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REVIEWS: CURRENT TOPICS

Mechanisms of high glucose-induced apoptosis and its relationship to diabetic complications

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Abstract

Cellular responses to high glucose are numerous and varied but ultimately result in functional changes and, often, cell death. High glucose induces oxidative and nitrosative stress in many cell types causing the generation of species such as superoxide, nitric oxide and peroxynitrite and their derivatives. The role of these species in high glucose-mediated apoptotic cell death is relevant to the complications of diabetes such as neuropathy, nephropathy and cardiovascular disease. High glucose causes activation of several proteins involved in apoptotic cell death, including members of the caspase and Bcl-2 families. These events and the relationship between high glucose-induced oxidative stress and apoptosis are discussed here with reference to additional regulators of apoptosis such as the mitogen-activated protein kinases (MAPKs) and cell-cycle regulators.
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Keywords: High glucose-induced apoptosis; Diabetic complications; Mitogen-activated protein kinases; Cell-cycle regulators

1. Overview

The prevalence of type 2 diabetes is increasing at an alarming rate, regardless of the population studied. The number of people with diabetes worldwide is expected to rise to well over 200 million by 2010 [1]. Perhaps more alarmingly still, cases of children with type 2 diabetes have been reported in Japan, the United Kingdom, the United States, Australia and elsewhere. Clearly, type 2 diabetes presents one of the major threats to global health in the 21st century. With the likelihood of complications of diabetes occurring at a far younger age than at present, knowledge of the body's response to glucose challenge is urgently needed. A number of important epidemiological studies have highlighted the relationship between hyperglycaemia and an increased risk of cardiovascular disease. Impaired glucose tolerance and the metabolic syndrome often lead to development of type 2 diabetes, although this is not universal. Micro- and macrovascular disease can be traced back to hyperglycaemia and the metabolic syndrome. The risk of cardiovascular disease is increased threefold in patients with established metabolic syndrome [2].

At the cellular level, much is now known about the deleterious effects of high ambient glucose concentrations, and, although the mechanisms seem complex, a number of key concepts are prevalent. Although many signalling intermediates contribute to high glucose-induced changes in cellular phenotype such as hypertrophy and altered function, it is their role as mediators of apoptosis that has received much attention in recent years. Cross-talk between an expanding network of intracellular proteins coupled with the variability of responses in different tissues has added to the complexity. However, a direct causal role for hyperglycaemia, osmotic and oxidative stress, activation and/or inhibition of intracellular signalling intermediates such as MAPKs in the overall pathogenesis of diabetes is as yet unproven.

2. Mechanisms of high glucose-induced cell death

2.1. High glucose-induced oxidative and nitrosative stress

One of the earliest detectable responses of a cell to high glucose challenge is the generation of reactive oxygen and nitrogen species. High glucose causes oxidative and nitrosative stress in numerous cell types. Generation of superoxide

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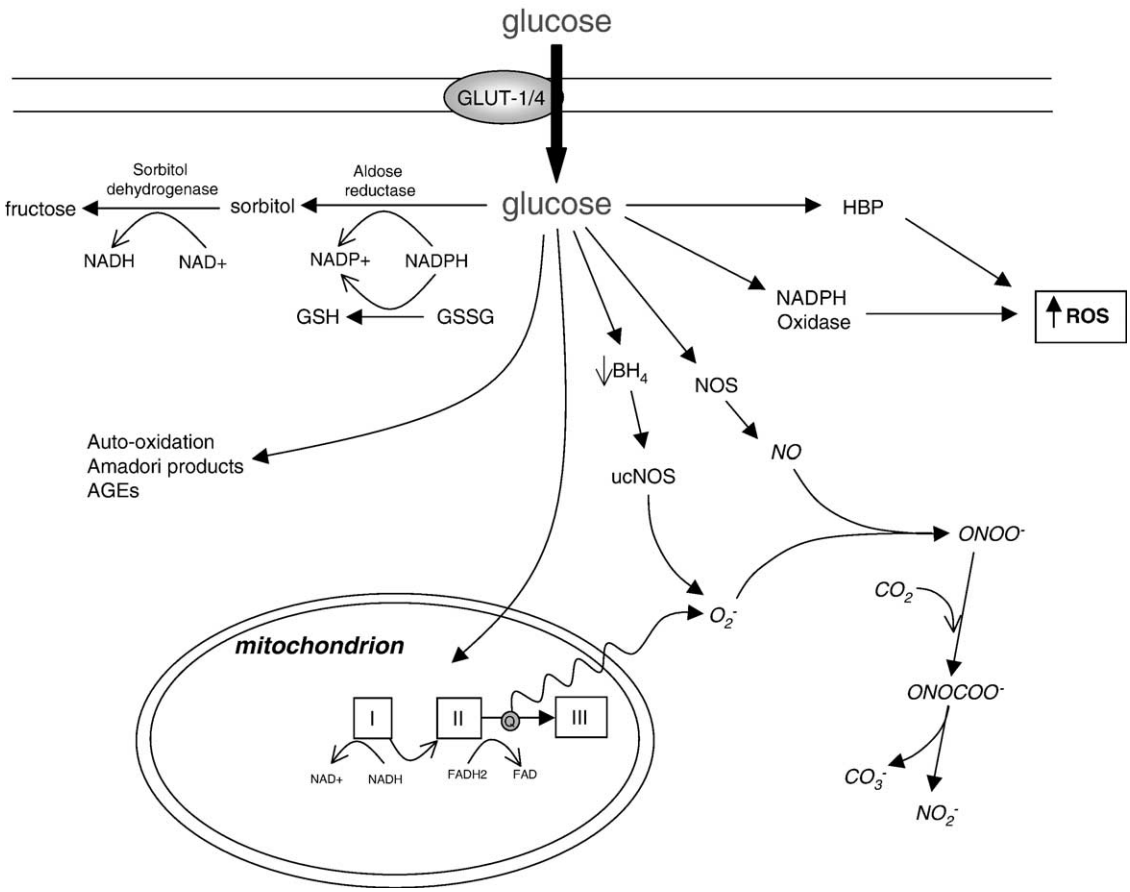

Fig. 1. Schematic diagram depicting possible routes for glucose-induced oxidative and nitrosative stress. Products of the polyol and hexosamine pathways, coupled with the activity of enzymes such as NADPH oxidase, contribute to the overall oxidative burden. The effects of glucose on mitochondrial superoxide generation and subsequent NOS activation can combine to generate peroxynitrite, a powerful oxidant. In turn, peroxynitrite can decompose to yield the carbonate anion and nitrogen dioxide. Glucose auto-oxidation and decomposition favor AGE formation that can cause further damage to cellular proteins and lipids.

by high glucose is a well-described phenomenon and arises principally via the mitochondrial electron transport chain. High glucose causes interruption of the electron transport chain at complex III, resulting in increased oxidation of molecular oxygen by coenzyme Q yielding superoxide anion (for review, see Ref. [3]). Thus, the normally efficient metabolism of glucose can, in situations of stress such as hyperglycemia, lead to excess free-radical generation and oxidative stress.

It is reasonable to assume that a combination of oxidative and nitrosative stress leads to apoptosis and necrosis of various cell types. Indeed, the concomitant generation of superoxide and nitric oxide favours the production of peroxynitrite ($ONOO^-$). Protonation of $ONOO^-$ yields peroxynitrous acid that can decompose to yield the hydroxyl radical. Oxidation of $ONOO^-$ by CO_2 generates nitrosoperoxycarbonate, an intermediate with an unknown half-life. Decomposition of this short-lived radical produces nitrogen dioxide and the carbonate anion leading to further oxidation and nitration reactions (Fig. 1).

Of the many studies to have demonstrated oxidative and nitrosative stress in cells following exposure to high ambient glucose concentrations [4–11], several have shown some beneficial effect of antioxidants. However, care should be taken when interpreting data from studies of this type. Many of the techniques used to detect free radical species such as superoxide are not specific nor are the antioxidants commonly used to scavenge them or prevent their generation. Moreover, the cellular half-life of these radicals is, at most, a few seconds given the number of possible reactants present. This is particularly true of peroxynitrite and its derivatives where oxidation and nitration reactions occur at very fast rates [12]. Despite these reservations, it is clear that oxidants do arise, albeit briefly, following exposure to high glucose, and the oxidation and nitration products can be relatively easily detected both in vivo and in vitro. The detection of 3-nitrotyrosine in tissue sections is often used as an indicator of ONOO-induced oxidative stress and cellular injury. Increased nitrotyrosine staining has been detected in the proximal tubules of patients with diabetic nephropathy [13], in the kidneys of rats following endotoxin treatment [14] and in the renal cortex of diabetic rats [15]. However, the use of 3-nitrotyrosine as a marker of ONOO-induced cellular damage is limited by the fact that

myeloperoxidase can also nitrate tyrosine residues. It has also been reported that nitration of tyrosine moieties occurs frequently due to the oxidation of nitrite by hydrogen peroxide. Peroxynitrite directly causes oxidative DNA damage such as point mutation and double-strand breaks as well as lipid peroxidation [16–18]. $ONOO^-$ was shown to activate caspases in HL-60 cells, but only caspase-3 was essential for apoptosis [19,20]. High glucose causes lipid peroxidation that can be prevented by antioxidants such as vitamin E [21], *N*-acetylcysteine and taurine [22]. In primary renal proximal tubular cells, high glucose induced lipid peroxidation via oxidative stress and was dependent on protein kinase C activity [23].

An additional source of high glucose-induced oxidative stress is via the polyol pathway. Here, glucose is reduced to sorbitol by aldose reductase in a process that consumes NADPH. Sorbitol is then converted to fructose by sorbitol dehydrogenase with the generation of NADH [24]. Thus, the consumption of NADPH impairs the NADPH-dependent generation of reduced glutathione, an essential cellular antioxidant. A lack of reduced glutathione has been proposed as one mechanism of high glucose-induced endothelial cell apoptosis. Powell et al. [25] demonstrated that high glucose reduced intracellular glutathione levels and contributed to DNA damage that could be prevented by supplementing the media with either α-lipoic acid or L-cystine. Importantly, L-cystine could not prevent the decrease in glutathione levels and DNA damage caused by an inhibitor of γ-glutamylcysteine synthetase, suggesting that, in high glucose, glutathione synthesis is dependent on the availability of cysteine. This mechanism, added to the diminished availability of NADPH for reduction of glutathione, provides another route for high glucose-induced oxidative stress. Similarly, it has been reported that high glucose-induced apoptosis in endothelial cells and retinal pericytes could be prevented by thiamine and benfotiamine [26].

High glucose-induced reactive-oxygen-species (ROS) generation increases the activity of nuclear factor-kappa B (NF-κB) in various cell types including endothelial [27], mesangial [28], pancreatic beta cells [29] and vascular smooth muscle cells [30]. This process is dependent on protein kinase C (PKC) activation, aldose reductase or ROS [31,32] and leads to apoptosis in a process that involves Bax and caspase activation. Conversely, activation of NF-κB by PI-3-kinase or p38 MAPK is anti-apoptotic in neuroblastoma cells [33]. It seems that, depending on the tissue or cell type, the mechanism by which NF-κB is activated determines its role in either cell survival or apoptosis. In macrophages, glycated albumin leads to oxidative stress-induced NF-κB activation and altered cell function, but these authors did not measure apoptosis. Interestingly, they showed that glycated albumin also activated extracellular signal-related kinase (ERK), which increased transforming growth factor-β production. Activation of MAPK by high glucose may facilitate cellular hypertrophy rather than apoptosis as has been shown in renal tubular cells [34] (see also Section 2.3).

Glucose can undergo auto-oxidation or decomposition via numerous intermediates to yield reactive dicarbonyls glyoxal, methylglyoxal and 3-deoxyglucosone [35]. These can then react with proteins to generate advanced glycation end products (AGEs) resulting in altered function that may lead, via ROS generation or NF-κB activation, to cell death. In glomerular mesangial cells, AGEs led to $p21^{WAF1}$ accumulation and growth arrest [36] as well as p38 MAPK-mediated nitric oxide synthase (NOS) expression [37]. Several studies implicate AGEs as mediators of diabetic pathophysiology including nephropathy [38], neuropathy [39] and microvascular disease [40].

2.2. Mitochondria-dependent and -independent mechanisms

Mitochondrial apoptosis is regulated by a large number of proteins that directly or indirectly activate or inhibit the activity of cysteine proteases. Several of these proteins share homology and comprise the bcl-2 family of apoptosis regulators, of which there are three main groups defined according to the number and type of Bcl-2 homology (BH) domains they contain [41]. Those that contain several BH domains (BH1-4) include the anti-apoptotic Bcl-2, Bcl-w, $Bcl\text{-}X_L$ and Mcl-1, whereas Bim, Bad and Bid contain only a single BH3 domain. The pro-apoptotic proteins Bax and Bak contain multiple BH domains. BH3-only proteins inhibit the anti-apoptotic actions of Bcl-2 directly by binding and preventing its inhibition of Bax homo-oligomer formation. Bax, a 25-kDa protein promotes mitochondrial membrane permeability, allowing the release of cytochrome *c* and formation of the apoptosome with Apaf-1. This leads to activation of caspase-9 and, subsequently, caspase-3. Some or all of these mechanisms are involved in high glucose-induced apoptosis depending on the cell type or tissue studied. The relationship between high glucose-induced oxidative stress and apoptosis is equally complex. In mesangial cells, high glucose initiates oxidative stress-induced apoptosis via Bax-mediated mitochondrial permeability and cytochrome *c* release. In addition, high glucose-induced apoptosis could be prevented by insulin-like growth factor-I (IGF-I), which caused phosphorylation of Bad at Ser^{112} in a process carried out by IGF-I-stimulated ERK [42]. This is interesting because the majority of data to date suggest that the anti-apoptotic effect of IGF-I is mediated predominantly by PI-3-kinase/Akt and is independent of MEK/ERK [43,44]. In practice, it is likely that one or both of these mechanisms is activated by IGF-I depending on cell type and duration of exposure to high glucose concentrations. Moreover, the considerable overlap between these pathways precludes definitive exclusion of MEK/ERK from the anti-apoptotic effects of IGF-I. It remains to be seen whether IGF-I-stimulated ERK plays a wider role in preventing high glucose-induced apoptosis in nonrenal cells.

High glucose causes mitochondrial membrane depolarisation and loss of uncoupling proteins (UCP), especially

UCP3, resulting in increased oxidative stress as well as release of cytochrome *c* and activation of caspases [45,46]. Uncoupling proteins are inner mitochondrial membrane proton carriers that can prevent ROS formation and maintain mitochondrial membrane potential. The role of UCPs in apoptosis is far from clear, however, and may depend on the nature of the stimuli and cell type. Ubiquitously expressed UCP2 can cause cell death in HeLa cells [47], whereas overexpression of UCP2 may protect neuronal cells against apoptosis [48]. Loss of UCP3 expression appears most closely associated with high glucose-induced apoptosis [49], and this may reflect its sensitivity to metabolic stress. Work by Du et al. [50] showed that high glucose-induced superoxide generation causes DNA strand breaks and activation of poly(ADP)-ribose polymerase (PARP). In turn, PARP inhibits glyceraldehyde phosphate dehydrogenase (GAPDH) and leads to cell death. In this model, overexpression of UCP1 prevented superoxide generation and PARP activation, illustrating the central role of mitochondrial ROS generation in high glucose-induced cell death. Indeed, high glucose-induced ROS generation and apoptotic cell death can be detected in the first 2 h of hyperglycaemia in neurons [51].

Further insight into the mechanism of oxidant-induced apoptosis comes from work by Yoon et al. [52] in which H_2O_2 induced an increase in apoptosis signal-regulating kinase-1 (ASK1) that caused down-regulation of Bcl-2, disruption of the mitochondrial membrane potential and activation of a caspase cascade. Interestingly, the antioxidant selenite blocked the apoptotic effects of ASK1 by activating PI-3-kinase/Akt pathways. Evidence for NO-induced apoptosis comes from work on gastric epithelial cells where the NO donor, NOC-18, induced mitochondrial membrane depolarisation, Bax-induced cytochrome *c* release and caspase activation. However, in this model high glucose raised intracellular ATP concentrations and resulted in resistance to apoptosis [53]. In contrast, in neuronal cells, high glucose prevented necrotic cell death whilst increasing apoptotic cell death in a PKC-dependent mechanism that involved Bax-induced cytochrome *c* release and activation of caspase-3 [54]. The effects of high glucose on apoptotic cell death appear to be independent of hyperosmolarity, which induced necrosis in endothelial cells; related effects on cell hypertrophy may involve TGFβ1 [55]. In renal tubular epithelial cells, high glucose caused a twofold increase in Bax expression and suppression of Bcl-2 expression [56]. In this study, the high glucose-induced ROS generation and apoptosis could be prevented by taurine. A similar effect of high glucose was observed in renal mesangial cells where high glucose caused activation of NF-κB that could be inhibited by antioxidants [57]. Moreover, high glucose reduced phosphorylation of Bad associated with an increase in the Bax/Bcl-2 ratio, cytochrome *c* release and caspase-3 activation. Apoptosis of pancreatic islet cells was observed in high glucose (albeit at lower concentrations to the studies above, 16.7 mM compared with 25–30 mM), and, as described before, the apoptotic mechanism involved Bax and could be prevented by an antioxidant (nicotinamide) [58]. High glucose-induced endothelial cell apoptosis has been implicated as a causal factor in the progression to atherosclerosis in diabetes. Nakagami et al. [59] showed that high glucose caused translocation of Bax to the mitochondrial membrane and subsequent caspase-3 and caspase-9 activation. Hepatocyte growth factor prevented high glucose-induced apoptosis by blocking Bax translocation from the cytosol to mitochondrial membrane, thus preventing caspase activation. The observation that high glucose induces a Bax-mediated apoptotic program extends to the retinal vasculature. High glucose activated NF-κB in retinal pericytes but not endothelial cells. Moreover, basal NF-κB appeared to be essential for survival, whereas high glucose-induced NF-κB activation is pro-apoptotic and acts via Bax [60]. The same group also showed that Bax expression was increased in vascular and neural cells of the retina in postmortem samples from diabetic patients [61]. In addition, Bax expression was increased in bovine retinal pericytes exposed to high glucose for up to 5 weeks. In a murine model of diabetes, Bax was up-regulated as early as the preimplantation blastocyst stage and caused apoptosis that appeared to be mediated, at least in part, by a process involving caspases and ceramide [62]. Moreover, Bax-deficient mice appeared resistant to hyperglycemia-induced apoptosis. Importantly, a report by Risso et al. [63] showed that intermittent high glucose (5 mM followed by 25 mM daily over 14 days) was more effective at inducing Bax-mediated apoptosis than stable high glucose (25 mM continuously), possibly reflecting the influence of high glucose on transient processes such as kinase activity. Of note, high glucose increases the expression of early response gene *c-myc* in a Ca^{2+}-dependent manner [64] and may induce an apoptotic program via c-myc-dependent mechanisms. High concentrations of glucose lead to glycation of HDL (gly-HDL), and incubation of endothelial cells with gly-HDL led to apoptosis via caspase-3 and caspase-9. The expression of both Bax and Bad was increased [65]. Ortiz et al. [66] showed that, in renal tubular epithelial cells, high glucose caused increased expression of Bax and down-regulated Bcl-2 and Bcl-X_L; results that were mirrored in vivo. Elegant studies by Russell et al. [67] demonstrated that high glucose caused early increase in ROS and mitochondrial membrane depolarisation in neurons. This led to ATP depletion and subsequent caspase-mediated apoptosis. Inhibiting ROS formation or components of the electron transfer chain could block apoptosis. High glucose can initiate apoptosis in pancreatic islet cells by transcriptional and posttranslational regulation of the BH3-only proteins Bad, Bid and, to a lesser extent, Bik [68]. In this study, expression of Bcl-X_L was much reduced compared with that of Bad and Bid. As in other studies of this type, the ratio of pro-apoptotic to anti-apoptotic Bcl-family proteins is altered in high glucose in a way that favors apoptosis.

The activity of the calcium-dependent cysteine protease calpain is increased in renal proximal tubular epithelial cells exposed to high glucose, and this precedes caspase activation (Harwood and Allen, unpublished data). Interestingly, Han et al. [69] showed that high glucose stimulated ATP-dependent Ca^{2+} uptake in the same cell type that was dependent on cAMP and protein kinase-C. It was recently reported that chronic high glucose-induced apoptosis of pancreatic β cells involved caspase-3 but not calpain 10, although there may be some degree of cross talk between caspase-3 and calpain [70]. In this study, calpain 10 mediated apoptosis induced by hypoglycaemia, reflecting the metabolic response of β cells to glucose fluctuations that may differ from that of other organs.

2.3. The role of MAPK in high glucose-induced apoptosis

The mitogen-activated protein kinases (MAPK) comprise a group of serine/threonine kinases that regulate cell survival, proliferation and apoptosis in response to a wide range of stimuli. The ERKs predominantly regulate cell proliferation and survival, p38 MAPK has a role in survival and cell death, and the c-Jun–NH_2-terminal kinases (JNKs) are classically associated with the stress response and are often termed stress-activated protein kinase (SAPK). MAPKs are activated by dual-specificity MAPKs such as MKK4/7 (JNK), MKK3/6 (p38) and MEK1/2 (ERK).

The role of p38 in high glucose-induced apoptosis was highlighted by Nakagami et al. [71]. Here, in endothelial cells, high glucose caused sustained phosphorylation of p38 MAPK, caspase-3 activation and Bax-mediated apoptosis. A p38 kinase inhibitor (SB203580) blocked p38 activation and cell death but did not attenuate caspase-3 activation, whereas caspase inhibitors blocked p38 activation and cell death. Importantly, high glucose caused MEK1 cleavage that was blocked by caspase inhibitors. In human umbilical vein endothelial cells, high glucose caused apoptosis that was mediated by a sequential activation of caspase-3 and JNK but did not involve activation of ERK1/2 or p38 MAPK and could be prevented by ascorbic acid [72]. Stress-induced coactivation of JNK and p38 could conceivably arise due to activation of the ASK1. ASK1 is a MAPKK kinase similar to Raf-1 and can dually activate JNK and p38 via MKK4 and MKK3/6, respectively [73]. In contrast, in streptozocin-induced diabetic rat sensory neurones, high glucose-induced JNK activation prevents cell death [74], whereas p38 causes conduction velocity defects [75].

Diabetic rat retinas showed higher expression of H-Ras and Raf-1 when compared to nondiabetic rats, and this was confirmed in endothelial cells in culture where inhibition of H-Ras blocked apoptosis [76]. High glucose causes mitochondrial superoxide and hydrogen peroxide generation in mesangial cells that can be inhibited by treatment with IGF-I. The mechanism of the cytoprotective effect of IGF-I requires the activation of both Akt/PKB and ERK. Work by D'Alessandris et al. [77] demonstrates that glucosamine, a product of the hexosamine pathway, impairs the ability of insulin to inhibit the expression of pro-apoptotic Bim. Thus, high glucose can directly modulate members of the bcl-2 family and induce apoptosis in pancreatic β cells. However, this may not hold true for other cell types since Raf-induced ERK activation can prevent up-regulation of Bim expression [78], and high glucose promotes ERK-dependent inhibition of apoptosis in coronary artery smooth muscle cells [79].

Of note, in PC-12 pheochromocytoma cells, initiation of apoptosis is dependent on the coactivation of p38 and JNK as well as the inhibition of ERK1/2 [80]. This may help to explain some of the apparently conflicting data in both in vivo and in vitro diabetic models. It seems that the role of MAPK in high glucose-induced apoptosis is not only dependent on cell type but also on the intensity and duration of the glucose challenge.

2.4. p53-Mediated apoptosis

A role for p53 in high glucose-induced ventricular myocyte apoptosis has been proposed by Fiordaliso et al. [81]. The *O*-glycosylation of p53 leads to angiotensin II accumulation and myocyte apoptosis via Bax activation. Interestingly, phosphorylation of p53 on Ser^{390} was dependent on p38 MAPK, whereas that on Ser^{18} was not. Phosphorylated p53 (Ser^{18}) activated JNK. Keim et al. [82] reported that blastocysts from wild-type, heterozygous and $p53^{-/-}$ mice exhibited differential induction of Bax and apoptosis in response to high glucose concentrations, suggesting that p53 is required for high glucose-induced apoptosis. Moreover, loss of p53 appears to reduce glucose transport when compared with wild-type mice. The relationship between p53 and Bax-induced apoptosis was recently illustrated by Ohtsuka et al. [83]. Following induction of apoptosis by DNA damage, p53-dependent induction of apoptosis-associated speck-like protein (ASC) mediated Bax-dependent cytochrome *c* release and activation of caspase-9, caspase-2 and caspase-3.

2.5. High glucose affects cell-cycle progression and apoptosis

Control of cell division is a tightly regulated process involving a family of proteins, termed cyclins and cyclin-dependent kinases (CDKs). Briefly, cyclins are proteins whose expression varies with the stage of the cell cycle. They have a very short half-life (~60 min), and they bind to constitutively expressed CDKs at pivotal points in the cell cycle. Cyclin-dependent kinase inhibitors (CKIs) are activated by phosphorylation and inhibit cell-cycle progression by blocking CDK activity. During apoptosis, cells exit the cell cycle and apoptosis is initiated prior to entry into S-phase. Entry into G_1/S phase is dependent on cyclin D/CDK4,6 and is negatively regulated by $p21^{cip1}$. High glucose promotes entry into G1 phase in renal tubular cells, which then undergo at least one complete round of mitosis before growth arrest and either hypertrophy or apoptosis. High glucose concentrations increase the expression and

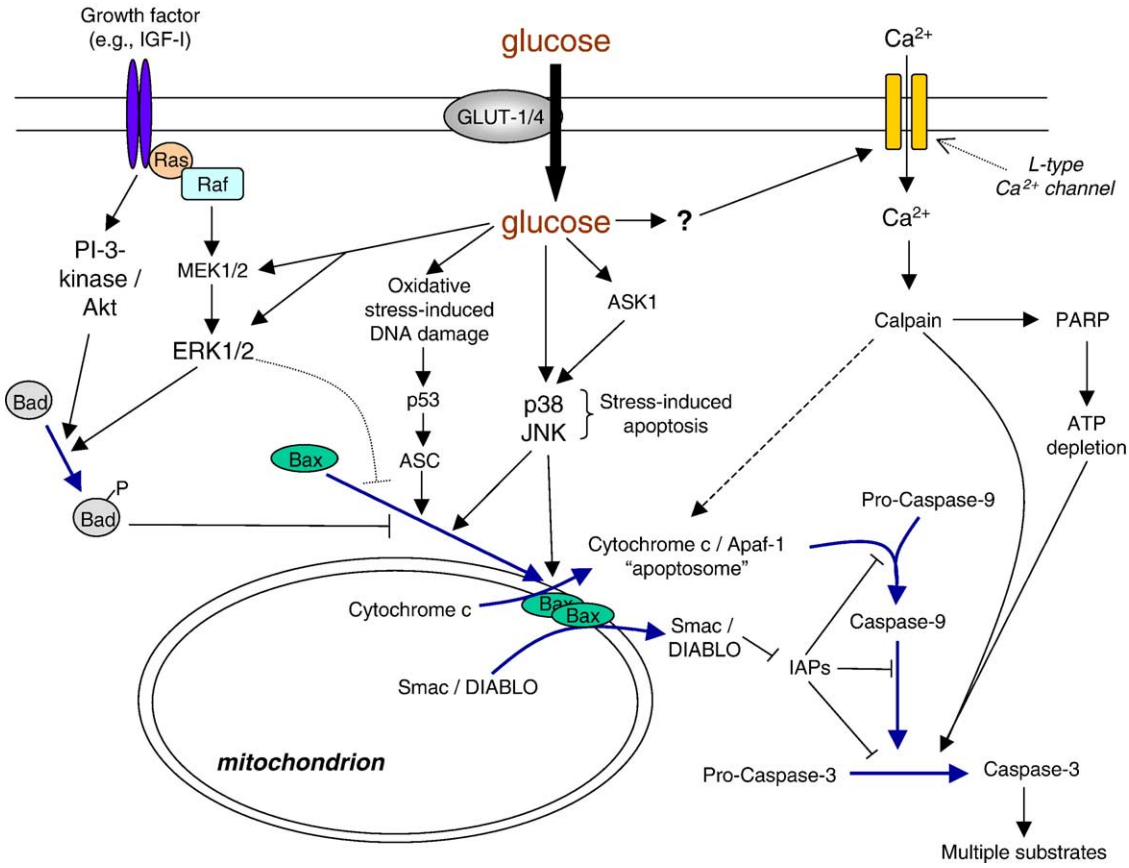

Fig. 2. Mechanisms of high glucose-induced apoptosis. High glucose concentrations activate, either directly or via ASK1, stress-induced MAPKs such as JNK and p38 that promote Bax oligomerisation and cytochrome *c* release from mitochondria (see Section 2.2) followed by the formation of the apoptosome and activation of caspase-9 and caspase-3. Additionally, increased mitochondrial outer membrane permeability results in the release of additional apoptosis regulators, further amplifying the caspase cascade. The contribution of Smac/DIABLO (an antagonist of IAPs) to high glucose-induced apoptosis is implied here but is as yet unproven. High glucose-induced oxidative stress causes DNA damage and can induce apoptosis via p53-related mechanisms that are mediated, in part, by ASC. High glucose activates the calcium-dependent protease calpain that can initiate apoptosis by caspase-dependent and -independent mechanisms. Growth factors such as IGF-I can prevent high glucose-induced apoptosis and appear to act through the Ras/Raf–MEK/ERK or PI-3-kinase/Akt pathway. IGF-I activated Akt or ERK can phosphorylate the pro-apoptotic BH3-only protein Bad, rendering it incapable of facilitating Bax oligomerisation. To complicate matters, in some cell types, high glucose can prevent apoptosis by activating ERK.

activation (phosphorylation) of $p21^{Cip1}$ and $p27^{Kip1}$. Additionally, high glucose-induced MAPK activity may stabilise the CKI protein. The tumour suppresser p53 increases the expression of $p21^{Cip1}$ in response to DNA damage and may provide a link between hyperglycaemia and apoptosis. In vivo, however, events leading to cell-cycle arrest and apoptosis may be more complex. In the kidney, hyperglycaemia causes G_1 arrest leading to hypertrophy of glomerular mesangial cells and tubular epithelial cells with an apparent absence of apoptosis. Moreover, apoptosis is increased in mesangial cells derived from $p27^{-/-}$ mice deprived of growth factor [84], and high glucose fails to induce mesangial cell and glomerular hypertrophy in mice lacking $p27^{Kip1}$ [85,86]. It appears that the cellular location of the cyclin A–cdk2 complex is pivotal for apoptosis to proceed. Nuclear cyclin A–cdk2 is proliferative, whereas cytoplasmic cyclin A–cdk2 promotes apoptosis. It is possible to imagine a cellular response to high glucose that involves cytoplasmic accumulation of cyclin A–cdk2 followed by apoptosis, but the mechanisms responsible for this effect are not clear. In addition, since high glucose causes phosphorylation of $p27^{Kip1}$ and cell-cycle arrest, this may enhance the apoptotic effect of cytoplasmic cyclin A–cdk2. Interestingly, high glucose-induced phosphorylation of $p27^{Kip1}$ appears to be dependent on oxidative stress and p44/42 MAPK activation. At present, the data suggest that both p21 and p27 are prosurvival proteins, and this is confirmed by the fact that loss of p21 leads to increased apoptosis in colon carcinoma [87]. Further, cytoplasmic $p21^{Cip1}$ can bind to pro-caspase-3 and prevent caspase activation [88], whereas active caspase-3 cleaves the nuclear import signal from p21 leading to accumulation of cdk2 [89]. CDC2 phosphorylates Bad at Ser^{128} causing apoptosis [90], in contrast to growth factor-induced phosphorylation of Bad at Ser^{136} that promotes survival. To further complicate matters, X-linked inhibitor of apoptosis protein (XIAP) down-regulates cyclins A and D_1 and up-regulates $p21^{Cip1}$ and $p27^{Kip1}$ in endothelial cells undergoing apoptosis [91]. The cleavage of XIAP by caspases caused a reduction in anti-apoptotic signalling

by NF-κB. A similar mechanism probably accounts for the high glucose-induced down-regulation of XIAP and increased apoptosis in renal proximal tubular epithelial cells [92,93]. Thus, at least in the kidney, an increase in cellular hypertrophy and apoptosis may both occur following exposure to high glucose. This would, in fact, mirror the clinical course of diabetic nephropathy in which tubular hypertrophy is followed by atrophy late in the disease, but both may occur simultaneously.

3. A model for high glucose-induced cell death

There are many reports of high glucose-induced cell death that display significant overlap in the mechanisms described. Indeed, a model for high glucose-induced apoptosis can be proposed (Fig. 2). The initial cellular response to high glucose challenge is the generation of ROS in various forms. This early and critical event in high glucose-induced cell death has several key stages beginning with the activation of key enzymes in the polyol pathway that may be linked to glucose transporters at the cell membrane. The glucose-induced polyol and hexosamine pathways may or may not be directly linked to the generation of ROS, but they certainly contribute to the overall oxidative burden. The generation of reactive nitrogen species, when combined with ROS, rapidly induces apoptotic and necrotic cell death via both mitochondria-dependent and -independent pathways. Bax-induced outer mitochondrial membrane permeability and release of cytochrome *c* are critical and irreversible stages in apoptotic signaling. It is clear that high glucose affects several stages in apoptotic signaling and, in most cell types studied, results in cell death by increasing oxidative and nitrosative stress, activating pro-apoptotic Bcl-2 family proteins and initiating a caspase cascade. Further research on these molecular pathways should lead to a better understanding of the causes of diabetic complications and realise new targets for intervention.

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