Vitamin K: Redox-modulation, prevention of mitochondrial dysfunction and anticancer effect

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ABSTRACT

This review is directed to the redox-modulating properties and anticancer effect of vitamin K. The concept is focused on two aspects: (i) redox-cycle of vitamin K and its effect on the calcium homeostasis, "oncogenic" and "onco-suppressive" reactive oxygen species and the specific induction of oxidative stress in cancer; (ii) vitamin K plus C as a powerful redox-system, which forms a bypass between mitochondrial complexes II and III and thus prevents mitochondrial dysfunction, restores oxidative phosphorylation and aerobic glycolysis, modulates the redox-state of endogenous redox-pairs, eliminates the hypoxic environment of cancer cells and induces cell death. The analyzed data suggest that vitamin C&K can sensitize cancer cells to conventional chemotherapy, which allows achievement of a lower effective dose of the drug and minimizing the harmful side-effects. The review is intended for a wide audience of readers - from students to specialists in the field.

1. Chemistry, transport and metabolism of vitamin K

The dietary trace element vitamin K was discovered in the early 1930s by Henrik Dam. During his studies on the cholesterol metabolism in the chicken, Dam has observed that chicks on a sterols-free and low-fat diet have developed large subcutaneous and intramuscular hemorrhages. Henrik Dam has designated this factor as "Koagulation vitamin" (from Danish) or vitamin K in short, because of its vital role for normal haemostasis [1,2]. Initial experiments with additions of lemon juice, cholesterol, cod-liver oil or ascorbic acid to the diet failed to prevent hemorrhages [1–3]. Later it has been discovered that vitamin K is an essential cofactor for post-translational modification of hepatic blood-coagulating proteins (as prothrombin, factors II, VII, IX and X) [3,4]. Vitamin K participates in the converting of their glutamic acid residues into γ-carboxyglutamic acid (Gla) residues. In last years, other functions of vitamin K, unrelated to coagulation, have attracted the attention of researchers, e.g., its role in bone metabolism, vascular calcification, regulation of cell growth and apoptosis [5–10]. There is also enormous interest in the anticancer activity of vitamin K in combination with vitamin C, which will be specifically addressed below.

Vitamin K is a group of structurally related molecules that have a 2-methyl-1,4 naphthoquinone ring and a variable aliphatic chain (Fig. 1A). The variable aliphatic chain distinguishes two naturally occurring forms: vitamin K1 (phylloquinone) and vitamin K2 (menaquinone). There is also a synthetic form of vitamin K without aliphatic chain (K3 or menadione), which is classified as a pro-vitamin. Vitamin K1 exists only in one phylloquinone structure, while vitamin K2 exists in multiple structures, which are distinguished by number of unsaturated isoprenyl groups in the aliphatic chain [10].

Vitamin K1 is found in green leafy vegetables: broccoli, lettuce, spinach, fermented soy (natto), spring onions, cabbage, etc. The various forms of vitamin K2 (MK-4, MK-7, MK-10) are mainly synthesized by bacteria, especially in nutrient products as natto and yogurt [11,12]. Vitamin K2 is also found in meat, eggs, curd and cheese [7,10,13].

Vitamins K1 and K2 are transported by triglyceride-rich lipoprotein (TGRLP) to the liver, after intestinal absorption [13,14] (Fig. 1B). Vitamin K1 is metabolized and more than half the amount is excreted by the organism, while vitamin K2 is transported from the low density
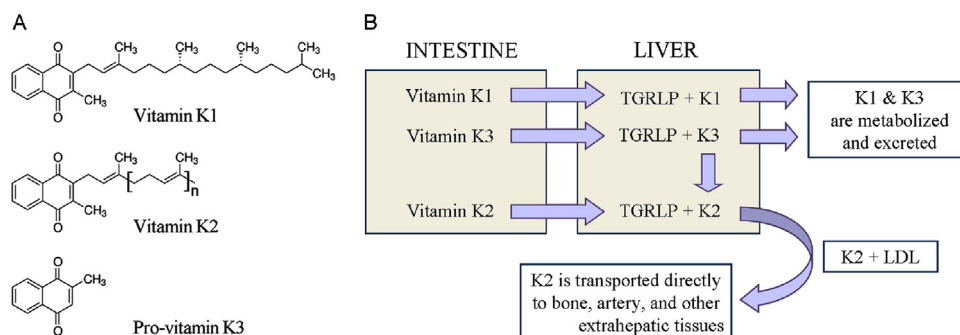
Fig. 1. (A) Chemical structures of vitamin K1, vitamin K2, and pro-vitamin K3 (menadione). (B) Transport and metabolism of vitamins K. TGRLP – triglyceride-rich lipoprotein; LDL – low density lipoproteins.

lipoproteins (LDL) to extra-liver tissues [13,14]. Vitamin K2 is accumulated preferentially in peripheral tissues. High levels are detected in the brain, aorta, pancreas, fat and low levels are detected in the liver [15–17].

Pro-vitamin K3 is a synthetic lipid-soluble vitamin precursor, which can be converted to active vitamin K2 (menaquinone) after its alkylation in the liver. In addition, pro-vitamin K3 could be metabolized to glucuronide and sulfate of reduced menadione, which are excreted in the urine [18–20]. Menadione has a relatively low toxicity and is approved by the Food and Drug Administration for therapeutic purposes. It has been shown that pro-vitamin K3 directly affects the redox status of thiols (including thiol-containing compounds) and calcium homeostasis [20]. The most intriguing property of menadione is its anticancer activity, which will be discussed in details below.

2. Metabolic cycle of vitamin K and its role in calcium homeostasis

It is generally accepted that the essential role of vitamin K is related to the post-translational γ-carboxylation of glutamate residues of proteins (Fig. 2). This process occurs in the lumen of the endoplasmic reticulum and involves two enzymes: γ-glutamyl carboxylase and vitamin K-dependent epoxide reductase complex (VKORC). Both enzymes together constitute the "vitamin K cycle", which is a redox-cycle in its biochemical essence [21,22]. In nature, vitamin K exists in an oxidized form (quinone), but γ-carboxylation of glutamate residues requires its reduced form (hydroquinone). In the organism, quinone is reduced to hydroquinone by NAD(P)H and/or glutathione (GSH). In turn, hydroquinone is converted to epoxide in the process of γ-carboxylation. The epoxide is converted to the initial oxidized quinone state by the

VKORC, which is accompanied by a consumption of other reducing equivalents (mainly thiol-groups). Obviously, the activity of vitamin K may affect redox-homeostasis of cells and tissues and can be considered as a regulatory factor in redox-signaling. This function of vitamin K is very important due to variety of glutamic acid-rich proteins in the bones, arteries and soft tissues. For example, vitamin K influences the degree of carboxylation of osteocalcin – a small calcium-binding protein, which is secreted by osteoblasts in bones and serves as an integral protein for the synthesis of bone matrix [23–25]. The biosynthesis of osteocalcin is regulated by hormones and growth factors, but its post-translational modification is regulated by vitamin K. Osteocalcin contains three Gla residues, which interact with calcium in the hydroxyapatite-crystal lattice of bones [24]. γ-Carboxylation of glutamic acid residues of osteocalcin increases its affinity to calcium and respectively to hydroxyapatite [25]. It was shown, that decarboxylated osteocalcin cannot bind calcium, which emphasizes the essential role of vitamin K in the γ-carboxylation process [26,27]. In addition, vitamin K inhibits the activity of osteoclasts, thus preventing the breakdown of bones [27,28].

Vitamin K participates in the carboxylation of the matrix Gla-containing protein (MGP) of the arterial walls and plays a crucial role in maintaining their elasticity [7,8,29,30]. MGP belongs to the group of vitamin K2-dependent Gla-containing proteins. MGP is produced in bones and vascular smooth muscle cells and inhibits vascular calcification [30–32]. Its function is affected by inflammatory factors [32]. The importance of MGP for vascular homeostasis was demonstrated on MGP-deficient animals – all of them died of massive arterial calcification within 6–8 weeks after birth [5,33]. It was established that non-carboxylated MGP forms calcium depositions in the vascular walls [8,27,34]. The calcium phosphate crystals in the arterial wall directly
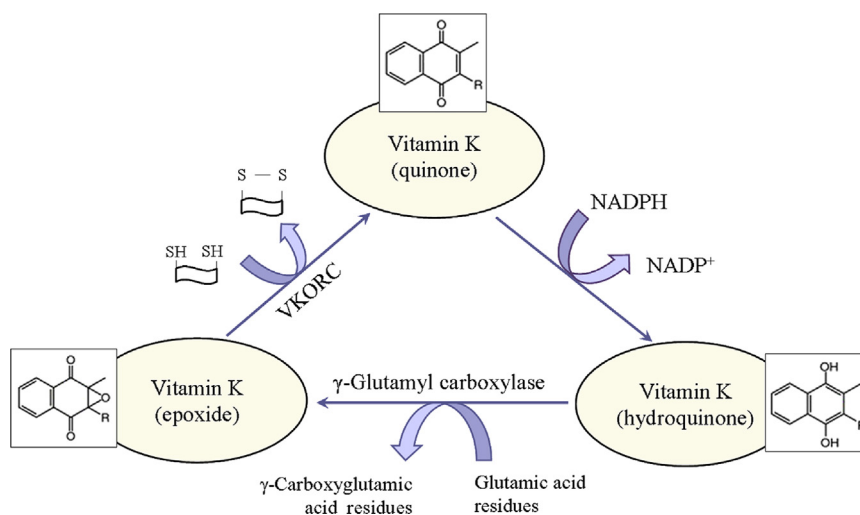

Fig. 2. Metabolic redox-cycle of vitamin K. VKORC – vitamin K-dependent epoxide reductase complex.
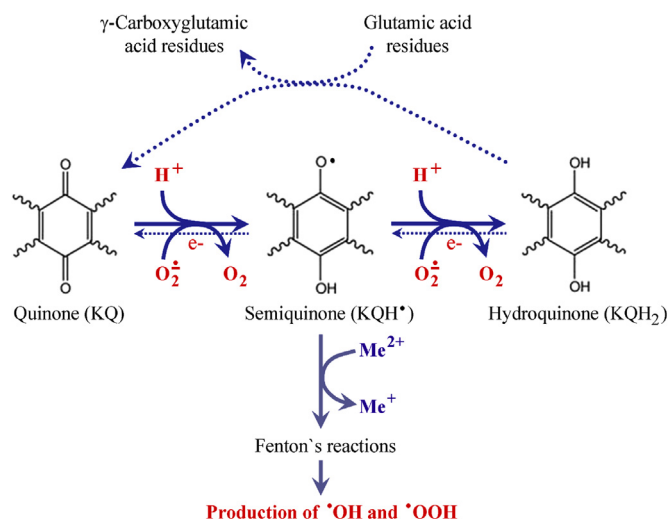
Fig. 3. Two-electron reduction of vitamin K and its effect on the levels of reducers and superoxide in biological objects. The γ-carboxylation of glutamic acid residues shifts the equilibrium towards production of hydroquinone, which is accompanied by consumption of reducers and superoxide. Alternative pathway for induction of Fenton's reactions by the intermediate (semiquinone) form of vitamin K is also given in the figure.

attract macrophages and induce inflammation [27,35,36]. Studies have demonstrated that rats with vitamin K-deficiency and chronic kidney disease have an enhanced vascular calcification, which decreases after vitamin K supplementation [31,37]. Warfarin, an inhibitor of vitamin K reduction, affects γ-glutamyl carboxylation of MGP and induces vascular calcification in experimental animals [4,31,34,38]. The effect is abolished by vitamin K treatment [4,31,38]. These findings suggest that vitamin K-deficiency affects calcium homeostasis, which leads to vascular calcification and bone disorders.

3. Redox-modulating effect of vitamin K and anticancer activity

A number of studies have shown a cytotoxicity of vitamin K towards cancer cells [10,39,40]. Various mechanisms, responsible for cell growth arrest and suppression of proliferation by vitamin K, have been described. However, almost all of them are focused on modulation of redox-balance and induction of oxidative stress in cancer cells due to quinone structure of vitamin K.

Quinones can undergo one-electron reduction, producing intermediate semiquinone radicals, as well as two-electron reduction with production of hydroquinones (Fig. 3) [41]. Both reactions are accompanied by consumption of superoxide radicals and reducing equivalents (as NADH, NADPH, glutathione), which are essential for cancer cell homeostasis [42,43]. It is generally accepted that superoxide is "oncogenic ROS", while hydrogen peroxide is "onco-suppressive ROS" [43–46]. Numerous studies suggest that cellular state, where the ratio tilts predominantly in favor of superoxide, inhibits apoptosis and promotes cell survival. If the ratio tilts in favor of hydrogen peroxide, this creates an intracellular environment suitable for induction of apoptosis and cell death. This hypothesis is well-grounded by experimental and empirical observations on cells, animals and humans, which are summarized in several excellent review articles [43,44,47,48]. In this context, the decrease of superoxide due to vitamin K redox-cycle may explain, at least partially, its anticancer activity. On the other hand, semiquinone radical of vitamin K can convert transition metal ions, e.g. Fe^{3+} to Fe^{2+}, thus inducing Fenton's reactions and production of highly reactive and cytotoxic hydroxyl and hydroperoxyl radicals [10,49].

It is shown that pro-vitamin K3 induces oxidative stress in cancer cells via production of hydroxyl radicals and DNA strand-breaks [50,51].

Superoxide dismutase does not influence the anticancer effect of

menadione [52,53]. However, antioxidant enzymes, involved in the depletion of "onco-suppressive" hydrogen peroxide (as catalase and glutathione peroxidase), decrease the anticancer effect of menadione [53,54]. Similar data have also been reported for transition metal chelators that suppress Fenton's reactions and menadione-mediated cytotoxicity in cancer [55]. The described data suggest that pro-vitamin K3 leads to depletion of "oncogenic" superoxide and probably induces apoptosis via production of "onco-suppressive" hydroperoxides and cytotoxic hydroxyl radicals. This explains, at least partially, the anticancer activity of menadione. However, it should be noted that the induction of Fenton's reactions and the production of hydroxyl radicals suggest for potential side-effects (cytotoxicity) of menadione on normal cells.

Other studies suggest that vitamin K induces apoptosis through different biochemical pathways, including alteration of intracellular calcium homeostasis and activation of the following pro-apoptotic factors: c-Jun N-terminal kinases (JNKs), Fas-dependent and Fas-independent pathways, and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [56–61].

Wu et al. have demonstrated that the expression of c-myc and c-fos proto-oncogenes is involved in the mechanism(s) for induction of apoptosis, differentiation and cell cycle arrest by vitamin K [57]. The authors have found that pro-vitamin K3 induces c-fos and c-myc expression in nasopharyngeal carcinoma (NPC) cell lines. Bouzahzah et al. have demonstrated that growth of hepatoma cells could be completely suppressed by addition of vitamin K to the suspension [62]. The authors have found that growth suppression is accompanied by an increased level of transcripts of the following genes: c-myc, c-jun, and prothrombin.

Pro-vitamin K3 is a potent growth inhibitor and an inducer of phosphorylation of the extracellular signal-regulated kinase (ERK) through specific pathway in the stomach cancer cell lines [10,63–66]. Osada et al. have shown that non-thiol antioxidants had no effect on pro-vitamin K3-induced tyrosine phosphorylation [63]. The anticancer effect is due to thiol-arylation of critical cysteine residues that mediate the tyrosine phosphorylation. Moreover, pro-vitamin K3 interacts directly with glutathione, which is essential for cancer cells [64]. The cell cycle arrest by pro-vitamin K3 induces also tyrosine phosphorylation of hepatocyte growth factor receptor (c-met) and epidermal growth factor receptors (EGFR), which in turn activate the ras-signaling pathway [63,64].

The ability of vitamin K to induce cell cycle arrest and cell death may also be explained by the inhibition of cyclin-dependent protein kinases (CDK) [67,68]. Pro-vitamin K3 inhibits CDK1 and CDK2 by their hyperphosphorylation, modification of the active sites of both proteins and abolishing the dephosphorylating activity of the enzymes. The anticancer effect of vitamin K2 and pro-vitamin K3 is most likely through regulation of p21 gene [69,70].

Different mechanisms of action of vitamin K on different cell types have been described. However, the potential cross-talk between these mechanisms still remains unclear. Most of the vitamin K-induced pro-apoptotic factors are inflammatory signals, inducing overproduction of ROS (mainly hydroperoxides) and oxidative stress in cancer cells and tissues [43,48]. The data also suggest that vitamin K decreases the levels of superoxide, NAD(P)H and glutathione, which are essential for hypoxic behavior of cancer cells and their homeostasis.

4. Vitamin C plus K3: A powerful redox-system to sensitize cancer cells towards chemotherapeutics

In vitro studies have reported a strong anticancer effect of the combination of vitamin C and pro-vitamin K3 (C&K3) on cancer cell lines [71–78]. Most of these studies have demonstrated that anticancer effect of the combination is most well expressed at ratio vitamin C:K3 = 100:1 (mol:mol). Different mechanisms of vitamin C&K3-mediated apoptosis have been discussed: production of free radicals
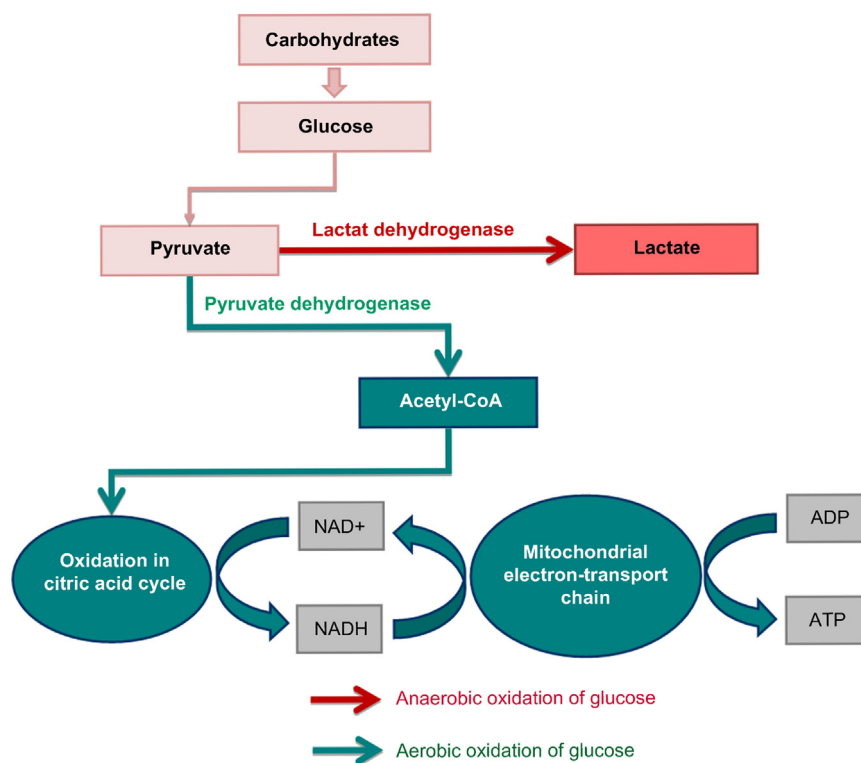
Fig. 4. Aerobic and anaerobic pathways for degradation of glucose and ATP synthesis.

under alkaline conditions, thiol depletion, lipid peroxidation, modulation of ATP level, calcium regulation, activation of NF-kB, etc. Some authors suggest a caspase-independent induction of apoptosis, and others suggest caspase-dependent cell death [71,73,74,76].

Zhang et al. have demonstrated that cancer cells (HSC-2, HSC-3, HL-60) are more sensitive to combined treatment with vitamin C&K3, as compared to normal cells [71]. Vita et al. have reported better cytotoxicity of vitamin C&K3 on glioma cells than on normal brain cells [77]. Our recent study on normal and leukemia lymphocytes, treated with vitamin C&K3, also supports the assumption that cancer cells are more sensitive to vitamin C&K3 than normal cells [78].

How vitamin C&K3 can activate the specific signaling pathways, involved in the cancer cells death? To answer this question, let's remind briefly the basic biochemical difference between normal and cancer cells, associated with the metabolism of glucose (Fig. 4).

As apposed to degradation of proteins and fats, the glucose metabolism is a unique pathway. It can utilize oxygen if available (aerobic pathway – via conversion of pyruvate to acetyl-CoA) or it can function in the absence of oxygen (anaerobic pathway – via conversion of pyruvate to lactate). Both pathways are necessary for the functioning of cells, tissues and organs, but in norm, the aerobic pathway dominates the anaerobic. In cancer cells, the anaerobic oxidation of glucose is a major source of energy. In many cancers, the major cause of anaerobic glycolysis is mitochondrial dysfunction and inhibition of oxidative phosphorylation. Cancer cells are characterized by high levels of glucose, anaerobic oxidation of glucose (Warburg effect) and accumulation of lactate, compared to normal cells [43,44,48,79]. In last years, lactate dehydrogenase is receiving a great deal of attention as a potential diagnostic marker or a predictive biomarker for many types of cancer and as a therapeutic target for development of new anticancer drugs [80–82].

Eleff at al. have reported that administration of vitamin C and K3 on the skeletal muscles of a 17-years-old patient with a severe defect in complex III of the mitochondrial electron transport chain (mitochondrial dysfunction) increases the recovery rate, compared to the recovery rate of the young female controls [83]. The defect includes a deficiency

of reducible cytochrome *b* (cyt. *b*), which is accompanied by an effective prevention of aerobic metabolism and oxidative phosphorylation. The authors bypass the deficient complex III by using pro-vitamin K3 and vitamin C as electron transfer mediators to carry the electrons from coenzyme Q (CoQ) to cyt. *c*. Thus, they succeeded to increase ATP production rate to about 65% of the normal state in mitochondria, as well as to decrease the lactic acidosis in the patient. The combination of vitamin C and K3 results in a production of more ATP, than in the case of vitamin C applied alone. The authors suggest that both vitamins can reduce directly cyt. *c,* since the reduction potential of cyt. *c* is over + 200 mV – more positive than those of menadione or ascorbate [83–87].

The possibility of improving oxidative phosphorylation by bypass of the cytochrome-deficient site in mitochondria by vitamin C and K3 was also reported by McCord and Fridovich many years ago [84]. Recent in vitro studies provide further evidence that treatment of cells with vitamin C and K3 results in spikes in ATP production due to a shunt around a defective area of complex III of the mitochondrial electron-transport chain (between CoQ and cyt. *c*) [74,75,88–91]. This reaction causes a shift from anaerobic (glycolytic) to aerobic (oxidative) metabolism, which diminishes hypoxia and lactic acidosis.

Based on the studies described above, we assume that vitamin C and K can serve as cyt. *c* reducers either directly or indirectly, but in both cases they can avoid mitochondrial dysfunction and restore the oxidative phosphorylation. The hypothetic mechanism of coupling between "vitamin C redox-cycle" and "vitamin K redox-cycle" and their effect on mitochondrial functionality is shown in Fig. 5. In the case of dysfunction in Complex-III (due to mutations in cyt. *b*), the electrons can not be transferred from CoQ to cyt. *c*. In the case of dysfunction in Complex-I (due to mutations in NADH dehydrogenase), the electrons are blocked on the first step of respiratory chain. It is well-known that both types of mutations are accompanied by superoxide production from complex-III and complex-I and suppression of ATP synthesis [43,48]. The oxidized vitamin K (KQ) can accept electrons from superoxide, converting consequently to semiquinone (KQH⁺) and hydroquinone (KQH₂). Finally, the reduced vitamin K can transfer electrons directly to cyt. *c*. In turn,
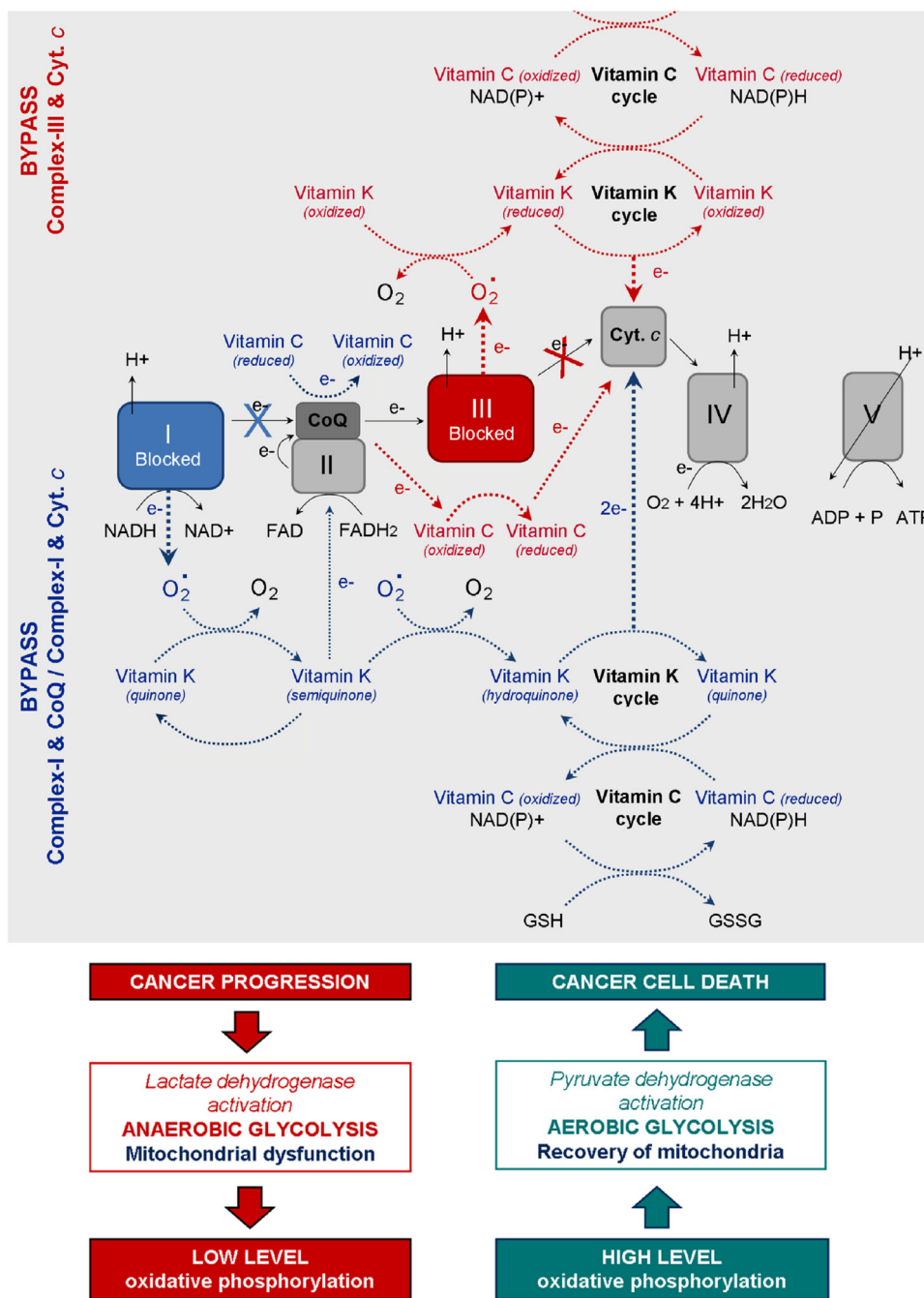
Fig. 5. Redox-cycles of vitamins C and K and prevention of mitochondrial dysfunction: recovery of mitochondrial oxidative phosphorylation in cancer cells – a possible mechanism for induction of apoptosis and cell death. Blue arrows indicate the possible vitamin K-mediated bypasses between complex-I and CoQ, as well as between complex-I and cyt. *c*, in the case of dysfunction in complex-I. Direct reduction of CoQ by vitamin C is also possible. Red arrows indicate the possible vitamin K- and vitamin C-mediated bypasses between complex-III and cyt. *c*, in the case of dysfunction in complex-III. NAD(P)H can reduce both vitamin K (quinone) and dehydroascorbate. Glutathione (GSH) can also reduce dehydroascorbate to ascorbate.

the oxidized vitamin C (dehydroascorbate) can accept electrons directly from Complex-I (NADH) or CoQ, converting consequently to semihydroascorbate (Asc$^{\bullet}$) and ascorbate (Asc). Finally, the reduced vitamin C can transfer electrons to cyt. *c*. The two redox-cycles lead to a bypass between Complex-I (or Complex-III) and cyt. *c*. This will restore the oxidative phosphorylation and ATP synthesis. ATP, generated due to the vitamin C&K-mediated bypasses, prevents the anaerobic conditions, decreases the levels of "oncogenic" superoxide, decreases the levels of lactate, eliminates the lactic acidosis and hypoxia, and allows the cancer cells to trigger autophagia and "to kill themselves".

Recent data indicate that the redox cycles of vitamins C and K are cross-linked – ascorbate can reduce pro-vitamin K3, which is accompanied by a production of hydrogen peroxide ("onco-suppressive" and cytotoxic ROS) [92,93]. The reduction of vitamin K by vitamin C allows also a direct (superoxide-independent) reduction of cyt. *c* by vitamin K (KQH$_2$). Silvera-Dorta et al. have also reported that vitamin C can reduce CoQ and the process is coupled with reduction of oxygen to hydrogen peroxide [93].

The redox-system vitamin C&K3 could also influence the ratio between the oxidized and reduced forms of the main endogenous redox-pairs: NAD$^+$/NADH, NADP$^+$/NADPH, GSH/GSSG, etc. (Fig. 5) [10,72,88]. These redox-pairs are enzymatic cofactors, involved in various biochemical reactions and serve as key regulators of cellular metabolism, including lactate dehydrogenase, pyruvate dehydrogenase,

glutathione-dependent enzymes, etc. The balance between oxidized and reduced forms is crucial for the cell behavior, as well as for cell survival or death. It is widely accepted that the increased mitochondrial oxidative stress and high levels of NAD(P)H and glutathione are distinctive features of the metabolic phenotype of cancer cells [43,44,47,48]. Since the reduction potentials of NAD(P)H, $FADH_2$ and GSH are very low (below -200 mV), all substances can directly reduce vitamins C and K.

The analyzed data suggest that the combination of vitamins C and K (in particular K3) is not just an antioxidant system. Vitamin C&K3 is a powerful redox-couple that can prevent mitochondrial dysfunction, restore oxidative phosphorylation, modulate redox-homeostasis, eliminate hypoxia, and induce apoptosis and cell death.

5. Conclusion

The redox-cycles of vitamins C and K are cross-linked and both vitamins can serve as associated redox-modulators (a redox-shuttle). The combination vitamin C&K can serve as a bypass between complex I (or complex-III) and cyt. *c* of mitochondrial respiratory chain and to donate electrons (directly or indirectly) to cyt. *c*, passing from reduced to oxidized form. Both vitamins can be also converted into reduced forms, consuming NAD(P)H and/or GSH. These processes are accompanied by an increase in ATP synthesis and accumulation of high levels of NAD(P) + and GSSH, which restores oxidative phosphorylation and aerobic glycolysis and eliminates the hypoxic environment. The specific behavior of the cancer cells is disrupted and apoptosis is initiated. It is assumed that changing the ratio NAD(P)+/NAD(P)H and/or GSH/GSSH in cancer cells could be the "switch mechanism" from survival to cell death. Targeting defective mitochondria in cancer cells and preventing their dysfunction, as well as modulating redox-state of endogenous redox-pairs can be a successful strategy for anticancer therapy. This strategy allows target cytotoxicity, which is not directed to normal cells and tissues [43,48].

There is one more interesting aspect of vitamin C&K-dependent modulation of the cellular redox-status. If the redox-system vitamin C&K can prevent mitochondrial dysfunction and change energy metabolism of cancer cells, we can expect a synergistic cytotoxicity after combining with conventional chemotherapeutics, targeting different oncogenes. Recently, we established that vitamin C&K3 sensitize leukemia lymphocytes (Jurkat) to variety of anticancer drugs [78]. Fifteen anticancer drugs were covered in this study. Almost all combinations showed synergistic cytotoxicity to leukemia cells, whereas the effect on normal lymphocytes was significantly less expressed. Thus, the combined treatment with anticancer drug and vitamin C&K3 can be considered as a new therapeutic approach – to sensitize cancer cells and to achieve a lower effective dose of the drug, minimizing the harmful side-effects of conventional chemotherapy. However, considering the fact that a significant number of people use anticoagulants due to concomitant diseases, the use of a procoagulant such as vitamin K should be done very carefully under the supervision of the physician.

Acknowledgements

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