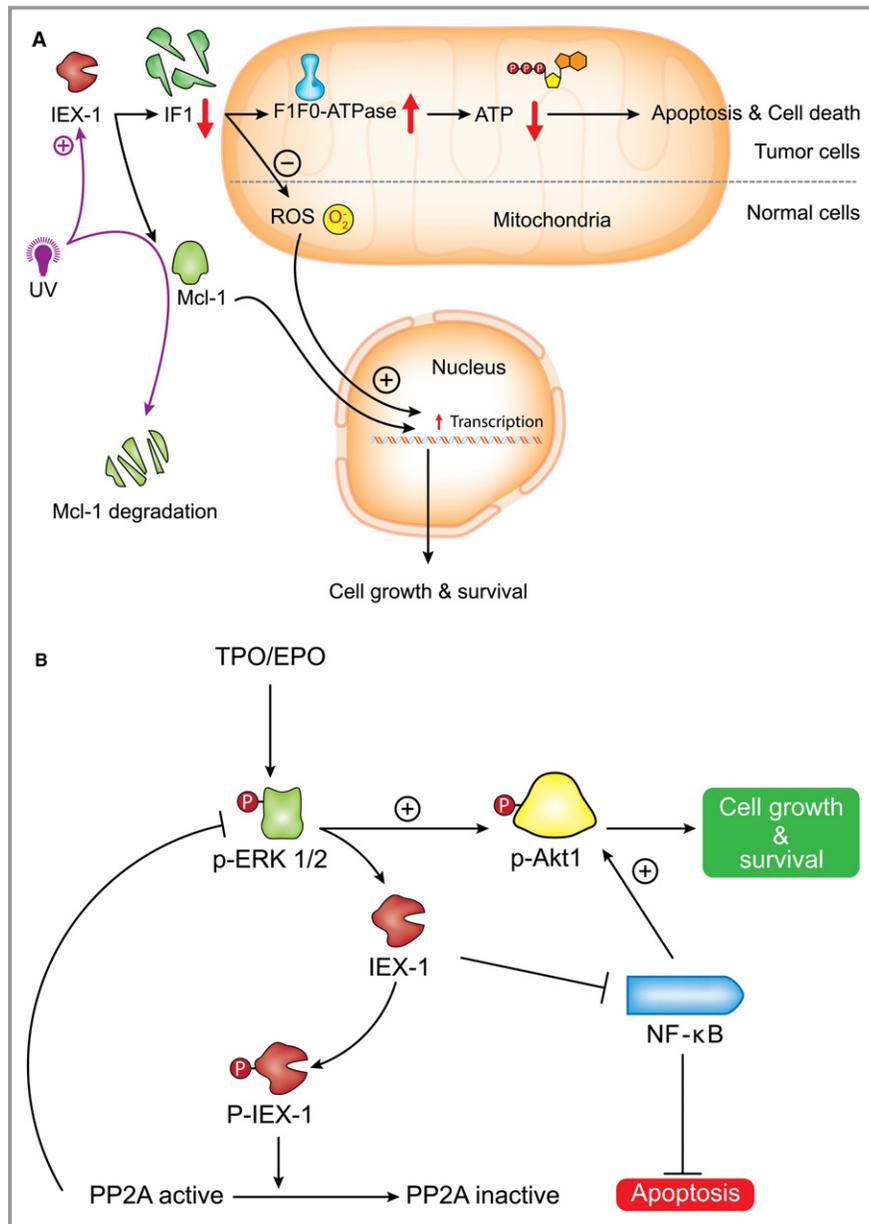
Figure 2. A, Schematic representation of the proposed mechanisms of survival and apoptotic actions of immediate early response gene X-1 (IEX-1). IEX-1 induces a survival action by reducing cellular reactive oxygen species (ROS) to a moderate level at which they act as signaling molecules to activate the transcription of various survival genes. In addition, IEX-1 promotes a survival action by facilitating nuclear translocation of antiapoptotic protein myeloid cell leukemia 1 (Mcl-1). In ATP compromised cells, such as tumor cells, IEX-1 overexpression immediately deprives the cells of their energy source by further accelerating ATP degradation as a result of increased F1F0-ATPase activity, producing a potent apoptotic action. B, Counterregulatory mechanism of IEX-1 in regulation of apoptosis via extracellular signal-regulated kinase (ERK) 1/2 and nuclear factor-κB (NF-κB) pathways. Phosphorylation/activation of ERK1/2 by thrombopoeitin (TPO) or erythropoietin (EPO) targets and augments the phosphorylation of IEX-1 that, in turn, interacts and inactivates protein phosphatase A 2 (PPA2). This effect inhibits PPA2-mediated dephosphorylation of ERK1/2, resulting in prolonged activation of ERK1/2 and activation of survival proteins, such as protein kinase B (Akt) 1. On the other hand, IEX-1 directly inhibits activation of NF-κB by several stimuli, such as tumor necrosis factor-α, and thereby promotes apoptosis by suppressing NF-κB–mediated antiapoptosis and activation of survival genes, such as Akt1/protein kinase B. IF1 indicates F1F0-ATPase inhibitor; O_2^-, superoxide radicals; p-, phosphorylated.

level.[53–56] This would not only make less Mcl-1 available to sequester the proapoptotic member of Bcl-2 family, but also reduce its translocation to nucleus. As a result, the proapoptotic member, such as Bcl-2 interacting mediator of cell death (BIM), would then be released and migrate into mitochondria to induce apoptosis. IEX-1 is required to interact with Mcl-1 for its nuclear translocation.[39] Conceivably, Mcl-1 degradation would result in increased IEX-1 bioavailability, which may, in turn, allow more efficient interaction of IEX-1 with IF1 at mitochondria, leading to a further increase in ATP hydrolysis and apoptosis. However, no study to date has been conducted to confirm this notion.

How IEX-1 switches its action from antiapoptotic to proapoptotic remains elusive. Several mechanisms may be involved for this disparate effect. IEX-1 plays a pivotal role in the regulation of extracellular signal-regulated kinase (ERK) activation by controlling its phosphorylation. IEX-1 contains 3 ERK-sensitive phosphorylation sites at threonine 18 and 123 and at serine 126. On activation, such as by treatment with thrombopoietin or erythropoietin, ERK1/2 phosphorylates IEX-1 at threonine residue.[32,33,57,58] ERK-mediated phosphorylation activates IEX-1 that, in turn, increases ERK phosphorylation back and prolongs ERK signaling, resulting in the induction of phosphorylation of ERK substrates, such as protein kinase B (alias protein kinase B 1), a prosurvival factor.[32] This effect is mediated by the direct interaction of IEX-1 with the protein phosphatase 2A via its subunit B56. Interaction of protein phosphatase 2A with IEX-1 results in its inactivation and, therefore, it would no longer be available to dephosphorylate ERK1/2. This produces sustained ERK-dependent phosphorylation of its prosurvival substrates, resulting in the increased survival of the cells, especially during growth-favoring conditions (Figure 2B).[57,58] In sharp contrast, IEX-1 has also been reported to reduce the expression of prosurvival factors phosphatidylinositol 3-kinase and protein kinase B via suppressing NF-κB expression. Although IEX-1 is a known direct downstream target of NF-κB, IEX-1 decreases the activation of NF-κB observed under inflammatory conditions (Figure 2B).[40,41] In this way, IEX-1 exerts a proapoptotic action via downregulating prosurvival proteins. Taken together, these findings demonstrate that IEX-1 is a negative feedback regulator of NF-κB and ERK pathways that provides highly effective counterregulatory mechanisms initiated by these factors to regulate apoptosis. Despite its varying, sometimes conflicting effects on cell survival and growth, IEX-1 appears to be essential for regulating cell survival and death, mainly through its action on mitochondria.[13,15] The key role of IEX-1 in regulation of mitochondrial function may also be the mechanism whereby deregulation of IEX-1 contributes to various cardiovascular disorders, such as hypertension,[21] atherosclerosis, and cardiac hypertrophy,[6,7] because of the importance of mitochondrial function in the control of cardiovascular activity and metabolism.

Role in Cardiovascular System

IEX-1 Counteracts Hypertrophy in Cardiovascular Tissues

A deregulated expression level of IEX-1 has been reported in vasculature, cardiac tissue, or blood cells in several pathological conditions, such as atherosclerosis, pressure overload, and myelodysplastic syndrome (MDS).[6,7,59] IEX-1 expression is rapidly induced in vascular smooth muscle cells (VSMCs), cardiac myocytes, and monocytes in response to mechanical stress or pressure overload.[6,7] Its expression is induced not only in response to mechanical strain but also on exposure to various neurohumoral factors, such as inflammatory cytokines interleukin 1-β, platelet-derived growth factor, phenylephrine, and phorbol 12-myristate 13-acetate.[6,7] This universal induction of IEX-1 in response to many nonspecific stimuli suggests a broader effect of this protein in the cardiovascular system, in addition to its ability to regulate apoptosis.

In an attempt to study the potential function of this gene in cardiovascular cells, Schulze et al and De Keulenaer et al found that overexpression of IEX-1 in VSMCs and in cardiac myocytes prevented the hypertrophic response induced by exposure to mechanical strain or various neurohumoral factors, suggestive of its antihypertrophic effect in these experimental settings.[6,7] In addition, in vivo vascular specific overexpression of IEX-1 in low-density lipoprotein receptor–deficient mice prevented the neointima formation in response to mechanical injury.[6] The observation that mechanical strain, a stress in association with hypertension, induces IEX-1 expression not only in VSMCs but also in macrophages is consistent with an importance of this gene in vascular pathological conditions, where these cell types are critically involved, such as atherosclerosis and systemic hypertension.[6,60]

IEX-1 Mediates Ischemic Preconditioning of the Heart

Brief episodes of ischemia-reperfusion elicit strong cardioprotection against the subsequently occurring prolonged and sustained ischemia-induced cardiac injury. This phenomenon is termed as ischemic preconditioning (IPC) because it preconditions the heart against the sustained myocardial ischemia-reperfusion injury. The expression of several immediate early-response genes, such as c-*Fos*, c-*Myc*, c-*Jun*, and *Egr-1*, has been reported to rapidly increase (within minutes) in the heart in response to IPC.[9–11] Consistent with this, Xu et al recently reported that IEX-1 expression in rat heart

markedly increased within 5 minutes of induction of episodes of IPC, consistent with the observation with other immediate early response genes.[61] In contrast, IEX-1 expression markedly reduced in the heart during sustained ischemia-reperfusion injury.[61] As a result, IPC-primed hearts were resistant to the sustained ischemic injury-induced downregulation of IEX-1. Furthermore, using the small-interfering RNA–mediated gene silencing approach, Xu et al[61] demonstrated that IEX-1 is required for the full protection conferred by IPC against ischemia-reperfusion injury. Conversely, they showed that cardiac overexpression of IEX-1 attenuated ischemia-reperfusion injury, as evident by reduction in infarct size and improvement in systolic function.[61] The authors concluded that IEX-1–dependent cardiac protection was mediated by phosphorylation and translocation of protein kinase Cϵ, a key molecule required for IPC, which reduced ROS production in mitochondria.[61] These effects inhibited cardiomyocyte apoptosis and necrosis and attenuated myocardial infarction during ischemic injury. These findings are in agreement with our previous reports where we demonstrated that IEX-1 deficiency specifically increased ROS production in mitochondria in VSMCs,[21] whereas its overexpression in T cells inhibited apoptosis, leading to T-cell lymphoma in mice.[30] Nevertheless, this new finding provides another strong evidence for a cardioprotective role of IEX-1 and reveals a new therapeutic strategy for the ischemic heart diseases.

IEX-1 Regulates Vascular Tone to Maintain Systemic Blood Pressure

In a quest to study the physiological role of IEX-1, we generated mice lacking a functional IEX-1 gene. These mice do not express a functional IEX-1 protein; however, they grow and breed normally under steady-state conditions.[21] These IEX-1–deficient mice also weigh similar to their wild-type littermates and appear devoid of any gross developmental abnormality. There was no significant difference in fasting blood glucose, insulin, and total cholesterol levels between IEX-1–deficient and wild-type mice under normal diet-fed conditions.[20,21] We also failed to detect any significant alteration in food intake and total motor activity in these mice, suggesting no major role of IEX-1 under normal physiological conditions. However, IEX-1 deficiency in mice was associated with elevated systemic blood pressure and cardiac hypertrophy as they aged.[3,21] In addition, IEX-1 paucity also increased ROS production in the vasculature of the mice.[3,15,21] Hypertension in IEX-1–deficient mice was not associated with salt sensitivity or abnormality in kidney function and did not progress with age.[3,21] Various vasoregulatory factors, such as angiotensin II, nitrites and nitrates, cAMP, and cGMP, that are implicated in the pathogenesis of hypertension appeared to be normal in these

mice.[3] Thus, hypertension in IEX-1–deficient mice may represent an early stage or a unique category of hypertension where its cause remains subtle. We asked why hypertension in IEX-1–deficient mice did not progress over time and what was the underlying mechanism of this unique form of hypertension. Because kidney function and histological features appeared to be normal in IEX-1–deficient mice, we next investigated the potential impact of IEX-1 deficiency on the vascular function that may contribute to elevated blood pressure if altered. We hypothesized that aggravated mROS production attributable to IEX-1 deficiency would enhance vascular tone by inactivating freely available vasodilator NO, causing an increase in blood pressure. To our surprise, even in the presence of increased mROS levels in aortic tissue and in isolated VSMCs, we failed to observe any significant alteration in NO-dependent signaling and vasodilation (acetylcholine-mediated vasodilation),[3,21] consistent with the previous observation of no difference in nitrite and nitrate levels.[3] On the contrary, we observed that cAMP-dependent vasorelaxation, as determined by vascular responses to β2 adrenergic receptor agonist *isoproterenol* and adenylyl cyclase activator *forskolin*, was significantly impaired in the vasculature of these mice. This defect remained intact in endothelium-denuded aortic vessel, suggesting a potential defect in VSMCs.[21] The cAMP production required for vascular relaxation is dependent on net adenylyl cyclase activity, which is tightly regulated by G proteins. Although stimulatory G proteins activate the enzyme, inhibitory proteins (G-protein α subunit (inhibitory) 2 or 3) inhibit its activity to reduce cAMP production. In this regard, ROS has been shown to specifically upregulate G-protein α subunit (inhibitory) transcription in spontaneously hypertensive rats by increasing its promoter activity via the reduction-oxidation–sensitive NF-κB and nuclear factor erythroid 2–related factor transcription factors.[17,62,63] Thus, to delineate the underlying mechanism of reduced cAMP-dependent vasorelaxation in the absence of IEX-1, we determined the expression levels of G-protein α subunit (inhibitory) in the vasculature of IEX-1–deficient mice. The molecular, cellular, and vascular analysis revealed that the IEX-1 deficiency markedly increased G-protein α subunit (inhibitory) 2 expression level in an mROS-dependent manner. Thus, inhibition of G-protein α subunit (inhibitory) 2 with pertussis toxin not only restored cAMP signaling and cAMP-dependent vasodilation, but also attenuated established hypertension in IEX-1–deficient mice (Figure 3).[21] The lack of vascular inflammation and intact NO signaling in these mice might explain why the hypertension in IEX-1 deficiency does not deteriorate over time. Collectively, these studies provide novel insight into how a defective mitochondrial function can induce hypertension, helping uncover an inflammation-independent mechanism in the onset of high blood pressure. Further study of the hypertension in IEX-1–deficient mice may help us understand how inflammation interacts with and
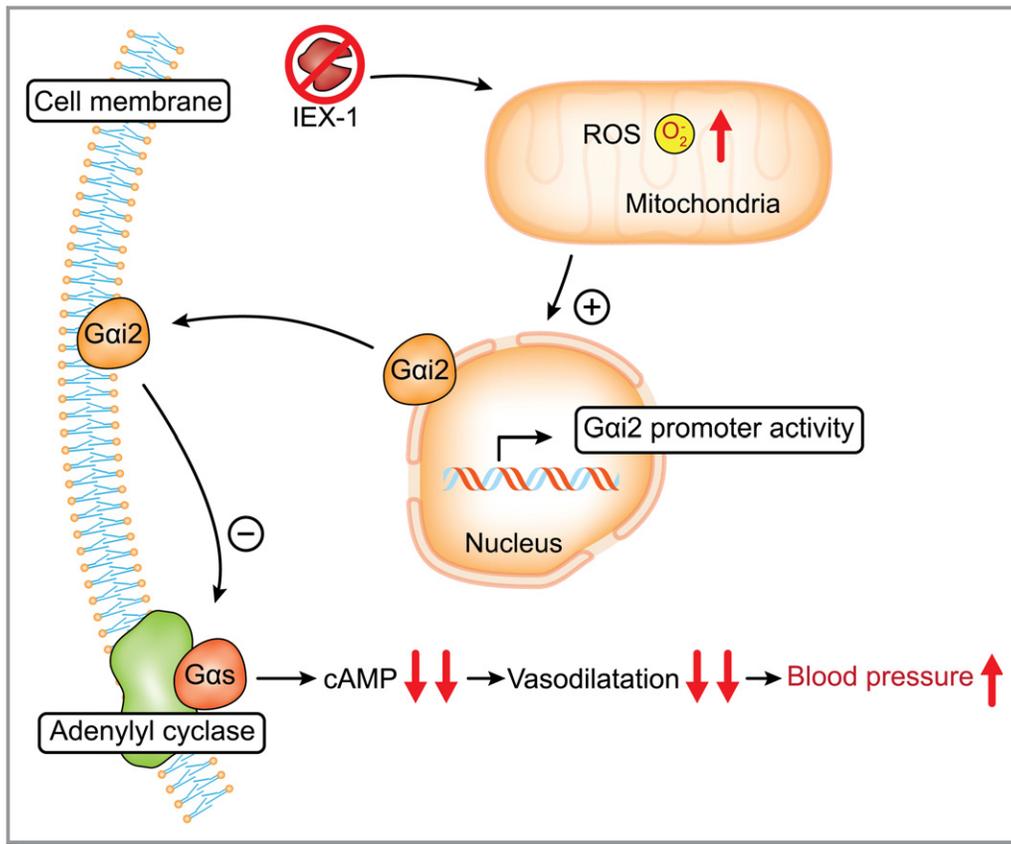
Figure 3. Proposed mechanism of hypertension induced by immediate early response gene X-1 (IEX-1) deficiency. IEX-1 deficiency upregulates G-protein α subunit (inhibitory) 2 (Gαi2) expression in a reactive oxygen species (ROS)–dependent manner at mitochondria. Overproduction of Gαi2 inhibits adenylyl cyclase–mediated synthesis of cAMP, decreasing cAMP-dependent vascular smooth muscle cell relaxation. This impairs vasodilation, causing an increase in systemic blood pressure (hypertension). Gαs indicates G-protein α subunit (stimulatory); O_2^-, superoxide radicals.

aggravates systemic hypertension. Taken together, the afore-mentioned studies provide strong evidence for a protective role of IEX-1 in cardiovascular tissues and identify IEX-1 as a novel therapeutic and diagnostic target for vascular and ischemic heart diseases.

IEX-1 Contributes to Diet-Induced Obesity and Insulin Resistance

The observation that IEX-1–deficient mice develop sustained hypertension with little or no sign of vascular or renal inflammation[21] led us to speculate the possible involvement of IEX-1 in inflammation. In addition, IEX-1 is one of the major direct downstream transcriptional targets of NF-κB, a central mediator of inflammation.[5–7] IEX-1 expression and many of its reported functions could be blocked by inhibiting the NF-κB pathway,[5–7] identifying activation of NF-κB as a necessary event in the induction of IEX-1 expression. Like NF-κB, IEX-1 is also capable of manipulating mutually exclusive processes, positively and negatively both (eg, cell survival versus cell death

or cell division versus cell cycle arrest, depending on the cell types).[5] Along this line, our group has previously reported that IEX-1 paucity impaired the ability of macrophages and T cells to induce inflammation in response to many stimuli, such as *Leishmania major* infection and dextran sodium sulfate–induced colitis.[64,65] Collectively, these data suggest that IEX-1 may be an inherent regulator of inflammation. Because NF-κB is the central inflammatory mediator that plays a critical role in the pathogenesis of lipid-driven inflammation and diet-induced insulin resistance, we reasoned that IEX-1 could be a down-stream mediator of NF-κB activation in metabolic disorder.

In a continued effort to explore the physiological roles of IEX-1, we discovered a key role of IEX-1 under metabolic stress. Although mice lacking IEX-1 exhibited normal metabolism on normal diet, they were remarkably resistant to high-fat diet (HFD)–induced insulin resistance and were protected against adipose and hepatic inflammation.[20] Unexpectedly, IEX-1–deficient mice were also highly resistant to HFD-induced weight gain. After 20 weeks of HFD consumption, knockout mice gained only ≈40% in their body weight,

whereas their wild-type littermates increased their body weight by ≈90% on the same diet.[20] Indirect calorimetry further revealed that lean phenotype of IEX-1–deficient mice on HFD was associated with enhanced energy expenditure, suggesting that IEX-1 paucity inhibits obesity development by increasing energy use. IEX-1–deficient mice grew and weighed normally when fed a regular diet, indicating that IEX-1 has little/no impact on energy metabolism under steady-state condition. To gain an insight into the underlying cellular mechanism in lean phenotype of IEX-1–deficient mice, we analyzed WAT of these mice, where we observed a significant increase in IEX-1 expression after HFD feeding. Consistent with other reports,[66–68] HFD strongly suppressed (Uncoupling Protein 1) UCP1 expression in WAT of wild-type mice, an effect that was not observed in knockout mice. Increased UCP1 expression in knockout mice was concomitant with induction of thermogenic genes and appearance of multilobular adipocytes, a hallmark of adipocyte beiging (browning).[66] These data provided initial cues suggesting that IEX-1 deficiency increases energy expenditure, likely by inducing adipocyte beiging.[66,69]

How IEX-1 deficiency induces beiging is not known yet. Our investigation has provided preliminary evidence that it could be linked to adipose tissue macrophages (ATMs). It has been well documented that HFD induces a robust transition in ATM phenotype from M2- to M1-like state, drastically decreasing the number of resident M2 macrophages in adipose tissue. We also observed such M2 to M1 switch in ATM phenotype of wild-type mice on HFD feeding. In sharp contrast, we failed to observe this phenomenon in IEX-1–deficient mice even after 20 weeks of HFD feeding. Thus, knockout mice sustained their M2-like macrophages in the adipose tissue, unlike wild-type littermates that lost a majority of those cells on HFD.[20] Sustenance of M2-like macrophages in WAT is likely the underlying mechanism by which IEX-1 paucity induces beiging because M2 macrophages are potent inducers of beiging program.[70] As opposed to M1-like macrophages, M2 polarization increases the production of catecholamines by increasing the expression of catecholamine-synthesizing enzymes tyrosine hydroxylase, dopa decarboxylase, and dopamine β-hydroxylase. Catecholamines released by M2-like cells present in WAT act on the surrounding adipocytes and induce beiging program.[70,71] On the basis of these findings, we propose that macrophage-specific IEX-1 is required for HFD-induced obesity and that preventing M2 polarization should reverse lean phenotype of IEX-1–deficient mice. This notion is the subject of current investigations in our laboratory.

IEX-1 exerts a strong survival action in several cell types, particularly under physiological conditions, as observed in vivo.[15,29–33] The observation that IEX-1 deficiency prevents HFD-induced M2 to M1 transition in ATMs suggests that IEX-1 promotes the polarization and survival of M1 macrophages as opposed to M2 cells.[20] This is in agreement with its differential role in T cells in a subset-specific manner. For example, IEX-1 deficiency protected mice against dextran sodium sulfate–induced colitis in mice by enhancing apoptosis specifically in proinflammatory type 1 helper T cells while promoting survival of the anti-inflammatory type 17 helper T cells.[65] Collectively, these data suggest that IEX-1 in general promotes the survival of proinflammatory immune cells and thereby contributes to inflammatory reaction. How IEX-1 exerts disparate effects in different subsets of T cells and macrophages is not known yet. In our preliminary investigation, we have found that HFD feeding in mice increases IEX-1 expression selectively in M1 ATMs but not M2 cells. Given a survival action of IEX-1, its increased expression in M1 as opposed to M2 may be the underlying mechanism by which HFD feeding promotes M1 polarization. As a result, IEX-1 deficiency prevents HFD-induced M2 to M1 transition and resists the development of obesity.[20] Similar to its action in macrophages, overexpression of IEX-1 increases the survival of cardiac myocytes by inhibiting ROS production and thereby reduces ischemia-reperfusion injury in mice.[61] Thus, selective expression of IEX-1 in different subsets of cells in response to stress, such HFD feeding or ischemia, may explain how IEX-1 exerts disparate effects in different pathological conditions. Our current investigation emphasizes a crucial role of IEX-1 in metabolic regulation of macrophage function and inflammatory process. Macrophages play a critical role in many lipid-driven inflammatory conditions, such as atherosclerosis, obesity, and type 2 diabetes mellitus. Understanding how IEX-1 controls macrophage phenotype to modulate inflammation and metabolism will provide novel insights into the pathogenesis of these diseases and may help develop novel therapeutic targets. This line of investigation becomes even more important when considering that inflammation and inflammatory pathways are increasingly targeted for therapeutics in treatment of cardiovascular disorders.

Potential Roles in Other Cardiovascular System–Related Disease Conditions

Myelodysplastic Syndrome

MDS is a heterogeneous disorder characterized by impaired hematopoiesis and altered peripheral blood cell morphological features that may progress to acute myeloid leukemia. First evidence for a potential role of IEX-1 in MDS emerged from a pioneer clinical study demonstrating a strong downregulation of IEX-1 expression in CD34^{+} stem cells in ≈60% of patients with MDS compared with healthy donors.[72,73] Interestingly, this aberrant IEX-1 expression was most prominent in the patients with low-risk or early stage of the disease and was associated with increased intramedullary apoptosis.

Subsequently, by using IEX-1–deficient mice, our group demonstrated that impaired IEX-1 expression in MDS contributed to, rather than being a consequence of, the disease. Specifically, IEX-1 deficiency induced apoptosis and a decrease in the proportion of hematopoietic stem cells (HSCs) in mice without affecting the absolute number under basal conditions.[74] However, transplantation of IEX-1–deficient bone marrow cells in wild-type mice or exposure of IEX-1–deficient mice to nonmyeloablative irradiation produced changes resembling MDS, such as thrombocytopenia, anemia, and dysplastic bone marrow morphological features. Similar to the observation in patients with MDS, IEX-1 deficiency was associated with abnormal increase in apoptosis accompanied by excessive proliferation in HSCs,[72–74] providing cues for how restricted hematopoiesis and impaired myeloid progenitor differentiation occur in patients with MDS, despite the enhanced repopulation capability of HSCs. These findings highlighted a previously unknown role of IEX-1 in maintenance of HSC quiescence and in the differentiation of multiple progenitors, including erythropoiesis and thrombopoiesis. Further mechanistic studies are warranted to raise the potential of IEX-1 as a promising therapeutic or diagnostic target in MDS. For example, studies should determine how IEX-1 expression decreases in stem cells in MDS, although no mutation or rearrangements in the gene are documented. Moreover, how IEX-1 exerted a myeloid-biased effect without affecting lymphoid lineage remains to be determined.

Lipopolysaccharide-Induced Sepsis

Sepsis is associated with multiple organ failure as a result of overactive systemic inflammation attributable to severe infection. It is the leading cause of mortality in the intensive care unit. Ramsey and Wu recently reported that mice lacking IEX-1 were susceptible to lipopolysaccharide-induced endotoxemia, liver and kidney damage, and subsequent death.[16] Because IEX-1 deficiency increases mROS production, they treated mice with mitochondrial-specific antioxidant MitoQ to determine a role of ROS in aggravated phenotype of IEX-1–deficient mice in response to lipopolysaccharide. Treatment with MitoQ protected knockout mice against pancytopenia and multiple organ failure and decreased the mortality rate.[16] These data suggest the downregulation of IEX-1 expression, and the consequent increase in mROS production may contribute to the cause of sepsis. Thus, targeting the IEX-1–mROS pathway may be a useful strategy for ameliorating sepsis and associated complications.

Summary and Future Direction

Despite a growing consensus supporting the causative role for inflammation in the development of hypertension, it is uncertain whether these 2 disorders are causally related or whether hypertension can develop independently of inflammation. The finding that persistent, but moderate, systemic hypertension developed in the absence of IEX-1, concurrent with little sign of inflammation, raises an intriguing possibility. It is that vascular inflammation is not required to initiate the pathogenesis of hypertension, but that it is involved in the full development of this disorder. Therefore, further investigation of the underlying molecular mechanisms in IEX-1 deficiency–induced hypertension may improve our understanding of this highly prevalent disease. Furthermore, besides its crucial role in mitochondrial respiration and regulation of apoptosis, for which it was originally discovered, IEX-1 appeared to play a significant role in diverse cellular functions in a variety of tissue types, including systemic blood vessels, WAT, macrophages, and HSCs, as we discussed above in this review. Macrophages are a major source of active inflammation associated with various chronic inflammatory diseases, such as obesity, atherosclerosis, hypertension, and bone loss. Although the role of macrophages in inflammatory conditions is undisputed, it is crucial to investigate for the factors that critically regulate their phenotype. Investigation from our laboratory suggests that IEX-1 could be one such factor that is required for classic activation of macrophages specifically in context of metabolic disorder. Understanding how IEX-1 regulates macrophage phenotype and modulates inflammatory and metabolic processes may offer promising opportunities for the development of novel therapeutic or diagnostic approaches for these disorders. Furthermore, findings from our laboratory using IEX-1–deficient mice raised many critical questions. For instance, how does IEX-1 deficiency render macrophages and T cells susceptible to apoptosis in a subset-dependent manner? What are the upstream mediators and downstream targets of IEX-1 in lipid-driven stress and inflammation? Answers to these fundamental questions will advance our understanding of how IEX-1 contributes to lipid-driven inflammatory conditions and metabolic disorder and may help raise the potential of IEX-1 as a potential therapeutic target for diseases, such as diabetes mellitus, obesity, and atherosclerosis.

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References

1. Feldmann KA, Pittelkow MR, Roche PC, Kumar R, Grande JP. Expression of an immediate early gene, IEX-1, in human tissues. *Histochem Cell Biol*. 2001;115:489–497.

2. Schilling D, Pittelkow MR, Kumar R. IEX-1, an immediate early gene, increases the rate of apoptosis in keratinocytes. *Oncogene*. 2001;20:7992–7997.

3. Sommer SL, Berndt TJ, Frank E, Patel JB, Redfield MM, Dong X, Griffin MD, Grande JP, van Deursen JM, Sieck GC, Romero JC, Kumar R. Elevated blood pressure and cardiac hypertrophy after ablation of the gly96/IEX-1 gene. *J Appl Physiol (1985)*. 2006;100:707–716.

4. Wu MX. Roles of the stress-induced gene IEX-1 in regulation of cell death and oncogenesis. *Apoptosis*. 2003;8:11–18.

5. Wu MX, Ao Z, Prasad KV, Wu R, Schlossman SF. IEX-1L, an apoptosis inhibitor involved in NF-kappaB-mediated cell survival. *Science*. 1998;281:998–1001.

6. Schulze PC, de Keulenaer GW, Kassik KA, Takahashi T, Chen Z, Simon DI, Lee RT. Biomechanically induced gene iex-1 inhibits vascular smooth muscle cell proliferation and neointima formation. *Circ Res*. 2003;93:1210–1217.

7. De Keulenaer GW, Wang Y, Feng Y, Muangman S, Yamamoto K, Thompson JF, Turi TG, Landschutz K, Lee RT. Identification of IEX-1 as a biomechanically controlled nuclear factor-kappaB target gene that inhibits cardiomyocyte hypertrophy. *Circ Res*. 2002;90:690–696.

8. Kondratyev AD, Chung KN, Jung MO. Identification and characterization of a radiation-inducible glycosylated human early-response gene. *Cancer Res*. 1996;56:1498–1502.

9. Das DK, Engelman RM, Kimura Y. Molecular adaptation of cellular defences following preconditioning of the heart by repeated ischaemia. *Cardiovasc Res*. 1993;27:578–584.

10. Zimmermann R, Andres J, Brand T, Frass O, Kluge A, Knoll R, Vogt A, Schaper W. Cardiac gene expression after brief coronary occlusion. *Z Kardiol*. 1995;84 (suppl 4):159–165.

11. Aebert H, Cornelius T, Ehr T, Holmer SR, Birnbaum DE, Riegger GA, Schunkert H. Expression of immediate early genes after cardioplegic arrest and reperfusion. *Ann Thorac Surg*. 1997;63:1669–1675.

12. Wu MX, Ustyugova IV, Han L, Akilov OE. Immediate early response gene X-1, a potential prognostic biomarker in cancers. *Exp Opinion Ther Targets*. 2013;17:593–606.

13. Shen L, Guo J, Santos-Berrios C, Wu MX. Distinct domains for anti- and pro-apoptotic activities of IEX-1. *J Biol Chem*. 2006;281:15304–15311.

14. Shen J, Chandrasekharan UM, Ashraf MZ, Long E, Morton RE, Liu Y, Smith JD, DiCorleto PE. Lack of mitogen-activated protein kinase phosphatase-1 protects ApoE-null mice against atherosclerosis. *Circ Res*. 2010;106:902–910.

15. Shen L, Zhi L, Hu W, Wu MX. IEX-1 targets mitochondrial F1Fo-ATPase inhibitor for degradation. *Cell Death Differ*. 2009;16:603–612.

16. Ramsey H, Wu MX. Mitochondrial anti-oxidant protects IEX-1 deficient mice from organ damage during endotoxemia. *Int Immunopharmacol*. 2014;23:658–663.

17. Nishida M, Maruyama Y, Tanaka R, Kontani K, Nagao T, Kurose H. G alpha(i) and G alpha(o) are target proteins of reactive oxygen species. *Nature*. 2000;408:492–495.

18. Cakir Y, Ballinger SW. Reactive species-mediated regulation of cell signaling and the cell cycle: the role of MAPK. *Antioxid Redox Signal*. 2005;7:726–740.

19. Haddad JJ. Antioxidant and prooxidant mechanisms in the regulation of redox (y)-sensitive transcription factors. *Cell Signal*. 2002;14:879–897.

20. Shahid M, Javed AA, Chandra D, Ramsey HE, Shah D, Khan MF, Zhao L, Wu MX. IEX-1 deficiency induces browning of white adipose tissue and resists diet-induced obesity. *Sci Rep*. 2016;6:24135.

21. Shahid M, Shen L, Seldin DC, Lu B, Ustyugova IV, Chen X, Zapol WM, Wu MX. Impaired 3′,5′-cyclic adenosine monophosphate-mediated signaling in immediate early responsive gene X-1-deficient vascular smooth muscle cells. *Hypertension*. 2010;56:705–712.

22. Zhi L, Ustyugova IV, Chen X, Zhang Q, Wu MX. Enhanced Th17 differentiation and aggravated arthritis in IEX-1-deficient mice by mitochondrial reactive oxygen species-mediated signaling. *J Immunol*. 2012;189:1639–1647.

23. Green DR, Kroemer G. The pathophysiology of mitochondrial cell death. *Science*. 2004;305:626–629.

24. Kadenbach B, Arnold S, Lee I, Huttemann M. The possible role of cytochrome C oxidase in stress-induced apoptosis and degenerative diseases. *Biochim Biophys Acta*. 2004;1655:400–408.

25. Atwal KS, Wang P, Rogers WL, Sleph P, Monshizadegan H, Ferrara FN, Traeger S, Green DW, Grover GJ. Small molecule mitochondrial F1F0 ATPase hydrolase inhibitors as cardioprotective agents: identification of 4-(N-arylimidazole)-substituted benzopyran derivatives as selective hydrolase inhibitors. *J Med Chem*. 2004;47:1081–1084.

26. Campanella M, Casswell E, Chong S, Farah Z, Wieckowski MR, Abramov AY, Tinker A, Duchen MR. Regulation of mitochondrial structure and function by the F1Fo-ATPase inhibitor protein, IF1. *Cell Metab*. 2008;8:13–25.

27. Luciakova K, Kuzela S. Increased content of natural ATPase inhibitor in tumor mitochondria. *FEBS Lett*. 1984;177:85–88.

28. Arlt A, Schafer H. Role of the immediate early response 3 (IER3) gene in cellular stress response, inflammation and tumorigenesis. *Eur J Cell Biol*. 2011;90:545–552.

29. Zhang Y, Finegold MJ, Porteu F, Kanteti P, Wu MX. Development of T-cell lymphomas in Emu-IEX-1 mice. *Oncogene*. 2003;22:6845–6851.

30. Zhang Y, Schlossman SF, Edwards RA, Ou CN, Gu J, Wu MX. Impaired apoptosis, extended duration of immune responses, and a lupus-like autoimmune disease in IEX-1-transgenic mice. *Proc Natl Acad Sci USA*. 2002;99:878–883.

31. Mittal A, Papa S, Franzoso G, Sen R. NF-kappaB-dependent regulation of the timing of activation-induced cell death of T lymphocytes. *J Immunol*. 2006;176:2183–2189.

32. Garcia J, Ye Y, Arranz V, Letourneux C, Pezeron G, Porteu F. IEX-1: a new ERK substrate involved in both ERK survival activity and ERK activation. *EMBO J*. 2002;21:5151–5163.

33. Hamelin V, Letourneux C, Romeo PH, Porteu F, Gaudry M. Thrombopoietin regulates IEX-1 gene expression through ERK-induced AML1 phosphorylation. *Blood*. 2006;107:3106–3113.

34. Jezek P, Hlavata L. Mitochondria in homeostasis of reactive oxygen species in cell, tissues, and organism. *Int J Biochem Cell Biol*. 2005;37:2478–2503.

35. Matsuzawa A, Ichijo H. Stress-responsive protein kinases in redox-regulated apoptosis signaling. *Antioxid Redox Signal*. 2005;7:472–481.

36. Sauer H, Wartenberg M, Hescheler J. Reactive oxygen species as intracellular messengers during cell growth and differentiation. *Cell Physiol Biochem*. 2001;11:173–186.

37. Morgan MJ, Liu ZG. Crosstalk of reactive oxygen species and NF-kappaB signaling. *Cell Res*. 2011;21:103–115.

38. Ushio-Fukai M. VEGF signaling through NADPH oxidase-derived ROS. *Antioxid Redox Signal*. 2007;9:731–739.

39. Pawlikowska P, Leray I, de Laval B, Guihard S, Kumar R, Rosselli F, Porteu F. ATM-dependent expression of IEX-1 controls nuclear accumulation of Mcl-1 and the DNA damage response. *Cell Death Differ*. 2010;17:1739–1750.

40. Arlt A, Grobe O, Sieke A, Kruse ML, Folsch UR, Schmidt WE, Schafer H. Expression of the NF-kappa B target gene IEX-1 (p22/PRG1) does not prevent cell death but instead triggers apoptosis in Hela cells. *Oncogene*. 2001;20:69–76.

41. Arlt A, Kruse ML, Breitenbroich M, Gehrz A, Koc B, Minkenberg J, Folsch UR, Schafer H. The early response gene IEX-1 attenuates NF-kappaB activation in 293 cells, a possible counter-regulatory process leading to enhanced cell death. *Oncogene*. 2003;22:3343–3351.

42. Grobe O, Arlt A, Ungefroren H, Krupp G, Folsch UR, Schmidt WE, Schafer H. Functional disruption of IEX-1 expression by concatemeric hammerhead ribozymes alters growth properties of 293 cells. *FEBS Lett*. 2001;494:196–200.

43. Osawa Y, Nagaki M, Banno Y, Brenner DA, Nozawa Y, Moriwaki H, Nakashima S. Expression of the NF-kappa B target gene X-ray-inducible immediate early response factor-1 short enhances TNF-alpha-induced hepatocyte apoptosis by inhibiting Akt activation. *J Immunol*. 2003;170:4053–4060.

44. Segev DL, Ha TU, Tran TT, Kenneally M, Harkin P, Jung M, MacLaughlin DT, Donahoe PK, Maheswaran S. Mullerian inhibiting substance inhibits breast cancer cell growth through an NFkappa B-mediated pathway. *J Biol Chem*. 2000;275:28371–28379.

45. Segev DL, Hoshiya Y, Hoshiya M, Tran TT, Carey JL, Stephen AE, MacLaughlin DT, Donahoe PK, Maheswaran S. Mullerian-inhibiting substance regulates NF-kappa B signaling in the prostate in vitro and in vivo. *Proc Nat Acad Sci USA*. 2002;99:239–244.

46. Segev DL, Hoshiya Y, Stephen AE, Hoshiya M, Tran TT, MacLaughlin DT, Donahoe PK, Maheswaran S. Mullerian inhibiting substance regulates NFkappaB signaling and growth of mammary epithelial cells in vivo. *J Biol Chem*. 2001;276:26799–26806.

47. Kruse ML, Arlt A, Sieke A, Grohmann F, Grossmann M, Minkenberg J, Folsch UR, Schafer H. Immediate early gene X1 (IEX-1) is organized in subnuclear structures and partially co-localizes with promyelocytic leukemia protein in HeLa cells. *J Biol Chem*. 2005;280:24849–24856.

48. Yamashita K, Nakashima S, You F, Hayashi S, Iwama T. Overexpression of immediate early gene X-1 (IEX-1) enhances gamma-radiation-induced apoptosis of human glioma cell line, U87-MG. *Neuropathology*. 2009;29:20–24.

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49. Xu RH, Pelicano H, Zhou Y, Carew JS, Feng L, Bhalla KN, Keating MJ, Huang P. Inhibition of glycolysis in cancer cells: a novel strategy to overcome drug resistance associated with mitochondrial respiratory defect and hypoxia. *Cancer Res*. 2005;65:613–621.

50. Carew JS, Huang P. Mitochondrial defects in cancer. *Mol Cancer*. 2002;1:9.

51. Semenza GL, Artemov D, Bedi A, Bhujwalla Z, Chiles K, Feldser D, Laughner E, Ravi R, Simons J, Taghavi P, Zhong H. "The metabolism of tumours": 70 years later. *Novartis Found Symp*. 2001;240:251–260; discussion 260–264.

52. Gerin I, Louis GW, Zhang X, Prestwich TC, Kumar TR, Myers MG Jr, Macdougald OA, Nothnick WB. Hyperphagia and obesity in female mice lacking tissue inhibitor of metalloproteinase-1. *Endocrinology*. 2009;150:1697–1704.

53. Nys K, Van Laethem A, Michiels C, Rubio N, Piette JG, Garmyn M, Agostinis P. A p38(MAPK)/HIF-1 pathway initiated by UVB irradiation is required to induce Noxa and apoptosis of human keratinocytes. *J Invest Dermatol*. 2010;130:2269–2276.

54. Zhao Y, Altman BJ, Coloff JL, Herman CE, Jacobs SR, Wieman HL, Wofford JA, Dimascio LN, Ilkayeva O, Kelekar A, Reya T, Rathmell JC. Glycogen synthase kinase 3alpha and 3beta mediate a glucose-sensitive antiapoptotic signaling pathway to stabilize Mcl-1. *Mol Cell Biol*. 2007;27:4328–4339.

55. Cross A, Moots RJ, Edwards SW. The dual effects of TNFalpha on neutrophil apoptosis are mediated via differential effects on expression of Mcl-1 and Bfl-1. *Blood*. 2008;111:878–884.

56. Wensveen FM, Alves NL, Derks IA, Reedquist KA, Eldering E. Apoptosis induced by overall metabolic stress converges on the Bcl-2 family proteins Noxa and Mcl-1. *Apoptosis*. 2011;16:708–721.

57. Rocher G, Letourneux C, Lenormand P, Porteu F. Inhibition of B56-containing protein phosphatase 2As by the early response gene IEX-1 leads to control of Akt activity. *J Biol Chem*. 2007;282:5468–5477.

58. Letourneux C, Rocher G, Porteu F. B56-containing PP2A dephosphorylate ERK and their activity is controlled by the early gene IEX-1 and ERK. *EMBO J*. 2006;25:727–738.

59. Prall WC, Czibere A, Grall F, Spentzos D, Steidl U, Giagounidis AAN, Kuendgen A, Otu H, Rong A, Libermann TA, Germing U, Gattermann N, Haas R, Aivado M. Differential gene expression of bone marrow-derived CD34+ cells is associated with survival of patients suffering from myelodysplastic syndrome. *Int J Hematol*. 2009;89:173–187.

60. Ohki R, Yamamoto K, Mano H, Lee RT, Ikeda U, Shimada K. Identification of mechanically induced genes in human monocytic cells by DNA microarrays. *J Hypertens*. 2002;20:685–691.

61. Xu MJ, Cai Y, Qu A, Shyy JY, Li W, Wang X. Immediate early response gene X-1 (IEX-1) mediates ischemic preconditioning-induced cardioprotection in rats. *Oxid Med Cell Longev*. 2017;2017:6109061.

62. Lappas G, Daou GB, Anand-Srivastava MB. Oxidative stress contributes to the enhanced expression of Gialpha proteins and adenylyl cyclase signaling in vascular smooth muscle cells from spontaneously hypertensive rats. *J Hypertens*. 2005;23:2251–2261.

63. Arinze IJ, Kawai Y. Transcriptional activation of the human Galphai2 gene promoter through nuclear factor-kappaB and antioxidant response elements. *J Biol Chem*. 2005;280:9786–9795.

64. Akilov OE, Ustyugova IV, Zhi L, Hasan T, Wu MX. Enhanced susceptibility to Leishmania infection in resistant mice in the absence of immediate early response gene X-1. *J Immunol*. 2009;183:7994–8003.

65. Ustyugova IV, Zhi L, Wu MX. Reciprocal regulation of the survival and apoptosis of Th17 and Th1 cells in the colon. *Inflamm Bowel Dis*. 2012;18:333–343.

66. Rao RR, Long JZ, White JP, Svensson KJ, Lou J, Lokurkar I, Jedrychowski MP, Ruas JL, Wrann CD, Lo JC, Camera DM, Lachey J, Gygi S, Seehra J, Hawley JA, Spiegelman BM. Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis. *Cell*. 2014;157:1279–1291.

67. Chiang SH, Bazuine M, Lumeng CN, Geletka LM, Mowers J, White NM, Ma JT, Zhou J, Qi N, Westcott D, Delproposto JB, Blackwell TS, Yull FE, Saltiel AR. The protein kinase IKKepsilon regulates energy balance in obese mice. *Cell*. 2009;138:961–975.

68. Zhang X, Xu A, Chung SK, Cresser JH, Sweeney G, Wong RL, Lin A, Lam KS. Selective inactivation of c-Jun NH2-terminal kinase in adipose tissue protects against diet-induced obesity and improves insulin sensitivity in both liver and skeletal muscle in mice. *Diabetes*. 2011;60:486–495.

69. Lee MW, Odegaard JI, Mukundan L, Qiu Y, Molofsky AB, Nussbaum JC, Yun K, Locksley RM, Chawla A. Activated type 2 innate lymphoid cells regulate beige fat biogenesis. *Cell*. 2015;160:74–87.

70. Nguyen KD, Qiu Y, Cui X, Goh YP, Mwangi J, David T, Mukundan L, Brombacher F, Locksley RM, Chawla A. Alternatively activated macrophages produce cate-cholamines to sustain adaptive thermogenesis. *Nature*. 2011;480:104–108.

71. Qiu Y, Nguyen KD, Odegaard JI, Cui X, Tian X, Locksley RM, Palmiter RD, Chawla A. Eosinophils and type 2 cytokine signaling in macrophages orchestrate development of functional beige fat. *Cell*. 2014;157:1292–1308.

72. Steensma DP, Neiger JD, Porcher JC, Keats JJ, Bergsagel PL, Dennis TR, Knudson RA, Jenkins RB, Santana-Davila R, Kumar R, Ketterling RP. Rearrangements and amplification of IER3 (IEX-1) represent a novel and recurrent molecular abnormality in myelodysplastic syndromes. *Cancer Res*. 2009;69:7518–7523.

73. Hofmann WK, de Vos S, Komor M, Hoelzer D, Wachsman W, Koeffler HP. Characterization of gene expression of CD34+ cells from normal and myelodysplastic bone marrow. *Blood*. 2002;100:3553–3560.

74. Ramsey H, Zhang Q, Brown DE, Steensma DP, Lin CP, Wu MX. Stress-induced hematopoietic failure in the absence of immediate early response gene X-1 (IEX-1, IER3). *Haematologica*. 2014;99:282–291.

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