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Kartogenin and Its Application in Regenerative Medicine*

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Summary: Regenerative medicine refers to the possibility of replacing aged/damaged cells with genetically similar young and functional cells to restore or establish normal function. Kartogenin (KGN), a small heterocyclic, drug-like compound was discovered in 2012, which is strongly associated with regenerative medicine. KGN has been applied in many regenerative fields, including cartilage regeneration and protection, tendon-bone healing, wound healing, and limb development. KGN could facilitate cartilage repair, promote formation of cartilage-like transition zone in tendon-bone junctions, stimulate collagen synthesis for wound healing, and regulate limb development in a coordinated manner. Considering the related mechanism, filamin A/CBFβ/RUNX1, Ihh, and TGFβ/Smad pathways have been reported to involve KGN. Therefore, KGN is proven a promising agent in regenerative medicine; however, studies conducted on the effect of KGN are limited to date and not convictive for long-term use. Further studies are recommended to explore the long-term effect and potential molecular mechanisms of KGN. Our investigations may motivate researchers to expand its applications in different forms and fields.
Key words: kartogenin; regenerative medicine; mechanism

Regenerative medicine can be defined as the possibility of replacing aged/damaged cells with genetically similar young and functional cells to restore or establish normal function[1, 2]. Although the efficacy and safety of regenerative medicine are still controversial, it has nevertheless begun to define a new perspective of contemporary science and future clinical practice[3].

Kartogenin (KGN), a small heterocyclic, drug-like compound is strongly associated with regenerative medicine. Hayek *et al*[4] named it "a game-changer in regenerative medicine" for its ability to stimulate endogenous somatic stem cells for cartilage regeneration. KGN was discovered in 2012 by Johnson *et al*[5]. They studied primary human bone marrow mesenchymal stem cells (MSCs) in an image-based high-throughput screening, assaying for chondrogenic nodule formation, and finally identified KGN from 22 000 drug-like molecules. Since then, a number of studies have reported its effective results associated with tissue repair or regeneration[6–8]. Herein, the aim of this review is to summarize the current application of KGN in regenerative medicine and describe its related mechanisms.

1 CURRENT APPLICATIONS

1.1 Cartilage Regeneration and Protection

Based on the first study of KGN reported by Johnson[5], KGN could promote chondrogenic differentiation of human MSCs (hMSCs) by forming cartilage nodules *in vitro*. The cartilage nodules contain proteoglycans and collagen Ⅱ, which are specific matrix components for hyaline cartilage. Interestingly, after a 21-day pellet culture of hMSCs, matrix metalloproteinase (MMP) activity, which is known to cause matrix degradation, does not increase. Instead, the protective tissue inhibitor level of MMP increases, implying the chondroprotective function of KGN. Moreover, the effect of KGN was evaluated in two animal models of surgery and non-surgery-induced

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osteoarthritis (OA). Compared with the control group, the results showed that intra-articular injection of KGN could facilitate cartilage repair. KGN has been regarded as both chondrogenic and chondroprotective agent and has been widely studied in the field of biological therapy for OA in recent years. Mohan *et al*[9] evaluated the effect of KGN on both cartilage and subchondral bone in a rat model of OA using multimodal imaging techniques, including magnetic resonance imaging and micro-computed tomography. They found that KGN could prevent not only cartilage degeneration, but also subchondral bone changes of OA. Besides, several innovative KGN release systems have been made by researchers to prolong the time of drug action. Kang *et al*[7] developed an intra-articular drug delivery system, consisting of KGN-conjugated chitosan (CHI-KGN). The release study *in vitro* proved the sustained and continuous release of KGN from CHI-KGN nano/microparticles. The CHI-KGN nano/microparticles were also applied by intra-articular injection in a rat model of OA. Lesser degenerative changes in the cartilage were observed in the experimental group than the untreated control group or rats treated with unconjugated KGN. Moreover, KGN is suitable not only for the treatment of OA, but also for the treatment of cartilage defect repair. Liu *et al*[10] applied the hydrogel incorporating KGN, BMSCs and chondrocytes (Gel/Cell/KGN) to a rat cartilage defect model and found that the International Cartilage Repair Society histological scores in the Gel/Cell/KGN group was higher than that in the Gel/Cell group at day 35 after surgery. Shi *et al*[11] used an ultraviolet-reactive, rapid cross-linkable scaffold integrated with KGN-loaded nanoparticles in the full-thickness cartilage defects of a rabbit model. The nanoparticles were injected into the defect site followed by ultraviolet (UV) irradiation to form a scaffold. The scaffold integrated with KGN also showed an excellent effect on cartilage regeneration. This study demonstrated the effect of KGN for cell homing, particularly for recruiting the host's endogenous cells without cell transplantation.

1.2 Tendon-bone Healing

Tendon-bone insertions can be divided into two types, indirect and direct insertions. The transition zone in the direct insertion site consists of four unique structures made of tendon, unmineralized fibrocartilage, mineralized fibrocartilage, and bone. The fibrocartilage structure can absorb shock and transfer stress between the tendon and bone[12]. However, once the tendon-bone junctions are injured, restoration of the native structures is difficult, often resulting in scar healing without or with little fibrocartilage formation[13, 14]. Therefore, researchers have sought new approaches to improve tendon-bone healing quality. Zhang *et al*[15] reported the effect of KGN on tendon-bone healing. *In vitro*, KGN could improve the proliferation and chondrogenic differentiation of rabbit bone MSCs and patellar tendon stem/progenitor cells (PTSCs). *In vivo*, KGN injection enhanced the healing process and quality of the wounded Achilles tendon-bone junction, which was proven by the immunostaining and immunohistochemical analyses for the formation of extensive cartilage-like tissues. Furthermore, considering injecting KGN alone into the bone tunnel might lead to diffusion and adverse effects, such as non-tendinous tissue formation in an otherwise healthy tendon tissue, Zhou *et al*[16] used platelet-rich plasma (PRP) gel as a carrier for KGN. The KGN-PRP gel was injected into a rat tendon graft-bone tunnel model. Histological evaluation and immunohistochemical analyses revealed better formation of cartilage-like transition zone in the KGN-PRP group than that in the PRP and saline control groups. Besides, the mechanical test showed that the pullout strength in the KGN-PRP group was significantly higher than that in the PRP (1.4 fold) and saline control (1.6 fold) groups. Wang *et al*[17] injected KGN beads into a murine model of rotator cuff repair and found that KGN could promote the formation and organization of collagen fibrils but not facilitate cartilage-like tissue formation at the healing enthesis. Accordingly, KGN combined with PRP gel may be a more effective bio-agent to promote tendon-bone healing *in vivo*.

1.3 Wound Healing

Wound healing is a complex process associated with many different tissues and cell lineages[18]. The primary goals of wound management are to achieve rapid wound closure with an aesthetically and functionally satisfactory scar[19]. Collagen-rich granulation bed tissue, manufactured by fibroblasts is noted to be important for wound closure[20]. Recently, biologic and synthetic collagen-embedded wound dressings have been commonly used as skin substitutes[21]. Wang *et al*[22] conducted a study to investigate if KGN could be applied to stimulate the collagen synthesis of fibroblasts for wound treatment. They found that KGN stimulated collagen synthesis of fibroblasts, but did not induce the expression of α-SMA, MMP1, and MMP9 *in vitro*. In addition, KGN exhibited no obvious cytotoxicity on the fibroblasts' viability, morphology, and survival. Moreover, an *in vivo* experiment proved that KGN could remarkably increase the collagen content in the skin and promote wound healing of the skin in mice. Compared with the control group, the wound healing time was significantly shortened (12.8±2.3 days *vs.* 16.4±3.4 days) in the group treated with KGN. Accordingly, the authors suggested that KGN might be widely used in wound healing or for aesthetic and reconstructive purposes of rejuvenation.

1.4 Limb Development

Limb development requires the coordinated

growth of many different tissue types, including bones, cartilage, joints, tendons, and ligaments[23]. Joint formation has played an important role in the limb development of vertebrate animals, leading to successful adaptation of animal limbs to a variety of ecological niches[24]. The first indication of joint formation is interzone formation, where highly condensed and flat mesenchymal cells emerge[25]. The interzone consists of three layers, a dense intermediate cell layer and two outer cell layers. The intermediate layer of the interzone is closely associated with the development of articular chondrocytes, whereas the outer layers are responsible for lengthening of the long bone anlagen by appositional growth[26]. Decker *et al*[8] conducted a study to test whether KGN could regulate the processes of limb development. They found that KGN could not only induce chondrogenesis in committed preskeletal mesenchymal cells in micromass culture, but also greatly facilitate overall limb growth, phalangeal elongation, and interdigit mesenchymal invagination of the limb buds in the explant culture system. Moreover, the expression of transcription factor scleraxis (*Scx*) increased more significantly in the limb buds responsive to KGN, indicating that tendon development and maturation seemed to be coordinately stimulated by KGN. More importantly, KGN has been proven to advance the processes of interzone formation and cavitation during synovial joint morphogenesis. As mentioned above, several limb developmental processes, including limb skeletal growth and elongation, interdigit invagination, tendon maturation, and interzone formation and cavitation could be harmoniously stimulated by KGN, and KGN is believed to be a potent tool for limb development and regeneration.

2 THE RELATED MECHANISMS

2.1 Filamin A/CBFβ/RUNX1 Pathway

KGN induces the chondrogenic differentiation of MSCs through its connection with filamin A (FLNA), an actin-binding protein. KGN binds to the FC-1 fragment of FLNA, displacing CBFβ from its cytoplasmic binding site[5, 6]. CBFβ is thus freed to enter the nucleus, binds the RUNX factors, and regulates transcription of proteins and genes associated with chondrogenesis via a CBFβ-RUNX1 transcription program[4, 27]. Meanwhile, RUNX2 is known to induce osteoblast differentiation and chondrocyte hypertrophy, which contributes to the pathogenesis of OA[28–30]. The CBFβ-RUNX1 transcription program may also keep RUNX2 at a relatively low level, thus maintaining the function of chondrogenesis[5].

2.2 Indian Hedgehog (Ihh) Pathway

KGN promotes the expression of *Ihh* and hedgehog target genes *Gli1* and *Ptch1* in the micromass culture of preskeletal limb bud cells[8]. Ihh is a member of the hedgehog family of secreted ligands and a key regulator of bone development, including chondrocyte proliferation, chondrocyte differentiation, and osteoblast differentiation[31]. *Ihh* acts later in the process of endochondral bone formation in limb development[32]. During endochondral bone development, *Ihh* is synthesized by chondrocytes in the growth plate. In vertebrates, when it binds to its trimeric receptor Patched-1 (*Ptch1*), Smoothened (*Smo*) inhibition is relieved. As a result, *Smo* becomes active and then translocates to the cilium, which, in turn, recruits the suppressor of fused homologue *Sufu-Gli* complex to the cilium. In this process, the active *Smo* plays a role in dissociating the bond between *Gli* and *Sufu*, thus allowing the activated *Gli2/3* to enter the nucleus and activate the expression of *Ihh* target genes, such as *Ptch1* and *Gli1* mentioned above[33, 34].

2.3 TGF-β/Smad Pathway

TGF-β/Smad pathway has been reported to be involved in numerous physiological and pathological processes, particularly in the regenerative process of many tissues and organs, including the cartilage, tendon, skin, and even limbs[35–38]. KGN could regulate the TGF-β/Smad pathway by increasing phosphorylation and subsequent activation of Smad family members. Decker *et al*[8] found that KGN showed little effect on Smad 1/5/8 phosphorylation, whereas TGF-β stimulation could enhance the phosphorylation of Smad 1/5/8 in MSCs. The receptor-activated Smads are divided into two groups, Smad2/3 and Smad1/5/8, which are activated by different molecular substances[39]. The phosphorylation of Smad1/5/8 may cause chondrocyte hypertrophy-like changes and OA pathogenesis, whereas Smad2/3p protects against chondrocyte hypertrophy[40, 41]. KGN seems to be an ideal agent for its specific action on the Smad2/3 pathway for chondrogenesis.

However, Wang *et al*[22] found that KGN could promote type- I collagen synthesis via TGF-β/Smad4/5 pathway in dermal fibroblasts. With the effect of KGN, the p-Smad5 protein is enhanced and completely translocated into the nucleus. Similarly, the Smad4 protein is also overexpressed and mostly translocated into the nucleus. The p-Smad5 and Smad4 proteins then trigger subsequent gene activation. This implies that KGN may act via different pathways, depending on the cell type.

3 CONCLUSIONS AND PERSPECTIVES

As summarized in fig. 1, KGN has been applied in many regenerative fields, including cartilage regeneration and protection, tendon-bone healing, wound healing, and limb development. The FLNA/CBFβ/RUNX1, Ihh, and TGF-β/Smad pathways have

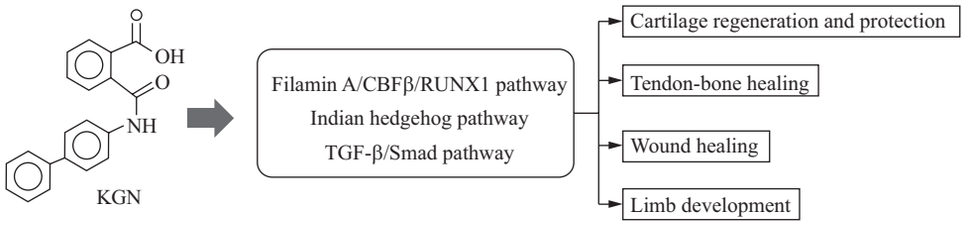

Fig. 1 Summary of the related pathways and applications of KGN

been reported to involve KGN. KGN is a promising agent in regenerative medicine; however, the overgrowth of tissues after activating endogenous stem cells with KGN should be taken into consideration. To date, studies conducted on the effect of KGN are limited and not convictive for long-term use. Further studies are recommended to explore the long-term effects and potential molecular mechanisms of KGN. Moreover, comparison of the effects that KGN exerts on different cell types and the synergistic effect of KGN with other cytokines are worth studying. In addition, combination of KGN with tissue engineering is an innovative way to maintain its sustained and continuous delivery as well as its efficacy, which may pave a new dimension for further studies. Collectively, it is reasonable to believe the excellent efficacy and safety of KGN on the regenerative tissues in the short term, and our investigations may motivate researchers to expand its applications in different forms and fields.

Conflict of Interest Statement

The authors declare that they have no competing interests.

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