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Review

Novel roles for GAPDH in cell death and carcinogenesis

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Growing evidence points to the fact that glucose metabolism has a central role in carcinogenesis. Among the enzymes controlling this energy production pathway, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is of particular interest. Initially identified as a glycolytic enzyme and considered as a housekeeping gene, this enzyme is actually tightly regulated and is involved in numerous cellular functions. Particularly intriguing are recent reports describing GAPDH as a regulator of cell death. However, its role in cell death is unclear; whereas some studies point toward a proapoptotic function, others describe a protective role and suggest its participation in tumor progression. In this study, we highlight recent findings and discuss potential mechanisms through which cells regulate GAPDH to fulfill its diverse functions to influence cell fate.
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Initially identified as a 'simple' glycolytic enzyme, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is tightly regulated at both transcriptional and posttranslational levels and has numerous cellular functions ascribed to it.[1,2] Among these, the role of GAPDH in cell death remains poorly understood. Initially identified as a proapoptotic agent, it is perhaps paradoxical that a majority of human tumors overexpress it. Recent findings underline its role in tumorigenesis, as well as in tumor progression and cell survival. The aim of this review is to provide an overview of how GAPDH functions, how it can be modulated and its different roles in controlling cell life and death.

GAPDH is a Multifunctional Protein

GAPDH is one of the enzymes involved in the ubiquitous process of glycolysis. GAPDH specifically catalyzes the simultaneous phosphorylation and oxidation of glyceraldehyde-3-phosphate to 1,3-biphosphoglycerate, using NAD^+ as the electron acceptor. GAPDH comprises a polypeptide chain of 335 amino acids. Structural studies identified two regions, namely the glyceraldehyde-3-phosphate catalytic site and the NAD^+ binding site, a primary structure known as the Rossmann fold, which is also required for the activity of other dehydrogenases.[1] The glycolytic function mainly relies on critical amino acids that include Cys^{152} and His^{179}, and on its tetrameric structure composed of four identical 37-kDa subunits.[1]

Besides its conventional metabolic role, a number of studies have identified the participation of GAPDH in diverse cellular functions. Many of these roles are dependent on the ability of GAPDH to bind different macromolecules in the cell. Early reports first identified the fusogenic properties of GAPDH,[3] whereas subsequent observations showed its physiological significance in endocytosis and nuclear membrane assembly.[4,5] Membrane transport between the endoplasmic reticulum and the Golgi complex has also been described to require the binding of GAPDH to the small GTPase Rab2 present in pre-Golgi intermediates.[6] In addition, an association of GAPDH with microtubules seems to have an essential role in tubulin bundling and cytoskeletal dynamics.[7,8] These studies show that ATP induces the dissociation of the GAPDH tetramer and inhibits its bundling activity.[8] Recently, *in vitro* microtubule binding assays showed that Rab2 associates with microtubules but only when GAPDH and atypical PKCτ are present.[9] This association stimulates the recruitment of the motor protein dynein, which in turn regulates microtubule motility and cargo transport.[9]

GAPDH also interacts with nucleic acids. Early studies noted the high affinity of the enzyme for transfer RNA (tRNA),

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and its ability to discriminate between wild-type and tRNA mutants that are defective in nuclear export, suggesting that GAPDH may participate in tRNA export.[10] Subsequent studies have further characterized GAPDH as an RNA-binding protein, with preference to AU-rich elements, and localized the binding activity to the Rossmann fold of the enzyme.[11] Recently, these findings have been confirmed and extended, demonstrating that, through this interaction, GAPDH regulates mRNA stability and consequently controls the expression of proteins, such as endothelin-1[12] and colony-stimulating factor-1 (CSF-1).[13]

In addition to its cytosolic and membrane roles, GAPDH shows several nuclear functions. It is part of the OCA-S complex, a multicomponent Oct-1 coactivator that is essential for S-phase-dependent histone-2B (H2B) transcription.[14] GAPDH binds directly to Oct-1, is selectively recruited to the H2B promoter in the S-phase and seems to have an intrinsic activation domain that interacts with an as yet unidentified component of the basal RNA polymerase II transcription machinery.[14] Studies in fission yeast showed that GAPDH interacts with the Rbp7 subunit of RNA polymerase II, further supporting its participation in transcription.[15] Its role in DNA repair was suggested by the observation that GAPDH monomers exhibit a uracyl DNA glycosylase activity that is cell-cycle regulated.[16] Further studies designed to elucidate cellular response to mercaptopurines showed the formation of a DNA–protein complex containing GAPDH that binds thioguanylated DNA and may act as a sensor of structural DNA alterations.[17] Moreover, it has recently been described that GAPDH may have a protective effect on telomere length after ceramide exposure.[18]

Mechanisms of GAPDH Regulation

The exact mechanisms by which GAPDH performs its non-glycolytic functions remain largely obscure. The GAPDH gene is localized on chromosome 12 in humans and on chromosome 6 in mice, and encodes a single mRNA species leading to the production of a single protein. Therefore, the functional diversity of GAPDH is probably not the result of differential RNA processing but is more likely a consequence of posttranslational events.

Although GAPDH is widely used as an internal control, its mRNA and protein levels vary in response to various stimuli. Indeed, the GAPDH gene and protein are actively regulated on cell proliferation.[19–21] GAPDH is the target of different transcription factors, and various control regions have been identified in its promoter, including hypoxia and insulin-responsive elements[22,23] (Figure 1). In the context of cancer, hypoxia is of particular interest. Tumor cells maintain a high proliferation rate and consumption of nutrients and oxygen that often overcomes the support capacity of existing local blood vessels.[24] As a result, areas with low oxygen develop in most solid tumors,[25,26] and cells have diverse mechanisms to adjust to hypoxia. Although adaptation to an acute decrease in oxygen levels is mainly mediated by reversible posttranscriptional modifications such as phosphorylation, under chronic deprivation, cells induce the expression of new genes that allow the tumor to adapt to these stressful conditions. Hypoxic gene transcription is mediated by various transcription factors, including AP-1, NF-κB, CREB and p53, among others. Particularly relevant is the transcription factor HIF-1,[27] of which more than 100 direct targets have been identified. Among these, the expression of glycolytic enzymes,[28,29] including GAPDH, is upregulated on oxygen deprivation.[30,31]

Protein levels can also be affected by changes in degradation. GAPDH is among the 30% of soluble cytosolic cellular proteins that contain a KFERQ amino-acid sequence that targets those proteins for lysosomal degradation[32] (Figure 1). This process of chaperone-mediated autophagy is part of the cellular quality control system that is essential for cellular response to stress.[33,34]

However, the wide functional diversity of GAPDH cannot be explained only by changes in protein levels; indeed, other posttranslational events may have a key role in regulating GAPDH glycolitic and non-glycolitic functions (Figure 1). In one study, phosphorylation by the muscle-specific isoform of Ca^{2+}/calmodulin-dependent protein kinase II increased GAPDH glycolitic activity by 3.4-fold and allowed the assembly of glycogen-mobilizing and glycolytic enzymes at the sarcoplasmic reticulum (SR) membrane in response to calcium signaling.[35] It was suggested that this could serve to modulate ATP and NADH levels at the SR, thereby allowing the regulation of calcium transport processes. GAPDH phosphorylation by PKCτ/λ has also been suggested to target the enzyme to pre-Golgi intermediates, enhancing its participation in microtubule dynamics.[6,36,37]

GAPDH is a key redox-sensitive protein, the activity of which is largely affected by covalent modifications by oxidants at its highly reactive Cys^{152} residue. The protein is inhibited when it undergoes *S*-nitrosylation by nitric oxide (NO),[38,39] NAD^+ covalent linkage on *S*-nitrosylation,[39] nitroalkylation by nitrated fatty acids,[40] *S*-glutathionylation by gluthatione and by NO,[41] as well as extensive oxidation by H_2O_2 or peroxynitrite.[42,43] These oxidative changes not only affect the glycolytic function but also stimulate the participation of GAPDH in cell death (described in the next section).

Another level of regulation is the translocation of the enzyme to the nucleus, which can be cell-cycle dependent[14,18] or triggered in response to cellular stress.[44–49] Similarly, serum withdrawal was described to induce an accumulation of GAPDH in the nucleus.[50] However, in contrast to other cell death stimuli, this nuclear translocation is a reversible process that could be recovered on serum addition, likely triggered by survival signals. Recently, a novel exportin1 or chromosome region maintenance (CRM)1-dependent nuclear export signal was identified in the C-terminal GAPDH domain.[51] Truncation or mutation of this sequence abrogated CRM1 binding and caused nuclear accumulation of GAPDH. Nuclear targeting can also be modulated by posttranslational changes. *O*-linked *N*-acetyl-glucosamine modifications of GAPDH (*O*-GlcNAcylation mainly on Thr^{227}) were reported as being able to disrupt the tetrameric form, enabling its nuclear translocation[52] (Figure 1). This finding contrasts with that of Hara *et al*.,[38] which described that *S*-nitrosylation of GAPDH allowed binding with Siah1, leading to the nuclear translocation of GAPDH dependent on Siah1 NLS. However, as the interaction of the *O*-GlcNAcylation form of GAPDH with Siah1 was not tested, it

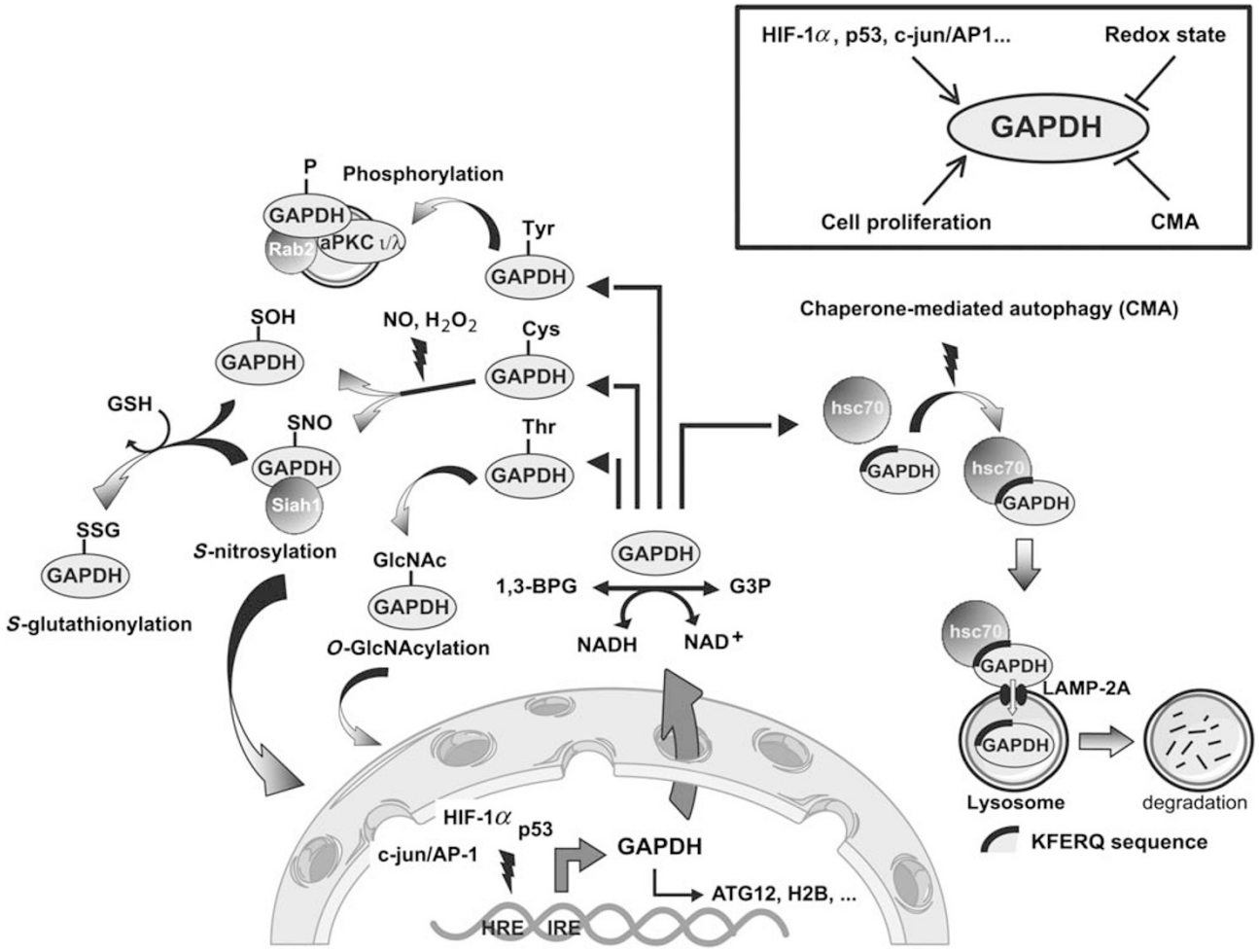

Figure 1 Regulatory mechanisms of GAPDH. Cellular GAPDH content is modulated by several biological processes (see inset). Its expression is highly dependent on the proliferative state of the cell and can also be regulated by transcription factors, such as hypoxia-inducible factor 1 (HIF-1α), p53 or c-jun/AP1. Indeed, different control regions have been identified on its promoter (HRE, hypoxia response element; IRE, insulin response element) that may regulate its levels in certain circumstances, such as low oxygen supply. In the cytosol, levels of the glycolytic enzyme, which catalyzes the conversion of glyceraldehyde-3-phosphate (G3P) into 1,3-biphosphoglycerate (1,3-BPG), can be affected by changes in its lysosomal degradation. Under conditions of cellular stress, chaperone-mediated autophagy is activated; GAPDH contains a KFERQ motif that is selectively recognized by the chaperone heat shock cognate protein of 70 kDa (hsc70). This interaction targets the complex to the lysosomal membrane, where it binds to the lysosome-associated membrane protein type 2A (LAMP-2A) that acts as a receptor for this pathway. At the posttranslational level, GAPDH can undergo different modifications, which may determine some of its non-glycolytic functions. Tyrosine (Tyr) phosphorylation by the atypical protein kinase Cτ/λ (aPKCτ/λ) is required for its association with Rab2 and for the transport between the endoplasmic reticulum and the Golgi complex. Hydrogen peroxide (H_2O_2) and nitric oxide (NO) both act on the active site cysteine 152 (Cys). The irreversible oxidation of the Cys residue can be prevented by *S*-glutathionylation, in which the sulfydryl group (–SH) conjugates with glutathione (GSH). *S*-nitrosylation facilitates its binding to Siah1 and also the translocation of the complex to the nucleus. In addition, GAPDH can undergo *O*-linked β-*N*-acetylglucosamine glycosylation (*O*-GlcNAcylation) at threonine residues, which also may mediate its nuclear migration by disrupting the tetrameric conformation of the enzyme. Once in the nucleus, GAPDH can participate in DNA repair mechanisms and in the transcriptional regulation of proteins, including ATG12 autophagy-related 12 homolog and histone H2B

is conceivable that a common mechanism of Siah1-dependent nuclear import of GAPDH may exist.

Overall, these data strongly support the notion that post-translational changes of GAPDH can affect its cellular functions, opening an important avenue that requires further work.

GAPDH as a Proapoptotic Agent

Evidence for the potential apoptotic role of GAPDH first came from studies on cultured cerebellar neurons,[46–48,53] in which an increase in GAPDH levels and its subsequent translocation to the nucleus preceded neuronal death induced by culture aging or cytosine arabinonucleoside exposure. Antisense oligonucleotides directed against GAPDH mRNA exerted a protective effect, and either antisense treatment or incubation with protein synthesis inhibitors prevented its appearance in the nucleus.[47,53] This suggested that nuclear GAPDH is mainly derived from new protein synthesis and may function in cell death. These initial observations have been extended to non-neuronal cells that are exposed to several apoptotic stimuli (summarized in Table 1).

Nuclear accumulation of GAPDH precedes apoptotic features that are antagonized by the overexpression of Bcl-2.[44,54] Once in the nucleus, GAPDH loses its catalytic

Table 1 Participation of GAPDH in cell death and carcinogenesis

Cell lines	Stimulus	Proposed mechanisms	References
Participation in apoptosis			
HEK293; Raw264.7; cerebellar neurons	Staurosporin; LPS/IFNγ; NMDA	*S*-nitrosylation of GAPDH and nuclear translocation	Hara *et al.*[38]
Cerebrocortical neurons	Culture aging	Increased expression of GAPDH	Ishitani *et al.*[45]
Cerebellar neurons	Culture aging; AraC; low K^+	Increased expression of GAPDH and nuclear translocation	Ishitani *et al.*[46]; Ishitani *et al.*[47]; Saunders *et al.*[48]; Ishitani and Chuang[53]; Saunders *et al.*[55]; Ishitani *et al.*[102]
S49; primary thymocytes; PC12; cerebrocortical neurons	Dexamethasone; NGF; culture aging	Nuclear translocation	Sawa *et al.*[49]
Neuroblastoma cells (mNB41A3); R6 fibroblasts	Staurosporin; MG132; H_2O_2; FeCN	Nuclear translocation	Dastoor and Dreyer[44]
Mesencephalic neurons	MPP+	Nuclear translocation	Fukuhara *et al.*[103]
Neuroblastoma cells (SH-SY5Y)	Dopaminergic neurotoxin	Nuclear translocation	Maruyama *et al.*[104]
Hepatocytes	TGF-β	Nuclear translocation	Barbini *et al.*[105]
HeLa	NO donor (NOC18)	Nuclear GAPDH aggregates	Nakajima *et al.*[56]
Raw264.7; macrophages; HEK293; neuroblastoma cells (SH-SY5Y)	LPS/IFNγ; NO donor (GSNO)	Induction of p53 by GAPDH-mediated activation of P300/CBP	Sen *et al.*[63]
Follicular thyroid cell; KTC2	TRAIL	*S*-nitrosylation of GAPDH and nuclear translocation	Du *et al.*[66]
HeLa; HEK293	Staurosporin; etoposide; Lonidamine	GAPDH-induced mitochondrial permeabilization	Tarze *et al.*[67]
Neuroblastoma cells (N2a)	Mutant huntingtin (mHtt)	GAPDH-mediated translocation of mHtt	Bae *et al.*[74]
HT22	Aβ peptides	Disulfide-linkage and nuclear accumulation of GAPDH	Cumming and Schubert[76]
Participation in carcinogenesis or protection from cell death			
HeLa, primary MEFs	Etoposide, staurosporine, actinomycine D, oncogenes	Protection from CICD by glycolysis increase and autophagy induction	Colell *et al.*[87]
K562, JURL-MK1	Imatinib mesylate	Inhibition of CICD	Lavallard *et al.*[81]
Jurkat, Molt4, primary ALL cells	Prednisolone	GAPDH downregulation sensitizes resistant cells to treatment	Hulleman *et al.*[95]
NOSE.1, Hey		GAPDH stabilizes CSF-1 mRNA	Zhou *et al.*[13]
HCT116, DLD1, primary lung fibroblastes (LF1)	Methyl methane sulfonate, bleomycin	GAPDH interaction with APE1	Azam *et al.*[97]

activity and becomes resistant to standard extraction procedures.[44,49] Further studies have shown that induction of cell death is accompanied by the appearance of nuclear alkaline forms of the enzyme distinct from cytosolic species.[55] Although these findings would suggest the involvement of novel GAPDH forms in cell death, it is worth noting that it has also been described that the presence of basic forms of GAPDH is cell-cycle regulated and exerts a protective role against telomere shortening induced by ceramide or chemotherapeutic agents.[18] Thus, it is conceivable that the participation of GAPDH in cell death may be determined by other posttranslational events. Further support for the existence of these regulatory mechanisms was the observation that apoptotic treatment markedly reduced sodium nitroprusside-induced NAD^+ labeling of nuclear GAPDH, suggesting that the active site of GAPDH may be covalently modified, denatured or improperly folded.[55] In this regard, recent studies have shown that sustained exposure to oxidants, through the formation of intermolecular disulfide bonds, induced insoluble amyloid-like GAPDH aggregates that promote cell death[56] (Figure 2). This irreversible oxidation can be prevented by protein *S*-thiolation, in which protein sulphydryl groups form mixed disulfides with low-molecular-weight thiols, such as glutathione.[57,58] Moreover, studies in *Caenorhabditis elegans* indicated that redox regulation of GAPDH can counteract oxidative stress by repressing the glycolytic pathway and consequently rerouting the metabolic flux to maintain an optimal $NADPH/NADP^+$ ratio, through the pentose cycle.[59] Strikingly, the redox-sensitive cysteine residue of the glycolytic enzyme also has an essential role in signaling pathways that sense oxidative stress in the phospho-relay signaling of the fission yeast *Schizosaccharomyces pombe*[60] (Figure 2). In this system, peroxide stress signals are transmitted from Mak2/3 sensor kinases to the Mpr1 histidine-containing phosphotransfer protein and finally to the Mcs4 response regulator, ultimately activating a MAP kinase cascade. In response to oxidative stress, the transient oxidization of GAPDH facilitates its association with the Mcs4 response regulator, and this represents an essential step for the interaction between Mcs4 and Mpr1.[60]

GAPDH has also been reported as a sensor of NO stress.[61,62] NO causes *S*-nitrosylation of GAPDH at its active site, increasing the binding to Siah1 (an E3 ubiquitin ligase)[38,62] (Figure 2), the nuclear localization signal of which mediates translocation of GAPDH. In turn, GAPDH in the nucleus stabilizes Siah1, facilitating its degradation of nuclear proteins.[38,62] Further studies have shown that nuclear *S*-nitrosylated GAPDH is acetylated at Lys^{160} by the acetyltransferase p300/CREB-binding protein (CBP),[63] which enhances the ability of GAPDH to stimulate auto-acetylation

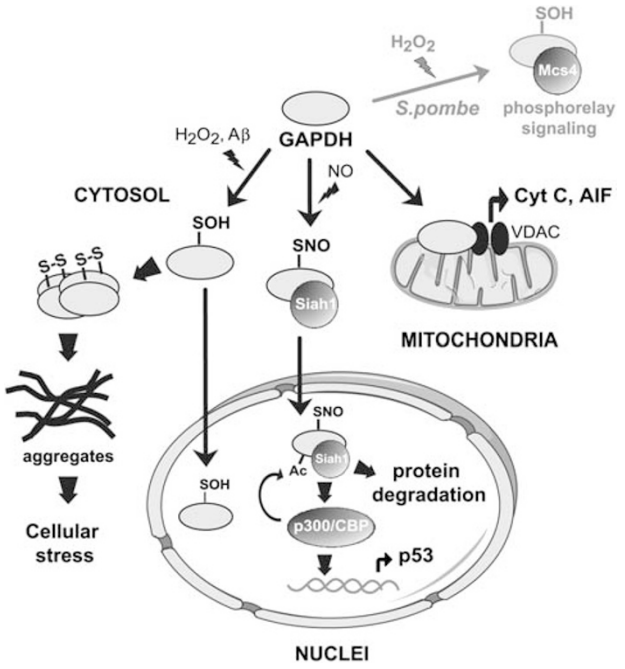

Figure 2 GAPDH participation in cell death. GAPDH can trigger oxidative stress-mediated cell death. Exposure to oxidants or amyloid-β peptides (Aβ) induces an irreversible oxidation of cysteine residues that favor intermolecular disulfide bonds and the subsequent formation of cytosolic aggregates. This insoluble protein may ultimately promote cellular stress. Oxidative modifications can also target GAPDH to the nucleus. *S*-nitrosylation of the enzyme increases binding to Siah1, which mediates its nuclear translocation. GAPDH stabilizes Siah1, enhancing the activity of this ubiquitin ligase and the proteasome-mediated degradation of nuclear proteins. Nuclear GAPDH is acetylated by the p300/CREB-binding protein (CBP), which in turn stimulates the catalytic activity of p300/CBP. Consequently, downstream targets of p300/CBP, such as p53, can be activated and cause cell death. GAPDH has been also localized in the mitochondria, in which its binding to the voltage-dependent anion channel (VDAC) has been suggested to promote the release of proapoptotic proteins, such as cytochrome c (CytC) and apoptosis-inducing factor (AIF). In prokaryotes, the glycolytic enzyme has been involved in signaling pathways that sense oxidative stress. The oxidation of GAPDH facilitates its association with the Mcs4 response regulator, an essential step in the phosphorelay signaling that ultimately activates a MAP kinase cascade

and catalytic activity of p300/CBP, and, consequently, downstream targets such as p53 are activated. Interestingly, both Siah-1, with a specific p53 response element within its second intron, and GAPDH have been shown to be upregulated by p53,[64,65] pointing to the existence of auto-amplifying loops. The involvement of *S*-nitrosylation mechanisms in GAPDH-mediated cell death is further supported by studies in thyroid cancer cell lines in which *S*-nitrosylation and nuclear translocation of GAPDH are observed after TNF-related apoptosis-inducing ligand (TRAIL) exposure.[66] Knockdown of GAPDH with small-interfering RNA partially prevented the apoptotic effect of TRAIL, although NO synthase stimulation and production of NO were not attenuated.[66]

After cell death induction, GAPDH levels were also found to apparently increase in the mitochondria;[47,48] however, its role within this organelle remains elusive. Recent studies in isolated mitochondria have suggested that GAPDH interacts with the voltage-dependent anion channel [67] (Figure 2), inducing a cyclosporin A-inhibitable permeability transition and the release of proteins, such as cytochrome *c* and apoptosis-inducing factor.[67] It is worth noting that these observations are based on the use of exogenous GAPDH at concentrations that may not reflect cellular levels. Indeed, only a small portion of total cellular GAPDH is found in the mitochondrial fraction after induction of cell death,[47,48,67] and therefore further work is required to confirm any direct role of GAPDH in the intrinsic mitochondrial pathway of apoptosis.

Evidence links GAPDH to several physiopathological models, particularly neurodegenerative disorders.[68] Nuclear GAPDH has been found in fibroblasts and in vulnerable neurons of postmortem samples from patients affected with polyglutamine-repeat disorders such as Huntington's disease (HD) or dentatorubral-pallidoluysian atrophy,[69,70] as well as from patients with Parkinson's disease[71] and Alzheimer's disease (AD).[72,73] Moreover, studies suggest that binding of the enzyme either to β-amyloid (Aβ) peptides or to mutant huntingtin, both directly involved in the development of these age-related disorders (AD and HD, respectively), regulates its cytotoxicity.[74–76] In cell culture models, mutant huntingtin, with polyglutamine tracts in the N-terminal region, elicits its cytotoxicity through the nuclear migration of its N-terminal fragments. Overexpression of GAPDH or Siah1 enhances huntingtin nuclear translocation and cytotoxicity, whereas GAPDH mutants that cannot bind Siah1 prevented the translocation.[74] Analyses of brain extracts from transgenic AD mice coexpressing the mutated forms of human amyloid precursor protein and presenilin-1 gene, or postmortem samples from AD patients, showed the accumulation of insoluble disulfide-linked multimers of GAPDH within neurons.[76] Furthermore, exposure of neuronal cells to Aβ promoted the formation of these insoluble aggregates of GAPDH to a similar extent as that induced by oxidative stress.[56,76] Although Aβ-mediated neurotoxicity occurs through multiple biological pathways, the effect of Aβ on disulfide binding and the subsequent aggregation of an abundantly expressed protein such as GAPDH are likely to influence a number of these processes. Studies in cell lines and animal models showed that the protective effect of anti-dementia drugs, such as the cholinesterase inhibitors tacrine or donezepil, and deprenyl, a selective monoamine oxidase B inhibitor widely used in the treatment of Parkinson's disease, largely relied on their ability to interact with the GAPDH apoptotic cascade.[73,77] These results prompted a clinical trial using the deprenyl derivative TCH346 in patients with amyotrophic lateral sclerosis;[78] however, the trial showed no evidence of a beneficial effect on disease progression.

GAPDH as a Prosurvival Factor

Given its role as a proapoptotic agent, it is perhaps paradoxical that GAPDH is overexpressed in most human cancers. Dramatically elevated levels of glycolytic enzymes, including GAPDH expression, were observed in most human cancer types tested,[79] and were often associated with reduced survival.[80–83] Since Warburg's work in 1929, it has been known that cancer cells frequently upregulate glucose metabolism, resulting in a high uptake and use of glucose but moderate rates of mitochondrial respiration under aerobic conditions. Upregulation of glycolytic enzymes will lead to an enhancement of cell metabolism that has been shown to

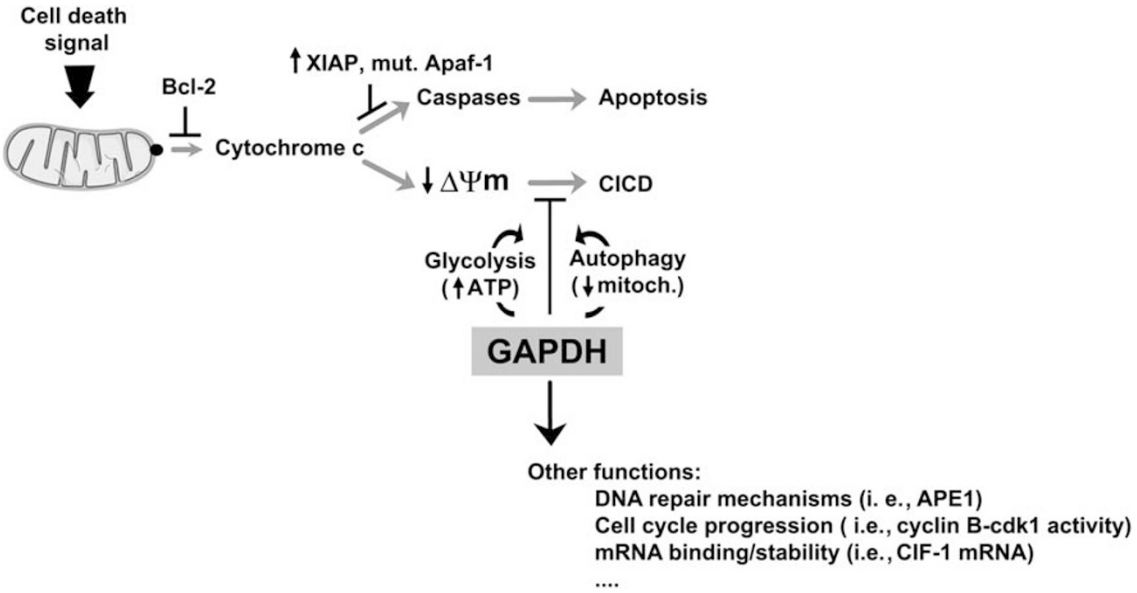

Figure 3 GAPDH as a prosurvival factor. Evasion of apoptosis is one of the hallmarks of human cancers, which promote tumor formation and progression, as well as treatment resistance. Signaling to cell death can be blocked by an increase in antiapoptotic molecules and/or by a decrease or defective function of proapoptotic proteins. The mitochondrial pathway may be impaired by overexpression of anti-apoptotic Bcl-2 proteins, as well as by Bax mutations or epigenetic silencing of Apaf-1. In addition, activation of downstream caspases can be blocked by high levels of IAPs (inhibitor of apoptosis proteins). However, besides caspase-dependent apoptosis, additional regulatory mechanisms of non-apoptotic modes of cell death must also be considered. When caspase activity is blocked, the decrease in mitochondrial membrane potential ($\Delta\psi m$) and function can result in caspase-independent cell death (CICD). Under these conditions, the increase in GAPDH levels inhibits cell death by simultaneously increasing ATP levels through glycolysis and stimulating autophagy-mediated clearance of permeabilized mitochondria. The involvement of GAPDH in other cellular processes may also contribute to its prosurvival role, including participation in DNA repair mechanisms, cell-cycle progression and mRNA binding and stability

correlate with increased tumor aggressiveness and poor patient prognosis in several cancers;[84,85] however, the reason for this switch in the cell energetic status is debated.[86]

In a recent study by Colell *et al*.,[87] a novel role of elevated GAPDH was suggested. In this study, the authors used an unbiased genomic screen to identify proteins that protected cells from caspase-independent cell death (CICD)[88] (Figure 3). Apoptosis has a key role in suppressing oncogenesis, but under some conditions in which apoptosis is inhibited, a 'back-up death mechanism' can manifest.[89] This alternative form of cell death was defined as CICD.[88] Both apoptosis and CICD are triggered by mitochondrial outer membrane permeabilization (MOMP).[89] Therefore, cells that resist cell death either do not undergo MOMP or have mechanisms to block or avoid both forms of cell death downstream of MOMP. Tumors frequently show such 'downstream defects', including mutation of caspases, lack of the adapter Apaf-1 or overexpression of the endogenous caspase inhibitor XIAP.[90–92]

Using a retroviral screen, GAPDH was identified as being able to protect cells from cell death after MOMP only when caspase activation was blocked or disrupted.[87] GAPDH mutants that discriminate between glycolytic and non-glycolytic functions of the enzyme did not protect, but were effective when expressed together, indicating that two separate functions may be involved. One of these involves a novel role for GAPDH in autophagy induction. On CICD induction, GAPDH translocated to the nucleus, where it participated in an upregulation of the autophagy protein ATG12.[87] In support of this, GAPDH upregulation induced by bacterial CpG motifs in colon carcinoma cells was correlated with an increase in autophagy *in vitro* and *in vivo*.[93] The elevated ATP levels as a result of glycolysis, and the function of autophagy to remove damaged mitochondria, both coordinated by GAPDH, cooperate in this protection toward CICD (Figure 3). However, in this study, protection from CICD involved GAPDH overexpression; therefore, further studies are required to unravel the mechanism whereby GAPDH protects cells from CICD under physiological conditions.

Very recently, under low-glucose conditions, GAPDH was found to inhibit mTORC1 signaling in an AMPK-independent manner by binding to Rheb.[94] This is of particular interest in the context of GAPDH protection from CICD, as mTORC1 inhibition causes autophagy induction (among other functions). Therefore, investigating the role of mTORC1 signaling in protection from CICD is of interest.

The role of GAPDH as an anti-death molecule was further investigated in a model of chronic myeloid leukemia (CML) showing resistance to imatinib mesylate treatment.[81] Imatinib mesylate is widely used for the treatment of patients with CML, acting to induce apoptosis by counteracting Bcr-Abl activity. In all, 20–25% patients develop resistance to imatinib for several reasons, but so far, the best-characterized mechanism is mutations in Bcr-Abl. Recently, imatinib was found to induce both apoptosis and CICD, and some imatinib-resistant cells showed a spontaneous overexpression of GAPDH. Moderate knockdown of GAPDH did not affect the cellular metabolism, but sensitized these resistant cells to imatinib. Therefore, targeting GAPDH-mediated protection from CICD may be an innovative way of sensitizing

imatinib-resistant CML patients.[81] In this regard, a recent study suggested that a modulation of GAPDH levels affects prednisolone resistance in acute lymphoblastic leukemia cells.[95]

Roles for GAPDH in tumorigenesis and tumor progression are further supported by other studies. In ovarian cancer metastases, a strong coexpression of CSF-1 and its receptor was associated with poor prognosis,[96] and GAPDH has recently been shown to bind and stabilize CSF-1 mRNA in this cell type.[13] The authors speculated that one function of GAPDH in ovarian cancer is to increase the levels of CSF-1, an important cytokine in tumor progression.

Another protective role for GAPDH involves DNA repair and response to cytotoxic drugs. GAPDH can physically interact with APE1, an enzyme involved in the repair of spontaneous or drug-induced abasic sites in damaged DNA.[97] This interaction results in a reactivation of APE1 endonuclease activity, thus preventing genomic instability resulting from aberrant structural changes caused by oxidative stress. Therefore, GAPDH can function to safeguard the genome by preventing APE1 inactivation on oxidative stress. Finally, GAPDH was found to be involved in cell-cycle regulation by modulating cyclin B-cdk1 activity, resulting in increased mitoses and accelerated cell-cycle progression.[98]

Most cancer cells exhibit increased glycolysis and use this metabolic pathway for ATP production. Therefore, inhibiting glycolysis could be an efficient way of targeting tumor cells while sparing normal tissue, and indeed several clinical trials have shown promising results using glycolytic inhibitors such as 2-deoxyglucose in combination with other anticancer agents (reviewed in Pelicano *et al.*[99] and Scatena *et al.*[100]). Recent studies have shown that koningic acid, a selective inhibitor of GAPDH, kills a broad range of highly glycolytic cell lines through growth inhibition and CICD.[101] Thus, understanding the biological functions of GAPDH beyond glycolysis will likely improve our ability to effectively target this enzyme in cancer therapy.

Concluding Remarks. There is mounting evidence that cell death is initiated after the synthesis of a new GAPDH protein and its subsequent nuclear appearance. However, the same events have also been observed during cell proliferation and/or transformation. Indeed, most cells exhibit some GAPDH translocation to the nucleus on cell cycle, and some, such as lymphocytes, naturally upregulate it on activation, without dying. How can this paradox be reconciled? It may well be that transformation requires that cells bypass a GAPDH-mediated metabolic checkpoint. One possible model would be that cells that normally derive energy from oxidation have a GAPDH-mediated checkpoint that can kill them if, because of sudden changes in signaling (indicative of transforming events), they engage elevated glycolysis. However, mechanisms must exist to overcome or bypass this in cells that make such a shift as a part of normal physiology. One of these might be linked to ROS production. GAPDH is a redox-sensitive protein that is inactivated by ROS. Therefore, in conditions that induce a mild ROS production (lymphocyte activation), GAPDH upregulation may be protective for the cell. However, in the presence of higher amounts of ROS, such as those observed under conditions of mitochondrial dysfunction, oxidation of Cys^{152} may lead to a GAPDH inhibition, participating in cell death. As we know very little regarding the manner in which GAPDH engages apoptosis or prevents CICD, the actual mechanisms involved in the above scenario remain obscure.

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