

Spotlight

Vitamin K: A new guardian against ferroptosis

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The dietary factor vitamin K has been found to protect against ferroptosis, a form of cell death driven by lipid peroxidation. This reveals new dietary links to cancers and degenerative conditions and a key factor involved in warfarin poisoning.

The little chicks were bleeding out. In 1929, Henrik Dam was working at the University of Copenhagen on nutritional deficiencies. Dam found that chicks that consumed certain low-fat diets had a severe bleeding disorder, with an inability of their blood to clot (Dam, 1934). He showed that the deficiency could not be cured by supplementing with known fat-soluble vitamins, and he named this new essential dietary factor vitamin K, for its key role in blood “koagulation” (Ferland, 2012). Nearly a century later, Mishima et al. (Mishima et al., 2022) have found an unexpected additional role for vitamin K as a guardian against ferroptosis, a form of regulated cell death driven by membrane lipid oxidation, and identified a key enzyme involved in regenerating the protective effect of vitamin K.

Ferroptosis is a regulated lethal process characterized by the accumulation of lipid-based reactive oxygen species that results in membrane damage and cell death. This common feature that defines the execution of ferroptotic cell death is in contrast to the numerous regulatory pathways that control ferroptosis. The importance of understanding the complexity of ferroptosis regulation is stressed by the emerging evidence of the central role for this death pathway in both pathophysiology and anti-cancer therapies (Stockwell, 2022).

Although several hallmark ferroptosis genes and metabolites have been identified in the past decade, sensitivity of cells to ferroptotic death is still considered to be highly dependent on the extrinsic and intrinsic cellular context. The lack of a single intrinsic genetic pathway that modulates cell sensitivity to ferroptosis was

recently concluded by integrating over 20 large-scale genetic screens for ferroptosis modulators (Magtanong et al., 2022). Mishima et al. (Mishima et al., 2022) now contribute to the understanding of metabolic modulation to ferroptosis sensitivity. These researchers identified vitamin K—and, more specifically, its reduced form, VKH₂—as an active inhibitor of ferroptosis through inhibition of lipid peroxidation. The main finding of the paper is that FSP1 is the vitamin K reductase that sustains VKH₂ levels to support ferroptosis suppression. Vitamin K is suggested to be the most ancient type of naturally occurring anti-ferroptotic quinone, which was evolutionarily replaced by the more abundant and potent version, ubiquinone (CoQ₁₀), which is also a target of reduction by FSP1.

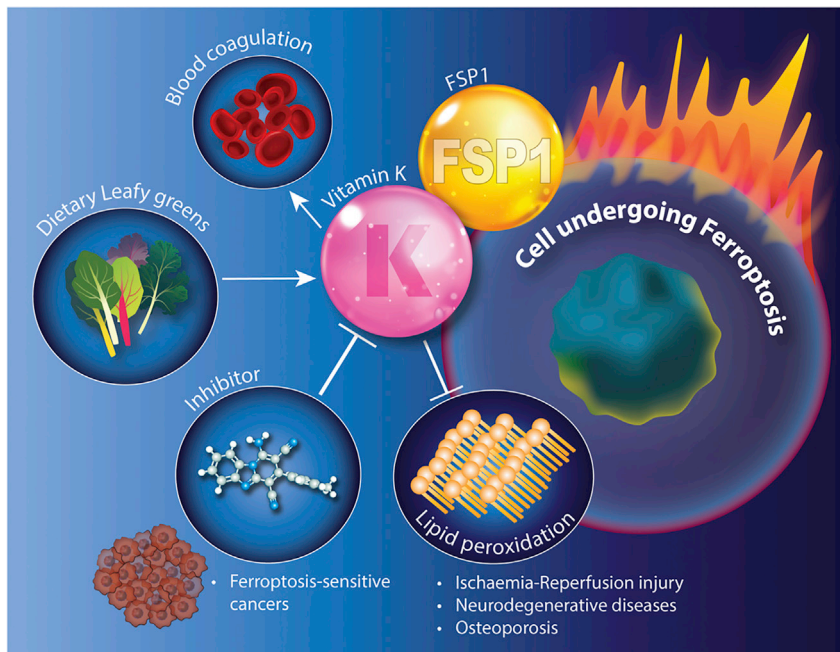
The identification of the vitamin K family as potent anti-ferroptotic agents emerged from a screen of naturally available vitamin compounds in *GPX4* knockout mouse embryonic fibroblasts, aimed to discover new ferroptosis-inhibitory mechanisms. Besides the well-established ferroptosis inhibitor vitamin E, only the three forms of vitamin K—phyloquinone, menaquinone-4 (MK-4), and menadione—were found to prevent cell death caused by TAM-induced *GPX4* deletion. The activity of vitamin K compounds as inhibitors of lipid peroxidation (rather than iron chelators) was found to rely on the quinone head group and was validated to inhibit ferroptosis markers in several human cell models. Importantly, pharmacological doses of vitamin K were shown to reduce ferroptosis-related tissue damage in three different *in vivo* mouse models in pathological contexts, pointing to the role of

vitamin K supplementation in the cellular defense mechanism against ferroptosis. In this context, vitamin K supplements may be proposed to reduce symptoms of neurodegenerative and other diseases where ferroptosis inhibition is beneficial therapeutically.

In contrast, in the context of cancer risk and vitamin K supplementation during anti-cancer treatment, most observational studies of clinical trials support the concept that vitamin K supplementation reduces risk of cancer development and mortality (Welsh et al., 2022), mostly attributed to vitamin-K-dependent carboxylation. However, in some subtypes of breast cancer, vitamin K intake was reported to increase the risk of incidence and death (Wang et al., 2021). The cancer-promoting effect in such breast cancers may be attributed to the role of vitamin K in radical trapping, by inhibiting cancer-suppressing ferroptosis in a similar manner as vitamin E dietary supplements were found to increase the risk for various cancer types, as well as cancer recurrence and patient mortality, especially amongst smokers who are more prone to oxidative damage (Harvie, 2014).

An additional context in which vitamin K may modulate physiological conditions by altering ferroptosis sensitivity is osteoporosis. Low vitamin K status was reported to contribute to osteoporosis, and vitamin K supplementation is typically recommended for prevention of osteoporosis (Welsh et al., 2022). This is in line with the recent association of ferroptosis with osteoporosis progression, which suggests targeting ferroptosis as a therapeutic strategy for this





chronic disease (Gao et al., 2022). Taken together, vitamin K status may serve as a novel biomarker for sensitivity to ferroptosis, and modulation of ferroptosis sensitivity through dietary vitamin K may be offered as a therapeutic approach in cancer, degenerative, and other chronic diseases.

The reduced form of vitamin K is a necessary cofactor for carboxylation of coagulation proteins; thus, vitamin K is required for healthy blood clotting and hemorrhage prevention. Concomitantly, warfarin, a specific inhibitor of canonical vitamin K epoxide reductase (VKOR), is the most commonly prescribed oral medication for blood clot prevention. The fact that warfarin poisoning can be treated with vitamin K supplementation points to an additional warfarin-resistant vitamin K reductase, which was previously attributed to an unknown protein. Mishima et al. identified FSP1 as the warfarin-resistant vitamin K reductase, responsible for coagulation control as well as manipulation of sensitivity to ferroptosis. The finding that one functional allele of *FSP1* was sufficient to fully rescue from warfarin toxicity *in vivo* suggests that FSP1 plays a central role in the canonical vitamin K cycle, comparable to the role of VKOR. Therefore, when vitamin K supplementation is considered for disease treat-

ment, FSP1 levels may be an important biomarker predicting its ability to protect from ferroptosis.

FSP1 was found to reduce and thus regenerate both vitamin K and CoQ₁₀, contributing to these cellular defense mechanisms against ferroptosis. This dual role of FSP1 explains the potent effect of FSP1 inhibition in ferroptosis sensitization and nominates this protein as a target for anti-cancer therapeutics by inducing selective cancer cell ferroptosis. Consistent with this discovery, Jin et al. recently identified FSP1 as vitamin K reductase by creating a vitamin-K-dependent cell line that allows for the identification of unknown genes involved in nonlethal post-translational modifications with no phenotypic consequences in cell growth, such as vitamin-K-dependent carboxylation of coagulation factors (Jin et al., 2022). This paper suggests that, although useful for sensitizing ferroptosis to kill cancer cells, suppression of the CoQ₁₀-related ferroptosis-defense system through FSP1 inhibition may induce bleeding risks due to the contribution of FSP1 to reduction of vitamin K. The authors suggest targeting the DHODH-dependent pathway of reducing CoQ₁₀ as an alternative therapeutic approach, as DHODH is a

CoQ₁₀ reductase that is not involved in the vitamin K cycle.

Mishima et al. highlight the involvement of ferroptosis in multiple physiological settings and contribute to understanding the complexity of the regulation on this lethal cellular process. The connections between ferroptosis and other areas of biology continue to expand and deepen.

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DECLARATION OF INTERESTS

B.R.S. is an inventor on patents and patent applications involving small-molecule drug discovery and ferroptosis; has co-founded and serves as a consultant to Inzen Therapeutics, Exarta Therapeutics, and ProJenX, Inc.; serves as a consultant to Weatherwax Biotechnologies Corporation and Akin Gump Strauss Hauer & Feld LLP; and receives sponsored research support from Sumitomo Dainippon Pharma Oncology.

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