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Review

Implication of a novel vitamin K dependent protein, GRP/Ucma in the pathophysiological conditions associated with vascular and soft tissue calcification, osteoarthritis, inflammation, and carcinoma

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ABSTRACT

Gla-rich protein (GRP) or unique cartilage matrix-associated protein (Ucma), the newest member of vitamin K dependent proteins, carries exceptionally high number of γ-carboxyglutamic acid (Gla) residues which contributes to its outstanding capacity of binding with calcium in the extracellular environment indicating its potential role as a global calcium modulator. Recent studies demonstrated a critical function of GRP in the regulation of different pathophysiological conditions associated with vascular and soft tissue calcification including cardiovascular diseases, osteoarthritis, inflammation, and skin and breast carcinomas. These findings established an important relationship between γ-carboxylation of GRP and calcification associated disease pathology suggesting a critical role of vitamin K in the pathophysiological features of various health disorders. This review for the first time summarizes all of the updated findings related to the functional activities of GRP in the pathogenesis of several diseases associated with vascular and soft tissue mineralization, osteoarthritis, inflammation, and carcinoma. The outcome of this review will improve the understanding about the role of GRP in the pathogenesis of tissue calcification and its associated health disorders, which should in turn lead to the design of clinical interventions to improve the condition of patients associated with these health disorders.

Contents

Abbreviations: VK, vitamin K; GRP, Gla rich protein; Ucma, unique cartilage matrix associated protein; VC, vascular calcification; CVD, cardiovascular disease; CAVS, calcific aortic valve stenosis; VSMC, vascular smooth muscle cell; Runx2, Runt-related *transcription factor* 2; Msx2, Msh homeobox 1; Sox9, SRY-box 9; Osx, Osterix; BMP-2, bone morphogenetic protein-2; OPN, Osteopontin; MGP, matrix gla protein; BSP, bone sialoprotein; ON, Osteonectin; OC, osteocalcin; ANK, Ankylosis; NPPS, nucleotide pyrophosphatase; Gas6, Growth Arrest Specific 6; VIC, valvular interstitial cells; CAVD, calcified aortic valve disease; PXE, pseudoxanthomaelasticum; OA, osteoarthritis; COX2, Cyclooxygenase 2; MMP13, Matrix Metallopeptidase 13; BCP, basic calcium phosphate; PGE2, prostaglandin E2; TNFα, Tumor Necrosis Factor α; αSM-22, Smooth muscle 22; αSMA, smooth muscle actin; EV, extracellular vesicle.

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1. Introduction

Vitamin K (VK) is well known for its critical function in activating blood coagulation factors, such as factor II, VII, IX, X, Protein C, S, and Z during the process of haemostasis. The enzyme, γ-glutamyl carboxylase (GGCX) causes the activation of these coagulation factors via post-translational modification of their glutamic acid (Glu) residues into corresponding γ-carboxyglutamic acid (Gla) residues by using VK as an essential cofactor [1]. Beyond these coagulation factors, several coagulation-unrelated Vitamin K dependent proteins (VKDPs), namely osteocalcin (OC), matrix gla protein (MGP), and growth arrest specific protein 6 (Gas6) are gaining scientific attention due to their diverse physiological activities in the regulation of obesity [2], type 2 diabetes [2,3], cardiovascular disease [4], bone metabolism [5], and vascular biology [6]. Insufficient γ-carboxylated VKDPs have been suggested as sensitive biomarkers for VK insufficiency [7]. The study of these proteins may open new avenues to design clinical interventions for the benefit of health disorders.

Gla-rich protein (GRP), the most recent member of VKDPs family, was discovered in calcified cartilage of adriatic sturgeon *Acipenser naccarii* by Viegas et al. in 2008 [8]. In the same year, another work by Tagariello et al. reported the identification of a novel distal chondrocyte specific transcript named as unique cartilage matrix-associated protein (Ucma) from a human fetal growth plate cartilage cDNA library and the protein appeared to be structurally similar to GRP [9]. Although GRP/Ucma has been predominantly observed in cartilaginous tissues of mouse, rat, sturgeon; it has also been found to accumulate in skin, bone, and vascular tissues of rat and human indicating a wide pattern of tissue localisation [10]. As suggested by the name, this 10.2 kDa protein exhibits the highest density of Gla residues of any known protein, i.e. 16 Gla residues out of its 74 residues sequence in its fully γ-carboxylated form [8]. GRP has been found to be highly conserved throughout evolution exhibiting high degree of sequence identity between sturgeon and human and it carries the highly conserved features specific to all VKDPs [8]. GRP1/UCMAa, the ortholog of GRP protein has been discovered in nearly all taxonomic groups of vertebrates whereas in teleost fish genome, a paralog named as GRP2/UCMAb has been found to exist together with GRP1/UCMAa [10]. It is thought that the presence of these two paralogs of GRP in the teleost fish genome is associated with a fish-specific whole genome duplication event that has reportedly occurred in the teleost fish lineage after divergence from tetrapods around 450 million years ago. Till now no homologs of GRP have been identified in invertebrate genome. Although GRP gene was identified in most classes of vertebrates, no GRP orthologs have been identified yet in some other vertebrate classes, such as chondricthyans, holosteans, caecilians, testudines, and archosaurians. Cancela et al. reported the general structural organization of vertebrate GRP gene which is arranged into five coding exons separated by phase-1 intron sequences and it encodes a prepropeptide of nearly 135 amino acids [10]. After the removal of the transmembrane signal peptide encoded by exon 2, proGRP is cleaved by a furin-like protease into a propeptide (38–39 amino acids) and a mature peptide (67–74 amino acids), which is characterized by a dense cluster of Gla residues. In addition to the original GRP transcript, three alternatively spliced variants of GRP (F2, F3, and F4) characterized by the absence of exon 2 and/or exon 4 (coding for most of the Gla domains) have been identified in mice [11] and zebrafish [12]. F2 and F4 isoforms lack exon 2 and so remain as intracellular aggregates whose physiological function is still unknown. The GRP-F1 (complete protein) and GRP-F3 (lacks exon 4 i.e. the principal Gla domain) are the secretary forms of GRP which have been mainly found to express during chondrogenesis and chondrocyte maturation respectively.

Because of its strong potential for binding to calcium ions or crystals through Gla residues, it has been proposed that GRP/Ucma might play a significant role in the regulation of calcium availability [8]. Neacsu et al. reported the structural organization and physiological roles of GRP/Ucma by using Zebrafish as a model organism [13]. The authors found that knockdown of Ucmaa isoform caused severe growth retardation and disturbance in the development of cartilaginous structures of embryonic zebrafish morphants. Similar abnormalities were also observed in the developing zebrafish upon inhibition of γ-carboxylation of gla residues of Ucma by warfarin treatment indicating that γ-carboxylation is crucial for Ucma function. Recent studies have reported the beneficial role of this novel VKDP in the pathology of the various health disorders related with vascular and soft tissue calcification, such as cardiovascular diseases, osteoarthritis, inflammation, and cancer [14–19]. This review for the first time provides a comprehensive and updated overview of all the currently available evidences about the functional activities of GRP in the pathogenesis of various health disorders associated with vascular and soft tissue mineralization (Table 1). The possible mechanisms underlying the association between GRP signaling and the pathogenesis of vascular and soft tissue calcification, cardiovascular diseases, osteoarthritis, inflammation, and cancer have also been discussed in this review (Fig. 1).

2. Role of GRP in vascular and soft tissue calcification

Vascular calcification (VC), an actively controlled multifactorial process, is often considered as a major cause of cardiovascular disease (CVD) worldwide [20]. VC has a frequent occurrence in the general population with advanced age and particularly in patients with atherosclerosis, diabetes, and chronic kidney disease [21,22]. Clinically VC is

Table 1
Role of GRP/Ucma in the pathophysiological conditions associated with vascular and soft tissue calcification, osteoarthritis, inflammation, and carcinoma.

Pathologies	Affected sites	Role of GRP	Ref
Calcified aortic valve disease	Valvular interstitial cells	Supplementation with cGRP resulted in dose-dependent inhibition of aortic calcification	[14]
Pseudoxanthomaelasticum/dermatomyositis	Skin tissues	GRP was highly accumulated at the sites of abnormal mineral deposition	[15]
Osteoarthritis	Tangential layer of cartilage and synovial membrane	ucGRP form was predominantly expressed in osteoarthritic cartilage matrix compared to cGRP	[16]
	Chondrocytes and synoviocyte	Addition of cGRP significantly reduced mineral deposition occurred during OA pathology	[17]
	Cartilage tissues	Ucma was overexpressed in mouse and human osteoarthritic cartilage tissues. Ucma promoted osteoclastogenesis and induced subchondral bone turnover during osteoarthritis. It protected the cartilage from proteolytic cleavage of aggrecan via inhibiting the aggrecanase activity.	[78]
Inflammation	Osteoarthritic chondrocytes and synoviocyte	cGRP/ucGRP progressively decreased the expression level of inflammatory cytokines	[17]
	THP1 monocytes/macrophages	Treatment with GRP resulted in release of decreased level of inflammatory cytokines independently of its γ-carboxylation status	[18]
Carcinoma	Skin and mammary gland tissues	ucGRP was found to be predominant form at the site of microcalcification of tumorous tissues with a decreased level of cGRP	[19]

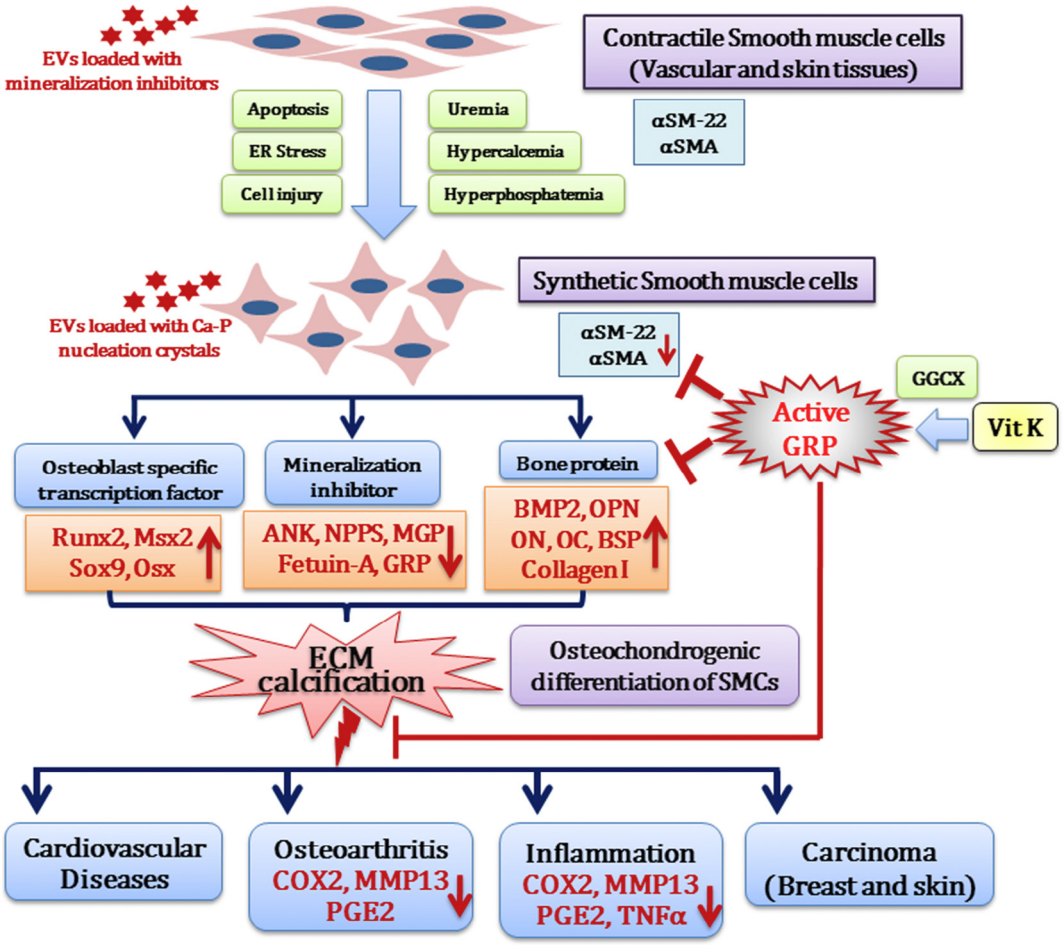

Fig. 1. Schematic representation of regulatory mechanism underlying the pathophysiological conditions associated with vascular and soft tissue calcification, cardiovascular diseases, osteoarthritis, inflammation, and carcinoma. Under physiological conditions, smooth muscle cells (SMC) maintain a contractile phenotype by the expression of contractile apparatus proteins (αSM-22, αSMA) and release of EVs loaded with mineralization inhibitors such MGP, GRP, Fetuin-A, ANK and NPPS. Under calcification promoting environments (uremia, hypercalcemia, hyperphosphatemia, ER stress, vitamin K deficiency, cell injury), SMCs undergo phenotypical changes and acquire a synthetic state due to down regulation of contractile apparatus proteins. SMCs, after getting differentiated to synthetic form, start expressing several bone associated proteins (BMP2, OPN, ON, OC, BSP, Collagen I), osteoblast specific transcription factors (Runx2, Msx2, Sox9, Osx) and release EVs loaded with hydroxyapatite nucleation crystals and decreased mineralization inhibitors. This whole event ultimately results on the osteochondrogenic differentiation of SMCs leading to ECM mineralization and degradation. In presence of Vitamin K, GRP gets activated due to post translational carboxylation in Glu residues and inhibits ECM calcification by downregulating bone associated proteins, such as OPN and upregulating the expression of contractile apparatus proteins, like α-SMA and thus in turn prevents the development of other disorders associated with SMC calcification [14].

nowadays considered as an important predictor of coronary heart disease [23]. In the aortic valve, calcification gives rise to life-threatening calcific aortic valve stenosis (CAVS), which is correlated with a very high risk of cardiovascular dysfunction and is one of the foremost causes of cardiovascular disease [24,25]. Vascular medial calcification is also responsible for calcific uremic arteriolopathy, a necrotizing skin condition associated with extremely high mortality rates among dialysis patients [26]. Studies have shown that intimal calcification is positively correlated with atherosclerotic plaque burden [27] and increased risk of myocardial infarction [28,29]. Idiopathic infantile arterial calcification, a disease characterized by blood vessel calcification, fibrosis, and stenosis, often leads to premature death in affected neonates [30].

Several mechanisms have been found to be involved in the VC pathogenesis, including reprogramming and differentiation of vascular smooth muscle cells (VSMC) to an osteoblast like phenotype and deposition of calcifying extracellular vesicles (EVs) released from VSMCs in the vessel walls [31]. The mechanism of VC has a strong similarity to that of physiological bio-mineralization occurred during skeletal development [32]. Like in osteogenesis, calcifying EVs are released from VSMCs and deposited in both medial and intimal layers during calcification initiation in vascular tissue [32]. Most importantly, under calcified conditions blood vessels have been found to express the major osteoblast transcription factors, such as Runt-related *transcription factor* 2 (Runx2), Msh homeobox 1 (Msx2), SRY-box 9 (Sox9), Osterix (Osx), and several bone matrix proteins, including bone morphogenetic protein-2 (BMP-2), Osteopontin (OPN), MGP, Fetuin-A, Bone sialoprotein (BSP), Osteonectin (ON), collagen I, and OC [33–38]. Several in vivo and in vitro studies have shown that blood vessels normally express inhibitors of mineralization, such as Ankylosis, a multipass transmembrane protein transporter (ANK), nucleotide pyrophosphatase (NPPS), MGP, or fetuin-A, and the absence of these molecules lead to spontaneous calcification and increased mortality [6,30]. This overall process has also been facilitated by increased SMC oxidant and/or endoplasmic reticulum stress, DNA damage response signaling, apoptosis, and disruption of calcium-phosphate homeostasis [39].

Effect of vitamin K on vascular calcification is widely established and several factors, such as inadequate intake of vitamin K, mutations in the γ-carboxylase enzyme, and warfarin treatment leading to reduced or abolished γ-carboxylation of VKDP, have been shown to induce arterial calcifications [40–43]. VKDP such as MGP, Gas6, and OC have been found to play an important role in the inhibition of VC [44,45]. Among them, MGP is considered as the most important player in the prevention of soft tissue and vascular calcification to date [46]. Recently some

studies reported the potential role of GRP in regulation of soft tissue and vascular calcification [14,15]. Studies reported that both the carboxylated GRP (cGRP) and undercarboxylated GRP (ucGRP) forms of GRP possess Ca/P mineral binding affinity and are present in healthy connective tissues, but an increased accumulation of ucGRP is associated with different pathological conditions, such as cancer and osteoarthritis [16,19]. Viegas et al. [14] reported that both cGRP and ucGRP accumulate at the site of osteogenic deposits, but ucGRP is predominantly found in calcified valvular interstitial cells (VIC) during the pathogenesis of calcified aortic valve disease (CAVD). More importantly, cGRP has been shown to inhibit VSMC calcification at the same extent as cMGP and fetuin-A in the ex vivo model of human vessel culture. Using the ex vivo model of aortic calcification, the authors observed that cGRP inhibits the calcification and osteochondrogenic differentiation via upregulating αSMA (α-smooth muscle actin) expression and downregulating OPN expression. Although a reduction in calcification was observed to some extent with ucGRP supplementation, supplementation with cGRP showed a clear dose-dependent inhibition of calcification suggesting that γ-carboxylation is essential for GRP mediated calcification inhibitory function. Moreover, this study also revealed that EVs released from normal VSMCs are enriched with GRP, MGP, and fetuin-A, whereas under calcifying conditions, these EVs carry higher amount of calcium and lower levels of GRP and MGP. Interestingly, during this study GRP was found to be part of an MGP-fetuin-A protein complex acting at the sites of valvular calcification. Altogether, these findings represent cGRP as an effective and potential inhibitor of vascular mineralization.

Viegas et al. also reported the expression and accumulation of GRP in soft tissues of rat and human, including skin and vascular system [15]. This study showed that GRP is evidently associated with calcification pathologies in both pseudoxanthomaelasticum (PXE) and dermatomyositis patients, being highly accumulated at the sites of abnormal mineral deposition suggesting that GRP may be an important player in modulating ectopic mineralization. Recently, one study has addressed the association of GRP with microcalcifications found in human skin and breast carcinomas [19], which implies that cGRP might be used as a possible marker for ectopic calcification due to its potential for inhibition of microcalcification occurrence in skin and breast carcinomas. Furthermore, GRP was also shown to be involved in the crosstalk between inflammation and calcification of articular tissues during osteoarthritis pathology [17].

3. Role of GRP in osteoarthritis pathology

Osteoarthritis (OA), the most frequent form of chronic joint disease worldwide, reportedly affects an estimated 10% of men and 18% of women over 60 years of age [47]. OA is primarily characterized by destruction and loss of articular cartilage of the joints. It can't be considered as a single disease but rather as a condition with a multifaceted aetiology that leads to a cascade of various events occurring within the whole joint resulting with progressive degeneration and functional failure [48,49]. The risk of developing OA is reported to increase substantially after the age of 45 years [50]. Commonly, OA develops in weight bearing joints such as the hips, knees, and ankle, but it can also occur in any synovial joint of the body, individually or simultaneously, with variable degree of intensities [51]. Among all the reported upper and lower extremity sites, medial compartment of the knee has been found to be most commonly affected in osteoarthritis [52]. This disease is clinically characterized by joint pain, tenderness, crepitus, stiffness, significant disability, and unpredictable levels of local inflammation [53,54]. Although the disease can be dependent on various genetic and epigenetic factors, sex, ethnicity and age, it has been also found to be associated with obesity, unhealthy lifestyle, sport injuries, and dietary factors [55–58]. The interplay among the various risk factors over time ultimately leads to the weakening of the articular cartilage and causes disturbances in the underlying bone and soft tissues of the joint and its surrounding muscles [51,53,59–61].

Due to the limited efficacy and various side effects of the currently available treatments of OA pathology, there is an urgent need to identify novel therapeutic and prophylactic agents for treatment and prevention. Since VK and VKDPs are widely known to play an important role in the regulation of bone and cartilage mineralization, the functionally active carboxylated gla proteins may be a potential target of interest in this regard. Various abnormalities, such as inappropriate or excessive mineralization of cartilage, hypertrophic chondrocytes, apoptotic chondrocytes, and endochondral ossification have been found to occur in absence of the functional forms of bone and cartilage Gla proteins [6,62]. Further, it has been also addressed that chondrocytes from human osteoarthritic joints produce less cMGP compared with chondrocytes from normal cartilage, suggesting that osteoarthritis may be associated with nonfunctional MGP [63]. Several studies have shown that insufficient intake of VK, over long periods of time, is a risk factor for development of a wide range of diseases, including OA, VC, and CVD, and even some types of cancer [40,64–71]. It has been reported that OA also results from the failure of chondrocytes to maintain the proper balance between synthesis and degradation of extracellular matrix (ECM) components, which in turn leads to the occurrence of ectopic calcification [16]. Various studies have showed that OA is directly associated with deposition of basic calcium phosphate (BCP) crystals in the OA affected tissues whether it be articular cartilage, synovial fluid, or synovial membrane [72–76].

Some studies have been performed to investigate the probable role of GRP in OA pathology. Gene expression studies have detected GRP as a novel gene primarily expressed in all cartilaginous tissues in sturgeon, human, and mouse [8,9,77,78]. However, Viegas et al. also discovered the presence of GRP transcripts in other type of tissues such as bone tissue, skin tissue and vasculature of rat and human inferring that expression of GRP is not always cartilage-specific [15]. Jeune et al. reported the existence of four alternatively spliced variants of GRP gene transcripts in mouse chondrocytes and also showed that the expression of all four isoforms is associated with the early stages of chondrogenesis [11]. They also addressed that although the four transcripts are co-expressed during chondrogenesis, the kinetics of their expression were observed to be different from each other, suggesting that the occurrence of each isoform is finely regulated during the process of chondrogenesis and chondrocyte differentiation. It is therefore assumed that imbalance in the expression pattern of GRP isoforms may contribute to skeletal pathology. By comparative analysis of control and OA associated tissues, Rafael et al. unveiled two novel splice variants, namely GRP-F5 and GRP-F6 in human along with already reported GRP-F1 transcript [16]. While GRP-F1 transcript occurred to be the most predominant splice variant during OA pathology, GRP-F5 and F6 were found to be mostly restricted to human fetal development. They reported that during OA pathology, both cGRP and ucGRP co-localise at the sites of ectopic calcification in the tangential layer of osteoarthritic cartilage and synovial membrane. Although both protein forms of GRP were found to be accumulated in osteoarthritic chondrocytes, ucGRP form was the predominant compared to cGRP in osteoarthritic cartilage matrix. On the other hand, in healthy subjects, there was no sign of mineral deposition in those types of tissues, and, moreover, accumulation of only cGRP form was found in their ECM and lining cells. Altogether, these results suggest that γ-carboxylation of GRP should be crucial for inhibition of calcium mineral formation occurred during ectopic calcification in OA. This study established a relationship between GRP γ-carboxylation deficiency and OA, suggesting that vitamin K metabolism may be associated with pathophysiological features of OA including cartilage mineralization and synovial membrane inflammation.

Cavaco et al. further demonstrated the association of GRP with two major processes of OA pathology, like mineralization and inflammation of articular tissue [17]. In their study, the authors found that the addition of cGRP significantly reduced the mineral deposition in both control or OA-derived chondrocytes and synoviocyte cell system, while treatment with ucGRP showed to have no role in the reduction of calcification.

Because tissue mineralization is known to trigger the subsequent inflammatory responses, the possible relationship between GRP and inflammation was further investigated under mineralizing condition. Results showed that GRP progressively decreased the expression level of inflammatory markers, such as cyclooxygenase2 (COX2) and matrix metallopeptidase13 (MMP13) in OA-derived chondrocytes and synoviocytes cell system. Moreover, treatment with GRP coated BCP crystals downregulated the expression levels of inflammatory cytokines in calcification-induced OA pathology independently of the carboxylation status of GRP. This study suggests that GRP can directly affect the OA-associated inflammation regardless of its γ-carboxylation status. Overall, these studies strengthen the calcification inhibitory function of GRP and also highlight the role of GRP as a novel anti-inflammatory agent in OA pathophysiology.

Very recently Stock et al. reported the critical role of GRP/Ucma in OA pathophysiology [78]. It has been observed that Ucma was overexpressed in mouse and human osteoarthritic cartilage tissues compared to control. Moreover, *Ucma*-deficient mice were found to be more prone to OA-triggered cartilage damage and chondrocytic death than wild type littermates and *Ucma*-deficiency led to the reduction of osteophyte formation and subchondral bone sclerosis. Ucma was also shown to promote osteoclastogenesis and to induce subchondral bone turnover during OA. Results further demonstrated that Ucma protected the cartilage from proteolytic cleavage of aggrecan via inhibiting the ADAMTS-dependent aggrecanase activity. Altogether, these significant findings suggested the protective role of Ucma in the cartilaginous tissues against the development of OA.

4. Role of GRP in inflammation

VK is known to play a significant role in the regulation of immune and inflammatory mechanisms. VK deficiency is associated with the inflammatory pathophysiology of various chronic metabolic disorders, including T2D, CVD, and osteoporosis [79,80]. Various studies depicted a beneficial role of VK supplementation in reducing the secretion of pro-inflammatory cytokines [81–83]. Recently, GRP is gaining attention as an important member of VKDPs involved in the regulation of various inflammatory events [17,18]. Cavaco et al. addressed the probable role of GRP during calcification induced inflammation in OA derived cell system [17]. In this study, the authors examined the anti-inflammatory effect of GRP as well as its γ-carboxylation status using OA derived chondrocytes and synoviocytes cell system. Results showed that there was an increased expression of COX2 and MMP13 in OA derived cells indicating the role of calcification stimulus in inflammatory responses. The authors further studied the possible relation between GRP and inflammation by inducing chondrocytes and synoviocytes with an inflammatory stimulus and evaluating GRP expression pattern. An increased expression of GRP gene simultaneously with an increase in COX2 and MMP13 expression was observed after interleukin-1β (IL-1β) treatment. Since BCP crystals deposition has been associated with inflammatory responses in OA, chondrocytes and synoviocytes were also treated with BCP crystals and BCP crystals coated with either cGRP/ucGRP to further confirm its anti-inflammatory response during OA pathology. Up-regulation of COX2 and MMP13 expression in BCP-treated cells confirmed the inflammatory response mediated by BCP crystals in both OA associated cell systems, while treatment of cells with BCP crystals, containing either cGRP or ucGRP, resulted in decreased COX2 and MMP13 expression. Moreover, treatment with either ucGRP/cGRP in IL-1β stimulated cells caused a decreased expression of COX2 and MMP13 relative to non-treated cells indicating an anti-inflammatory effect of GRP.

Viegas et al. further investigated the anti-inflammatory mechanism of GRP using both freshly isolated human leucocytes and THP-1 monocyte cell culture model [18]. In calcification dependent diseases, like atherosclerosis and OA, monocytes and macrophages are the key players in the disease initiation and progression. Results showed that treatment of cells with inflammatory inducers, like lipopolysaccharide (LPS) and hydroxyapatite (HA) upregulated the GRP expression. The authors also studied the effect of purified cGRP and ucGRP or BCP crystals coated with either cGRP or ucGRP in THP1 macrophage cell lines. The treatment with either cGRP or ucGRP resulted in decreased level of inflammatory markers like tumor necrosis factor-α (TNFα) or prostaglandin E2 (PGE2). In addition, the authors also performed over expression studies with GRP and found that GRP overexpression downregulated the inflammatory responses of THP1 stimulated with LPS or HA. Various studies demonstrated a potential role of VK in the regulation of vascular inflammation; however, the role of γ-carboxylated VKDP on regulating the inflammatory pathways remains unexplored, and further mechanistic studies are necessary to understand the role of GRP in inflammatory pathophysiology.

5. Role of GRP in carcinoma

Although extensive research has investigated the role of VK in haemostasis, calcification and cellular metabolism, much remains to be discovered regarding its function in promotion or suppression of tumor growth. Vitamin K1, K2, and K3 have been found to independently affect mitogen activated protein kinase (MAPK) signaling cascade in different pathways resulting with in vitro tumor suppression for colon cancer [69], leukemia [70], and melanoma [71]. Vitamin K2 has also been shown to down-regulate proto-oncogene c-MYC involved in cell cycle progression and carcinogenesis to human leukemia cell lines [84]. When administered together with cotelynin A, a synergistic effect was obtained showing a significant suppression of c-MYC gene expression [84]. Furthermore, in vitro studies with vitamin K3 and K5 have been shown to inhibit pyruvate kinase M2 (PKM2), which is the rate-limiting enzyme in glycolysis commonly expressed by tumor cells [85], suggesting a beneficial role of vitamin K3 and K5 as adjuvants after chemotherapy.

A number of studies have been conducted to date to explore the involvement of VK and VKDPs, such as PIVKA-II, MGP, Gas6, and OC in cancer biology [86–94]. These studies have demonstrated both the tumor promoting and inhibitory effects of VKDPs associated with different types of cancer. Discovery of new VK targets in cancer, such as GRP, may unravel the underlying mechanism associated with the functional role of vitamin K in the development of various types of cancer. Recently, Viegas et al. has addressed the possible function of GRP, the newest member of the VKDPs, regarding its association with microcalcifications occurred in human skin and breast carcinomas [19]. Microcalcification is known to play a crucial role in early breast cancer diagnosis, which is the second leading cause of cancer death among women [95]. Breast carcinomas with calcifications are predicted to be more aggressive and have worse prognosis than those without calcifications [96–99]. Approximately 50% of non-detectable breast cancer incidences are diagnosed by mammography exclusively through microcalcification patterns [100] revealing up to 90% of ductal carcinoma in situ [101]. Although, the association of microcalcification with breast cancer aetiology is a well-known phenomenon, the molecular mechanisms behind the formation of microcalcifications during the occurrence of breast cancer are still unknown. Recently, it has been suggested that ectopic mineralization in pathological conditions might be regulated by mechanisms similar to those occurring in normal physiological conditions during skeletal development [102]. The study conducted by Viegas et al. regarding the possible effect of GRP in preventing microcalcification during breast and skin carcinoma may help to better understand the process of microcalcification in cancer pathologies. They investigated the γ-carboxylation status of GRP during the incidence of microcalcification in both basal cell carcinoma and invasive ductal carcinoma. This study demonstrated the differential accumulation pattern of both cGRP and ucGRP forms in healthy tissues as well as in the tumorous tissues. It was observed that both the cGRP and ucGRP are co-localized in healthy skin and mammary gland tissues indicating an incomplete status of GRP γ-carboxylation under normal

physiological conditions. On the contrary, ucGRP was found to be the predominant form at the site of microcalcification of tumorous tissues with a decreased level of cGRP. These findings imply GRP as a potential target for the anticancer potential of VK, and the cGRP might be used as a possible marker for ectopic calcification occurrence in carcinoma.

6. Conclusion

The information presented in this review represents GRP as a critical player in the regulation of various pathophysiological conditions associated with vascular and ectopic calcification, such as cardiovascular diseases, osteoarthritis, inflammation, and breast and skin carcinoma. Results demonstrated that cGRP acts as a potential inhibitor of VSMC calcification and osteochondrogenic differentiation. GRP has also been proposed to be a potent modulator of soft tissue mineralization due to its high accumulation at the sites of abnormal calcification of human skin tissue in both PXE and dermatomyositis patients. In addition, ucGRP form was also found to be predominantly expressed in the calcified tissues associated with osteoarthritis as well as breast and skin carcinoma; however, among healthy subjects only cGRP was detected in ECM and lining cells. The relationship between GRP and inflammation in calcification related pathologies also indicates the anti-inflammatory role of GRP. Altogether, these findings established a relationship between GRP γ-carboxylation deficiency and calcification related disease pathology, which suggests an important role of VK metabolism associated with the pathophysiological features of various health disorders.

Many questions concerning the molecular mechanisms underlying the action of GRP remains unclear. The outcome of this review will increase the understanding regarding the role of GRP in the pathogenesis of vascular and soft tissue calcification, cardiovascular diseases, osteoarthritis, inflammation, and cancer. Further long term research and clinic trials are warranted to elucidate and support the molecular mechanisms underlying the role of GRP in calcification associated disease pathology and to establish its role as a biomarker.

Conflict of interest

The authors have declared that no conflict of interest exists.

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