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Diverse biological functions of vitamin K: from coagulation to ferroptosis

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Vitamin K is essential for several physiological processes, such as blood coagulation, in which it serves as a cofactor for the conversion of peptide-bound glutamate to γ-carboxyglutamate in vitamin K-dependent proteins. This process is driven by the vitamin K cycle facilitated by γ-carboxyglutamyl carboxylase, vitamin K epoxide reductase and ferroptosis suppressor protein-1, the latter of which was recently identified as the long-sought-after warfarin-resistant vitamin K reductase. In addition, vitamin K has carboxylation-independent functions. Akin to ubiquinone, vitamin K acts as an electron carrier for ATP production in some organisms and prevents ferroptosis, a type of cell death hallmarked by lipid peroxidation. In this Perspective, we provide an overview of the diverse functions of vitamin K in physiology and metabolism and, at the same time, offer a perspective on its role in ferroptosis together with ferroptosis suppressor protein-1. A comparison between vitamin K and ubiquinone, from an evolutionary perspective, may offer further insights into the manifold roles of vitamin K in biology.

Vitamin K is an essential micronutrient involved in a variety of physiological processes. Chiefly, vitamin K is essential to the blood clotting cascade by serving as a cofactor for the γ-carboxylation of vitamin K-dependent (VKD) coagulation factors (including factors II, VII, IX and X) generated in the liver. In addition, several other VKD proteins have been identified, totalling 19 in mammals[1]. The discovery of these additional proteins has expanded our understanding of vitamin K's importance beyond coagulation, with roles in bone formation and vascular mineralization. Furthermore, vitamin K has γ-carboxylation-independent functions, such as transcriptional regulatory effect[2]. Additionally, similar to ubiquinone, vitamin K—a redox-active naphthoquinone—serves as an electron carrier for ATP production in plants and bacteria[3] and was recently shown to prevent ferroptosis[4], a type of cell death triggered by unrestrained (phospho) lipid peroxidation[5,6]. Examining the redox-based function of vitamin K compared to ubiquinone may provide insight into its evolutionary role and may serve as a springboard for the future therapeutic use of vitamin K, beyond its role in haematology.

History of vitamin K and warfarin

Discovery of vitamin K

In 1935, Danish scientist Henrik Dam discovered the coagulation vitamin (vitamin K), which was given the letter K from the German word 'Koagulation' due to the observation that chickens on a fat-free diet tended to bleed easily[7] (Fig. 1). In 1939, Dam and Doisy independently purified both the yellow oil form (vitamin K_1) and crystalline form (vitamin K_2) of the vitamin K from alfalfa and putrefied fish meal, respectively[8,9] (Fig. 2a). Due to its central role in coagulation, vitamin K has been used to prevent haemorrhagic disease in newborns since the 1940s[10]. In the 1970s, it was found that vitamin K acts as a cofactor for the microsomal enzyme γ-carboxyglutamyl carboxylase (GGCX), an enzyme catalysing the conversion of distinct peptide-bound glutamate (Glu) to γ-carboxyglutamate (Gla)[11]. This posttranslational modification of Glu to Gla is critical for the biological activity of VKD proteins. In 1974, the specific role of vitamin K in coagulation was further elucidated when it was discovered that prothrombin, a coagulation factor, is a VKD protein[12]. Subsequent research has identified several other VKD

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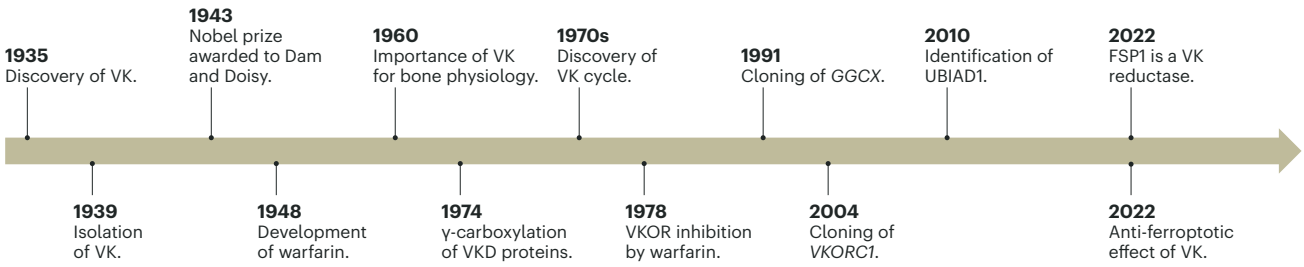

Fig. 1 | History of vitamin K research. Vitamin K (VK) was discovered as a blood coagulation vitamin in 1935. In the following decades, it was established that VK functions as a cofactor for GGCX, which catalyses the γ-carboxylation in VKD proteins, including coagulation factors. Among the key players in the VK cycle, the genes encoding GGCX and VKOR were identified in 1991 and 2004, respectively. In 2022, FSP1 was identified as the long-postulated but unidentified warfarin-resistant VK reductase. The anticoagulant drug warfarin was developed in 1948, and the anticoagulation mechanism as an inhibitor of VKOR was identified in 1978. Identification of FSP1 as the warfarin-resistant 'antidotal' enzyme clarified the molecular mechanism of the antidotal effect of VK against warfarin intoxication.

proteins, such as the anticoagulant proteins protein C and S[13,14]. Among extrahepatic VKD proteins, osteocalcin, matrix Gla protein and growth arrest-specific protein 6 (Gas6) play various roles in processes like bone homeostasis and vascular mineralization[15]. These findings established the mechanistic link between vitamin K and bone metabolism that had been suggested as early as in the 1960s[16]. In addition to vertebrates, VKD carboxylation is observed in non-vertebrate organisms[17]. Toxins called conantokins produced by marine cone snails have been found to be VKD proteins[18]. VKD carboxylase activity has also been reported in *Drosophila*, although the specific VKD proteins remain to be identified in this organism[19].

Discovery of warfarin

In tandem with investigations into vitamin K, the mechanism and function of warfarin, a vitamin K antagonist and the most widely used anticoagulant drug globally, were studied. The early 1920s witnessed an outbreak of cattle haemorrhaging disease in parts of the United States and Canada. In 1921, Frank Schofield found that the cattle had ingested mouldy silage made from sweet clover, which acted as a potent anticoagulant[20]. In 1941, the haemorrhagic agent in the spoiled hay, dicoumarol, was isolated[21]. Based on dicoumarol, warfarin, a more potent anticoagulant, was developed[22]. Warfarin was first commercially utilized in 1948 as a rodenticide. However, the use of warfarin for humans was not approved due to the risk of bleeding in case of overdosing. After an incident in 1951, in which an army inductee attempted suicide with warfarin in rodenticide, but fully recovered after treatment with vitamin K[23] (which was by then known as a specific antidote), research into the use of warfarin as a therapeutic anticoagulant was initiated. The US Food and Drug Administration approved warfarin for medical use in humans in 1954, and it was subsequently widely used as an anticoagulant for the treatment and prevention of thrombosis worldwide. The precise mechanism of warfarin's anticoagulant action was clarified in 1978 when it was identified to inhibit the enzyme vitamin K epoxide reductase (VKOR), thereby disrupting the vitamin K cycle and decreasing the rate of carboxylation in VKD coagulation factors[24].

Canonical vitamin K cycle for γ-carboxylation

GGCX and VKOR

Vitamin K, more specifically its fully reduced form, vitamin K hydroquinone (VKH_2), acts as a cofactor for the γ-carboxylation of VKD proteins[25]. During this process, VKH_2 is being oxidized and converted to vitamin K epoxide. Because the amount of vitamin K in the body is limiting, the epoxide form must be recycled through the vitamin K cycle to prevent its depletion. This concept of the cyclic interconversion of vitamin K and vitamin K epoxide was first postulated during the 1970s and 1980s[26,27]. The cycle involves three chemical reactions mediated by GGCX, VKOR and vitamin K reductase[28] (Fig. 2b). Vitamin K, a redox-active naphthoquinone, exists in nature in its chemically stable quinone form. GGCX uses VKH_2 as a cofactor in γ-carboxylation, during which VKH_2 is oxidized to an epoxide and then converted back to vitamin K quinone by VKOR, whose activity is inhibited by warfarin (Fig. 2c). After the initial reports of the enzymatic activities of GGCX and VKOR in the 1970s, it took several decades to clone the genes encoding GGCX (*GGCX*) and VKOR (*VKORC1*), which occurred in 1991 and 2004, respectively[29–31]. Moreover, the highly sophisticated cell-based assays using chimeric VKD reporter proteins afforded to gain precise insights into the mechanistic and phenotypic properties of these enzymes[28].

FSP1: long-sought-after warfarin-resistant vitamin K reductase

Despite the identification of GGCX and VKOR in the canonical vitamin K cycle, the identity of the enzyme(s) responsible for reducing vitamin K quinone to VKH_2 in this cycle had remained unknown. It was suggested that two independent enzymes, a warfarin-sensitive reductase and a warfarin-resistant NAD(P)H-dependent reductase, are responsible for this step (Fig. 2b)[32,33]. VKOR was shown to be able to reduce vitamin K to VKH_2 at least in vitro[34], indicating that it also functions as a warfarin-sensitive vitamin K reductase. However, even when VKOR activity is extremely inhibited by high doses of warfarin, an impaired coagulation status could be restored by administering an appropriate dose of vitamin K, suggesting the existence of an alternative warfarin-resistant enzyme for vitamin K reduction. This enzyme, known as the 'antidotal' vitamin K reductase[35], remained obscure although it first was described more than half a century ago[32,36]. NAD(P)H:quinone reductase 1 (NQO1) has been repeatedly proposed as a candidate enzyme exerting this role, albeit it was shown to have low reducing activity against natural forms of vitamin K[37] (it can, however, reduce menadione, a synthetic form of vitamin K[38]). In addition, supplementation of vitamin K rescues warfarin intoxication in Nqo1-deficient mice[37], indicating that NQO1 does not play a major role in reducing vitamin K required for the carboxylation of VKD proteins.

In 2022, Mishima et al. identified ferroptosis suppressor protein-1 (FSP1), also known as extra-mitochondrial NAD(P)H:ubiquinone reductase or apoptosis-inducing factor mitochondria-associated 2 (AIFM2)[39,40], as the warfarin-resistant vitamin K reductase rather serendipitously when studying its role on ferroptosis[4]. Initially, Mishima et al. performed a cell-based compound screening of naturally occurring antioxidants and discovered that fully reduced vitamin K exhibits a potent anti-ferroptotic effect by acting as a so-called radical-trapping antioxidant. By comparing the structural similarities between vitamin K and ubiquinone, the authors further discovered that the NAD(P)H:ubiquinone reductase FSP1 can function as a vitamin K reductase, thereby regenerating VKH_2 and averting lipid peroxidation and associated ferroptosis. Notably, because the enzymatic activity of FSP1 is not affected by warfarin unlike VKOR, the study additionally unveiled

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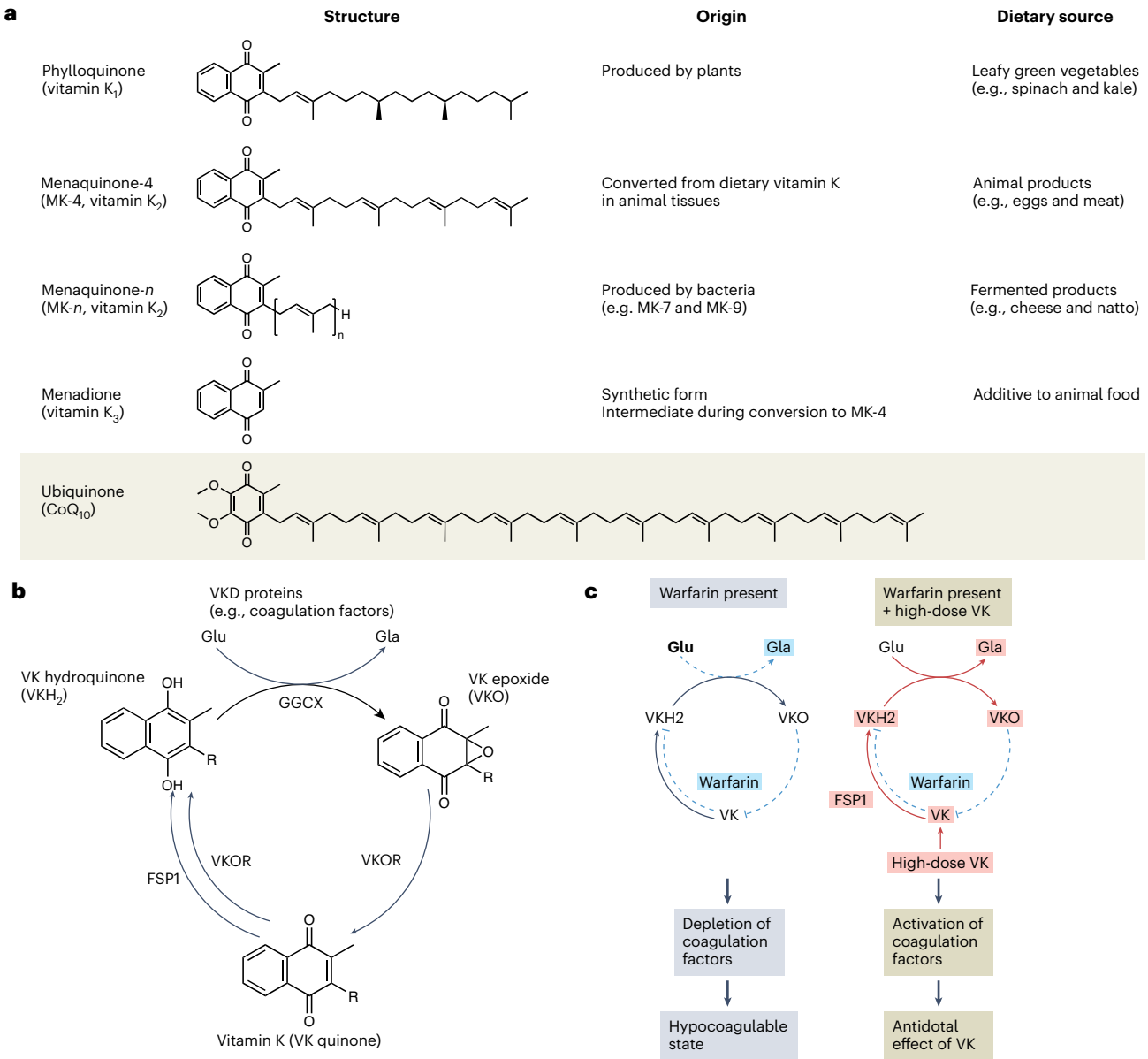

Fig. 2 | Vitamin K cycle for carboxylation of vitamin K-dependent proteins and chemical structures of vitamin K. a, Structures and characteristics of VK and ubiquinone. **b**, The canonical VK cycle. This cycle enables the γ-carboxylation of VKD proteins and the recycling of VK. The enzyme GGCX, in conjunction with the cofactor VKH_2, facilitates the conversion of peptide-bound Glu to Gla in VKD proteins. This γ-carboxylation reaction produces VK epoxide (VKO), which is then reduced to VK quinone by VKOR. VK quinone is reduced to VKH_2 through either the warfarin-resistant FSP1-mediated pathway or the warfarin-sensitive VKOR pathway, thus completing the cycle. **c**, In the presence of the anticoagulant warfarin, inhibition of VKOR ultimately leads to the depletion of VKD coagulation factors. Supplementation of a sufficient dose of VK into the system enables the alternative warfarin-resistant FSP1 pathway to bypass the dysfunctional VKOR pathway, thereby providing the necessary VKH_2 for the GGCX-mediated carboxylation reaction. This is the antidotal mechanism of VK against warfarin intoxication.

FSP1 as the antidotal enzyme overcoming warfarin poisoning. Indeed, in Fsp1-deficient mice, the reduction of vitamin K was impaired under warfarin treatment as well as in cells with a genetic deletion of FSP1 (*AIFM2*). Consequently, the antidotal effect of vitamin K against warfarin intoxication was absent in Fsp1-deficient mice[4]. In clinical settings, warfarin therapy is susceptible to causing warfarin poisoning as a result of interactions with other drugs and large variations in the dosage required to achieve its anticoagulant effect among individuals[41,42], thereby potentially leading to life-threatening bleeding. The identification of FSP1 as the warfarin-resistant vitamin K reductase eventually clarified the molecular mechanism of the antidotal effect of vitamin K against warfarin poisoning (Fig. 2c).

This discovery was independently corroborated by another group using an entirely different experimental approach. By performing an elegant genome-wide CRISPR–Cas9 knockout screening, Jin et al. also showed that FSP1 is the warfarin-resistant vitamin K reductase[43]. Specifically, they established a VKD apoptotic reporter cell line to identify enzymes linked to VKD carboxylation, whereby the reporter cell only undergoes apoptosis when the VKD protein is carboxylated. Thus, loss of function of the enzymes contributing to the vitamin K cycle would prevent apoptosis. In the genome-wide loss-of-function screening using the reporter cell line treated with vitamin K and warfarin, FSP1 was then identified as one of the top candidates. They could also show that FSP1 reduces vitamin K, including phylloquinone, menaquinone-4

(MK-4) and menaquinone-7 (MK-7) in a warfarin-insensitive manner. Hence, these results reinforce the findings that FSP1 is responsible for warfarin-resistant vitamin K reduction (Fig. 2b).

Furthermore, both studies uncovered several important findings about FSP1. FSP1 showed comparable substrate preference towards vitamin K and ubiquinone[4]. Although the protein structure of FSP1 remains to be elucidated, an initial detailed site-directed mutational analysis indicates that FSP1 uses the same predicted binding pocket to reduce both vitamin K and ubiquinone[43]. While N-terminal myristylation, a posttranslational protein modification facilitating membrane binding, is essential for the anti-ferroptotic activity of FSP1 (refs. 4,39), it was shown to be dispensable for FSP1-mediated vitamin K reduction required for VKD γ-carboxylation[43].

Disorders by deficits in vitamin K cycling

The enzymes GGCX and VKOR are crucial in carrying out the vitamin K cycle for the carboxylation of VKD proteins. Accordingly, the genetic loss-of-function mutation of GGCX or VKOR causes hereditary bleeding disorders, known as vitamin K coagulation factor deficiencies (VKCFDs), with those caused by mutations in *GGCX*, referred to as subtype 1 (VKCFD1) and mutations in *VKORC1* as subtype 2 (VKCFD2)[44,45]. VKCFDs often present with excessive bleeding and bruising from birth. In addition to bleeding issues, individuals with VKCFD1 exhibit skin symptoms such as pseudoxanthoma elasticum and skeletal and ophthalmological manifestations[46]. Individuals with VKCFD2 display a comorbid skeletal abnormality[47] similar to the teratogenic effects of warfarin use in early pregnancy[48]. These non-haemostatic symptoms are thought to be due to impaired carboxylation of certain VKD proteins such as osteocalcin and matrix Gla protein. In people with VKCFDs, daily oral supplementation with phylloquinone (1–10 mg) can restore and sustain VKD coagulation activities within the normal reference range[47]. Hence, this highlights the importance of providing sufficient vitamin K to the FSP1-dependent alternative vitamin K reduction pathway in overcoming dysfunctional VKOR, in the same way as warfarin poisoning can be treated with an appropriate dose of VK.

The physiological roles of GGCX and VKOR have also been examined in genetically engineered mice. Ggcx-deficient mice die from intra-abdominal haemorrhage shortly after birth due to severe depletion of VKD coagulation factors[49,50]. Osteoblast-specific and Sertoli cell-specific Ggcx-deficient mice show abnormal mineralization in bone formation and male fertility due to spermatogenic arrest, respectively[51,52]. Like *Ggcx*-null mice, *Vkorc1*-null mice also die shortly after birth due to extensive bleeding[53]. In contrast to these animal models, *Fsp1*$^{-/-}$ mice show no bleeding abnormalities and normal growth[4], suggesting that vitamin K reduction by the VKOR pathway alone is sufficient to maintain the coagulation factors at least under steady-state conditions in mice.

Structure and metabolism of vitamin K

Structure of vitamin K

Vitamin K describes a group of lipophilic molecules that share a 2-methyl-1,4-naphthoquinone head group and a polyisoprenoid side chain, varying in both length and hydrophobicity (Fig. 2a). In nature, there are two forms of vitamin K: phylloquinone (also known as vitamin K_1), which is found in photosynthetic organisms such as green plants, cyanobacteria and algae; and menaquinones (also referred to as vitamin K_2), which are found in animal and bacterial sources. There are different types of menaquinones, classified based on the length of their unsaturated side chains (from MK-1 to MK-15). The most common form of menaquinones in animal-derived foods is MK-4, a short-chain menaquinone and long-chain menaquinones MK-7 through MK-10, which are produced by bacteria including gut microbiota[54]. Menadione, also known as vitamin K_3, is a synthetic hydrophilic variant lacking the polyisoprenoid side chain and is the intermediate in the biosynthesis of MK-4.

Intake and metabolism of vitamin K

Most dietary vitamin K is phylloquinone, which is abundant in green leafy vegetables. Menaquinones (MK-*n*), present in fermented foods such as cheese and natto (traditional Japanese foods made from fermented soybeans), are another major source of vitamin K. Animal foods such as meat and eggs contain MK-4, although in smaller amounts as compared to phylloquinone. Phylloquinone is ingested and first incorporated into mixed micelles with bile salts in the proximal region of the small intestine and is subsequently absorbed in the proximal intestine by enterocytes and transferred across the brush-border membrane[55] (Fig. 3a). Niemann–Pick C1-like 1 (NPC1L1), previously known as a transporter for dietary cholesterol and alpha-tocopherol[56], is a key transporter of intestinal phylloquinone absorption[57]. Previous research further suggested that other cholesterol transporter proteins (scavenger receptor class B type I and CD36) may play a potential role in intestinal vitamin K absorption[58].

While phylloquinone is a major form of dietary vitamin K, MK-4 is more prevalent in some organs than phylloquinone. This is because dietary ingested phylloquinone and MK-*n* can be converted to MK-4 locally within tissues (Fig. 3a). After being ingested, the side chain of phylloquinone is cleaved within enterocytes yielding menadione, which in turn is transported via the lymphatic system to other tissues[59]. In the target tissues, menadione is prenylated, and the side chain is added to form MK-4 through the action of the enzyme, UbiA prenyltransferase domain-containing protein-1 (UBIAD1)[60]. UBIAD1 transfers geranylgeranyl moieties from geranylgeranyl pyrophosphate, a product in the mevalonate pathway, to menadione thereby generating MK-4.

The distribution of phylloquinone and MK-*n* is tissue specific and variable[61]. For example, MK-4 is not evenly distributed among different mammalian tissues, with particularly high concentrations found in the exocrine and endocrine glands as well as in the kidney and brain[61,62]. In addition to the main target organs for vitamin K function, each organ is capable of locally producing MK-4, suggesting that MK-4 may play important and specific roles in different tissues, although the reasons for this are not yet fully understood.

Vitamin K deficiency

The well-established result of a deficiency in vitamin K on health is a hypocoagulable state resulting from an insufficient amount of VKD coagulation factors, similar to the condition when treated with warfarin. In adults, bleeding due to nutritional deficiency of vitamin K is rare and is almost always associated with a pathological condition, such as a status of malabsorption especially owing to cholestatic liver disease[63]. In contrast, breastfed newborns have a high risk of life-threatening bleeding caused by deficiency of vitamin K due to the low pool of vitamin K in the body, low levels of vitamin K in breast milk and an immature gut microbiome, an important source of vitamin K[64]. For this reason, there is a consensus that all newborn infants should receive vitamin K prophylaxis[65]. In addition to the hypocoagulable state, impaired γ-carboxylation of VKD proteins due to vitamin K insufficiency has been investigated in relation to bone and cardiovascular health, glucose metabolism and cognition[66,67].

Toxicity of vitamin K

There have been almost no reported cases of systemic toxicity by natural vitamin K except allergic reactions due to solubilizing reagents[1]. As such, natural forms of vitamin K are not toxic when consumed orally, even in large quantities. In contrast, the administration of menadione causes toxicity, such as haemolytic anaemia and jaundice[68,69], and is therefore prohibited for use in treating vitamin K deficiency in humans. However, a low dose of menadione is frequently supplemented as a dietary source of vitamin K in animal feed due to its low production costs. Under in vitro conditions, menadione generates reactive oxygen species and can induce reactive oxygen species-dependent cell death[70]. Vitamin K is highly unstable in light, and the photo-degradation product

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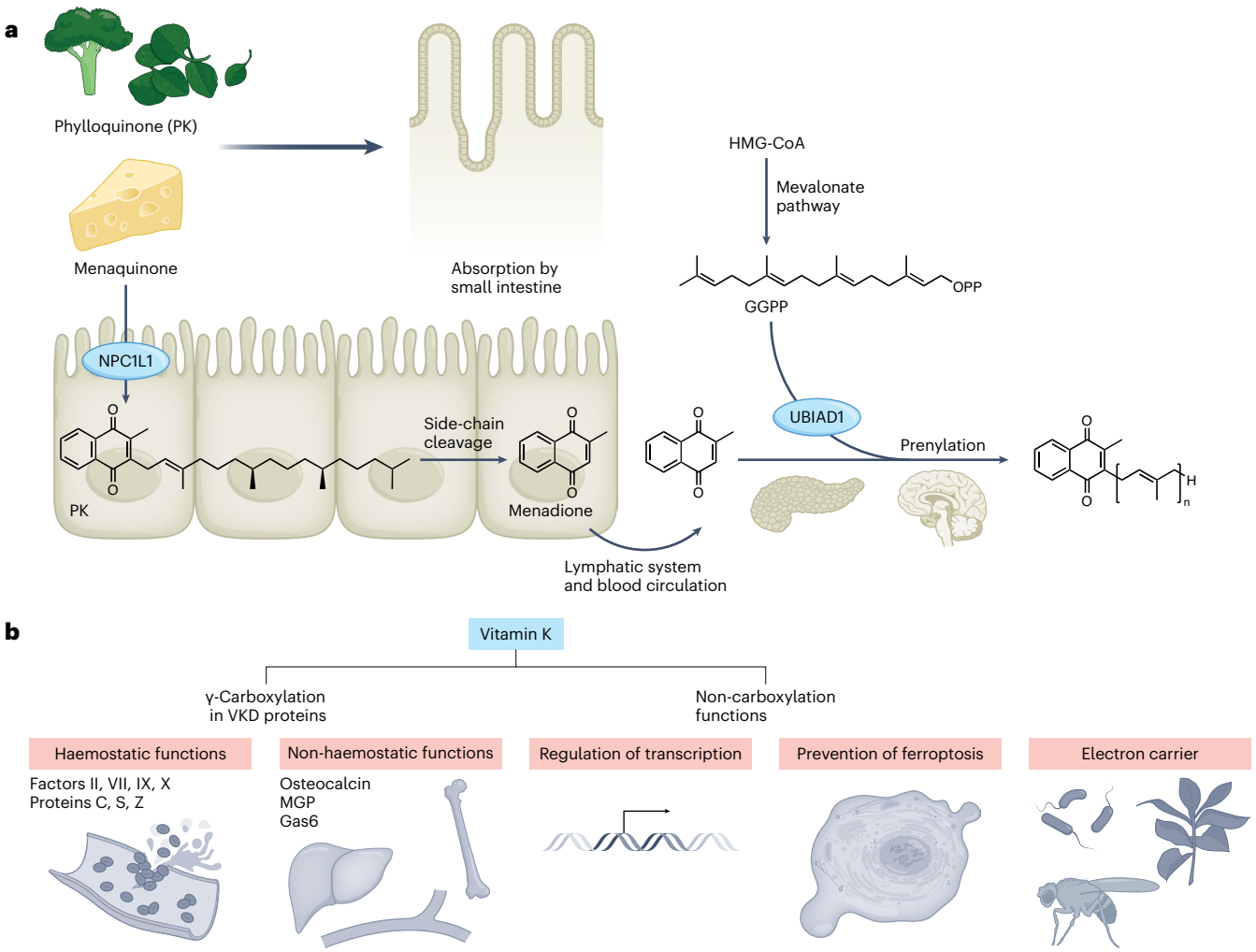

Fig. 3 | Metabolism and biological function of vitamin K. a, Ingestion of vitamin K and interconversion into MK-4. Dietary phylloquinone (PK) is absorbed through NPC1L1 in the small intestine. In enterocytes, the side chain of PK and menaquinones is cleaved to form menadione, which is transferred to each target organ. In the respective tissue, menadione is prenylated by UBIAD1 using geranylgeranyl diphosphate (GGPP) as a source of the side chain to generate MK-4. **b**, The biological functions of vitamin K. Figure adapted from images created with BioRender.com.

of vitamin K exhibits phototoxicity to cultured cells following exposure to ultraviolet light[71]. This physicochemical property limits its use in the supplementation of vitamin K in cosmetics.

Non-carboxylation function of vitamin K

Vitamin K has various biological effects independent of its role as a cofactor for γ-carboxylation of VKD protein (Fig. 3b). Vitamin K has transcription regulatory effects. For instance, MK-4 acts as a ligand for the steroid and xenobiotic receptor (SXR)[2], which is a nuclear hormone receptor activated by a diverse array of steroid hormones, drugs and xenobiotic compounds. MK-4 was also reported to show its transcriptional regulatory action via the activation of protein kinase A[72] and its anti-inflammatory effect by suppressing nuclear factor-κB (NF-κB), a family of transcription factors that play crucial roles in inflammation[73]. In addition, vitamin K has been reported to show a modest activity in driving the differentiation of neural stem cells[74].

Prevention of ferroptosis

Recently, Mishima et al. demonstrated that vitamin K has a function in preventing ferroptosis[4], a type of non-apoptotic, iron-dependent cell death characterized by excessive lipid peroxidation of cellular membranes[5,6]. The term ferroptosis was first coined in 2012 (ref. 5), and manipulating this process holds the potential to treat a range of diseases, including acute organ injury, neurodegeneration and cancers[75]. Unrestrained lipid peroxidation and lipid radicals formed due to physicochemical stress and during the lipid peroxidation chain reaction are considered the hallmark of ferroptosis (Fig. 4a). Among the cell-intrinsic mechanisms that prevent ferroptosis, glutathione peroxidase 4 (GPX4) is the prime regulator of ferroptosis by catalysing the reduction of potentially toxic (phospho)lipid hydroperoxides to non-toxic phospholipid alcohols[76]. The GPX4-independent defence mechanism involves FSP1 that suppresses lipid peroxidation and ferroptosis through its NAD(P)H:ubiquinone oxidoreductase activity[39,40]. Mechanistically, by consuming NAD(P)H, FSP1 reduces extra-mitochondrial ubiquinone (also known as coenzyme Q_{10} (CoQ_{10})) to its reduced form, ubiquinol. Ubiquinol in turn acts as a potent lipophilic radical-trapping antioxidant and directly reduces lipid radicals in membranes, thereby preventing the lipid peroxidation chain reaction. Mishima et al. discovered that FSP1 reduces vitamin K in a manner like ubiquinone. VKH_2 then acts as a potent radical-trapping antioxidant and inhibitor of (phospho)lipid peroxidation[4] (Fig. 4b). Notably, this reaction is independent of GGCX and VKOR, and therefore warfarin does not affect the anti-ferroptotic action of vitamin K. Among the forms of vitamin K, MK-4 showed the most potent anti-ferroptotic effect. Taken together, FSP1-mediated vitamin K reduction protects cells against detrimental lipid peroxidation and ferroptosis in the non-canonical vitamin K cycle,

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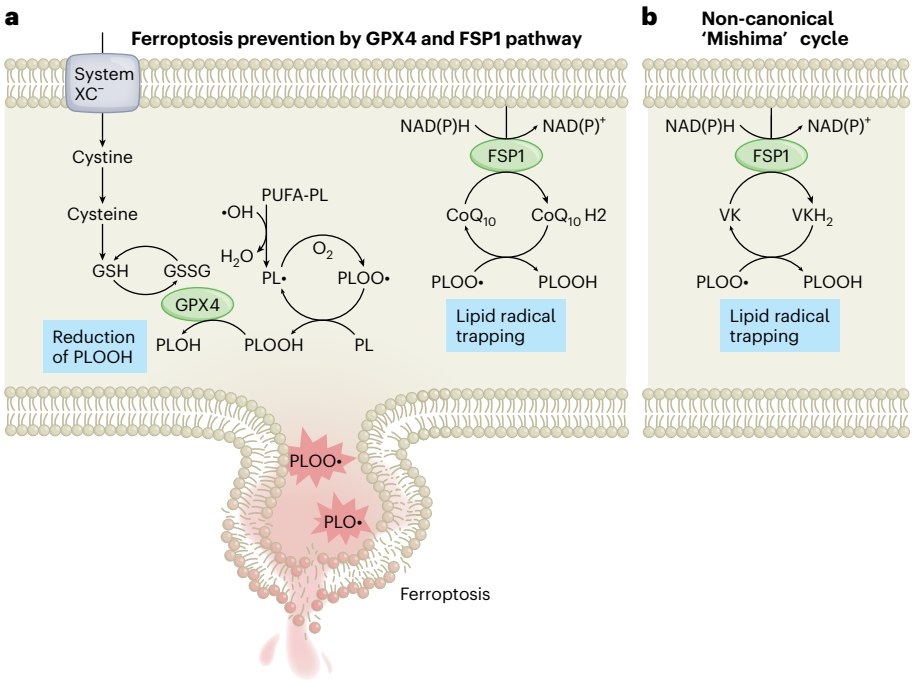

Fig. 4 | Mechanisms of ferroptosis and its suppression pathways. a, Lipid peroxidation, the hallmark of ferroptosis, may be induced by Fenton-type chemistry including hydroxyl radical (•OH) or peroxyl radicals (•OOH). These radicals may remove a bisallylic hydrogen atom from a polyunsaturated fatty acid (PUFA) incorporated in phospholipids (PL), the main building blocks of lipid bilayers, leading to the formation of a phospholipid radical (PL•). In a subsequent reaction with molecular oxygen (O_2), a phospholipid peroxyl radical (PLOO•) is formed, which in turn removes hydrogen from another PUFA-PL to form phospholipid hydroperoxide (PLOOH). Uncontrolled and extensive (phospho) lipid peroxidation and the generation of lipid radicals, such as PLOO• and phospholipid alkoxyl radical (PLO•), damages membrane integrity, eventually leading to plasma membrane rupture and ferroptosis. The main pathways for ferroptosis prevention are the cyst(e)ine/GSH/GPX4 and FSP1/ubiquinone (CoQ_{10}) pathways. Glutathione (GSH) is synthesized from cysteine, derived from cystine taken up via system Xc^-. Using GSH, GPX4 reduces toxic PLOOHs, yielding PLOH. Oxidized GSH (GSSG) is recycled to GSH. In the FSP1/CoQ_{10} pathway, FSP1 reduces CoQ_{10} to ubiquinol ($CoQ_{10}H_2$) using electrons from NAD(P)H. $CoQ_{10}H_2$ in turn suppresses phospholipid peroxidation of lipid bilayers by trapping lipid radicals such as PLOO• forming PLOOH. **b**, Anti-ferroptotic function of VK. By consuming NAD(P)H, FSP1 reduces VK quinone to VK hydroquinone (VKH_2), which acts as a powerful radical-trapping antioxidant. The reaction of VKH_2 with lipid radicals generates VK quinone, which can be reduced by FSP1 using two electrons coming from NAD(P)H. We tentatively name this non-canonical VK cycle the 'Mishima' cycle.

which we tentatively name as the Mishima cycle (Fig. 4b), in analogy to the canonical vitamin K cycle required for VKD carboxylation. These findings thus clarify the previously reported, yet poorly understood, antioxidant and cell-protective mechanism of vitamin K[77] in oxidative glutamate toxicity in cells[78], now widely known as ferroptosis[5].

Because the abundance of ubiquinone in animals and humans is much higher than that of vitamin K, the FSP1/ubiquinone pathway is likely the prevailing mechanism for intrinsic ferroptosis defence as compared to the FSP1/vitamin K pathway. Yet, MK-4 hydroquinone has a more potent effect on suppressing lipid peroxidation than the reduced form of ubiquinone in vitro[4]. In this context, high-dose vitamin K supplementation might be a way forward to reduce symptoms of neurodegenerative and other diseases, where ferroptosis inhibition is therapeutically beneficial[75]. Indeed, a supraphysiological dose of vitamin K ameliorated organ injuries induced by genetic ablation of *Gpx4* or by ischaemia–reperfusion injury in wild-type mice, which are in vivo conditions directly associated with increased ferroptosis[4,79].

Sensitization of cancer cells to ferroptosis by FSP1 inhibition is considered a promising strategy for anticancer treatment because therapy-resistant cancers show high vulnerability towards ferroptosis[80]. FSP1 inhibitors were in fact shown to sensitize cancer cells to ferroptosis[39,81]. As described above, the deletion of FSP1 had no overt effect on the coagulation status at least in mice[4], although FSP1 is essential for the antidotal effect of vitamin K against warfarin poisoning. Therefore, when FSP1 inhibitors ever become clinically available, caution should be taken when using in combination with warfarin, as warfarin overdose under FSP1-inhibiting conditions may not be overcome by vitamin K administration.

Electron carrier

Vitamin K acts as an electron carrier in the synthesis of ATP in certain organisms. Unicellular organisms generally do not express GGCX, indicating that in these organisms the primary functions of vitamin K do not relate to the γ-carboxylation of VKD proteins. In eukaryotes, ubiquinone plays a role in intracellular electron transfer in the mitochondrial respiratory chain, while in certain bacteria, including *Escherichia coli*, *Streptomyces coelicolor*, *Bacillus subtilis* and *Helicobacter pylori*, MK-*n* serves as an important electron carrier and is even essential for growth[82]. These bacteria synthesize MK-*n* from chorismate, an intermediate in the production of aromatic amino acids, via the shikimate or futalosine pathway[83]. The shikimate pathway involves MenA, which is a prenyltransferase with homology to mammalian UBIAD1, and other enzymes (MenB to MenG)[83]. In plants, phylloquinone serves as an electron acceptor during photosynthesis as part of the electron transport chain of photosystem I[84]. Chorismate serves as a precursor for the formation of the naphthoquinone ring in *Arabidopsis*, while phytyl diphosphate, produced through the phosphorylation of phytol, provides the phytyl moiety as the side chain of phylloquinone[85]. In eukaryotes, Vos et al. demonstrated that MK-4 can function as an electron carrier required for ATP production via the mitochondrial electron transport chain in *Drosophila* and that supplementation of MK-4 improves the efficiency of the electron transport chain and ATP production in flies with mitochondrial defects[86]. By contrast,

in mammalian cells, MK-4 fails to restore electron flow in the respiratory chain of cells with ubiquinone deficiency[87].

Evolutionary considerations

Vitamin K exhibits several similarities with ubiquinone in terms of structure, metabolism and biological function (Fig. 2a). Both vitamin K and ubiquinone are composed of a quinone head group and a polyisoprene side chain. The MK-4 synthesis enzyme, UBIAD1, displays homology with the ubiquinone synthesis enzyme COQ2 (ref. 88). During the biosynthesis of both MK-4 and ubiquinone, geranylgeranyl pyrophosphate, which is the product in the mevalonate pathway, is used as the source of the side chain. The intestinal vitamin K transporter, NPC1L1, also contributes to the resorption of ubiquinone[89]. As described above, both vitamin K and ubiquinone act as electron carriers for ATP production. FSP1 reduces and consequently regenerates both vitamin K and ubiquinone, contributing to defence mechanisms against ferroptosis.

While plants and certain bacteria utilize vitamin K as electron carriers, eukaryotes use ubiquinone. In light of the evolution of life, it appears that vitamin K was replaced by ubiquinone as an electron carrier due to its higher redox potential when environmental oxygen concentrations increased following the great oxidation event on primordial Earth[3,90]. Menaquinones possess a lower redox midpoint potential (~−70 mV) compared to ubiquinone (~+100 mV) due to the characteristics of the different quinone structures[91]. Notably, the reduced forms of vitamin K are readily and non-enzymatically oxidized to their oxidized forms in an aerobic atmosphere due to its low redox potential, rendering these compounds incapable of efficiently functioning in an oxygen-rich environment[3].

Ferroptosis, originally studied in mammalian systems, has been meanwhile observed in diverse species, such as plants[92], cyanobacteria[93], protozoa[94], fungi[95] and *Caenorhabditis elegans*[96], indicating that ferroptosis is an evolutionarily conserved cell death mechanism[97]. Among these organisms, the FSP1-mediated lipophilic quinone cycle likely performs a protective role against environmental ferroptotic stress, such as iron, heat and ultraviolet exposure[92,98], dating back to the earliest period of Earth's history. It thus follows that vitamin K might be the oldest naturally occurring anti-ferroptotic/antioxidant quinone and, considering this, it can be rationalized that ubiquinone and vitamin K are sibling metabolites. Vitamin K may have evolved to serve ATP production and anti-ferroptotic functions in certain organisms, in analogy to the roles of mitochondrial and extra-mitochondrial ubiquinone in ATP production and ferroptosis suppression in mammals, respectively. When considering the role of vitamin K in the context of evolution, gut microbiota may provide some interesting insights, as several strains of microbiota are known to produce vitamin K and the oxygen concentration in the lumen of the distal colon is quite low[99] similar to conditions in the primordial earth. Thus, it is tempting to speculate that vitamin K beyond its electron carrier function may serve as an important anti-ferroptotic agent in these organisms.

Vitamin K has been traditionally linked with blood coagulation and the physiological function of VKD proteins. However, research into vitamin K biology has unveiled an increasing number of nonclassical functions of vitamin K, with ferroptosis suppression as the most recently identified example. Further clarification of the biological roles of vitamin K in the context of the evolution of life across various species will likely connect ferroptosis to other areas of vitamin K-related biology.

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Author contributions

E.M., A.W., T.S. and M.C. jointly wrote this article.

Competing interests

E.M. has filed a patent related to the treatment of ferroptosis-associated diseases with vitamin K (WO2022075444A1). M.C. is a cofounder and shareholder of ROSCUE Therapeutics, which is developing ferroptosis inhibitors. The other authors declare no competing interests.

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