Minireview

Regulating cell survival by controlling cellular energy production: novel functions for ancient signaling pathways?

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Abstract Cell survival is maintained by growth factors and critically depends on sufficient energy supply. New evidence suggests that a rise in cellular energy production is not merely a homeostatic response to increased demand but subject to regulation by extrinsic factors. The mechanisms operating in this control are largely enigmatic. Work on transformed cells identified direct targeting of glycolytic enzymes by signaling proteins as one possibility. But mitochondrial oxidative phosphorylation and biogenesis may also be subject to regulation by growth and survival factors. Both, positive and negative regulators of cell survival impinge on the processes of cellular energy production to regulate growth and survival versus death.

Keywords: Growth; Survival; Metabolism; Signaling; Glycolysis; Oxidative phosphorylation

1. Introduction

Apoptosis, the predominant form of physiological cell death during development and adult life, occurs via a tightly orchestrated sequence of events. Central to this process is the activation of caspases through death receptor stimulation (extrinsic pathway) or the breakdown of mitochondrial integrity and the release of apoptogenic factors (intrinsic pathway) [1]. By degrading structural and other proteins, caspases initiate and execute the demise of the cell. The main components of the apoptotic machinery are constitutively expressed in all cells and guarded against the accidental activation through spatial separation and the expression of inhibitory proteins. Bcl-2 maintains the intactness of the outer mitochondrial membrane to prevent the release of apoptogenic factors [2], whereas the protective function of IAP proteins extends beyond caspase activation [3]. Extrinsic factors play a key role in the control of cell survival and anti-apoptotic activity has been demonstrated for all canonical signaling pathways. Two of the best studied survival pathways, the cytoplasmic (Ras-Raf-MEK-ERK) [4] and the PI3K (PI-3 kinase)-PKB (protein kinase B) [5] pathway, exert their effects through transcriptional and post-transcriptional regulation of these and other survival proteins (Fig. 1A and B). Suppression of caspase activation or activity is critical for survival in the face of an immediate threat to the cell. However, the maintenance of cellular energy homeostasis during routine as well as situations of enhanced demand is equally important for cell survival. An increasing set of data supports the idea that survival pathways critically regulate the processes of cellular energy production and management.

2. Sensing nutrients and oxygen

Availability of nutrients and oxygen determines the competence of a cell to respond to exogenous stimuli with proliferation, survival, differentiation or even death. Work of the last years has laid out the framework for our understanding of the sensory systems, which allow the cell to decipher environmental clues. Cellular energy production occurs through the conversion of ADP to ATP during glycolysis and oxidative phosphorylation. Maintenance of a high ATP to ADP ratio is essential and changes in the ADP/ATP and more importantly the AMP/ATP ratio are sensitive indicators of the cellular energy status [6] (Fig. 1C). A rise in the AMP/ATP ratio triggers the activation of AMPK (AMP-activated protein kinase), which terminates ATP-consuming metabolic pathways and switches on ATP-generating systems, including glycolysis, fatty acid oxidation and glucose uptake [7]. AMPK interacts with the tumor suppressor TSC2 (hamartin), which together with TSC1 (tuberin) functions as an antagonist of the mTOR signaling pathway [8], a central controller of cell growth in response to growth factors, cellular energy and nutrient levels [9]. The survival kinase PKB in turn activates mTOR signaling by directly phosphorylating and inactivating TSC2 [10,11]. AMPK, on the contrary, binds, phosphorylates and thereby enhances TSC2 function [12]. This mechanism represents a convergence point of nutrient sensing and survival signaling. Little is known about the upstream regulators of AMPK. LKB1, whose inactivation provides the genetic basis for the Peutz–Jeghers syndrome, a familial colorectal polyp disorder [13], was shown to phosphorylate and activate AMPK [14]. Mouse embryonic fibroblasts deficient in LKB1 display hypersensitivity towards apoptosis induction by treatments that elevate AMP. Moreover, these cells exhibit elevated signaling downstream of mTOR, demonstrating that LKB1 functions in negative regulation of mTOR [15].

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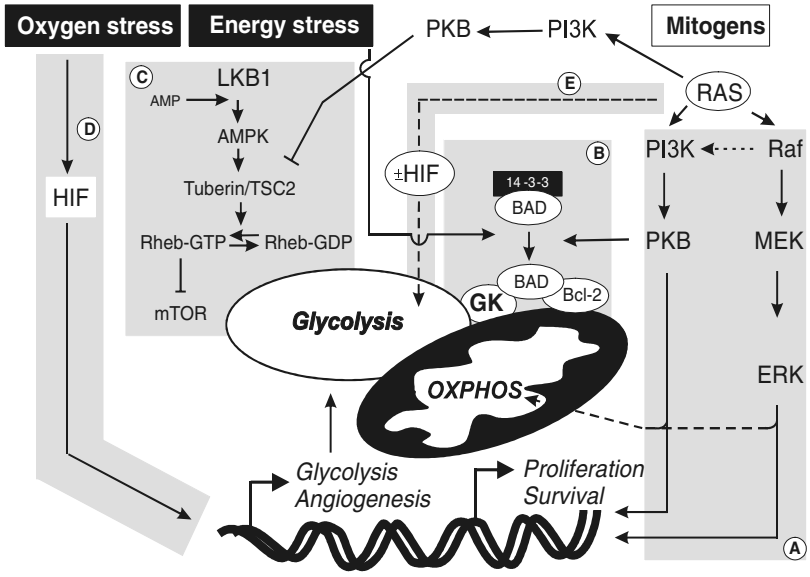

Fig. 1. Mitogenic signaling cascades govern cell survival by controlling cellular energy homeostasis. Individual signaling entities discussed for their involvement in the control of cellular energy production and consumption are grouped (gray shaded areas) and labeled (A)–(E). (A) Mitogenic signaling cascades control cell survival through transcription-dependent and -independent mechanisms and increasing evidence, presented in this review, suggests that they also directly govern cellular energy metabolism. The dashed arrow at the bottom of these signaling cascades illustrates that proteins signaling through these pathways or being part of them frequently are encountered in or at the mitochondria. (B) Phosphorylation of the protein BAD, normally residing at the outer mitochondrial membrane, has been described for C-Raf and PKB. Modified BAD binds to cytoplasmic 14-3-3 proteins and thereby looses its negative impact on cell survival. New work suggest that mitochondrial BAD is required in addition for the assembly of a large protein complex that also contains glucokinase. (C) Lack of nutrients leads to an increase in the AMP/ATP and activation of AMPK. As a consequence, ATP-consuming processes are shut down and ATP generation is stimulated. Signaling downstream of AMPK results in the repression of mTOR, a central controller of growth, which can be antagonized by mitogen-activated PKB. (D) Lack of oxygen also results in the HIF-1 dependent compensatory upregulation of factors required for increased glycolysis and formation of new blood vessels. (E) These processes are also regulated through oxygen-independent regulation of HIF-1 via mitogenic signaling cascades.

HIF-1 (hypoxia inducible factor) is a heterodimeric transcription factor composed of a constitutively expressed HIF-1β and a HIF-1α subunit, whose expression is highly regulated by oxygen-dependent (mainly affecting the degradation of the protein) and oxygen-independent (regulating mainly the synthesis) mechanisms [16,17] (Fig. 1D). HIF-1 target genes are involved in organismic responses to condition of low oxygen (e.g., the formation of new blood vessels or the expansion of erythrocytes) and alterations in cellular metabolism [16]. Degradation of HIF-1-α under normoxic conditions results from an O_2-dependent prolyl-hydroxylation of HIF-1α, interaction with the tumor suppressor protein VHL and proteasomal targeting of HIF-1α. Since O_2 is a limiting substrate in this reaction, this post-translational regulation of HIF-1α levels directly links oxygen availability to HIF-dependent regulation of gene products involved in the response to hypoxia [17]. Oxygen-independent regulation of HIF-1 through mitogens is the second major mechanism regulating the expression of this protein and allows the cells to respond to increasing energy demands during growth processes (Fig. 1E). Both, PI3K/PKB and the cytoplasmic signaling cascade have been implicated in the expression of the HIF-1 [17] and they may also contribute to increased HIF-1 protein levels seen in tumors [17].

3. Evidence for extrinsic control of cellular energy production

A first strong hint for a link between mitogenic signaling and metabolism came from the analysis of tumor cells. Mutations in components of mitogenic cascades render them largely independent of the control by extrinsic factors and result in constitutive activation of survival and growth pathways. Increase in tumor size limits oxygen supply through mere diffusion [17]. This lack is compensated by the transcriptional upregulation of angiogenic factors via mitogen signaling and by metabolic alterations. As first realized by Warburg [18], tumors commonly rely on glycolysis under aerobic conditions as a means for energy production. This glycolytic switch is driven by the deregulated mitogenic cascades, which through HIF-1-dependent and HIF-1-independent mechanisms upregulate the expression of components of the glycolytic machinery [19]. Additionally, oncogenes may directly regulate the activity of key metabolic enzymes [20,21]. The critical role for HIF-1 in cellular transformation is underscored by the resistance of HIF-1α-deficient ES cells to teratocarcinoma formation [22].

Mitochondrial oxidative phosphorylation is the predominant mechanism for energy generation under aerobic conditions in the organism, but possibly not in all cultured cells [23]. ATP is produced in a process in which electrons, initially generated from NADH and $FADH_2$, are passed along a series of carrier molecules and enzymes (electron transport chain) and ultimately are transferred to molecular oxygen [24]. Cytochrome *c* oxidase (COX) is the terminal oxidase of cell respiration. NADH and $FADH_2$ are derived from the metabolism of nutrients such as glucose and fatty acids. Several published observations support the view that extracellular signals directly participate in the regulation of oxidative phosphorylation. Mitochondrial localization has been dem-

onstrated for Lyn [25], a member of the Src family [26] and Miyazaki et al. have shown that c-Src is present within the mitochondria of osteoclasts, where it phosphorylates COX. This Src-induced COX activity is required for the normal function of these cells but not for their survival [27]. Mitochondrial presence has been reported for A-Raf [28] as well as components of the NF-κB pathway [29].

With the advancement of organelle proteomics, an extensive catalog of the mitochondrial proteome generated from highly purified mitochondria of the normal human heart has been compiled [30]. Among the 615 proteins identified in a recent screen, a significant portion is involved in cellular signaling. This group comprises members as well as regulators of the small GTPases of the Ras superfamily, subunits of heterotrimeric G-proteins, mitogen-activated protein kinases (MAPK), lipid kinases, and RSK [30]. As exemplified by the PKB-dependent association of hexokinase (HK) with the mitochondria [31], mitochondrial protein composition and perhaps also posttranslational modification of these proteins may be subject to regulation by extrinsic signals. The reasons for the extension of primarily cytoplasmic signaling cascades to and into the mitochondria are currently unclear, and in almost all cases we lack an understanding of the processes regulated by them. For most non-mitochondrial proteins, the mitochondrial interaction partners and the mechanisms regulating their interaction or their uptake remain to be defined. Analysis of Ras signaling showed that this protein, which normally resides at the inner surface of the cell membrane, also can be targeted to the ER and the Golgi and initiate signaling from there [32]. The possibility thus has to be considered that mitochondria are not only the endpoint of signaling pathways initiated at the cell membrane or the cytoplasm, but that signaling proteins residing at or in the mitochondria participate in mitochondrial retrograde signaling and thereby affect non-mitochondrial processes [33].

4. Integrating energy homeostasis and pro-survival signaling

Events, which precede the onset of apoptosis following growth factor removal, are impaired glucose metabolism as a result of glucose transporter downregulation and a reduction in the flux through the glycolytic pathway via direct effect on the localization or activity of glycolytic enzymes, collapse of the inner mitochondrial membrane potential, release of cytochrome *c*, and a drop in mitochondrial oxygen consumption. Two different mechanisms have been suggested to counteract these events: cells protected by the antiapoptotic protein Bcl-2 adapt to lower rate in metabolism, PKB counteracts a decline in metabolism, e.g., by increasing glucose uptake and stimulating glycolysis [34]. Furthermore, PKB inhibits early apoptotic events by increasing the mitochondria-bound HK activity. Mitochondrial HK catalyzes the first committed step of glucose metabolism by phosphorylating glucose to glucose-6-phosphate using ATP produced by mitochondria, thereby coupling glycolysis and mitochondrial phosphorylation [31]. Also, the survival activity of oncogenic C-Raf may in part depend on its effects on mitochondrial energy production through interference with the flux of metabolites in and out of the mitochondria. VDAC (voltage-dependent anion channel/mitochondrial porin) [35] has been shown to interact with C-Raf [36]. This work pointed to a potential negative regulatory effect of C-Raf on the mitochondrial reconstitution of VDAC, a mechanism, which may limit the exchange of metabolites and thereby indirectly gear cellular energy production towards glycolysis [4,36].

Not only do survival pathways converge upon energy producing processes but also proteins involved in the negative control of survival. The pro-apoptotic function of BAD, a BH3-only domain pro-apoptotic member of the Bcl-2 family, is normally inactivated through phosphorylation by survival kinases resulting in cytoplasmic relocalization [2] (Fig. 1B). When residing at the mitochondria BAD is part of a large protein complex that also contains glucokinase, which catalyzes the first step of glucose metabolism at the start of glycolysis or storage as glycogen. BAD is required to assemble this complex and lack of BAD results in reduced mitochondrial respiration in response to glucose. Glucose deprivation in turn results in dephosphorylation of BAD and apoptosis [37]. These findings again support the close interplay of cellular metabolism and survival control (Fig. 1).

5. Long-term adjustments

Short-term alterations in the composition and function of mitochondrial proteins are instrumental in cell death/survival decisions, but also enhanced mitochondrial biogenesis allows cells to adapt to an increased demand for energy production (long-term effect). Mitochondrial protein composition is of hybrid origin. Apart from a limited number of mitochondrially synthesized proteins, the overwhelming majority of proteins is encoded in the nucleus, synthesized in the cytoplasm and requires mitochondrial import. Formation of new mitochondrial proteins derived from nuclear and mitochondrial genomes is tightly regulated by transcription factors, such as nuclear respiratory factors 1,2 (NRF-1,2) or muscle-specific CREB and YY1 [38]. Of particular importance are their co-activators, PGC-1 (PPARγ/peroxisome proliferators-activated receptor coactivator-1) and PRC (PGC-1 related coactivator). Expression of PCG-1α by itself is sufficient to increase the number of mitochondria in cardiac and skeletal muscle in transgenic mice [39]. Expression and phosphorylation of PCG-1α are regulated in response to key physiological regulators [38]. p38, a member of the MAPK family, has been shown to phosphorylate PCG-1α on three residues in a negative regulatory domain adjacent to the transcriptional activation domain, resulting in the relief on transcriptional repression. Since many cytokines stimulate p38, this activation may increase PCG-1α activity allowing the cells to cope with the increased energy expenditure [40]. These coactivators thus could provide a link between extrinsic signals and mitochondrial biogenesis.

Nitric oxide (NO), which is an important regulator of proliferation and differentiation, also increases the expression of PCG-1α leading to mitochondrial biogenesis [41]. Given the important role of NO as a second messenger, this mechanism thus may connect cytoplasmic signaling with the regulation of mitochondrial processes. Most recently, it has been shown that NRF-1 regulated genes are also functional targets of the oncogene class transcription factor c-Myc [42]. c-Myc expression is rapidly induced by mitogenic signaling [43], and it is possible that this physiological response may establish a link between mitogen signaling and mitochondrial biogenesis.

5.1. Outlook

The outcome of signals transmitted in the cells is dictated by the effectors. To this end a mitochondria may represent a much more heterogeneous population than previously anticipated. Several published reports suggest that they not only differ in size, shape and number [44], but are biochemically and functionally different. Heterogeneity has not only been described for mitochondria originating from different cells and organs [45], but also for mitochondria within the same cell [45–47]. The reasons for this are best understood in cases where this reflects mitochondrial adaptation to specific energy demands of the cell or to tissue-specific functions (steroidogenesis in adrenal cortex; heme biosynthesis in bone marrow; and energy production for contraction in oxidative muscles or for various metabolic transformations in liver). Comparative proteomics of mitochondria from heart, brain, kidney and liver revealed that out of all potential or verified mitochondrial proteins only 50% are present in each individual tissue, suggesting the existence of a vast number of proteins, which allow mitochondria to adjust to organ-specific tasks. In the discussion of the mitochondrial heterogeneity within a single cell, the question arises whether mitochondria are independently responding units or functionally coupled parts of a network [46,48]. In the context of the question(s) discussed here, it will be interesting to see how intracellular functional compartmentalization can be achieved and which mechanisms regulate subset-specific responses to extrinsic challenges.

Signaling pathways postulated to function in the regulation of cellular energy production are frequently altered in diseases including cancer and other patho-physiological conditions (e.g., ischemia/reperfusion). But also mutations in mitochondrially encoded proteins can be the underlying cause of cardiomyopathies and other cytopathies, including diabetes, obesity, neurodegeneration and even cancer [49,50]. Understanding how these changes affect cellular metabolism and thus also survival and proliferation, metabolic alterations contributing to these diseases may be instrumental for the design of new therapeutic strategies in the treatment of these ailments.

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References

[1] Wang, X. (2001) Genes. Dev. 15, 2922–2933.
[2] Cory, S., Huang, D.C. and Adams, J.M. (2003) Oncogene 22, 8590–8607.
[3] Liston, P., Fong, W.G. and Korneluk, R.G. (2003) Oncogene 22, 8568–8580.
[4] Troppmair, J. and Rapp, U.R. (2003) Biochem. Pharmacol. 66, 1341–1345.
[5] Brazil, D.P. and Hemmings, B.A. (2001) Trends Biochem. Sci. 26, 657–664.
[6] Hardie, D.G., Scott, J.W., Pan, D.A. and Hudson, E.R. (2003) FEBS Lett. 546, 113–120.
[7] Carling, D. (2004) Trends Biochem. Sci. 29, 18–24.
[8] Li, Y., Corradetti, M.N., Inoki, K. and Guan, K.L. (2004) Trends Biochem. Sci. 29, 32–38.
[9] Fingar, D.C. and Blenis, J. (2004) Oncogene 23, 3151–3171.
[10] Manning, B.D., Tee, A.R., Logsdon, M.N., Blenis, J. and Cantley, L.C. (2002) Mol. Cell 10, 151–162.
[11] Inoki, K., Li, Y., Zhu, T., Wu, J. and Guan, K.L. (2002) Nat. Cell Biol. 4, 648–657.
[12] Inoki, K., Zhu, T. and Guan, K.L. (2003) Cell 115, 577–590.
[13] Boudeau, J., Sapkota, G. and Alessi, D.R. (2003) FEBS Lett. 546, 159–165.
[14] Stein, S.C., Woods, A., Jones, N.A., Davison, M.D. and Carling, D. (2000) Biochem. J. 345 (Pt 3), 437–443.
[15] Shaw, R.J., Bardeesy, N., Manning, B.D., Lopez, L., Kosmatka, M., DePinho, R.A. and Cantley, L.C. (2004) Cancer Cell 6, 91–99.
[16] Schofield, C.J. and Ratcliffe, P.J. (2004) Nat. Rev. Mol. Cell Biol. 5, 343–354.
[17] Semenza, G.L. (2003) Nat. Rev. Cancer 3, 721–732.
[18] Warburg, O. (1931) (Warburg, O., Ed.) Richard P. Smith Inc.
[19] Dang, C.V. and Semenza, G.L. (1999) Trends Biochem. Sci. 24, 68–72.
[20] Zwerschke, W., Mazurek, S., Massimi, P., Banks, L., Eigenbrodt, E. and Jansen-Durr, P. (1999) Proc. Natl. Acad. Sci. USA 96, 1291–1296.
[21] Le Mellay, V. et al. (2002) Adv. Enzyme Regul. 42, 317–332.
[22] Ryan, H.E., Lo, J. and Johnson, R.S. (1998) EMBO J. 17, 3005–3015.
[23] Gnaiger, E. and Kemp, R.B. (1990) Biochim. Biophys. Acta 1016, 328–332.
[24] Saraste, M. (1999) Science 283, 1488–1493.
[25] Salvi, M., Brunati, A.M., Bordin, L., La Rocca, N., Clari, G. and Toninello, A. (2002) Biochim. Biophys. Acta 1589, 181–195.
[26] Thomas, S.M. and Brugge, J.S. (1997) Annu. Rev. Cell Dev. Biol. 13, 513–609.
[27] Miyazaki, T., Neff, L., Tanaka, S., Horne, W.C. and Baron, R. (2003) J. Cell Biol. 160, 709–718.
[28] Yuryev, A., Ono, M., Goff, S.A., Macaluso, F. and Wennogle, L.P. (2000) Mol. Cell Biol. 20, 4870–4878.
[29] Cogswell, P.C. et al. (2003) J. Biol. Chem. 278, 2963–2968.
[30] Taylor, S.W. et al. (2003) Nat. Biotechnol. 21, 281–286.
[31] Gottlob, K., Majewski, N., Kennedy, S., Kandel, E., Robey, R.B. and Hay, N. (2001) Genes Dev. 15, 1406–1418.
[32] Chiu, V.K. et al. (2002) Nat. Cell Biol. 4, 343–350.
[33] Butow, R.A. and Avadhani, N.G. (2004) Mol. Cell 14, 1–15.
[34] Plas, D.R. and Thompson, C.B. (2002) Trends Endocrinol. Metab. 13, 75–78.
[35] Colombini, M., Blachly-Dyson, E. and Forte, M. (1996) in: Ion Channels (Narahashi, T., Ed.), vol. 4, pp. 169–202, Plenum Publishing Corporation, New York, NY.
[36] Le Mellay, V., Troppmair, J., Benz, R. and Rapp, U.R. (2002) BMC Cell Biol. 3, 14.
[37] Danial, N.N. et al. (2003) Nature 424, 952–956.
[38] Scarpulla, R.C. (2002) Biochim. Biophys. Acta 1576, 1–14.
[39] Lin, J. et al. (2002) Nature 418, 797–801.
[40] Puigserver, P. et al. (2001) Mol. Cell 8, 971–982.
[41] Nisoli, E. et al. (2003) Science 299, 896–899.
[42] Morrish, F., Giedt, C. and Hockenbery, D. (2003) Genes Dev. 17, 240–255.
[43] Kerkhoff, E., Houben, R., Loffler, S., Troppmair, J., Lee, J.E. and Rapp, U.R. (1998) Oncogene 16, 211–216.
[44] Jensen, R.E., Dunn, C.D., Youngman, M.J. and Sesaki, H. (2004) Trends Cell Biol. 14, 215–218.
[45] Mootha, V.K. et al. (2003) Cell 115, 629–640.
[46] Collins, T.J., Berridge, M.J., Lipp, P. and Bootman, M.D. (2002) EMBO J. 21, 1616–1627.
[47] Kuznetsov, A.V., Usson, Y., Leverve, X. and Margreiter, R. (2004) Mol. Cell Biochem. 256–257, 359–365.
[48] Skulachev, V.P. (2001) Trends Biochem. Sci. 26, 23–29.
[49] Wallace, D.C. (1999) Science 283, 1482–1488.
[50] Polyak, K. et al. (1998) Nat. Genet. 20, 291–293.