Redox Biology 12 (2017) 208–215

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Review article

Mitochondrial composition and function under the control of hypoxia

Dominik C. Fuhrmann[a], Bernhard Brüne[a,b,*]

[a] Institute of Biochemistry I, Faculty of Medicine, Goethe-University Frankfurt, 60590 Frankfurt, Germany
[b] Project Group Translational Medicine and Pharmacology TMP, Fraunhofer Institute for Molecular Biology and Applied Ecology, 60596 Frankfurt, Germany

ARTICLE INFO

Keywords:
Mitochondria
ROS
Hypoxia
HIF
Respiratory chain

ABSTRACT

Hypoxia triggers several mechanisms to adapt cells to a low oxygen environment. Mitochondria are major consumers of oxygen and a potential source of reactive oxygen species (ROS). In response to hypoxia they exchange or modify distinct subunits of the respiratory chain and adjust their metabolism, especially lowering the citric acid cycle. Intermediates of the citric acid cycle participate in regulating hypoxia inducible factors (HIF), the key mediators of adaptation to hypoxia. Here we summarize how hypoxia conditions mitochondria with consequences for ROS-production and the HIF-pathway.

1. Introduction

Hypoxia imposes stress to cells and organisms, which occurs under both, pathological and non-pathological conditions. The lack of oxygen is linked to diseases like cancer, diabetes, or inflammation but constitutes also a challenge for people living at high altitude. Cells within organisms adapt to hypoxia by altering their metabolism. This is facilitated by changes in protein expression occurring at the transcriptional or translational level, mRNA- or protein stability as well as enzyme activity. Sensors and adaptors towards decreased oxygen availability are prolylhydroxylases (PHD), also known as Egl nine homolog 1 proteins (EGLN). Their loss of activity stabilizes the transcription factors hypoxia inducible factors (HIF) under hypoxia. HIFs enter the nucleus to enhance transcription of a variety of target genes, including mitochondrial components.

Major consumers of oxygen in the cell are mitochondria. Consequently, they are severely affected by decreased oxygen availability. Along those lines, hypoxia alters mitochondrial fusion and fission, mitophagy, and oxidative phosphorylation (OXPHOS). OXPHOS is adapted to hypoxia by remodeling the electron transport chain (ETC) as well as the activity of the TCA cycle. The mitochondrial respiratory chain was originally described as flavin- and cytochrome-containing proteins in the inner mitochondrial matrix [1]. This model proposed the four major complexes, i.e. NADH-coenzyme Q reductase (complex I), succinate-coenzyme Q reductase or succinate dehydrogenase (complex II or SDH), ubiquinol cytochrome c reductase (complex III), and cytochrome c oxidase (complex IV) of the respiratory chain randomly dispersed in the matrix, being connected by the redox active enzymes coenzyme Q (CoQ) and cytochrome c [2,3]. This model was refined with complex I, III, and IV forming supercomplexes that allow an effective electron transport with a minimum of superoxide (O_2^-) production. Nevertheless, ROS (if not specified the term refers to both, superoxide and H_2O_2) from complex I, II, and III appear not only as an accidental escape of electrons from the ETC and their transfer to molecular oxygen, but are now considered as important mediators in physiological cell signaling. ROS production needs to be tightly controlled to avoid its overproduction, provoking damage of mitochondrial and extramitochondrial macromolecules and eliciting cell death [4]. Considering that ROS signaling is linked to the HIF system, it allows anticipating multiple layers of reciprocal interaction. Each ETC complex adapts to hypoxia by replacing distinct proteins, which alter the function of the complex. Using this system, it is not necessary to build an entire new complex to adapt, making these processes fast, reversible, and highly effective. Another adaptive response to hypoxia is the reduction of mitochondrial mass, by mitophagy [5]. This specialized form of autophagy involves factors such as nucleoporin p62, beclin1, microtubule-associated protein1A/1B-light chain 3 (LC3), BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3), or BNIP3 ligant [6,7]. These components are part of the phosphatidylinositide 3-kinase class III complex-mediated autophagosome formation. The autophagosome docks to lysosomes, followed by fusion and subsequent digestion of trapped macromolecules/organelles. Some proteins eliciting autophagosome formation like BNIP3 are HIF-regulated, suggesting that mitophagy under hypoxia is at least partly HIF-driven [8–10]. In this review, we address fundamental changes and adaptive mechanisms of mitochondria and mitochondrial ROS production to hypoxia and refer to the crosstalk between mitochondria and the HIF-system (Fig. 1).

* Corresponding author at: Goethe-University Frankfurt, Faculty of Medicine, Institute of Biochemistry I, Theodor-Stern-Kai 7, 60590 Frankfurt, Germany.
E-mail address: b.bruene@biochem.uni-frankfurt.de (B. Brüne).

http://dx.doi.org/10.1016/j.redox.2017.02.012
Received 3 January 2017; Received in revised form 31 January 2017; Accepted 18 February 2017
Available online 24 February 2017

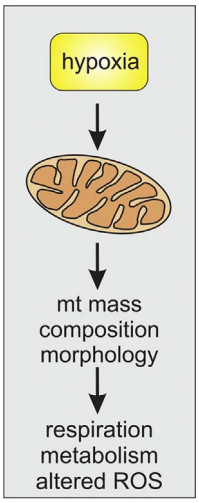

Fig. 1. Hypoxia and mitochondria. Hypoxia acts on mitochondria in multiple ways. It changes the protein composition of the electron transport chain, mitochondrial morphology, and mitochondrial mass. Consequences comprise an altered rate of respiration, changes in the mitochondrial intermediary metabolism, and an adjusted increased or decreased ROS-producing capacity. These changes determine mitochondrial biology with an outreaching impact on cell physiology. *Abbreviations: mt: mitochondrial, ROS: reactive oxygen species.*

2. Mitochondrial ROS production in brief

Besides NADPH oxidases, mitochondria emerged as a powerful source of ROS. Fig. 2 provides an overview towards ROS-production by the ETC and ROS-detoxifying enzymes. Deficiencies in assembling ETC components, mutations in distinct ETC subunits, or the specific inhibition of the ETC can increase ROS production. The molecular details of ROS production at distinct ETC complexes are elaborated in review articles [11–16]. Individual targets, experiencing oxidative modifications in response to complex I and III ROS have been identified [17]. Complex I ROS oxidize TCA cycle associated proteins like subunits of pyruvate dehydrogenase or isocitrate dehydrogenase as well as 2-oxoglutarate dehydrogenase complex component E2 [17]. Apparently, complex I ROS are predominantly oxidizing proteins that are localized in the matrix. Complex III ROS in turn oxidize proteins such as voltage-dependent anion channel 3 and mitochondrial import inner membrane translocase subunit TIM50 but also NDUFB10 and NDUFS3, both components of complex I, which are located in the mitochondrial inner membrane [17]. The voltage-dependent anion channel 3 in the outer mitochondrial membrane was proposed as a marker for oxidative stress, occurring in the intermembrane space [18]. Conclusively, distinct sources of ROS modify divergent proteins and thus, alter discrete signaling pathways (Fig. 3).

3. Regulation of HIF

HIF proteins are key determinants of a cellular response to hypoxia. Once stabilized, they induce multiple target genes, thereby affecting intermediary metabolism e.g. by increasing the expression of proteins involved in glycolysis and decreasing oxygen-dependent pathways by altering the ETC complex structure and activity. The fundamental concepts of HIF regulation are outlined in Fig. 4.

4. Fusion and fission

Besides changes at the protein level, that affect the quarterly structure of protein aggregates, hypoxia impacts on the mitochondrial morphology, including cristae structure (Fig. 5) [19]. Under normoxia,

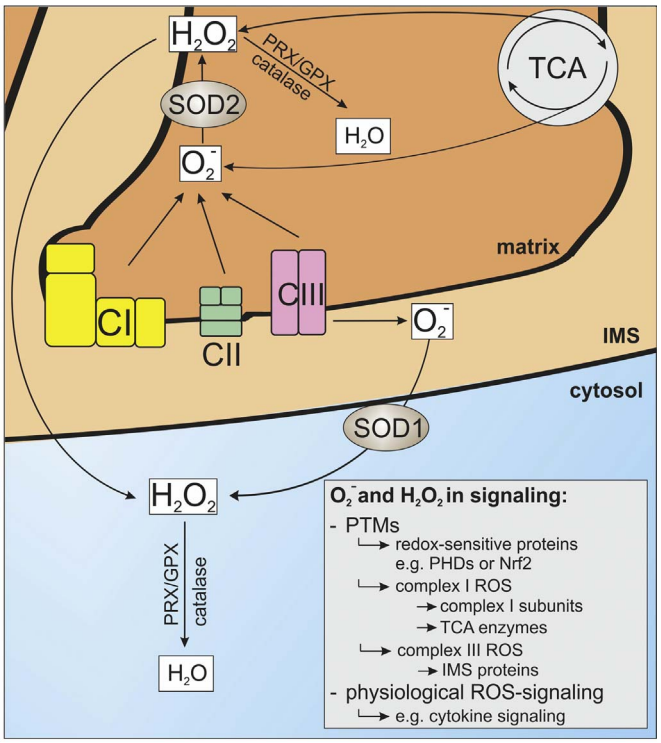

Fig. 2. Mitochondrial ROS. Complexes I, II, and III of the respiratory chain are shown to release O_2^- either into the matrix (CI, CII, CIII) or into the intermembrane space (CIII) [11]. To avoid damage of mitochondrial proteins, lipids, or DNA, effective defense mechanisms are in place. Mitochondrial or cytosolic SOD disproportionate O_2^- to molecular oxygen and H_2O_2. In turn, H_2O_2 is efficiently removed by catalase and/or the PRX/GPX system. Nevertheless, ROS may cause PTMs of redox-sensitive proteins, e.g. components of the ETC or transcriptional elements such as Nrf2 or PHDs. ROS cannot only be regarded as damaging agents, as they appear critical for physiological redox-signaling, e.g. during cytokine formation to protect cells from pathogen invasion. Interestingly, cancer cells were shown to protect mitochondria under hypoxic conditions by an increased expression of antioxidative proteins [28]. Thus, cytoplasmic and intermembrane space SOD1 and matrix SOD2 are transcriptionally upregulated under hypoxia, enhancing superoxide detoxification but producing H_2O_2. Although this review concentrates on ROS produced by the ETC, it should be mentioned that NAD-linked dehydrogenases of the TCA cycle such as 2-oxoglutarate dehydrogenase and pyruvate dehydrogenase are potential sites of electron leakage and thus, ROS formation [29]. *Abbreviations: ETC: electron transport chain, GPX: glutathione peroxidase, Nrf2: Nuclear factor erythroid derived 2, IMS: mitochondrial intermembrane space, PHD: prolyl hydroxylase, PRX: peroxiredoxin, PTM: posttranslational modification, ROS: reactive oxygen species, SOD: superoxide dismutase, TCA: citric acid cycle.*

mitochondria form tubular networks favoring OXPHOS and ATP production. Under hypoxia, mitochondria undergo fission and appear as single organelles, possibly to promote mitophagy, to keep ROS production at a physiological low level, and to maintain integrity by a decrease in respiratory activity.

5. Crosstalk between HIF and mitochondria

Ongoing research in the areas of metabolomics, assembly, and structure of ETC components provide multiple links between mitochondria and the HIF pathway, as depicted in Fig. 6. HIF gets stabilized by a decreased PHD activity, an enzyme demanding metabolites of the mitochondrial TCA cycle for catalysis. In turn, HIF induces the expression of proteins, which impinge on both, metabolism and structure of mitochondria as described in detail in the following figures.

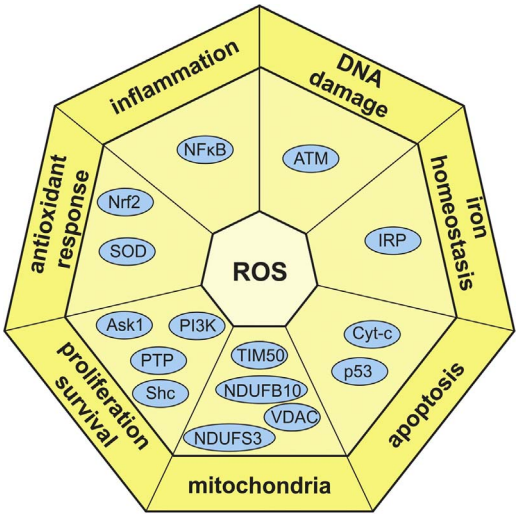

Fig. 3. ROS in cellular signaling. The picture of ROS has changed within the last decades from being considered a harmful substance to an important signaling molecule [30]. Especially mitochondrial-derived ROS are mediators of diverse signaling pathways to provoke immune response, elicit antioxidative signaling, initiate DNA damage responses, affect iron homeostasis, stimulate apoptosis, or signal towards cell survival and proliferation [31–33]. The apparently contradictory roles linked to ROS signaling can be explained by the distinct species, amount, and duration of ROS production as well as antioxidative capacities. For example, a high ROS production rate causes cell death, while moderate ROS levels provoke cytokine production (inflammation). Importantly, spacial and subcellular ROS production by mitochondria and NADPH oxidases create oxidative microenvironments within the cell, affecting distinct proteins or organells. This review focuses on mitochondrial ROS in connection with hypoxic adaptation. Fig. 3 shows several proteins (blue), which are influenced by ROS (inner heptagon), and their role in cellular signaling (outer ring). *Abbreviations: Ask1: apoptosis signal-regulating kinase 1, ATM: ataxia telangiectasia mutated, Cyt c: cytochrome c, IRP: iron-related protein, NADPH: nicotinamide adenine dinucleotide phosphate, NDUFB10: NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 10, NDUFS3: NADH dehydrogenase [ubiquinone] 1 iron-sulfur protein 3, NFkB: nuclear factor (NF) kappa B, Nrf2: Nuclear factor erythroid derived 2, p53: cellular tumor antigen p53, PI3K: phosphatidylinositol 3-kinase, PTP: protein-tyrosine phosphatase, ROS: reactive oxygen species, Shc: SHC-transforming protein, SOD: superoxide dismutase, TIM50: mitochondrial import inner membrane translocase subunit TIM50, VDAC: voltage-dependent anion channel.*

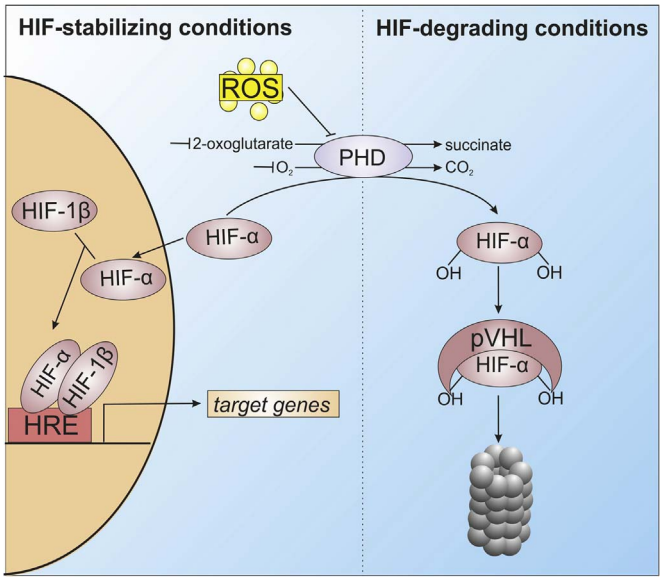

Fig. 4. Basics of the HIF-system. Under hypoxic conditions HIF is a master regulator for adaptation to low oxygen content and causes the expression of roughly 400 target genes [34]. The heterodimeric transcription factor consists of the constitutively expressed HIF-1β also known as ARNT and a labile α-subunit, belonging to the basic helix-loop-helix/Per ARNT SIM transcription factor family. Three HIF-α isoforms are known in mammals. HIF-1α and HIF-2α share high sequence homology and regulatory features, containing an oxygen-dependent degradation domain, with two conserved prolyl residues [35]. Under normoxia the α-subunit prolyl residues are continuously hydroxylated by PHD 1–3. This reaction is catalyzed by a set of non-haem Fe(II)- and 2-oxoglutarate-dependent dioxygenase. During catalysis, the splitting of molecular oxygen is coupled to the hydroxylation of HIF, while the oxidative decarboxylation of 2-oxoglutarate gives succinate and CO_2. PHDs are inhibited (ł) by the lack of oxygen or metabolites such as fumarate or succinate (further information in Fig. 6). Following hydroxylation, pVHL binds and marks HIF for ubiquitination and thus, proteasomal degradation [36–38]. Under hypoxic conditions, PHDs are inhibited, HIF-α gets stabilized, translocates to the nucleus, dimerizes with its corresponding β-subunit, and recognizes HREs within a promoter or enhancer of distinct target genes to initiate transcription by recruitment of cofactors like p300 or CBP [39,40]. Inactivation of PHD enzymes may be promoted by ROS e.g. due to oxidation of the central Fe(II) to Fe(III), especially if the antioxidative capacity of the cell is low. It remains a matter of discussion to define concentrations, distinct ROS species and/or secondary metabolites that interfere with PHD activity. Recent studies revealed that PHDs are not only sensitive to iron oxidations but also contain redox-sensitive cysteines, which are subjected to multiple modifications. This highlights diverse possibilities of PHD activity regulation and thus HIF stabilization [41]. *Abbreviations: ARNT: Aryl hydrocarbon receptor nuclear translocator, CBP: CREB-binding protein, HIF: hypoxia inducible factor, HRE: hypoxia response element, p300: histone acetyl transferase p300, PHD: prolyl hydroxylase, pVHL: tumor suppressor protein von Hippel-Lindau, ROS: reactive oxygen species.*

6. Adaptation of the respiratory chain to hypoxia

ROS generation is a tightly controlled process and has pivotal roles in patho-physiological signaling. Thus, adaption processes have evolved to maintain mitochondrial membrane potential and ATP production, at the same time avoiding uncontrolled ROS production. This becomes obvious when oxygen availability declines. A lack of oxygen induces reductive carboxylation, which was shown to increase ROS production [20–23]. Moreover, hypoxia causes multiple changes in the composition of ETC complexes. These changes are required to keep mitochondria intact under low oxygen conditions and to prevent excessive ROS formation. Most of the changes in complex composition occur within complex I, a dominant acceptor of electrons, and complex IV, facilitating electron transport to molecular oxygen. Some of these alterations comprise the exchange of subunits within ETC complexes others modify complex structure, while subunit depletion also occurs. Remarkably, the amount of ROS formation or its reduction strongly depends on the subunit being affected. Figs. 7 and 8 summarize distinct modifications of ETC complexes under hypoxia and outline consequences for ROS production. Besides hypoxia, reoxygenation appears as a trigger for ROS production influencing complex I. Thus, complex I subunits like NDUFV1, NDUFV2, NDUFS2, and NDUFS3 are oxidized after reoxygenation provoking a decrease in protein stability [24].

Another form of oxidative stress sensed and handled predominantly by mitochondria is ischemia and reperfusion (I/R). During I/R an oxidative burst occurs, which is generated by enhanced succinate dehydrogenase activity and Ca^{2+} influx, which opens the mitochondrial permeability transition pore. An overview of ROS generating mechanisms and consequences of I/R is depicted in Fig. 9.

7. Conclusion

Research over the last decades highlighted the impact of hypoxia on mitochondria and mitochondrial metabolism, including changes in ROS production and signaling. It is becoming apparent that ETC complexes adapt by posttranslational modification of distinct proteins in individual complexes or by altering subunit composition to keep

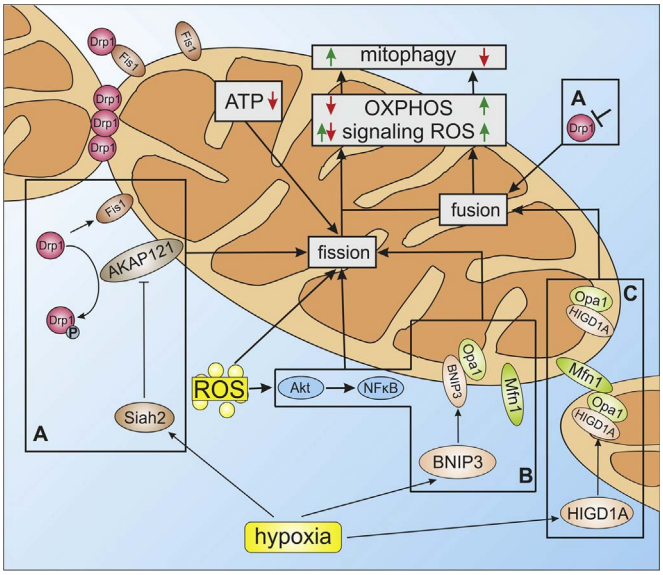

Fig. 5. Fusion and fission under hypoxia. A Fission: The dynamic mitochondrial network structure is controlled by fusion and fission. Fission is mediated by Drp1 and Fis1 [42]. Drp1 is a target of diverse posttranslational modifications. AKAP121 phosphorylates Drp1 in a PKA-dependent manner, inhibiting its function. Phosphorylation of Drp1 is indirectly attenuated by Siah2, which is expressed under severe hypoxic conditions, i.e., ischemia thereby promoting fission [43]. Interestingly, Siah2 is also described as a HIF-α-stabilizing protein by regulating PHD stability [44], arguing for a feed-forward amplification loop in regulating fission. Other reports demonstrated that Drp1 is upregulated under hypoxia to provoke migration of U251 cells [45]. Thus, decreased Drp1-mediated fission reduced migration of MDA-mb-231 cells, linking Drp1 to tumor progression and metastasis [46]. Furthermore, a knockdown (ŀ) of Drp1 enhanced fusion and reduced fission-mediated ROS production. ROS may foster mitophagy, indicating that the process of mitochondrial fusion is linked to physiological ROS generation [47]. B BNIP3 promotes fission: Opa1 is a dynamine of the inner mitochondrial membrane and interaction partner of Mfn1 that together promote fusion. Binding of the hypoxia-inducible protein BNIP3 to Opa1 promotes Opa1 oligomer disassembly, thereby fostering fission and further on, pushing cells towards apoptosis [48]. Besides, fission can be induced by ROS via the Akt- and NFκB-pathway [49]. However, at present it remains unclear, whether ROS generated by ETC defects induce fission under hypoxia. **C** HIGD1A promotes fusion: When the HIF-induced protein HIGD1A targets Opa1, it is protected from cleavage, promotes the interaction with Mfn1 and thus, fusion [50]. Logically, a loss of HIGD1A increases fission, cristae disorganization, and reduces cell growth. Thus, HIGD1A and BNIP3 exert opposing functions in fusion and fission under hypoxia. Besides Opa1-cleavage, a loss of ATP, likely due to an impaired ETC under hypoxia, is discussed to induce mitochondrial fission [51]. *Abbreviations: AKAP121: A-kinase anchoring protein 121, Akt: RAC-alpha serine/threonine-protein kinase, BNIP3: BCL2/adenovirus E1B 19 kDa protein-interacting protein 3, Drp1: dynamin-related protein 1, ETC: electron transport chain, Fis1: fission 1, HIF: hypoxia inducible factor, HIGD1A: hypoxia inducible gene 1, Mfn1: mitofusin, NFkB: nuclear factor NF kappa B, Opa1: Optic atrophy 1, OXPHOS: oxidative phosphorylation, PHD: prolyl hydroxylase, PKA: protein kinase A ROS: reactive oxygen species, Siah2: E3 ubiquitin-protein ligase Siah2.*

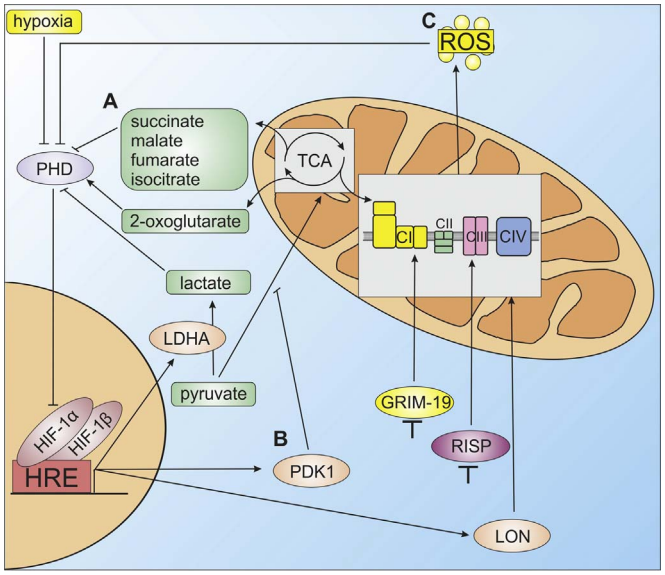

Fig. 6. Crosstalk between mitochondria and HIF. A TCA cycle metabolites regulate PHD activity: Succinate, fumarate, pyruvate, oxalacetate, and isocitrate all attenuate PHD activity and thus, favor accumulation of HIF-α subunits [52–54]. In contrast, 2-oxoglutarate constitutes the cosubstrate for PHD activity. B HIF inhibits the TCA cycle: HIF-1 lowers the activity of the TCA cycle by inducing PDK1 [55–57]. PDK1 inhibits pyruvate dehydrogenase activity to reduce levels of acetyl CoA, which under normoxia is processed to citrate and fuels the TCA cycle. LDHA increases under hypoxia, followed by enhanced conversion of pyruvate to lactate [57,58]. In turn, lactate stabilizes HIF, especially in tumor cells that are characterized by a shift from OXPHOS to glycolysis [59–61]. C Mitochondrial ROS interfere with PHD activity: Mitochondrial respiratory chain complexes are an established source of ROS. ROS, especially H_2O_2 inhibits PHD activity, thereby interfering with HIF-degradation [62]. A rapid increase in ROS production and thus, PHD inhibition was observed within the first minutes of hypoxic incubations, corroborating stabilization of HIF under acute hypoxic conditions [63]. A knockdown (ŀ) of GRIM-19 (NDUFA13), a subunit of complex I, induces ROS, with the consequence to stabilize HIF-1 and to enhance autophagy [64,65]. Also complex II gained some interest. A loss of SDHB, the iron-sulfur cluster containing subunit of complex II, provoked a ROS-dependent stabilization of HIF and stimulated tumorigenesis [64–66]. A study by Guzy et al. documented that exclusively the loss of SDHB, but no other subunit of complex II, triggered ROS formation with the subsequent ability to stabilize HIF [64]. Also, complex III ROS were identified to stabilize HIF, which enhanced invasiveness in melanoma cells [67]. However, there is some controversy whether complex III ROS are a prerequisite to induce HIF even under hypoxia [68,69]. A knockdown of the RISP of complex III reduced HIF-1 expression and was claimed to prove the connection between complex III ROS and HIF [70]. Diaz et al. showed that a knockdown of RISP also changes ETC supercomplex formation as well as the abundance of complex I and IV [71]. This leaves the possibility that a knockdown of RISP has consequences for the entire respiratory chain and possibly for mitochondrial metabolism in general, raising the question whether secondary effects explain the observations by Brunelle et al. Hypoxia also upregulates the mitochondrial protease Lon, which degrades mitochondrial proteins as part of the mitochondrial protein quality control system, thereby increasing ROS production followed by apoptosis [72]. Abbreviations: ETC: electron transport chain, HIF: hypoxia inducible factor, HRE: hypoxia response element, LDHA: lactate dehydrogenase A, Lon: Lon protease homolog, NDUFA13: NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 13, OXPHOS: oxidative phosphorylation, PDK1: pyruvate dehydrogenase kinase 1, PHD: prolyl hydroxylase, RISP: Rieske iron-sulfur protein, ROS: reactive oxygen species, SDHB: succinate dehydrogenase [ubiquinone] iron-sulfur subunit, TCA: citric acid cycle.

ROS production at a low, physiological level (Fig. 10). However, cell type specific responses, the presence of various amounts of detoxifying components and ROS-facilitated oxidation in micro-compartments such as the inner membrane space versus the inner mitochondrial membrane or the mitochondrial matrix make it difficult to generalize observations. Another issue of controversy is stabilization of HIF by mitochondrial ROS and the production of ROS under hypoxia itself. Very likely, the different mechanisms that allow adaption of the ETC complexes to hypoxia evolved to keep ROS at a balanced level. Low physiological mitochondrial ROS are needed to guarantee cytokine formation during defense against invading pathogens, at the same time keeping mitochondrial integrity intact, e.g. avoiding destruction of mitochondrial DNA, lipids, or macromolecules [25]. Moreover, adaption may preserve other functions of mitochondria during intermediary metabolism, like amino acid metabolism or the delivery of building blocks needed for cell proliferation under hypoxia. The advantage of

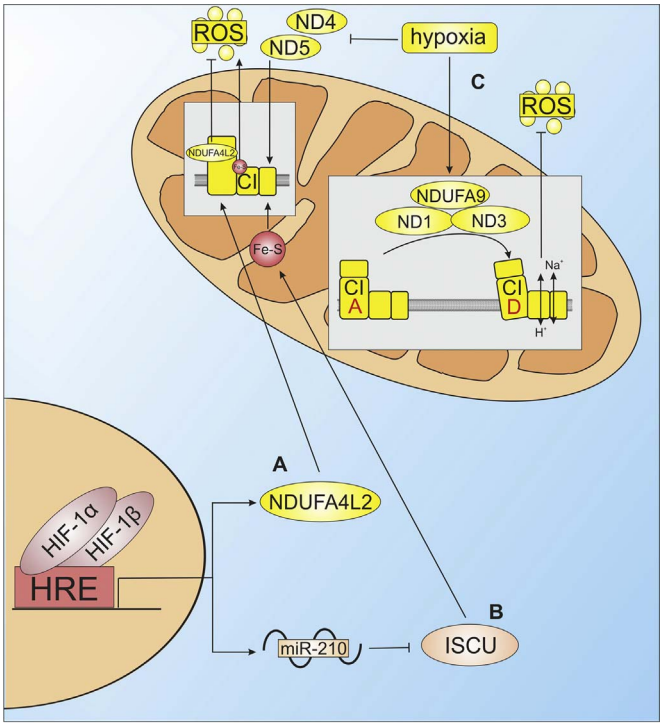

Fig. 7. Hypoxic adaptation of complex I. A NDUFA4L2 decreases complex I activity: NDUFA4L2 was characterized by Tello et al. as a HIF-1 target gene, which reduced complex I activity under hypoxic conditions [73]. Induction of NDUFA4L2 under hypoxia decreased respiration, prevented an increase in ROS production and preserved the membrane potential. Lai et al. confirmed the HIF-1-dependent induction of NDUFA4L2 and connected its enhanced expression in hepatocellular carcinoma to poor patient survival [74]. Along those lines, a knockdown of NDUFA4L2 suppressed tumor growth as well as metastasis *in vivo*. B miR-210 decreases ISCU: miR-210 is an established HIF-target and one of the major regulators of metabolism under hypoxia [75,76]. Furthermore, miR-210 reduces levels of complex I and IV together with their enzyme activity [77]. Thus, it is not surprising that miR-210 plays a central role in regulating ETC complex formation. Importantly, miR-210 attenuates ISCU [78,79]. ISCU functions as a scaffold for maturation of iron-sulfur containing proteins, such as NDUFS in complex I. A defect in an iron-sulfur containing protein disrupts the electron flow within this complex. Thus, a miR-210-mediated decrease in ISCU induces ROS formation under hypoxia [80]. Both, NDUFA4L and ISCU are HIF- regulated proteins. While NDUFA4L2 is increased, ISCU is decreased by mR-210. Interestingly, they have opposing roles in ROS production. NDUFA4L2 induction decreases ROS, but a reduction of ISCU increases ROS formation. These findings underscore the relevance of the site for ROS production and highlight the difficulties in predicting how modifications or differently composed ETC complexes affect ROS production. C Hypoxia provokes complex I transition: Under hypoxia, respectively during ischemia, complex I changes its conformation from the active (A) to the dormant (D) form [81]. The D-form is considered to be silent in term of ROS formation and thus, does not create a burst in ROS following reoxygenation [82]. The precise mechanism is not fully understood, but the proteins NDUFA9, ND1, and ND3 at the connection of the hydrophilic and the membrane arm of complex I, apparently are involved [83]. After I/R, Cys39 of ND3 is exposed and can be S-nitrosated by different S-nitrosating species, maintaining complex I in the D-form and reducing ROS production upon reoxygenation [84]. Other proteins of the membrane arm, ND4 and ND5, were decreased under hypoxia at mRNA level, decreasing complex I activity [85]. Raising the question whether those subunits might also be involved in complex I transition. *Abbreviations: ETC: electron transport chain, HIF: hypoxia inducible factor, HRE: hypoxia response element, I/R: ischemia/reperfusion, ISCU: iron sulfur cluster assembly enzyme, miR: micro RNA, ND1: NADH-ubiquinone oxidoreductase chain 1, ND3: NADH-ubiquinone oxidoreductase chain 3 ND4: NADH-ubiquinone oxidoreductase chain 4, ND5: NADH-ubiquinone oxidoreductase chain 5, NDUFA9: NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9, NDUFA4L2: NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 4-like 2, ROS: reactive oxygen species.*

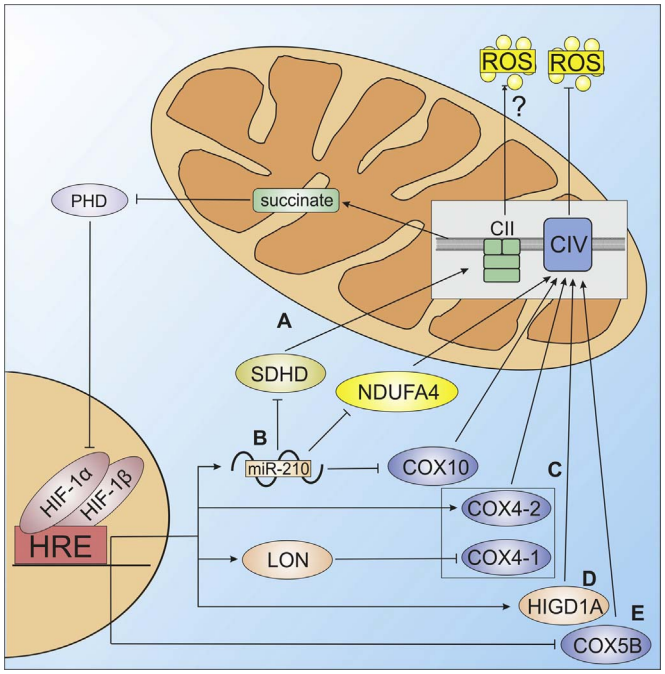

Fig. 8. Hypoxic adaptation of complex II and IV. A Complex II: As part of the TCA cycle and the respiratory chain complex II (succinate dehydrogenase) is integral to mitochondrial metabolism. HIF-1, via the induction of miR-210, modulates complex I, as described in Fig. 7 [86]. In addition, miR-210 targets SDHD, one of the membrane bound and haem *b* containing subunits of complex II [87]. In A549 cells transfected with miR-210 SDHD expression decreased, accompanied by a lower complex II activity. Recent studies identified complex II, besides complex I and III, as a generator of ROS, opening the possibility that complex II serves as an additional regulator of ROS under hypoxia [88]. B miR-210 regulates complex IV abundance and activity: Besides ISCU and SDHD, the complex IV subunit COX10 was identified as a miR-210 target either by transfecting HCT116 cells with miR-210 under normoxia or with an antagomir under hypoxia [89]. These experiments linked miR-210 expression to increased ROS production. Moreover, a screening approach also identified NDUFA4 as a miR-210 regulated complex IV subunit [87,90]. C Subunit exchange in complex IV: Complex IV is regulated by a HIF-dependent exchange of its subunits COX4-1 and COX4-2 under hypoxic conditions [91]. HIF-1 induces COX4-2 together with the Lon protease, which in turn degrades COX4-1. The complex subsequently incorporates COX4-2. This modification optimizes the efficiency of the complex to transfer electrons to molecular oxygen under low oxygen conditions and thus, to decrease ROS formation, maintain ATP production, and to preserve the integrity of complex IV. D HIGD1A enhances complex IV activity: As an early hypoxic inducible and HIF-dependent protein, HIGD1A apparently amalgamates many functions as already described for its function in fusion and fission. Among others, it ensures optimal performance of complex IV [92]. Hayashi et al. showed that HIGD1A binds to complex IV *in vivo*, while recombinant HIGD1A directly integrated into purified bovine complex IV. Precisely, HIGD1A exerts a protective function. It accumulated around the haem *a* containing active center of complex IV, a protein cluster, which is responsible for the proton pumping activity of complex IV. E COX5B decreases HIF-1α-dependently: In white adipose tissue COX5B decreases during aging due to HIF-1α mediated transcriptional repression [93]. The lack of COX5B decreases complex IV assembly and activity. Additionally, inhibition of COX5B facilitated lipid accumulation by a reduction in fatty acid oxidation, which in turn enlarged white adipocytes. *Abbreviations: COX4: cytochrome c oxidase subunit 4, COX5B: cytochrome c oxidase subunit 5B, COX10: protoheme IX farnesyltransferase, ETC: electron transport chain, HIF: hypoxia inducible factor, HIGD1A: hypoxia inducible gene 1, HRE: hypoxia response element, Lon: Lon protease homolog, ISCU: iron sulfur cluster assembly enzyme, miR: micro RNA, NDUFA4: NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 4, PHD: prolyl hydroxylase, ROS: reactive oxygen species, SDHD: succinate dehydrogenase [ubiquinone] cytochrome b small subunit, TCA: citric acid cycle.*

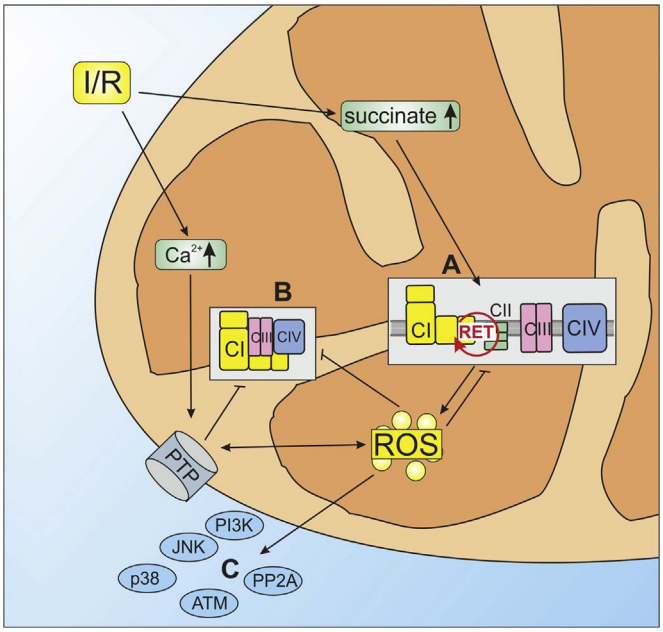

Fig. 9. Ischemia and Reperfusion. A I/R induces ROS: During I/R, the activity of ETC complexes is reduced by an extraordinary increase in ROS, which is a result of succinate accumulation under ischemia. Upon reperfusion, succinate is rapidly oxidized resulting in a burst of ROS, probably mediated by a reverse electron transport from complex II to complex I [94]. The increase of ROS together with an I/R-induced calcium influx into mitochondria opens the mitochondrial PTP, further increasing ROS formation, decreasing ETC activity, and finally provoking cell death [95]. B An open PTP decreases supercomplex formation: An open PTP causes supercomplex degradation after reperfusion. After I/R, supercomplex abundance was reduced but could be recovered either by the PTP inhibitor sanglifehrin or the ROS and electron scavenger XJB-5–131 [95]. C Consequences of mitochondrial ROS: Mitochondrial ROS production during I/R may activate survival mechanisms, i.e. mitogen-activated protein kinases such as JNK, p38, or PI3K, promote apoptosis-associated mechanisms such as PLC1- and PP2A-activation, or induce cell proliferation via ATM [96–98]. The distinct, in part contradictory, signaling qualities of ROS may be explained by dissimilar sources of ROS. Along those lines, complex I ROS are described to be harmful in cardiac I/R, while complex III ROS appeared to be cardio-protective [99]. *Abbreviations: ATM: ataxia telangiectasia, ETC: electron transport chain, I/R: ischemia/reperfusion, JNK: c-Jun N-terminal kinase, p38: mitogen-activated protein kinase p38, PI3K: phosphatidylinositol 3-kinase, PP2A: Serine/threonine-protein phosphatase PP2A, PLC1: 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase, PTP: mitochondrial permeability transition pore, RET: reverse electron transport, ROS: reactive oxygen species.*

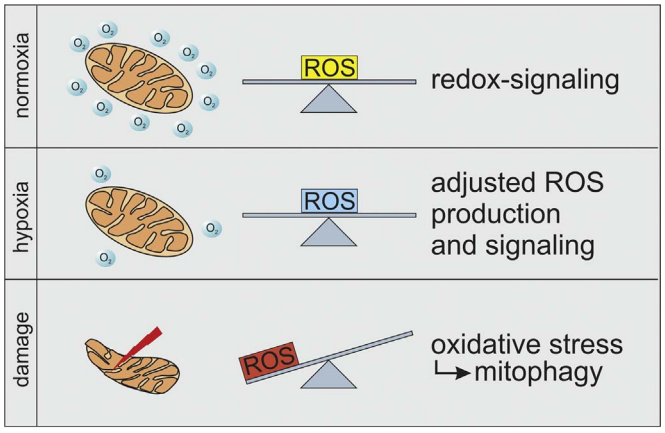

Fig. 10. Conclusion. Current concepts provide evidence for a complex interaction between a hypoxic mitochondrial biology and their ability to produce ROS. Mitochondrial ROS production cannot only be seen as an accidental escape of electrons from the ETC and its transfer to molecular oxygen with the production of superoxide and/or hydrogen peroxide but should also be considered an essential component of physiological cell communication. In a hypoxic environment, often found in tumors, mitochondrial biology is altered. It is generally accepted that OXPHOS is slowed down because reducing equivalents generated in the TCA cycle are decreasing. Nevertheless, hypoxia massively modulates ETC composition and activity. Interestingly, hypoxia, mostly via the HIF-transducing system, produces subtle changes by modifying individual proteins in complex I to IV or by replacing distinct proteins with either more effective (e.g. complex IV) or less efficient (e.g. complex I) variants. Energetically, this is more efficient rather than replacing entire ETC complexes and probably allows a much faster return to basal complex composition when returning to normoxia. Overall, these changes adjust the ability of ROS production under hypoxia, often reducing the ability to generate ROS. It can be speculated that under hypoxia, despite a reduced electron flow through the individual redox centers, mitochondria try to minimize ROS formation in order to lower the risk of macromolecular damage. One can also argue that changes are preventive in order to suppress a burst in ROS once mitochondria return from hypoxia back to normoxia. Along these lines, mitochondrial fission certainly may eliminate damaged mitochondria that often show increased ROS production. Fission observed during hypoxia may be considered as a preventive mechanism to lower ROS production and to exit to mitophagy once environmental conditions get worse or to fuse when mitochondria face normoxia again. We certainly have to learn more about the communicating ability and distinct targets of ROS under hypoxia and explore how a gradual and time-dependent decrease of oxygen affects mitochondrial biology and what happens upon the shift from hypoxia back to normoxia. *Abbreviations: ETC: electron transport chain, HIF: hypoxia inducible factor, HIGD1A: hypoxia inducible gene 1, OXPHOS: oxidative phosphorylation, ROS: reactive oxygen species, TCA: citric acid cycle.*

exchanging single subunits or modifying distinct proteins only may preserve the structure and composition of the entire ETC and guarantee a fast and high degree of reversibility upon the return to normoxia. What is missing at present, are detailed studies how mitochondria functionally, structurally, and ETC composition wise respond to acute and/or chronic hypoxia as well as the return to normoxia. It remains to be elucidated, whether mitophagy removes mitochondria, which failed to adapt, or diminishes mitochondria independently of their state of adaption as part of the metabolic adjustment to hypoxia. Techniques like complexome profiling coupled to 2-dimensional blue native electrophoresis, metabolomics, and cell respirometry will help to answer these question [26,27].

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This work was supported by Deutsche Forschungsgemeinschaft (SFB 815, project A8).

References

[1] D.E. Green, A. Tzagoloff, The mitochondrial electron transfer chain, Arch. Biochem. Biophys. 116 (1966) 293–304.
[2] B. Chance, G.R. Williams, A method for the localization of sites for oxidative phosphorylation, Nature 176 (1955) 250–254.
[3] T. Friedrich, B. Bottcher, The gross structure of the respiratory complex I: a lego system, Biochim. Biophys. Acta 1608 (2004) 1–9.
[4] G.S. Shadel, T.L. Horvath, Mitochondrial ROS signaling in organismal homeostasis, Cell 163 (2015) 560–569.
[5] D.C. Fuhrmann, I. Wittig, H. Heide, N. Dehne, B. Brune, Chronic hypoxia alters mitochondrial composition in human macrophages, Biochim. Biophys. Acta 1834 (2013) 2750–2760.
[6] M.C. Maiuri, E. Zalckvar, A. Kimchi, G. Kroemer, Self-eating and self-killing: crosstalk between autophagy and apoptosis, Nat. Rev. Mol. Cell Biol. 8 (2007) 741–752.
[7] D.A. Kubli, A.B. Gustafsson, Mitochondria and mitophagy: the yin and yang of cell

death control, Circ. Res. 111 (2012) 1208–1221.
[8] G. Bellot, R. Garcia-Medina, P. Gounon, J. Chiche, D. Roux, J. Pouyssegur, N.M. Mazure, Hypoxia-induced autophagy is mediated through hypoxia-inducible factor induction of BNIP3 and BNIP3L via their BH3 domains, Mol. Cell Biol. 29 (2009) 2570–2581.
[9] H. Zhang, M. Bosch-Marce, L.A. Shimoda, Y.S. Tan, J.H. Baek, J.B. Wesley, F.J. Gonzalez, G.L. Semenza, Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia, J. Biol. Chem. 283 (2008) 10892–10903.
[10] R. Anand, T. Langer, M.J. Baker, Proteolytic control of mitochondrial function and morphogenesis, Biochim. Biophys. Acta 1833 (2013) 195–204.
[11] L. Bleier, S. Drose, Superoxide generation by complex III: from mechanistic rationales to functional consequences, Biochim. Biophys. Acta 1827 (2013) 1320–1331.
[12] S. Drose, Differential effects of complex II on mitochondrial ROS production and their relation to cardioprotective pre- and postconditioning, Biochim. Biophys. Acta 1827 (2013) 578–587.
[13] A.D. Vinogradov, V.G. Grivennikova, Oxidation of NADH and ROS production by respiratory complex I, Biochim. Biophys. Acta 1857 (2016) 863–871.
[14] C. Wirth, U. Brandt, C. Hunte, V. Zickermann, Structure and function of mitochondrial complex I, Biochim. Biophys. Acta (2016).
[15] G. Lenaz, A. Baracca, G. Barbero, C. Bergamini, M.E. Dalmonte, M. Del Sole, M. Faccioli, A. Falasca, R. Fato, M.L. Genova, G. Sgarbi, G. Solaini, Mitochondrial respiratory chain super-complex I-III in physiology and pathology, Biochim. Biophys. Acta 1797 (2010) 633–640.
[16] G. Lenaz, G. Tioli, A.I. Falasca, M.L. Genova, Complex I function in mitochondrial supercomplexes, Biochim. Biophys. Acta (2016).
[17] L. Bleier, I. Wittig, H. Heide, M. Steger, U. Brandt, S. Drose, Generator-specific targets of mitochondrial reactive oxygen species, Free Radic. Biol. Med. 78 (2015) 1–10.
[18] S. Reina, V. Checchetto, R. Saletti, A. Gupta, D. Chaturvedi, C. Guardiani, F. Guarino, M.A. Scorciapino, A. Magri, S. Foti, M. Ceccarelli, A.A. Messina, R. Mahalakshmi, I. Szabo, V. De Pinto, VDAC3 as a sensor of oxidative state of the intermembrane space of mitochondria: the putative role of cysteine residue modifications, Oncotarget 7 (2016) 2249–2268.
[19] L. Plecita-Hlavata, P. Jezek, Integration of superoxide formation and cristae morphology for mitochondrial redox signaling, Int. J. Biochem. Cell Biol. 80 (2016) 31–50.
[20] L. Jiang, A.A. Shestov, P. Swain, C. Yang, S.J. Parker, Q.A. Wang, L.S. Terada, N.D. Adams, M.T. McCabe, B. Pietrak, S. Schmidt, C.M. Metallo, B.P. Dranka, B. Schwartz, R.J. DeBerardinis, Reductive carboxylation supports redox homeostasis during anchorage-independent growth, Nature 532 (2016) 255–258.
[21] C.M. Metallo, P.A. Gameiro, E.L. Bell, K.R. Mattaini, J. Yang, K. Hiller, C.M. Jewell, Z.R. Johnson, D.J. Irvine, L. Guarente, J.K. Kelleher, M.G. Vander Heiden, O. Iliopoulos, G. Stephanopoulos, Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia, Nature 481 (2011) 380–384.
[22] A.R. Mullen, W.W. Wheaton, E.S. Jin, P.H. Chen, L.B. Sullivan, T. Cheng, Y. Yang, W.M. Linehan, N.S. Chandel, R.J. DeBerardinis, Reductive carboxylation supports growth in tumour cells with defective mitochondria, Nature 481 (2011) 385–388.
[23] D.R. Wise, P.S. Ward, J.E. Shay, J.R. Cross, J.J. Gruber, U.M. Sachdeva, J.M. Platt, R.G. DeMatteo, M.C. Simon, C.B. Thompson, Hypoxia promotes isocitrate dehydrogenase-dependent carboxylation of alpha-ketoglutarate to citrate to support cell growth and viability, Proc. Natl. Acad. Sci. USA 108 (2011) 19611–19616.
[24] A. Guaras, E. Perales-Clemente, E. Calvo, R. Acin-Perez, M. Loureiro-Lopez, C. Pujol, I. Martinez-Carrascoso, E. Nunez, F. Garcia-Marques, M.A. Rodriguez-Hernandez, A. Cortes, F. Diaz, A. Perez-Martos, C.T. Moraes, P. Fernandez-Silva, A. Trifunovic, P. Navas, J. Vazquez, J.A. Enriquez, The CoQH2/CoQ ratio serves as a sensor of respiratory chain efficiency, Cell Rep. 15 (2016) 197–209.
[25] J.W. Zmijewski, E. Lorne, X. Zhao, Y. Tsuruta, Y. Sha, G. Liu, G.P. Siegal, E. Abraham, Mitochondrial respiratory complex I regulates neutrophil activation and severity of lung injury, Am. J. Respir. Crit. Care Med. 178 (2008) 168–179.
[26] H. Heide, L. Bleier, M. Steger, J. Ackermann, S. Drose, B. Schwamb, M. Zornig, A.S. Reichert, I. Koch, I. Wittig, U. Brandt, Complexome profiling identifies TMEM126B as a component of the mitochondrial complex I assembly complex, Cell Metab. (2012).
[27] I. Wittig, H.P. Braun, H. Schagger, Blue native PAGE, Nat. Protoc. 1 (2006) 418–428.
[28] P. Li, D. Zhang, L. Shen, K. Dong, M. Wu, Z. Ou, D. Shi, Redox homeostasis protects mitochondria through accelerating ROS conversion to enhance hypoxia resistance in cancer cells, Sci. Rep. 6 (2016) 22831.
[29] M.D. Brand, Mitochondrial generation of superoxide and hydrogen peroxide as the source of mitochondrial redox signaling, Free Radic. Biol. Med. 100 (2016) 14–31.
[30] D.P. Jones, H. Sies, The redox code, Antioxid. Redox Signal. 23 (2015) 734–746.
[31] Z. Li, X. Xu, X. Leng, M. He, J. Wang, S. Cheng, H. Wu, Roles of reactive oxygen species in cell signaling pathways and immune responses to viral infections, Arch. Virol. (2016).
[32] M. Redza-Dutordoir, D.A. Averill-Bates, Activation of apoptosis signalling pathways by reactive oxygen species, Biochim. Biophys. Acta 1863 (2016) 2977–2992.
[33] E.S. Hanson, E.A. Leibold, Regulation of the iron regulatory proteins by reactive nitrogen and oxygen species, Gene Expr. 7 (1999) 367–376.
[34] J. Schodel, S. Oikonomopoulos, J. Ragoussis, C.W. Pugh, P.J. Ratcliffe, D.R. Mole, High-resolution genome-wide mapping of HIF-binding sites by ChIP-seq, Blood 117 (2011) e207–e217.
[35] C.W. Pugh, J.F. O'Rourke, M. Nagao, J.M. Gleadle, P.J. Ratcliffe, Activation of hypoxia-inducible factor-1; definition of regulatory domains within the alpha subunit, J. Biol. Chem. 272 (1997) 11205–11214.
[36] R.J. Appelhoff, Y.M. Tian, R.R. Raval, H. Turley, A.L. Harris, C.W. Pugh, P.J. Ratcliffe, J.M. Gleadle, Differential function of the prolyl hydroxylases PHD1, PHD2, and PHD3 in the regulation of hypoxia-inducible factor, J. Biol. Chem. 279 (2004) 38458–38465.
[37] N. Fujita, D. Markova, D.G. Anderson, K. Chiba, Y. Toyama, I.M. Shapiro, M.V. Risbud, Expression of prolyl hydroxylases (PHDs) is selectively controlled by HIF-1 and HIF-2 proteins in nucleus pulposus cells of the intervertebral disc: distinct roles of PHD2 and PHD3 proteins in controlling HIF-1alpha activity in hypoxia, J. Biol. Chem. 287 (2012) 16975–16986.
[38] N.A. Smirnova, D.M. Hushpulian, R.E. Speer, I.N. Gaisina, R.R. Ratan, I.G. Gazaryan, Catalytic mechanism and substrate specificity of HIF prolyl hydroxylases, Biochemistry (Mosc.) 77 (2012) 1108–1119.
[39] X. Xia, M.E. Lemieux, W. Li, J.S. Carroll, M. Brown, X.S. Liu, A.L. Kung, Integrative analysis of HIF binding and transactivation reveals its role in maintaining histone methylation homeostasis, Proc. Natl. Acad. Sci. USA 106 (2009) 4260–4265.
[40] R.H. Wenger, D.P. Stiehl, G. Camenisch, Integration of oxygen signaling at the consensus HRE, Sci. STKE 2005 (2005) re12.
[41] K.J. Briggs, P. Koivunen, S. Cao, K.M. Backus, B.A. Olenchock, H. Patel, Q. Zhang, S. Signoretti, G.J. Gerfen, A.L. Richardson, A.K. Witkiewicz, B.F. Cravatt, J. Clardy, W.G. Kaelin Jr., Paracrine induction of HIF by glutamate in breast cancer: EglN1 senses cysteine, Cell 166 (2016) 126–139.
[42] L.L. Lackner, J.M. Nunnari, The molecular mechanism and cellular functions of mitochondrial division, Biochim. Biophys. Acta 1792 (2009) 1138–1144.
[43] H. Kim, M.C. Scimia, D. Wilkinson, R.D. Trelles, M.R. Wood, D. Bowtell, A. Dillin, M. Mercola, Z.A. Ronai, Fine-tuning of Drp1/Fis1 availability by AKAP121/Siah2 regulates mitochondrial adaptation to hypoxia, Mol. Cell 44 (2011) 532–544.
[44] K. Nakayama, I.J. Frew, M. Hagensen, M. Skals, H. Habelhah, A. Bhoumik, T. Kadoya, H. Erdjument-Bromage, P. Tempst, P.B. Frappell, D.D. Bowtell, Z. Ronai, Siah2 regulates stability of prolyl-hydroxylases, controls HIF1alpha abundance, and modulates physiological responses to hypoxia, Cell 117 (2004) 941–952.
[45] Y.Y. Wan, J.F. Zhang, Z.J. Yang, L.P. Jiang, Y.F. Wei, Q.N. Lai, J.B. Wang, H.B. Xin, X.J. Han, Involvement of Drp1 in hypoxia-induced migration of human glioblastoma U251 cells, Oncol. Rep. 32 (2014) 619–626.
[46] X.J. Han, Z.J. Yang, L.P. Jiang, Y.F. Wei, M.F. Liao, Y. Qian, Y. Li, X. Huang, J.B. Wang, H.B. Xin, Y.Y. Wan, Mitochondrial dynamics regulates hypoxia-induced migration and antineoplastic activity of cisplatin in breast cancer cells, Int. J. Oncol. 46 (2015) 691–700.
[47] D. Santos, A.R. Esteves, D.F. Silva, C. Januario, S.M. Cardoso, The impact of mitochondrial fusion and fission modulation in sporadic Parkinson's disease, Mol. Neurobiol. 52 (2015) 573–586.
[48] T. Landes, L.J. Emorine, D. Courilleau, M. Rojo, P. Belenguer, L. Arnaune-Pelloquin, The BH3-only Bnip3 binds to the dynamin Opa1 to promote mitochondrial fragmentation and apoptosis by distinct mechanisms, EMBO Rep. 11 (2010) 459–465.
[49] Q. Huang, L. Zhan, H. Cao, J. Li, Y. Lyu, X. Guo, J. Zhang, L. Ji, T. Ren, J. An, B. Liu, Y. Nie, J. Xing, Increased mitochondrial fission promotes autophagy and hepatocellular carcinoma cell survival through the ROS-modulated coordinated regulation of the NFKB and TP53 pathways, Autophagy 12 (2016) 999–1014.
[50] H.J. An, G. Cho, J.O. Lee, S.G. Paik, Y.S. Kim, H. Lee, Higd-1a interacts with Opa1 and is required for the morphological and functional integrity of mitochondria, Proc. Natl. Acad. Sci. USA 110 (2013) 13014–13019.
[51] X. Liu, G. Hajnoczky, Altered fusion dynamics underlie unique morphological changes in mitochondria during hypoxia-reoxygenation stress, Cell Death Differ. 18 (2011) 1561–1572.
[52] K.S. Hewitson, B.M. Lienard, M.A. McDonough, I.J. Clifton, D. Butler, A.S. Soares, N.J. Oldham, L.A. McNeill, C.J. Schofield, Structural and mechanistic studies on the inhibition of the hypoxia-inducible transcription factor hydroxylases by tricarboxylic acid cycle intermediates, J. Biol. Chem. 282 (2007) 3293–3301.
[53] P. Koivunen, M. Hirsila, A.M. Remes, I.E. Hassinen, K.I. Kivirikko, J. Myllyharju, Inhibition of hypoxia-inducible factor (HIF) hydroxylases by citric acid cycle intermediates: possible links between cell metabolism and stabilization of HIF, J. Biol. Chem. 282 (2007) 4524–4532.
[54] H. Lu, C.L. Dalgard, A. Mohyeldin, T. McFate, A.S. Tait, A. Verma, Reversible inactivation of HIF-1 prolyl hydroxylases allows cell metabolism to control basal HIF-1, J. Biol. Chem. 280 (2005) 41928–41939.
[55] F. Dupuy, S. Tabaries, S. Andrzejewski, Z. Dong, J. Blagih, M.G. Annis, A. Omeroglu, D. Gao, S. Leung, E. Amir, M. Clemons, A. Aguilar-Mahecha, M. Basik, E.E. Vincent, J. St-Pierre, R.G. Jones, P.M. Siegel, PDK1-dependent metabolic reprogramming dictates metastatic potential in breast cancer, Cell Metab. 22 (2015) 577–589.
[56] H. Semba, N. Takeda, T. Isagawa, Y. Sugiura, K. Honda, M. Wake, H. Miyazawa, Y. Yamaguchi, M. Miura, D.M. Jenkins, H. Choi, J.W. Kim, M. Asagiri, A.S. Cowburn, H. Abe, K. Soma, K. Koyama, M. Katoh, K. Sayama, N. Goda, R.S. Johnson, I. Manabe, R. Nagai, I. Komuro, HIF-1alpha-PDK1 axis-induced active glycolysis plays an essential role in macrophage migratory capacity, Nat. Commun. 7 (2016) 11635.
[57] J.W. Kim, I. Tchernyshyov, G.L. Semenza, C.V. Dang, HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia, Cell Metab. 3 (2006) 177–185.
[58] G.L. Semenza, B.H. Jiang, S.W. Leung, R. Passantino, J.P. Concordet, P. Maire, A. Giallongo, Hypoxia response elements in the aldolase A, enolase 1, and lactate dehydrogenase A gene promoters contain essential binding sites for hypoxia-inducible factor 1, J. Biol. Chem. 271 (1996) 32529–32537.
[59] C.J. De Saedeleer, T. Copetti, P.E. Porporato, J. Verrax, O. Feron, P. Sonveaux, Lactate activates HIF-1 in oxidative but not in Warburg-phenotype human tumor cells, PLoS One 7 (2012) e46571.

[60] G.L. Semenza, Oxygen-dependent regulation of mitochondrial respiration by hypoxia-inducible factor 1, Biochem. J. 405 (2007) 1–9.
[61] G.L. Semenza, Regulation of metabolism by hypoxia-inducible factor 1, Cold Spring Harb. Symp. Quant. Biol. 76 (2011) 347–353.
[62] B. Brune, N. Dehne, Sensors, transmitters and targets in mitochondrial oxygen shortage - a HIF relay story, Antioxid. Redox Signal. (2012).
[63] P. Hernansanz-Agustin, A. Izquierdo-Alvarez, F.J. Sanchez-Gomez, E. Ramos, T. Villa-Pina, S. Lamas, A. Bogdanova, A. Martinez-Ruiz, Acute hypoxia produces a superoxide burst in cells, Free Radic. Biol. Med. 71 (2014) 146–156.
[64] R.D. Guzy, B. Sharma, E. Bell, N.S. Chandel, P.T. Schumacker, Loss of the SdhB, but not the SdhA, subunit of complex II triggers reactive oxygen species-dependent hypoxia-inducible factor activation and tumorigenesis, Mol. Cell. Biol. 28 (2008) 718–731.
[65] X. Yue, P. Zhao, K. Wu, J. Huang, W. Zhang, Y. Wu, X. Liang, X. He, GRIM-19 inhibition induced autophagy through activation of ERK and HIF-1alpha not STAT3 in Hela cells, Tumour Biol. 37 (2016) 9789–9796.
[66] Y. Saito, K.A. Ishii, Y. Aita, T. Ikeda, Y. Kawakami, H. Shimano, H. Hara, K. Takekoshi, Loss of SDHB elevates catecholamine synthesis and secretion depending on ROS production and HIF stabilization, Neurochem. Res. 41 (2016) 696–706.
[67] G. Comito, M. Calvani, E. Giannoni, F. Bianchini, L. Calorini, E. Torre, C. Migliore, S. Giordano, P. Chiarugi, HIF-1alpha stabilization by mitochondrial ROS promotes Met-dependent invasive growth and vasculogenic mimicry in melanoma cells, Free Radic. Biol. Med. 51 (2011) 893–904.
[68] T. Klimova, N.S. Chandel, Mitochondrial complex III regulates hypoxic activation of HIF, Cell Death Differ. 15 (2008) 660–666.
[69] Y.L. Chua, E. Dufour, E.P. Dassa, P. Rustin, H.T. Jacobs, C.T. Taylor, T. Hagen, Stabilization of hypoxia-inducible factor-1alpha protein in hypoxia occurs independently of mitochondrial reactive oxygen species production, J. Biol. Chem. 285 (2010) 31277–31284.
[70] J.K. Brunelle, E.L. Bell, N.M. Quesada, K. Vercauteren, V. Tiranti, M. Zeviani, R.C. Scarpulla, N.S. Chandel, Oxygen sensing requires mitochondrial ROS but not oxidative phosphorylation, Cell Metab. 1 (2005) 409–414.
[71] F. Diaz, J.A. Enriquez, C.T. Moraes, Cells lacking Rieske iron-sulfur protein have a reactive oxygen species-associated decrease in respiratory complexes I and IV, Mol. Cell. Biol. 32 (2012) 415–429.
[72] C.Y. Kuo, Y.C. Chiu, A.Y. Lee, T.L. Hwang, Mitochondrial Lon protease controls ROS-dependent apoptosis in cardiomyocyte under hypoxia, Mitochondrion 23 (2015) 7–16.
[73] D. Tello, E. Balsa, B. Acosta-Iborra, E. Fuertes-Yebra, A. Elorza, A. Ordonez, M. Corral-Escariz, I. Soro, E. Lopez-Bernardo, E. Perales-Clemente, A. Martinez-Ruiz, J.A. Enriquez, J. Aragones, S. Cadenas, M.O. Landazuri, Induction of the mitochondrial NDUFA4L2 protein by HIF-1alpha decreases oxygen consumption by inhibiting Complex I activity, Cell Metab. 14 (2011) 768–779.
[74] R.K. Lai, I.M. Xu, D.K. Chiu, A.P. Tse, L.L. Wei, C.T. Law, D. Lee, C.M. Wong, M.P. Wong, I.O. Ng, C.C. Wong, NDUFA4L2 Fine-tunes oxidative stress in hepatocellular carcinoma, Clin. Cancer Res. 22 (2016) 3105–3117.
[75] P. Yu, S. Fan, L. Huang, L. Yang, Y. Du, MIR210 as a potential molecular target to block invasion and metastasis of gastric cancer, Med. Hypotheses 84 (2015) 209–212.
[76] S.Y. Chan, Y.Y. Zhang, C. Hemann, C.E. Mahoney, J.L. Zweier, J. Loscalzo, MicroRNA-210 controls mitochondrial metabolism during hypoxia by repressing the iron-sulfur cluster assembly proteins ISCU1/2, Cell Metab. 10 (2009) 273–284.
[77] S. Muralimanoharan, A. Maloyan, J. Mele, C. Guo, L.G. Myatt, L. Myatt, MIR-210 modulates mitochondrial respiration in placenta with preeclampsia, Placenta 33 (2012) 816–823.
[78] R.I. McCormick, C. Blick, J. Ragoussis, J. Schoedel, D.R. Mole, A.C. Young, P.J. Selby, R.E. Banks, A.L. Harris, miR-210 is a target of hypoxia-inducible factors 1 and 2 in renal cancer, regulates ISCU and correlates with good prognosis, Br. J. Cancer 108 (2013) 1133–1142.
[79] P. Ullmann, K. Qureshi-Baig, F. Rodriguez, A. Ginolhac, Y. Nonnenmacher, D. Ternes, J. Weiler, K. Gabler, C. Bahlawane, K. Hiller, S. Haan, E. Letellier, Hypoxia-responsive miR-210 promotes self-renewal capacity of colon tumor-initiating cells by repressing ISCU and by inducing lactate production, Oncotarget, 2016.
[80] E. Favaro, A. Ramachandran, R. McCormick, H. Gee, C. Blancher, M. Crosby, C. Devlin, C. Blick, F. Buffa, J.L. Li, B. Vojnovic, R. Pires das Neves, P. Glazer, F. Iborra, M. Ivan, J. Ragoussis, A.L. Harris, MicroRNA-210 regulates mitochondrial free radical response to hypoxia and krebs cycle in cancer cells by targeting iron sulfur cluster protein ISCU, PLoS One 5 (2010) e10345.
[81] M. Babot, A. Birch, P. Labarbuta, A. Galkin, Characterisation of the active/de-active transition of mitochondrial complex I, Biochim. Biophys. Acta 1837 (2014) 1083–1092.
[82] S. Drose, A. Stepanova, A. Galkin, Ischemic A/D transition of mitochondrial complex I and its role in ROS generation, Biochim. Biophys. Acta 1857 (2016) 946–957.
[83] M. Ciano, M. Fuszard, H. Heide, C.H. Botting, A. Galkin, Conformation-specific crosslinking of mitochondrial complex I, FEBS Lett. 587 (2013) 867–872.
[84] E.T. Chouchani, C. Methner, S.M. Nadtochiy, A. Logan, V.R. Pell, S. Ding, A.M. James, H.M. Cocheme, J. Reinhold, K.S. Lilley, L. Partridge, I.M. Fearnley, A.J. Robinson, R.C. Hartley, R.A. Smith, T. Krieg, P.S. Brookes, M.P. Murphy, Cardioprotection by S-nitrosation of a cysteine switch on mitochondrial complex I, Nat. Med. 19 (2013) 753–759.
[85] J.I. Piruat, J. Lopez-Barneo, Oxygen tension regulates mitochondrial DNA-encoded complex I gene expression, J. Biol. Chem. 280 (2005) 42676–42684.
[86] C. Devlin, S. Greco, F. Martelli, M. Ivan, miR-210: more than a silent player in hypoxia, IUBMB Life 63 (2011) 94–100.
[87] M.P. Puissegur, N.M. Mazure, T. Bertero, L. Pradelli, S. Grosso, K. Robbe-Sermesant, T. Maurin, K. Lebrigand, B. Cardinaud, V. Hofman, S. Fourre, V. Magnone, J.E. Ricci, J. Pouyssegur, P. Gounon, P. Hofman, P. Barbry, B. Mari, miR-210 is overexpressed in late stages of lung cancer and mediates mitochondrial alterations associated with modulation of HIF-1 activity, Cell Death Differ. 18 (2011) 465–478.
[88] C.L. Quinlan, I.V. Perevoshchikova, M. Hey-Mogensen, A.L. Orr, M.D. Brand, Sites of reactive oxygen species generation by mitochondria oxidizing different substrates, Redox Biol. 1 (2013) 304–312.
[89] Z. Chen, Y. Li, H. Zhang, P. Huang, R. Luthra, Hypoxia-regulated microRNA-210 modulates mitochondrial function and decreases ISCU and COX10 expression, Oncogene 29 (2010) 4362–4368.
[90] E. Balsa, R. Marco, E. Perales-Clemente, R. Szklarczyk, E. Calvo, M.O. Landazuri, J.A. Enriquez, NDUFA4 is a subunit of complex IV of the mammalian electron transport chain, Cell Metab. 16 (2012) 378–386.
[91] R. Fukuda, H. Zhang, J.W. Kim, L. Shimoda, C.V. Dang, G.L. Semenza, HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells, Cell 129 (2007) 111–122.
[92] T. Hayashi, Y. Asano, Y. Shintani, H. Aoyama, H. Kioka, O. Tsukamoto, M. Hikita, K. Shinzawa-Itoh, K. Takafuji, S. Higo, H. Kato, S. Yamazaki, K. Matsuoka, A. Nakano, H. Asanuma, M. Asakura, T. Minamino, Y. Goto, T. Ogura, M. Kitakaze, I. Komuro, Y. Sakata, T. Tsukihara, S. Yoshikawa, S. Takashima, Higd1a is a positive regulator of cytochrome c oxidase, Proc. Natl. Acad. Sci. USA 112 (2015) 1553–1558.
[93] I. Soro-Arnaiz, Q.O. Li, M. Torres-Capelli, F. Melendez-Rodriguez, S. Veiga, K. Veys, D. Sebastian, A. Elorza, D. Tello, P. Hernansanz-Agustin, S. Cogliati, J.M. Moreno-Navarrete, E. Balsa, E. Fuertes, E. Romanos, A. Martinez-Ruiz, J.A. Enriquez, J.M. Fernandez-Real, A. Zorzano, K. De Bock, J. Aragones, Role of mitochondrial complex IV in age-dependent obesity, Cell Rep. 16 (2016) 2991–3002.
[94] E.T. Chouchani, V.R. Pell, E. Gaude, D. Aksentijevic, S.Y. Sundier, E.L. Robb, A. Logan, S.M. Nadtochiy, E.N. Ord, A.C. Smith, F. Eyassu, R. Shirley, C.H. Hu, A.J. Dare, A.M. James, S. Rogatti, R.C. Hartley, S. Eaton, A.S. Costa, P.S. Brookes, S.M. Davidson, M.R. Duchen, K. Saeb-Parsy, M.J. Shattock, A.J. Robinson, L.M. Work, C. Frezza, T. Krieg, M.P. Murphy, Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS, Nature 515 (2014) 431–435.
[95] S. Jang, T.S. Lewis, C. Powers, Z. Khuchua, C.P. Baines, P. Wipf, S. Javadov, Elucidating mitochondrial electron transport chain supercomplexes in the heart during ischemia-reperfusion, Antioxid. Redox Signal. (2016).
[96] H. Kamata, S. Honda, S. Maeda, L. Chang, H. Hirata, M. Karin, Reactive oxygen species promote TNF alpha-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases, Cell 120 (2005) 649–661.
[97] J. Sun, G. Sun, X. Meng, H. Wang, M. Wang, M. Qin, B. Ma, Y. Luo, Y. Yu, R. Chen, Q. Ai, X. Sun, Ginsenoside RK3 prevents hypoxia-reoxygenation induced apoptosis in H9c2 cardiomyocytes via AKT and MAPK pathway, Evid. Based Complement. Altern. Med. 2013 (2013) 690190.
[98] F. Bagheri, V. Khori, A.M. Alizadeh, S. Khalighfard, S. Khodayari, H. Khodayari, Reactive oxygen species-mediated cardiac-reperfusion injury: mechanisms and therapies, Life Sci. 165 (2016) 43–55.
[99] N.B. Madungwe, N.F. Zilberstein, Y. Feng, J.C. Bopassa, Critical role of mitochondrial ROS is dependent on their site of production on the electron transport chain in ischemic heart, Am. J. Cardiovasc. Dis. 6 (2016) 93–108.