Recent advances in vitamin K-dependent Gla-containing proteins and vitamin K nutrition

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Abstract

Vitamin K is a multi-functional nutrient and various tissues modify their function in response to vitamin K bioavailability mainly through post-translational modification of vitamin K-dependent (VKD) proteins. In this review, we discuss five clinical topics of vitamin K nutrition and vitamin K-dependent Gla-containing proteins. Although the physiological roles of these VKD proteins need further elucidation, study of these proteins may open new avenues for therapy in the clinical field. The topics discussed in the review are focused on des-gamma-carboxyprothrombin (DCP) in relation to the pathogenesis of hepatocellular carcinoma, osteocalcin (OC) and undercarboxylated OC (ucOC) in relation to bone fractures and insulin sensitivity, matrix Gla protein (MGP) in relation to vascular calcification, and growth arrest-specific protein-6 (Gas6) in relation to inflammation and platelet aggregation. Finally, interaction among vitamins were discussed.

Keywords: Vitamin K; Vitamin K dependent proteins; PVKA-II; Osteocalcin; Matrix Gla protein; Growth arrest-specific protein-6

1. Introduction

Vitamin K is a vitamin known to activate not only blood coagulation factors, but also tissue-specific vitamin K-dependent (VKD) proteins through post-translational modification of γ carboxylation, which converts Glu residues to Gla. Insufficient γ carboxylated VKD proteins are tissue-specific sensitive markers for vitamin K insufficiency. Vitamin K actions are also accomplished via the binding to the nuclear receptor, SXR. However, until now, useful markers for vitamin

Abbreviations: AAC, aortic artery calcification; ABCC6, large ATP-binding cassette (ABC) gene subfamily C; AFT4, activating transcription factor 4; Axl, AXL receptor tyrosine kinase; BMC, bone mineral content; BMD, bone mineral density; cOC, carboxylated osteocalcin/Gla-osteocalcin; CRP, C-reactive protein; CTx, cortical thickness; CTS, collagen type I cross-linked C-telopeptide; DCP, des-gamma-carboxyprothrombin; Esp, embryonic stem cell protein; FoxO, forkhead family transcription factor; Gas6, growth arrest-specific (protein/gene) 6; GGCX, gamma-glutamyl carboxylase; Glu, glutamic acid; GPRC6A, G-protein coupled receptor family C, group6, member A.; HAP, hydroxyapatite; HCC, hepatocellular carcinoma; ICAM-1, intercellular adhesion molecule-1; IL-6, interleukin-6; INR, international normalized ratio; InsR, insulin receptor; IOC, intact osteocalcin; IRI, immuno-reactive insulin; MGP, matrix Gla protein; MK-4, menaquinone-4; MK-7, menaquinone-7; MRP6, multidrug resistance-associated protein-6; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; OX-PTP, osteotesticular protein tyrosine phosphatase; PIVKA-II, protein induced by vitamin K absence II; PTH, parathyroid hormone; PXE, pseudoxanthoma elasticum; RTKs, receptor tyrosine kinases; OC, osteocalcin; SXR, xenobiotic nuclear receptor; TAM, tyrosine kinase receptors including TYRO3, AXL and MERTK; TLR, Toll-like receptor; TNFR2, tumor necrosis factor receptor-2; tOC, total osteocalcin; uGas6, undercarboxylated growth arrest-specific protein-6; ucMGP, undercarboxylated matrix Gla protein/uncarboxylated matrix Gla protein; ucOC, undercarboxylated osteocalcin; ucVKD protein, undercarboxylated vitamin K-dependent protein; VKD protein, vitamin K-dependent protein; VKORC1, vitamin K epoxide reductase; VSMCs, vascular smooth muscle cells; WT, wild-type.

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K function through nuclear receptors have not been available in clinical settings. Therefore, vitamin K functions are evaluated by the measurements of tissue-specific VKD proteins in human studies. One of the typical VKD proteins, osteocalcin (OC), is considered to be a bone-specific protein and the function of OC was believed to be protein accretion, which may stabilize bone hydroxyapatite. The role of OC has been expanded and it has been shown to be a bone-derived hormone, which regulates energy expenditure. Breakthroughs regarding the function of vitamin K-dependent proteins in immunology, inflammation and atherosclerosis have now been achieved. As a result, several VKD proteins have been independently well characterized. However, there is a lack of knowledge regarding common features of VKD proteins and of vitamin K nutrition. In this review, the main functions of each vitamin K-dependent protein are summarized and possible new biological actions are discussed.

2. Vitamin K-dependent (VKD) proteins as sensitive markers for vitamin K status

Genetic absence or gene mutations of VKD proteins, dietary vitamin K inadequacy, and vitamin K deficiency induced by chronic anti-coagulant therapy are all linked to age-associated conditions. For example, bone fragility after menopause is linked to OC; arterial calcification connected to cardiovascular disease involves matrix Gla protein (MGP) [1], while growth arrest-specific protein-6 (Gas6) is related to thrombotic disease [2] or inflammation.

Table 1 summarizes VKD proteins and their functions.

Hepatic VKD proteins are essential for survival because bleeding is an immediate threat. Vitamin K preferentially accumulates in the liver and subsequently vitamin K is transported to extra-hepatic tissues [3]. Thus, the appearance of hepatic undercarboxylated VKD is less frequent than the other organ-specific ucVKDs. Since ucVKD proteins are tissue-specific, the values of ucVKD proteins such as DCP (PIVKA II), uncarboxylated-OC (ucOC) and uncarboxylated-MGP (ucMGP) are indicators of tissue-specific vitamin K bioavailability [4] in liver, bone and vessels, or cartilage, respectively. Gas6 (growth arrest-specific protein 6) plays important roles in a variety of organs such as vessels, fat tissue and brain as mentioned in the latter part of the review. However, until now, there has been no report on whether ucGas6 shows significant clinical manifestations.

Using such sensitive markers in a depletion-repletion study, Booth et al. [5] established that a vitamin K-restricted diet taken by volunteers for 15 days resulted in a sufficient lowering of tissue stores of vitamin K, which caused an increase in DCP and %ucOC (ratio of ucOC to intact (c)OC). The increase in ucVKD proteins after vitamin K depletion was reversed by a subsequent 10-day repletion phase with vitamin K; at a dose of 200 μg/day [5]. Therefore, vitamin K depletion and repletion in tissues was achieved with intake of a vitamin K deficient or sufficient diet, respectively.

3. Clinical topics of ucVKD/cVKD proteins

3.1. PIVKA II (protein induced by vitamin K absence II) or des-γ-carboxyprothrombin (DCP) in hepatocellular carcinoma

Des-γ-carboxyprothrombin (DCP) is known as a marker of hepatocellular carcinoma (HCC) [6]. Excessive production of DCP is associated with tumor expansion or metastasis of HCC. Therefore, DCP is a good marker for the diagnosis of HCC and is a predictor of the prognosis in patients bearing HCC. Although several causes of the production of DCP in HCC cells have been postulated, the exact mechanism(s) of how DCP is produced by HCC cells is yet to be confirmed. Four possible mechanisms may be involved in the DCP production process. First, low γ-glutamyl carboxylase (GGCX) activity was observed in a rat model of Morris hepatoma tumor [7]. Vitamin K epoxide reductase subunit C1 (VKORC1) haplotypes also influenced the performance characteristics of DCP for screening hepatocellular carcinomas [8]. Second, the
availability of vitamin K may deteriorate in HCC cells. Okuda et al. [9] reported that a HCC cell line produced DCP in a time- and cell number-dependent manner in the absence of vitamin K, but not in the presence of vitamin K. This finding clearly indicated that DCP production is enhanced by vitamin K insufficiency in HCC cells. Third, DCP production is not a specific event in HCC since severe vitamin K deficiency in normal hepatic cells also produced DCP [5]. However, the amount of DCP produced in normal cells is less than HCC cells. Therefore, tumor-specific metabolic processes such as hypoxia may stimulate DCP production. Fourth, excessive production of the pro-thrombin precursor was observed in HCC cell lines [10] and excess pro-thrombin precursor may lead to excessive DCP production.

Since DCP is associated with HCC growth and metastasis, it is reasonable to expect that vitamin K supplementation may have a beneficial effect on HCC. Another possible role of vitamin K in suppression of HCC has been reported. Over-expression of SXR in vitamin K2 treated HCC cell (HuH17) resulted in reduced proliferation and motility of the cells. These results suggest that the activation of SXR could contribute to tumor suppressive effects of vitamin K2 on HCC cells [11].

In fact, an open label randomized study regarding vitamin K2 effects on curative recurrence-free rates in patients with HCC revealed that vitamin K2 significantly suppressed the recurrence of HCC [12,13]. However, a double blinded placebo-controlled study between the groups with vitamin K2 and placebo failed to reduce the recurrence of HCC and to prolong survival [14]. This study ignored the baseline vitamin K nutritional state of the participants. Since vitamin K2 may be expected to have beneficial effects only in vitamin K-deficient subjects, trials should be focused on patients with low vitamin K intake.

In conclusion, vitamin K deficiency or insufficiency induces production of DCP in patients with HCC and this biomarker is useful to monitor HCC growth and metastasis. However, vitamin K nutrition in the assessment of blood levels of DCP will be required. Although the intervention of vitamin K failed to show beneficial effects in patients with HCC, further clarification of the role of vitamin K on the progression of HCC may be necessary.

3.2. Undercarboxylated osteocalcin (ucOC)/carboxylated osteocalcin (cOC)

3.2.1. Determinants of $\gamma$-carboxylation in OC

OC is a bone-specific vitamin K-dependent protein and is synthesized by osteoblasts during the latter phase of bone calcification [15]. Osteocalcin is the most abundant non-
collagenous protein in bone and its concentration in blood is tightly connected to osteoblast function, differentiation and vitamin K sufficiency. OC contains three Glu residues in the molecule, which need vitamin K-dependent post-translational \( \gamma \)-carboxylation to Gla residues. The post-translational modification of OC is regulated by several factors. The strongest regulator of carboxylation is vitamin K intake from food [16–21]. Administration of vitamin K homologues (Phylloquinone/Vitamin K\(_1\) [22,23], Menaquinone-4 (MK-4) [24–26] and Menaquinone-7 (MK-7) [27,28] enhanced the carboxylation process. Both dietary intake of vitamin K [5] and pharmacological dose of MK-4 [24] induced rapid alteration of \( \gamma \)-carboxylation of Glu residues in OC molecules. Fig. 1 shows the effect of MK-4 treatment on serum levels of intact osteocalcin (IOC) (Fig. 1a), Gla-OC (Fig. 1b) and ucOC (Fig. 1c). A rapid increase in Gla-OC and concomitant decrease in ucOC within 1 month after the MK-4 administration, and a subsequent increase in IOC 3–6 months after treatment, were observed [24]. These findings might indicate that MK-4 administration induced rapid enhancement of \( \gamma \)-carboxylation and at the latter phase, MK-4 may induce OC expression from osteoblasts.

Since serum levels of vitamin K\(_1\) are negatively correlated with ucOC/IOC ratio in postmenopausal women [19], the ratio of ucOC to IOC (ucOC/IOC) is a sensitive marker for the sufficiency of vitamin K in bone. Carboxylation efficiency is deteriorated by advancing age. The regression line between plasma vitamin K\(_1\) levels and the ucOC/IOC ratio in the older group shifted toward a higher ucOC/IOC ratio at a given serum vitamin K\(_1\) level [21] (Fig. 2). This means that older people require more vitamin K than younger people in terms of \( \gamma \)-carboxylation of OC. The reasons why the vitamin K requirement is increased with aging are discussed below. Bone turnover rate or the bone remodeling process may influence the ucOC/IOC ratio. Children, who have a very high turnover rate of bone, showed markedly higher levels of the ucOC/IOC ratio than healthy adults [27,29]. In contrast, treatment of bisphosphonate in osteoporotic patients induced a larger reduction of serum ucOC than other bone markers suggesting that suppressed bone turnover with bisphosphonate treatment was linked to enhanced carboxylation of OC [30] or lowered vitamin K requirement of bone [26]. Therefore, the bone requirement of vitamin K may depend on the rate of bone turnover.

A second possible explanation is the change in the enzymatic activity of \( \gamma \)-carboxylase as age advances. The metabolic vitamin K cycle involves VKORC1 and GGCX. These two key enzymes in the vitamin K cycle are critically responsible for the biological activity of vitamin K. Thus, it is possible that aging might influence enzymatic activities. However, the mechanistic details that may be involved in aging have not yet been elucidated.

These two key enzymes are known to have functionally relevant gene polymorphisms and the efficiency of the vitamin K cycle depends on these gene polymorphisms. Therefore, it can be hypothesized that functionally relevant polymorphisms of these enzymes modulate the inverse relationship between dietary vitamin K intake and serum ucOC [18,31,32]. Table 2 summarizes the confirmed determinants of \( \gamma \)-carboxylation in the OC molecule.

### 3.2.2. Undercarboxylated osteocalcin (ucOC), \( \gamma \)-carboxylated osteocalcin (cOC) and bone fractures

\( \gamma \)-carboxylated Gla residues are responsible for the specific affinity of OC to the hydroxyapatite (HAP) molecule [33,34]. As a result, Gla-containing osteocalcin (cOC) normalizes bone crystal nucleation [34]. The bone phenotype of OC knockout mice (OC\(^{-/-}\)) had higher bone formation than wild-type (WT) mice. Bone strength was also superior in OC\(^{-/-}\) mice than in WT mice. However, bone mineral density (BMD) in OC\(^{-/-}\) mice was decreased rapidly after ovariectomy suggesting that OC modulates bone formation to protect from hyper-mineralization and rapid bone loss after menopause [35]. In addition to the OC\(^{-/-}\) mice, long-term warfarin-treated rats showed higher bone mineral content (BMC), bone area, and cortical thickness (Cth) than controls [36]. This suggests that long-term OC deficiency in bone caused increased bone size. Ovariectomy in long-term warfarin-treated rats induced a significantly faster reduction in BMD, bone area and Cth than controls [36]. Therefore, OC-deficient bone shows hyper-mineralized bone before menopause, but bone is rapidly reduced when estrogen is lacking.

In 1985, Hart et al. first reported low serum vitamin K\(_1\) levels in patients with hip fractures [37]. Several subsequent studies also reported that low vitamin K intake was associated with hip or vertebral fractures both in Caucasian and Asian populations [38–45]. Tanaka et al. reported that bone OC content in patients with hip fractures was significantly lower than controls who had osteoarthritis [46]. In addition to the deterioration of vitamin K nutrition in hip fracture patients, the close relationship between vitamin K-dependent proteins and incident fractures has been reported extensively. Szulc et al. first reported that serum levels of ucOC were higher in patients with hip fractures than controls [47]. Moreover, serum levels of ucOC predicted future fractures in elderly people [48–50] and even in osteoporosis patients treated with
vitamin K2 stimulated osteoblastogenesis and suppressed glandin production in an organ culture system [55] and might inhibit bone resorption through inhibition of prostaglandin production of the other kinds of ligands. Moreover, vitamin K exhibit loss of function of vitamin K but also shows loss of function of extracellular matrix-related genes, some of which are involved in collagen assembly [53]. However, in contrast to steroid receptors such as estrogen receptor, which binds to estrogen specifically, the SXR/PXR can bind to endogenous or exogenous toxic chemicals to reduce their toxicity. This means that vitamin K is a transcriptional regulator of extracellular matrix-related genes, some of which are involved in collagen assembly [53]. In contrast to steroid receptors, the SXR/PXR can bind to endogenous or exogenous toxic chemicals to reduce their toxicity. This means that vitamin K binds to the xenobiotic nuclear receptor (SXR/PXR) leading to enhanced expression of several components of the bone matrix [52,53]. Furthermore, systemic SXR/PXR knockout mice displayed marked reduction in bone mass through reduction of bone formation together with increase in bone resorption [54]. These reports indicated that vitamin K is a transcriptional regulator of extracellular matrix-related genes, some of which are involved in collagen assembly [53]. In contrast to steroid receptors, which binds to estrogen specifically, the SXR/PXR can bind to endogenous or exogenous toxic chemicals to reduce their toxicity. This means that the phenotypes of SXR/PXR knockout mouse not only exhibit loss of function of vitamin K but also shows loss of function of the other kinds of ligands. Moreover, vitamin K might inhibit bone resorption through inhibition of prostaglandin production in an organ culture system [55] and vitamin K2 stimulated osteoblastogenesis and suppressed osteoclastogenesis [56,57] by suppressing NF-κB activation [58]. However, it has been still not elucidated whether the effects of vitamin K on bone metabolism is a reflection of SXR/PXR mediated process or not. The SXR-mediated metabolic effects of vitamin K on bone could not be assessed clinically because there are no biological marker(s) that reflect vitamin K actions via a nuclear receptor. Effects of vitamin K on osteoblastogenesis may be explained by increased OC levels after vitamin K2 administration [24]. However, other evidence of vitamin K effects on osteoclastogenesis or osteoblastogenesis, or on collagen metabolism, in humans has not been established clinically.

3.2.3. Effects of vitamin K treatment on bone

There are several reports regarding vitamin K intervention trials on bone markers, bone mineral density (BMD) and fractures. Table 4 summarizes the findings of the 9 articles that met the inclusion criteria mentioned at the table legend. From these studies, it might be concluded that vitamin K administration did not reduce bone resorption markers, but did reduce ucOC in both postmenopausal women and osteoporotic women. However, changes in total OC concentration after vitamin K treatment were inconsistent among the reports. The change in BMD after vitamin K administration was also found to be inconsistent. A meta-analysis indicated that modest overall treatment effects for vitamin K on BMD were observed, but the reports had significant bias. Therefore, the effects of vitamin K on BMD should be interpreted with caution [67].

The reported data are also inconsistent in regard to the effect of vitamin K on bone fractures, as shown in Table 4. The most surprising observation was the Osteoporotic Fracture study (OF study) [65], in which the incidence of fracture between the control and the vitamin K2-treated groups did not differ significantly (RR 1.03, 95% confidence interval was 0.87−1.22). However, another two trials [59,63] showed a significant reduction in incident fracture rate in vitamin K2 [59] and K1 [63]-treated groups. The reason(s) for these disparate results is uncertain. The meta-analysis indicated that several plausible factors may account for the discrepancy in results in the OF study. There was a less specific diagnostic criteria for osteoporosis and incident fracture [68], and a very low incident fracture rate in the control group (13.6% during 3 years). This suggested that there was an inadequate count of incident fracture occurrence [68,69].

It has been reported that vitamin K2 administration either with calcium and risedronate [70], or vitamin K1 with vitamin D plus calcium [71], significantly reduced non-vertebral fractures in Alzheimer's disease, which is a disease known to have significantly higher rates of hip fractures [72]. In addition to Alzheimer's disease, fractures in Parkinsonism and cerebrovascular disease were also reduced by the administration of vitamin K2 [73]. Therefore, it might be tentatively concluded that vitamin K has some beneficial effect on bone health probably through reduction of ucOC.

Vitamin K intake has large demographic differences [37]. Elderly people [74] living in institutions [75] showed a higher susceptibility to vitamin K deficiency. These subjects may be good candidates to evaluate the anti-fracture efficacy of vitamin K [76]. Children, who have very high bone turnover...
rates with relative insufficient vitamin K, are other candidates to assess vitamin K benefits on bone. Although serum ucOC concentration in puberty, where bone turnover is increased, is much higher than in adults, a clear association is observed between vitamin K status and serum ucOC concentration in puberty [77]. Van Summeren et al. reported an improvement of vitamin K status over 2 years that was associated with a marked increase in total body BMC in peri-pubertal children [29,78].

3.2.4. ucOC and bone in warfarin users.

Since many epidemiological studies indicated that vitamin K deficiency in bone was connected to the incidence of bone fracture in humans, it is reasonable to expect that patients treated with warfarin might have a higher susceptibility to bone fractures. However, divergent results have been reported regarding warfarin use and low BMD or fracture risk. Binkley et al. reported long-term warfarin treatment altered neither serum bone turnover markers, urinary excretion of calcium nor BMD despite an increase in ucOC in male rhesus monkeys [79]. Jamal et al. first reported the effects of warfarin treatment on bone health in 149 warfarin users versus 6052 non-users. There was neither a significant difference in BMD at the hip or heel, nor was there a difference in the fracture incidence between patients with and without warfarin treatment [80]. Another two studies also reported negative results in fracture risk with warfarin treatment [81,82]. On the other hand, Caraballo et al. reported a significant increase in fracture risk of vertebrae and ribs in long-term warfarin treated patients in a time-dependent manner [83]. Two other studies also reported a positive association [84,85]. These studies used a nationwide study design suggesting that a case—control study between warfarin users and non-users needs a huge number of subjects because of the presence of numerous confounding factors. Generally, warfarin users may have lower physical activity, frailty and existing comorbidities, which are all additional potential risks for fractures. Therefore, the increased risk of fractures in warfarin users may be over-estimated. Recently, anti-coagulants that do not affect vitamin K metabolism have become available and it is now possible to investigate whether warfarin affects bone health or not by comparing patients treated with these new anti-coagulants such as dabigatran. Past research suggested that the bone status of warfarin users should be carefully assessed and patients should be guided as to the proper dietary interventions concerning calcium and vitamin D to maintain bone health [86]. A recent large-scale

<table>
<thead>
<tr>
<th>Author</th>
<th>Y</th>
<th>Subjects</th>
<th>Reference group (no)</th>
<th>Test group (no)</th>
<th>Observation</th>
<th>BTM</th>
<th>BMD</th>
<th>Fracture</th>
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<tbody>
<tr>
<td>Shiraki</td>
<td>2000</td>
<td>PMO</td>
<td>Ca (121)</td>
<td>K₂ 45 mg (120)</td>
<td>2 y</td>
<td>No DIF in DPD. Decrease in ucOC.</td>
<td>Sustained</td>
<td>RR 0.44</td>
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<td>Braam</td>
<td>2003</td>
<td>PMW</td>
<td>D + Ca (124)</td>
<td>K₁ 1 mg (133)</td>
<td>3 y</td>
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<tr>
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<td>PMW</td>
<td>No (124)</td>
<td>K₂ 45 mg (133)</td>
<td>3 y</td>
<td>Increased ucOC. Decreased ucOC in K groups. Decreased PTH in D groups</td>
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<tr>
<td>Bolton-Smith</td>
<td>2007</td>
<td>PMW</td>
<td>Placebo (61) D + Ca (62)</td>
<td>K₂ (60) K₁ + Ca D (61)</td>
<td>2 y</td>
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<tr>
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<td>Osteopenia</td>
<td>Placebo (202) K₁ 5 mg (198)</td>
<td>2 y</td>
<td>No DIF in CTX. Decrease in % ucOC</td>
<td>No DIF in BMDs at any sites</td>
<td>RR 0.45</td>
<td></td>
</tr>
<tr>
<td>Booth</td>
<td>2008</td>
<td>Men and PMW</td>
<td>D + Ca (223) K₁ 500 µg (229)</td>
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<td>Decrease in %ucOC. No DIF in OC, NTX.</td>
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</tr>
<tr>
<td>Inoue</td>
<td>2009</td>
<td>OP</td>
<td>Ca (2016)</td>
<td>K₂ 45 mg (1999)</td>
<td>3 y</td>
<td>N/A</td>
<td>No DIF in BMDs, geometry or US parameters</td>
<td>N/A</td>
</tr>
<tr>
<td>Emaus</td>
<td>2010</td>
<td>PMW</td>
<td>Placebo (153) MK-7360 µg (153)</td>
<td>1 y</td>
<td>ucOC decreased, cOC increased. N-mid OC decreased</td>
<td>No DIF at any sites of bone</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>


The selection criteria of the literature are as follows.
1) Subjects: Healthy volunteers or postmenopausal osteoporosis patients.
2) Method of intervention: Randomized placebo or no treatment controlled prospective study.
3) Number of subjects: Over 50 participants per group.
4) End points: Bone markers, BMD or incident fractures.

Table 4

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</table>
Table 5
Summary of the clinical investigations on the relationship between vitamin K or osteocalcin and glucose or fat metabolism. The literature demonstrated the relationship between vitamin K or osteocalcin and glucose or fat metabolism in human study, were summarized in the table. A total of 30 references were cited and the design of the studies were mostly consisted of cross-sectional study. Some of them were intervention study using vitamin K administration.

<table>
<thead>
<tr>
<th>Author (ref. No.)</th>
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<th>Method</th>
<th>Subjects</th>
<th>Results</th>
</tr>
</thead>
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<td>Intervention using MK-4 (90 mg/day) for 1 week on glucose tolerance test</td>
<td>Healthy male divided by the serum level of DCP</td>
<td>IRI response at glucose load improved after MK-4 treatment in the high baseline DCP group.</td>
</tr>
<tr>
<td>Im [101]</td>
<td>2008</td>
<td>Cross-sectional study</td>
<td>PMW (339) Quartile analysis using t-OC level</td>
<td>t-OC correlated with plasma glucose, HbA1c and insulin resistance</td>
</tr>
<tr>
<td>Yoshida [102]</td>
<td>2008</td>
<td>Cross-sectional study (Framingham Offspring Cohort)</td>
<td>1247 men and 1472 women</td>
<td>Higher vitamin K1 intake was associated with greater insulin sensitivity and glycemic status.</td>
</tr>
<tr>
<td>Yoshida [103]</td>
<td>2008</td>
<td>Intervention using vitamin K1 500 µg/day versus control in elderly non-diabetic subjects. End point was HOMA-IR.</td>
<td>95 men and 134 women received vitamin K, and 90 men and 133 women were given no supplementation.</td>
<td>Vitamin K1 improved HOMA-IR in men at 36 months, but not in women.</td>
</tr>
<tr>
<td>Kindblom [104]</td>
<td>2009</td>
<td>Cross-sectional study (MrOS Sweden study)</td>
<td>Non-DM (857) T2DM [153]</td>
<td>t-OC related with BMI, fat mass, FPG.</td>
</tr>
<tr>
<td>Shea [105]</td>
<td>2009</td>
<td>Cross-sectional and longitudinal (3-year observation)</td>
<td>Non-DM (348 men &amp; women)</td>
<td>ucOC not associated with HOMA-IR. cOC and tOC were associated with lower insulin resistance. Baseline cOC was associated with a 3-year change in HOMA-IR.</td>
</tr>
<tr>
<td>Hwang [106]</td>
<td>2009</td>
<td>Cross-sectional</td>
<td>199 men (mean age, 47 years)</td>
<td>Vitamin K intake and CHD incidence. Dietary intake of Vitamin K2 was associated with CHD.</td>
</tr>
<tr>
<td>Pittas [107]</td>
<td>2009</td>
<td>Cross-sectional and longitudinal (3 years observation)</td>
<td>199 men and 246 women (mean age, 71 years)</td>
<td>tOC was associated with FPG, fasting insulin, insulin resistance, hCRP, IL-6, BMI and body fat. NTX showed no relationship.</td>
</tr>
<tr>
<td>Kanazawa [108]</td>
<td>2009</td>
<td>Cross-sectional</td>
<td>179 men and 149 women with or without T2DM</td>
<td>tOC was associated with FPG, HbA1c, %Fat, baPWV, IMT and adiponectin.</td>
</tr>
<tr>
<td>Kanazawa [109]</td>
<td>2009</td>
<td>Intervention on glycemic control</td>
<td>ucOC/tOC were measured in 50 poorly controlled T2DM before and after glycemic control.</td>
<td>tOC was increased and ucOC/tOC ratio was decreased after glycemic control.</td>
</tr>
<tr>
<td>Zhou [110]</td>
<td>2009</td>
<td>Cross-sectional</td>
<td>254 men and 246 women with or without T2DM</td>
<td>tOC correlated with glucose and fat metabolic parameters. Cross-sectional: tOC positively correlated with insulin sensitivity. Intervention study: Diet + Exercise group showed increase in tOC with improvement in fat metabolism.</td>
</tr>
<tr>
<td>Fernández-Real [111]</td>
<td>2009</td>
<td>Cross-sectional and a dietary and exercise intervention study</td>
<td>149 men for cross-sectional study. In the intervention study, control (n = 7), diet induced weight loss (n = 8) and weight loss by diet + exercise (n = 11) in obese subjects</td>
<td>Low dietary intake of PK1 is associated with hyperglycemia.</td>
</tr>
<tr>
<td>Pan [112]</td>
<td>2009</td>
<td>Cross-sectional</td>
<td>5800 participants to NHANES, aged 20—45. Association study between PK1 intake and MetS.</td>
<td>Dietary intake of Vitamin K2 was associated with CHD regardless of race. ucOC/tOC ratio was associated with HOMA-β and adiponectin. tOC was higher in gestational diabetic women than normal glucose tolerance pregnant women. tOC was associated with the components of MetS.</td>
</tr>
<tr>
<td>Gast [113]</td>
<td>2009</td>
<td>Prospective</td>
<td>16,057 female participants in the Prospect-EPIC cohort Vitamin K intake and CHD incidence</td>
<td>Changes in ucOC concentration do not alter glucose metabolism.</td>
</tr>
<tr>
<td>Saleem [114]</td>
<td>2010</td>
<td>Cross-sectional</td>
<td>1284 black and 1209 white. Association study between tOC and MetS</td>
<td>Dietary intake of Vitamin K2 was associated with CHD regardless of race. ucOC/tOC ratio was associated with HOMA-β and adiponectin. tOC was higher in gestational diabetic women than normal glucose tolerance pregnant women. tOC was associated with the components of MetS.</td>
</tr>
<tr>
<td>Yeap [117]</td>
<td>2010</td>
<td>Cross-sectional</td>
<td>Population based cohort of men aged over 70 years (n = 2765). tOC and components of MetS</td>
<td>Changes in ucOC concentration do not alter glucose metabolism.</td>
</tr>
<tr>
<td>Kumar [118]</td>
<td>2010</td>
<td>Intervention with 1 mg of vitamin K1 for 12 months on insulin secretion and resistance.</td>
<td>End points: ucOC, insulin secretion and resistance.</td>
<td>Changes in ucOC concentration do not alter glucose metabolism.</td>
</tr>
</tbody>
</table>
Bao [119] 2011 Cross-sectional Men who underwent coronary angiography (n = 181) tOC was lower in MetS and CHD.

Kanazawa [120] 2011 Intervention on glycemic control T2DM (n = 50) End point: Changes in glycemic control and toC.

Iglesias [121] 2011 Cross-sectional 64 obese patients. End points: bone markers and glucose tolerance test tOC only was correlated with glucose tolerance, but other bone parameters were not.


Kanazawa [123] 2011 Cross-sectional 180 men and 109 PMW. End points: Changes in glycemic control and tOC change also correlated with atherosclerotic index in T2DM.

Kanazawa [124] 2011 Cross-sectional 101 PMW and 152 men with T2DM. End points: Correlation between bone markers and diabetic parameters and fat mass. tOC is associated with MetS and the components.

Tan [125] 2011 Cross-sectional 2344 men (20–69 years) tOC is associated with MetS and the components.


Schafer [127] 2011 Intervention using alendronate and PTH (n = 97) Comparative study between alendronate (n = 33) and daily PTH (n = 64). End point: Changes in ucOC, body fat and glucose metabolism. PTH treatment induced the increase in ucOC, while alendronate treatment decreased ucOC. The change in ucOC correlated with the changes in body weight, fat mass and adiponectin.

Rochefort [128] 2011 Intervention 27 pre-pubertal obese children with or without physical training. Physical training increased BMD and OC. Insulin level was lowered by training.

Hwang [129] 2012 Cross-sectional 425 subjects. tOC, glycemic status and adiponectin. tOC level is associated with improved glucose tolerance and insulin sensitivity and secretion independently from adiponectin.

Levinger [130] 2014 Exercise Intervention 11 obese men tOC and ucOC before and after exercise ucOC was increased, while glucose decreased. No change in tOC phosphorylated Akt. AS160 in muscle increased.

randomized comparative study between dabigatran and warfarin patients indicated that stroke and the risk of bleeding were reduced in the dabigatran-treated group regardless of INR (International Normalized Ratio) values [87]. Thus, it is possible that the new alternative treatments may have beneficial effects on bone health compared to warfarin treatment [87]. Warfarin use during gestation has been reported to have skeletal and central nervous system abnormalities called as warfarin embryopathy (DiSaia syndrome) [88]. The skeletal manifestation of warfarin embryopathy was nasal hypoplasia, and calcific stippling of secondary epiphyses. It is difficult to distinguish the cause of these manifestations owed to congenital malformations or to drug induced one. Inhibition of calcium binding of osteocalcin during a critical period of ossification, could explain the nasal hypoplasia and stippled calcification seen in the warfarin embryopathy [88]. However, these manifestations are quite different from the findings of osteocalcin knockout mouse [35]. Therefore, the congenital elimination of osteocalcin and the lack of carboxylated osteocalcin during development may display different manifestations.

3.2.6. Osteocalcin-insulin relationship in humans [98–129]

In 1999, Sakamoto et al. reported that a low vitamin K diet induced glucose intolerance in rats [98]. Subsequently, an intervention trial in young male volunteers using menaquinone-4 (MK-4) at a dose of 90 mg/day for 1 week was performed [99]. MK-4 increased the immunoreactive insulin (IRI) response in pre-existing vitamin K-deficient subjects [99]. Furthermore, vitamin K$_1$ or MK-4 administration in rats induced a significant reduction in fat accumulation with increased BMD [100]. No researchers followed up on these reports until the publication of Lee et al. [89]. After publishing reports about the skeleton-pancreas loop, it has been questioned whether OC levels are associated with glucose and/or fat metabolism in humans.

In a rodent model, it has been clearly shown there is a close relationship between OC or vitamin K levels and glucose and fat metabolism. In humans, a similar relationship has been partly demonstrated. In Table 5, we have summarized the literature that investigated the roles of vitamin K or OC on fat and glucose metabolism. Almost all of the investigations support the hypothesis in rodent models that ucOC secretion from bone stimulates glucose tolerance through enhanced insulin secretion and sensitivity, or fat metabolism. However, in contrast to the rodent models, cOC or tOC may also have a close relationship with fat or glucose metabolism in humans.

In a rodent model, Lee et al. [89] found that the embryonic stem cell protein (Esp) gene plays a key role in the OC-pancreas relationship. When the Esp gene was deleted in mice (Esp$^{-/-}$), the phenotype of this mouse was completely opposite to that of OC$^{-/-}$ mice. Esp$^{-/-}$ mice showed lower plasma glucose, higher insulin secretion and higher insulin content in β-cells through increasing ucOC. The authors hypothesized that osteotesticular protein tyrosine phosphatase (OST-PTP) was responsible for inactivating ucOC through γ-carboxylation [89]. However, the Esp gene does not produce a functional product in humans [130]. Therefore, the metabolism of OC in humans may differ from rodents and the role of OC on insulin or fat mass may also differ.

In many cross-sectional studies, OC was clearly correlated with glycemic control or the size of body fat mass. If the OC de-carboxylation process is responsible for improvement of glucose intolerance or of fat mass in humans, vitamin K treatment or inhibition of de-carboxylation of OC may have negative effects on glucose or fat metabolism. In the intervention studies listed in Table 4, however, the effects of using vitamin K or dietary intake of vitamin K did not provide clear results compared to the cross-sectional studies and they were also be involved in anabolic actions in multiple tissues [95]. As for transcriptional factors in this bone-pancreas loop, forkhead family transcription factor (FoxO1) and activating transcription factor 4 (AFT4) were reported to be key modulators [96,97]. These experiments using mouse models have shown that OC is a bone-derived hormone affecting glucose levels through insulin secretion and sensitivity. OC was also shown to increase energy expenditure at least in the mouse.
opposite to the rodent model. The intervention studies were carried out in the general population or in patients with diabetes or obesity regardless of vitamin K status, which may partly explain this discrepancy. A previous report [98] indicated that the beneficial effect of vitamin K2 on insulin secretion during glucose load was significant only in subjects with a vitamin K deficiency. Thus, future investigations on whether vitamin K intervention has direct effects on glucose or fat metabolism should be carried out in patients divided into groups based on their vitamin K status.

In addition to these intervention studies, the effects of warfarin treatment on glucose metabolism should also be investigated. Since warfarin does not affect bone turnover, warfarin effects are probably limited to the carboxylation process in OC. If the rodent model is applicable to humans, it is expected that warfarin treatment may have a beneficial effect on glucose metabolism because warfarin treatment markedly increases serum ucOC. However, until now, there is no available data investigating the beneficial effects of warfarin on glucose metabolism.

In the treatment of osteoporosis, Schafer et al. [126] reported the relationship between changes in ucOC and body weight among the patients treated with alendronate or 1–84 parathyroid hormone (PTH). Participants who received PTH treatment experienced a small but significant decrease in mean body weight over 12 months (–0.8 Kg, 95% CI -1.5 to –0.1 Kg, p = 0.03). Conversely, those in the alendronate group had no significant change in weight. ucOC levels in the PTH and alendronate groups demonstrated a 242% increase and 29% decrease, respectively. Therefore, changes in ucOC or tOC induced by osteoporosis treatment may induce weight change. A recent study focused on the role of ucOC on skeletal muscle, a modulator of glucose metabolism. Levinger et al. reported that exercise increased serum ucOC levels, but not tOC, concomitant with lower blood glucose and an increase in insulin sensitivity in obese men [129].

In summary, the relationship between ucOC and glucose or fat metabolism in humans is fundamentally the same as rodents. However, the molecular specificity may be different between rodents and humans because both tOC and ucOC are associated with glucose and fat metabolism in humans, while ucOC was the candidate molecule in rodents. Although vitamin K administration reduced ucOC, the increase in eOC or tOC [24] may compensate for the decrease in ucOC in humans. Thus, vitamin K intervention may not induce retardation of glucose and fat metabolism through decreasing the serum ucOC levels. The roles of vitamin K and osteocalcin on various tissues are summarized in the Fig. 3.

3.3. Undercarboxylated/uncarboxylated matrix Gla protein (ucMGP)

3.3.1. Role of vitamin K metabolism in pseudoxanthoma elasticum

A significant relationship between undercarboxylated VKDs and tissue calcification has been observed in MGP knockout mice [131]. These knockout mice suffered from spontaneous and ultimately fetal calcification of arteries and cartilage. The cause of soft-tissue calcification may result in an imbalance of both systemic and tissue-specific inhibitors and promoters of calcium precipitation in tissues such as cartilage or vessels. Several candidate factors related to soft-tissue calcification have been reported including fetuin-A (α2-Schmid-Herrmans glycoprotein inhibitor) [132], MGP, osteopontin [133], and bone morphogenetic protein-2 [134]. Proteins primarily involved in the regulation of bone calcification, such as OC or osteonectin, have also been linked to soft tissue calcification [130,135]. Among these regulators of soft-tissue calcification, MGP is a key physiological inhibitor of soft-tissue calcification. The activity of MGP as an inhibitor of tissue calcification depends on two post-translational modifications of the molecule. These include γ-carboxylation, catalyzed by endoplasmic γ-glutamyl carboxylase (GGCX), and subsequent phosphorylation at a tandemly repeated Ser-X-Glu sequence at the N-terminus [136].

Pseudoxanthoma elasticum (PXE) in humans is defined as a dystrophic and progressive mineralization of elastic fibers in cutaneous, ocular and vascular tissues. This autosomal recessive multisystem disorder derives from a loss-of-function mutation in the human ABCC6 (the large ATP-binding cassette (ABC) gene subfamily C6) gene. This mutation was found by positional cloning analysis [137]. The ABCC6 gene encodes a 1503 amino acid transmembrane protein, ABCC6 (MRP6), which uses ATP hydrolysis to drive organic anion transport across cellular membranes (efflux transporter) [138]. However, this finding raised an apparent dilemma concerning the pathogenesis of PXE [137]. Because the ABCC6 protein is mainly expressed in liver, how do mutations in the gene result in mineralization of the skin, eyes, and blood vessels?
Mineralized blood vessels develop arteriosclerosis leading to intermittent claudication and muscle cramps in the legs and arms. Hypertension, early occurrence of myocardial infarction and gastro-intestinal bleeding are the other complications of PXE. The vascular manifestations of PXE are caused by degeneration of the elastic laminae of medium-size arteries and excessive calcification. Since fragmentation of elastic fibers and tissue calcification are the most predominant manifestations of PXE, one postulates that the disease revolves around vitamin K dependent MGP. Gheduzzi et al. described that PXE patients have a lower serum concentration of MGP and a higher ratio of ucMGP [139] suggesting that modifier gene(s) or environmental factors may be involved in tissue calcification in PXE [140,141]. Since bleeding diathesis is another clinical manifestation of PXE, deficiency of vitamin K-dependent coagulation factor(s) may be attributed because the ABCc6 mutation may induce retardation of vitamin K transport to the liver. Serum levels of vitamin K₁ in PXE or PXE-like syndrome were reported to be significantly lower than controls [142]. However, vitamin K administration in the knockout animal model did not prevent vascular calcification [141,143,144], although tissue vitamin K content was increased [144]. Therefore, it was expected that the clinical manifestations of PXE might result from an ABCc6 mutation with vitamin K deficiency and also retardation of vitamin K utilization. In this context, the functional abnormalities in GGCX have been examined in a rodent model [144]. The soft-tissue calcification of Abcc6<sup>−/−</sup>/Ggcx<sup>+/−</sup> double-mutant mice was evaluated compared to Abcc<sup>−/−</sup>/Ggcx<sup>+/+</sup> mice. The Abcc<sup>−/−</sup>/Ggcx<sup>+/−</sup> mice had soft tissue calcification at a younger age than the single-mutant counterparts. Furthermore, the mineralization process was accelerated in Abcc<sup>−/−</sup>/Ggcx<sup>+/−</sup> mice when the mice were fed a diet rich in phosphate (2-fold higher than the control diet) and vitamin D₃ (22-fold higher), but low in calcium (25% of the control diet) and magnesium (18%). These findings suggest a role for both the GGCX gene as well as dietary factors in modulating the phenotypic severity of PXE caused by loss-of-function mutations in ABCc6 [144].

3.3.2. Vascular calcification and ucMGP

Vascular calcification is commonly observed in elderly people and in patients with chronic hemodialysis, and morbidity is associated with vascular accident through atherosclerosis. Although the complete pathogenesis of vascular calcification is not yet understood, multifactorial processes are certainly involved in mineral apposition. Currently, the efficacy of compounds such as bisphosphonates, phosphate binders, selective vitamin D receptor activators or calcium sensing receptor modulators [145] to prevent vascular calcification in uremic patients has been investigated with the caution of possible adverse events [146]. Vitamin K is also postulated as a candidate to prevent vascular calcification [147] because vitamin K activates MGP, which is a local potent inhibitor of calcification in vessels [148]. Circulating ucMGP is associated with vascular calcification [149] and with vitamin K nutrition [150]. Vitamin K₁ administration slowed the progression of coronary artery calcification in healthy older adults [150] and arterial calcification induced by warfarin was reversible by high vitamin K intake in rats [151]. Therefore, the fortified intake of vitamin K may be a promising approach to prevent vascular calcification. Furthermore, the measurement of ucMGP may have potential value for identifying patients at high risk for developing cardiovascular disease [152].

3.4. Gas6

Gas6 is the newest member of the family of vitamin K-dependent proteins [153]. The structure of Gas6 is similar to protein S and it functions as a growth factor-like molecule as it interacts with receptor tyrosine kinases (RTKs) of the TAM family (Tyrosine kinase receptors including TYRO3, AXL and MERTK). The main role of Gas6, protein S and the TAM receptors is the stimulation of cell proliferation and survival. In addition, the Gas6-TAM system may also play roles in inflammation, homeostasis of cultured cells, vascular diseases such as thromboembolic disease, cancer and adiposity [153].

3.4.1. Gas6 and vascular disease

Gas6 is a growth-potentiating factor of vascular smooth muscle cells (VSMCs) and one of the Gas6 receptors, Axl, was cloned from rat carotid artery after mechanical damage. Both Gas6 and Axl increased their expression in the damaged vessels [153,154,155] in order to proliferate and migrate VSMCs to recover the damaged area. Gas6<sup>−/−</sup> mice were protected against fetal thrombosis suggesting that the Gas6-Axl system promotes platelet aggregation [2]. Since both MGP and Gas6 are carboxylated within the vasculature, warfarin inhibited the activation of MGP and Gas6 and induced vascular calcification in animals [156] and in humans [157]. Dietary intake of vitamin K₂ was inversely related to severe aortic calcification [158]. Serum levels of Gas6 at admission were reported to be lower in patients with acute coronary syndrome than in controls [159]. Gas6 gene polymorphisms were significantly associated with stroke [160]. Although warfarin treatment reduced carboxylation of Gas6, there is no available data on whether ucGas6 can bind to TAM receptors.

In general, vitamin K-dependent proteins such as MGP and Gas6 are essential for the prevention of vascular calcification through γ-carboxylation of these molecules. Gas6 may enhance platelet aggregation in vessels leading to vascular embolism. Warfarin is used to prevent embolism, although it carries a greater risk of bleeding, fracture and vascular calcification through inhibition of activation of prothrombin, OC, MGP and Gas6, respectively. Warfarin inhibition of carboxylation of the Gas6 molecule may reduce hyper-aggregation of platelets. Newly developed anti-coagulant drugs, such as dabigatran, a direct thrombin inhibitor, and rivaroxaban, a direct factor-Xa inhibitor, offer alternative treatments to warfarin [87]. In fact, a randomized study showed that dabigatran was more effective at reducing stroke and bleeding than warfarin irrespective of INR (International Normalized Ratio).
controls [87]. In addition, these newly developed compounds may not develop vascular calcification because of the lack of inhibition in γ-carboxylation of MGP or Gas6. The benefits of the new treatments are therefore obvious [161].

3.4.2. Role of Gas6 on systemic inflammation and insulin resistance

Gas6 was originally found in growth-arrested fibroblasts suggesting a role in protection from apoptosis. Gas6 expression is observed in many types of cells such as immune cells, endothelial cells, vascular smooth muscle cells, and adipocytes [162–164]. In addition to its role in vascular health, circulating Gas6 protein levels are positively associated with adiposity after adjustment for age, gender and other potential confounders in adolescents [165]. Hsiao et al. simultaneously reported a strong positive association between Gas6 levels and the inflammatory marker, C-reactive protein (CRP), tumor necrosis factor-α levels and insulin resistance in obese people [165]. They utilized a polyclonal antibody for Gas6, which meant that they could not distinguish ucGas6 and γ-carboxylated Gas6. This is particularly important because adolescents may have a higher requirement for vitamin K to promote sufficient γ-carboxylation of VKD proteins.

According to the Framingham Offspring Cohort study, pro-inflammatory cytokines such as IL-6, intercellular adhesion molecule-1 (ICAM-1), and tumor necrosis factor receptor 2 (TNFR2) were negatively correlated with serum levels of vitamin K1 and vitamin K intake [166]. The percentage of ucOC was not associated with overall inflammation, but was associated with CRP [166]. The same trend between CRP and vitamin K deficiency (%ucOC) was also observed in an Asian population [167]. The effect of vitamin K on inflammation is transduced by TAM receptors, Tyro3, Axl and Mer, which also are receptors for Gas6. In fact, TAM receptor activation by Gas6 or protein S inhibited TLR (Toll-like receptor)-driven cytokine production [168]. Therefore, the relationship between vitamin K and VKD proteins may play an important role in autoimmune diseases. The Framingham cohort study strongly suggested that sufficient vitamin K intake promotes beneficial effects on the inflammation process. However, Gas6 levels showed a positive association with CRP and adiposity. Gas6 deficient mice (Gas6<sup>−/−</sup>) had significantly reduced fat mass than their WT counterparts when fed a high-fat diet [164].

The roles of vitamin K and/or VKD proteins on vascular health and inflammation need further clarification. One possible explanation for these controversies may depend on the lack of information on vitamin K sufficiency and Gas6 measurements.

3.4.3. Vitamin K and Gas6 in the nervous system

A strong relationship between the K vitamins and sphingolipids in the brain was recognized 40 years ago. Among the K vitamins, MK-4 was identified as the principal vitamin K in brain. Certain sphingolipids in the brain are highly correlated with MK-4 content suggesting that MK-4 plays an important role on sphingolipids construction. A link between sphingolipid metabolism and Alzheimer’s disease [169] and Parkinson’s disease [170] was recently reported. In addition, the discovery of Gas6 and the characterization of its signaling actions in neurons and various glial cell types through TAM receptors is expected to have a relevant effect on cognition [171]. The functions of Gas6 in various tissues are summarized in Fig. 4.

4. Interaction of vitamin K and vitamin D

Vitamins D and K are thought to be highly synergistic as they both affect production and activation of osteocalcin molecule in bone. Namely, vitamin D enhances gene expression of osteocalcin [172] and vitamin K activates osteocalcin molecule through γ-carboxylation, subsequently. In cell culture system, vitamin K addition to bone cells augmented a vitamin D induced osteocalcin production [173] and both vitamins may play an important role in extracellular mineralization [173]. Therefore, the sufficiency of both vitamins may have beneficial effects on bone health. Vitamin K deficiency induced vascular calcification through under carboxylated MGP production. On the other hand, vitamin D excess induced vascular calcification. Thus, vitamin D toxicity and vitamin K deficiency are similar in phenotype. It can be hypothesized that vitamin K deficiency may enhance vitamin D toxicity.

5. Summary of the clinical significance of vitamin K and VKD proteins

Since vitamin K is considered to be a pleiotropic nutrient, the functions of various tissues and systems are modified by changes in vitamin K bioavailability. In this review, we indicated five clinical topics, which need further elucidation, but may open new therapeutic avenues in the clinical field:

1) DCP (PIVKA II) is associated with the progression and metastasis of hepatocellular carcinoma (HCC) and the measurement of DCP is useful to monitor the recurrence of HCC. Although vitamin K administration failed to inhibit the recurrence of HCC, the role of vitamin K
nutrition on the occurrence of HCC has not yet been fully investigated.

2) OC or ucOC are markers for bone formation and vitamin K insufficiency in bone, respectively. Poor vitamin K nutrition undoubtedly deteriorates bone health. Vitamin K administration seemed to prevent incident fractures, but the data are still insufficient to prove it. Vitamin K action through SXR nuclear receptor activation needs more clarification in patients.

3) OC and ucOC are both related to insulin secretion and sensitivity as well as secretion of fat cell cytokines in humans and rodents. Although the molecular specificity between humans (tOC) and rodents (ucOC) was different, the loop between the bone, pancreas, and fat tissue may be of central interest in the near future. The biological effects of OC may extend not only to bone, but also to energy expenditure.

4) MGP is a potent inhibitor of vascular calcification. The measurement of ucMGP is useful as a surrogate marker for vascular calcification. Gas6, and its receptor complex, may be a marker for vascular occlusion in the heart and central nervous system. Whether vitamin K administration can prevent vascular disease or not is still under investigation. A larger scale prospective study will be required to reach a definitive conclusion. The role of ucGas6 is still completely unknown.

5) Numerous studies have shown that warfarin can prevent embolism. However, the studies indicated that warfarin treatment was associated with an increased risk of vascular calcification, fractures and bleeding. Recent development of anti-coagulants such as dabigatran or rivaroxaban has shown that they prevent embolism. A comparative study between warfarin and dabigatran clearly showed the benefits of dabigatran in terms of prevention of stroke and drug-induced bleeding.

6) Gas6, one of the VKD proteins, plays a role in inflammation, homeostasis of cultured cells, vascular diseases such as thromboembolic disease, cancer growth and signal transduction in the brain. However, there have been no tools to investigate the direct relationship between vitamin K nutrition and the function of Gas6 because we could not measure ucGas6.

7) Both vitamin K and D play an important role on bone mineralization and vascular calcification. It may be important to keep sufficient levels of these two vitamins regarding bone and vascular health.

Conflict of interest

Masataka Shiraki received an honorarium for a lecture from Eisai Pharmaceutical Co., which is a vitamin K vendor.

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