天然产物多糖在骨质疏松中的应用及其分子机制研究进展

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摘要 骨质疏松症是一种全身性骨代谢疾病, 其特征是骨量的降低和骨组织微结构的破坏, 最终导致骨脆性和骨折风险增加。骨质疏松严重影响着人类的生命周期和生活质量, 并对社会造成巨大的经济负担。目前市场上的抗骨质疏松药物主要是化学合成药物, 可以较有效地改善患者骨质疏松的症状, 但长期服用会产生不良的副作用。研究发现, 某些多糖能够促进成骨细胞形成、抑制破骨细胞活性, 进而影响骨骼重塑过程, 且因其副作用较少, 更适合长期使用而受到大众青睐。该文通过对大量文献信息的整合, 介绍了近年来研究较多的与改善骨骼健康状况有关的多糖。实验表明, 多糖主要通过调节成骨细胞和破骨细胞的活性保护骨骼健康, 多条信号通路如Wnt/β-catenin信号通路、BMP/Smad信号通路和OPG/RANKL/RANK信号通路等参与调节过程。该综述对多糖抗骨质疏松的作用及其分子机制最新研究成果进行归纳和总结, 旨在为进一步推进更加安全有效的抗骨质疏松症新药物的开发提供理论依据和研究方向。

关键词 骨质疏松; 天然产物; 多糖; 成骨细胞; 破骨细胞

Application and Molecular Mechanism of Polysaccharide from Natural Product in Osteoporosis

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Abstract Osteoporosis is a disease of bone characterized by a reduction in bone mass and disruption of bone tissue microarchitecture, ultimately leading to bone fragility and increased fracture risk. Osteoporosis seriously affects the life cycle and quality of life of human beings, and causes a huge economic burden to the society. Current drugs for anti-osteoporosis are mainly synthetic drugs, which are indeed effective in preventing bone loss but with adverse side effects. It has been reported that certain polysaccharides can promote the formation of osteoblasts and inhibit the activity of osteoclasts, thereby affecting the process of bone remodeling. Since polysaccharides from natural products show fewer side effects and are more suitable for long-term use, they are favored by the public. We performed a comprehensive review of the literature to consolidate studies about polysaccharides improving bone health in recent years. *In vivo* and *in vitro* experiments have demonstrated that polysaccharides mainly protect bone health by regulating the activities of osteoblasts and osteoclasts. Moreover, multiple signaling pathways such as

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Wnt/β-catenin signaling pathway, BMP/Smad signaling pathway and OPG/RANKL/RANK signaling pathway, are involved in improving bone homeostasis. This review summarizes the latest research about the anti-osteoporosis effect and molecular mechanism of polysaccharides, aiming to provide theoretical basis and direction for searching safer and more effective anti-osteoporosis drugs.

Keywords osteoporosis; natural products; polysaccharides; osteoblasts; osteoclasts

骨质疏松是一种全身性骨代谢疾病, 其特征是骨量的降低和骨组织微结构的破坏, 最终导致骨脆性和骨折风险增加[1]。在中国, 骨质疏松症是我国排名第四位的慢性疾病。近年来, 中国人口老龄化问题日益突出。目前, 我国是世界上老年人口绝对数最大的国家。60岁以上人口超过2.1亿, 其中包括近1.4亿的65岁以上人口[2]。中国国家卫生健康委员会于2018年进行了首次骨质疏松症流行病学调查, 结果显示, 50岁以上人口的骨质疏松症发病率为19.2%, 其中, 女性患病率32.1%, 男性为6.0%。65岁以上的女性患病率更是高达51.6%[3]。骨质疏松症患者临床表现为疼痛、驼背、身高降低甚至骨折, 且骨质疏松性骨折不仅发生率高, 再发风险也较高。近几年来, 骨质疏松性骨折导致的死亡率和病残率随着患者总数的增加而显著提高, 其中有一大部分是老年人群体[4]。除了较高的患病率和死亡率外, 骨质疏松症还会给患者家庭和社会医疗保健系统带来沉重的经济负担[5]。

骨质疏松症多采用药物治疗, 有效的抗骨质疏松症药物可以极大地改善骨质疏松症患者的生活质量, 延长患者的生命周期。但目前传统的治疗骨质疏松症的药物疗效有限, 且长期服用会给身体带来一些副作用(表1)。近年来, 多糖抗骨质疏松的作用逐渐得到关注, 但由于起步较晚, 研究仍处于初级阶段。本文将从自然界的三大类多糖——高等植物多糖、微生物多糖、海洋藻类多糖中, 有针对性地选取近年来被认为在治疗骨质疏松症方面具有较大应用潜力的几种多糖, 简要概述其对骨骼健康、骨再生等方面的积极作用及其分子机制的研究进展。

1 常用抗骨质疏松症药物

目前常用的几类抗骨质疏松症药物, 根据其作用机制, 可分为骨吸收抑制剂、骨形成促进剂和其他机制类药物。包括双膦酸盐类、选择性雌激素受体调节剂类(selective estrogen receptor modulators, SERMs)、降钙素类、锶盐类(雷奈酸锶)、甲状旁腺激素(特立帕肽)、迪诺塞麦[核因子-κB受体活化因子配体抑制剂, RANKL(receptor activator of nuclear factor-κ B ligand)抑制剂]等。这些药物都能够从一定程度上缓解骨质疏松患者的病情, 但长期服用会对人体产生明显的副作用。关于传统抗骨质疏松药物的作用机理及副作用介绍(表1)。

2 多糖概述

2.1 多糖的结构

多糖, 又称多聚糖, 通常指由10个以上的单糖通过不同类型的糖苷键以支链或直链的形式连接而成的一类生物大分子。多糖在自然界中广泛存在, 可来源于高等光合植物、真菌、藻类、细菌等。多糖的功能复杂多样, 与其复杂的空间结构和较大的分子量密切相关。多糖糖链之间可通过氢键相连, 形成多糖的二级结构; 糖残基上的基团, 又可通过非共价键的相互作用, 形成特异的空间构象, 即多糖的三级结构; 若干条糖链通过非共价键的相互作用, 形成具有复杂空间结构的聚集体则被称为多糖的四级结构[12]。多糖的分子量相差巨大, 组成多糖的单糖单元的数量, 被称为聚合度(degree of polymerization, DP)。DP随着多糖类型的变化而变化, 只有少数天然存在的多糖的DP小于100, 其中, 大于10个且小于20个单糖的又可称为寡糖; 大多数多糖DP在300~3 000; 分子量较大的多糖, 例如纤维素, 其DP为7 000~15 000。超大分子量多糖, DP可超过10^7。若一种多糖的糖基单元都是相同的, 这种多糖被称为均聚糖, 如葡聚糖。若一种多糖, 由两种或多种单糖构成, 则被称为杂聚糖, 如粘多糖。

2.2 多糖具有广泛的生物活性和巨大的药用价值

长久以来, 多糖一直被视为生物体中维持细胞组织结构和提供能量来源的物质, 但没有得到足够的重视, 其发展较蛋白质、核酸晚得多。然而, 越来越多的研究表明, 多糖具有复杂的生物活性和多种重要的生理功能, 对人类的身体健康具有十分积极的作用, 并且, 由于多糖属于天然产物, 相对于目前

表1 治疗骨质疏松症常用药物介绍

Table 1 Introduction of common drugs for anti-osteoporosis

药物名称 Name of drug	类别 Category	作用机制 Mechanism	副作用 Side effect	参考文献 Reference
Bisphosphonates	Pyrophosphate analogues with a nitrogen-containing component	Inhibit osteoclast activity and increase osteoblast activity	Osteonecrosis of the jaw, severe musculoskeletal pain, and atrial fibrillation and esophageal cancer	[6]
SERMs (selective estrogen receptor modulators)	Nonste-roidal agents	Have positive effects on BMD (bone mineral density) and reduction of fracture risk	Stroke, venous thromboembolic disease	[7]
Calcitonin	Endogenous polypeptide hormone	Decrease osteoclast formation and suppress osteoclast attachment	Increase the risk of tumors, continuous use for no more than 3 months	[8]
Strontium ranelate	Ranelic acid and two atoms of stable nonradioactive strontium	Increase bone formation and inhibited the bone resorption activity of osteoclasts	Not suitable for patients with ischemic heart disease, peripheral vascular disease and/or cerebrovascular disease	[9]
Teriparatide (TPTD)	1–34 amino acid peptide, a human PTH analog	Stimulate bone formation and resorption, depending on the mode and dose of administration	Continuous administration can lead to deleterious consequences for the skeleton	[10]
Denosumab	Human monoclonal antibody	Inhibit osteoclast activity and decreasing bone resorption	Not suitable for patients with hypocalcemia	[11]

临床使用的大部分化学合成药而言, 其副作用较少, 更适合长期使用。目前, 多糖已成为天然药物和保健品研发的重要组成部分。据不完全统计, 世界上至少有50多种多糖被证实对人体健康有积极的保健作用, 其中大部分多糖正在被开展涉及抗肿瘤、抗病毒、糖尿病治疗等标准临床试验[13]。

3 多糖在骨质疏松中的应用

近年来, 关于天然产物多糖与骨骼健康的关系越来越受到重视, 且已经有研究证明, 多糖通过促进成骨细胞形成、抑制破骨细胞活性, 阻碍骨吸收等过程, 对骨代谢的调节具有深远的积极影响。以下将对高等植物多糖、微生物多糖和海洋藻类多糖三大类多糖在骨质疏松中的应用进行详细总结(表2)。

3.1 高等植物中的多糖在骨质疏松中的应用

3.1.1 仙茅(*Curculigo orchioides* Gaertn)多糖 仙茅属于石蒜科仙茅属植物, 在中国, 仙茅是一种历史悠久的知名中药, 被广泛用于治疗腰椎和膝关节关节炎等疾病。治疗骨质疏松症的中药方剂如健骨颗粒、二仙汤、骨松康胶囊等, 其中的重要成分便是仙茅[63]。仙茅苷(*Curculigoside*)是一种酚苷类化合物, 临床上具有抗骨质疏松作用, 研究人员对仙茅苷在大鼠体内的代谢产物进行鉴定, 发现仙茅多糖是其中的有效成分[64]。严春艳教授实验团队[14-15]从仙茅中分离出粗多糖CO70、CO90, 发现其具有抗骨质疏松活性, 并进一步从CO70、CO90中分离并纯化出新型的均质杂多糖COP70-3和COP90-1。体内实验证明, COP70-3能够显著提高卵巢切除大鼠的骨小梁连接密度和骨密度, 使其骨强度和骨韧性得到恢复, 骨质疏松症状缓解, 其效果甚至优于目前市场上的阳性药——雌二醇[14]。体外细胞实验进一步表明, 仙茅多糖能促进前成骨细胞系MC3T3-E1细胞和原代成骨细胞中成骨标志物碱性磷酸酶(alkaline phosphatase, ALP)的表达, 并且显著提高MC3T3-E1细胞和原代成骨细胞的矿化速率[14-15]。

3.1.2 黄精(*Polygonatum sibiricum Delar. ex* Redoute)多糖 黄精多糖是百合科植物黄精的主要活性成分, 由多种单糖, 主要包括甘露糖、半乳糖、葡萄糖、果糖等组成[65]。黄精多糖因具有降血糖、降血脂、抗肿瘤和增强免疫等功能, 受到市场的青睐。近年来, 黄精多糖抗骨质疏松的作用逐渐得到关注。前期的动物实验证明, 黄精多糖能预防卵巢切除大鼠骨质疏松症的发生[66]。最新的研究表明, 黄精多糖促进小鼠骨髓间充质干细胞(bone mesenchymal stem

表2 多糖对骨重塑的调节作用

Table 2 Effects of polysaccharide on bone remodeling

多糖 Polysaccharide	作用机制 Mechanism	参考文献 References
Curculigo orchioides polysaccharide	Evaluate ALP activity and enhance the differentiation and mineralization of MC3T3-E1 cells	[14-15]
Polygonatum sibiricum polysaccharide	Promote osteoblastic differentiation and suppresses osteoclastogenesis	[16-17]
Achyranthes bidentata polysaccharide	Exhibit favorable effects on the proliferation and differentiation of osteoblasts	[18-19]
Morinda officinalis polysaccharide	Promote the proliferation, differentiation, and mineralization of MC3T3-E1 cells Reduce deterioration of trabecular microarchitecture and lower levels of bone turnover markers in ovariectomized rats	[20-23]
Cibotium barometz polysaccharide	Promote the differentiation and mineralization of MC3T3-E1 cells	[24]
Astragalus polysaccharide	Activate BMP-2/Smads signaling pathway to protect the bone mineral density and bone mass in ovariectomized rats	[25]
Angelica sinensis polysaccharide	Increase the number of trabeculae and promoted the repair of bone injury in type 2 diabetic rats	[26]
Cistanche deserticola polysaccharide	Remarkably ameliorated bone histopathological damage and promoted the formation of new bone in SAMP6 mice	[27]
Gastrodia elata polysaccharide	Increase BMD in the OVX model and inhibit osteoclastic differentiation in the early stage	[28]
Agrimonia pilosa polysaccharide	Enhance ALP activity, promoted proliferation and differentiation of OB, and increased OB differentiation marker proteins BMP2, Runx2, Osterix, and Osteocalcin	[29]
Lycium barbarum polysaccharide	Significantly increased BMD in OVX rats and glucocorticoid-induced osteoporosis rats	[30]
Saposhnikovia divaricate polysaccharide	Increase BMD and serum Ca^{2+}, Mg^{2+}, and P^{2-} levels in the OVX model	[31-32]
Dendrobium officinale polysaccharide	Prevent the degradation of trabecular microstructural and improved BS/TV, Tb. N, and the reduction in Tb.Sp age-induced osteoporosis model	[33]
Epimedium polysaccharid	Stimulate and improve the proliferation and differentiation of OB cells induced by dexamethasone	[34]
Safflower polysaccharide	Enhance the proliferation of primary OB and mineralization	[35]
Hedysari polysaccharide	Improve the proliferation of OB and enhance ALP activity in a dose-dependent manner	[30]
Dioscoreae polysaccharide	Inhibit bone degeneration, increase BMD and BMC levels in ovariectomized rats	[36]
Tamarind polysaccharide	Chemical modification for adhesion and growth of osteoclast-precursor, induce bone cell differentiation	[37]
Saccharomyces cerevisiae β-glucan	Prevent alveolar bone loss in streptozotocin-induced diabetes model with periodontitis/ ligature-induced periodontitis	[38-40]
Aureobasidium pullulans β-glucan	Attenuate alveolar bone loss, osteoclast numbers, and concentrations of inflammatory cytokines	[41-43]
Curdlan	Suppress nfatc1 activation/ RANKL expression on osteoblasts	[44-47]
Poria cocos polysaccharides	Impair RANKL-induced OC formation in RAW264.7 and BMMS cells and attenuated resorption activity	[48]
Coriolus versicolor polysaccharide	Mitigate DM-induced bone deterioration by increasing the bone volume of the proximal tibia, trabecular number	[49]
Lichenan	Obvious inhibitory effect on osteoclast differentiation from bone marrow cells	[50]
Fucoidan	Induce proliferation of bone cells and osteoblastic differentiation by promoting the expression of ALP, collagen type 1, osteocalcin and BMP-2	[51-54]
Alginate	Chemical modification with adhesion ligands and release of tissue induction factors such as BMP, Tra TGF-β	[55-58]
Polysaccharide from *Sargassum horneri*	Promote OB formation and inhibit bone resorption	[59-60]
Chitin	Widely used for bone tissue engineering	[61]
Chitosan	Widely used for bone tissue engineering	[61]
Carrageenan	Widely used for bone tissue engineering, enhance protein expressions of RUNX2, COL, and OPN	[62]
Laminarin	Obvious inhibitory effect on osteoclast differentiation from bone marrow cells	[50]

cells, BMSCs)的成骨分化。经黄精多糖处理的BMSCs, 成骨标志物ALP、胶原蛋白(collagen, COL)、矮小相关转录因子2(runt-related transcription factor 2, Runx2)、骨钙素(osteocalcin, OCN)的表达水平显著提高, 而破骨细胞特异性基因如抗酒石酸酸性磷酸酶(tartrate resistant acid phosphatase, *TRAP*)、基质金属蛋白酶-9(matrix metalloprotein-9, *MMP-9*)、组织蛋白酶K(cathepsin K, *CTSK*)等的表达量显著下调[16]。

3.1.3 牛膝(*Achyranthes bidentata* Blume)多糖 牛膝属于苋科植物, 中药牛膝指的是牛膝的干燥根, 是一味用于治疗骨质疏松的传统中药。牛膝多糖是牛膝的重要成分之一。在糖皮质激素诱导的骨质疏松斑马鱼模型中, 对斑马鱼头部骨骼进行钙黄绿素染色发现, 牛膝多糖以浓度依赖性方式显著增加斑马鱼头部骨骼的相对荧光强度[67]。在去卵巢大鼠骨质疏松症模型中, 研究人员发现, 牛膝多糖可以显著提高去卵巢大鼠的骨密度、骨矿物质含量、骨小梁厚度、骨小梁数量和骨生物力学特性, 其疗效与阳性药物17β-雌二醇相当[18,68]。此外, 新兴的代谢组学还提供了一种新颖且可靠的方法来评估牛膝多糖对骨质疏松的治疗效果, 并试图阐明其潜在的机制。研究人员通过一项基于超高相液相色谱分析的代谢组学研究发现, 正常大鼠和骨质疏松大鼠的代谢物存在明显差异。通过代谢组学分析发现, 牛膝多糖可以通过调节骨质疏松的潜在生物标志物甘油磷脂代谢物包括LysoPC(18:1)和戊二酰肉碱来治疗骨质疏松[19]。实验数据还表明, 牛膝多糖可能通过调节脂质代谢物影响骨代谢[19]。

3.1.4 巴戟天(*Morinda officinalis* How)多糖 巴戟天是茜草科, 巴戟天属植物。巴戟天是中国传统中药, 数千年来被广泛用于治疗各种骨骼疾病, 如腰痛、肢体疼痛、坐骨神经痛和风湿性关节痛等, 具有巨大的药用价值[69]。ZHU等[20]从巴戟天中提取分离的粗多糖被证实对卵巢摘除大鼠的骨质流失具有预防作用。此外, 巴戟天总多糖参与调节骨形成, 可提高成骨细胞的增殖速率和成骨细胞中ALP的活性[21]。这些实验结果都表明, 巴戟天多糖在预防和治疗骨丢失相关疾病方面具有巨大潜力。近年来, JIANG等[22]从巴戟天根部分离出两种多糖(MOP70-1和MOP70-2), 并且证明这两种多糖都能够明显促进MC3T3-E1细胞的增殖、分化和矿化。进一步研究发现, 巴戟天多糖对大鼠由于卵巢切除术引起的骨流失和骨生物力学功能紊乱有明显的保护作用。实验证明, 巴戟天多糖能够改善卵巢切除大鼠的骨小梁微结构退化和骨转换标志物如ALP水平降低的现象[23]。

3.1.5 黄芪(*Astragali radix*)多糖 黄芪为豆科植物, 黄芪多糖是黄芪的主要功能成分。作为中国的传统中药, 黄芪多糖的功效正逐步被科学证实。据报道, 黄芪多糖具有免疫调节、抗炎、抗氧化、抗衰老和保肝活性。此外, 黄芪多糖抗骨质疏松研究也取得了一定进展。在地塞米松诱导的骨质疏松大鼠模型中, 黄芪多糖能够改善模型大鼠的骨密度和修复骨微结构的损伤[70]。同时, 有证据表明, 黄芪多糖通过诱导肠道微生物的结构和功能的变化, 从而改善模型大鼠的骨质疏松状况[70]。黄芪多糖对卵巢切除大鼠的骨骼健康状况同样具有改善作用。实验表明, 黄芪多糖能够增加模型大鼠血清中的ALP和血清骨钙素(bone gla protein, BGP)水平, 以剂量依赖性方式逆转模型鼠骨量的减少[25]。

3.1.6 其他植物多糖 此外, 还有大量植物来源的多糖被证实具有抗骨质疏松效果, 其中大部分是中国的传统中药材。有数据表明, 从大型树状陆生蕨类金毛狗脊[*Cibotium barometz* (L.) J. Sm]中提取的新型多糖(CBBP-2、CBBP-3、CBP70-1-1、CBP70-1-2)具有诱导成骨活性[24]; 当归(*Angelica sinensis*)多糖能增加2型糖尿病大鼠骨小梁的数量, 促进大鼠骨损伤修复[26]; 肉苁蓉(*Cistanche deserticola Ma*)多糖显著改善了衰老加速小鼠(SAMP6小鼠)的骨损伤, 并促进新骨形成[27]; 铁皮石斛(*Dendrobium officinale Kimura et Migo*)多糖能够显著增加老年鼠的骨量并减少骨髓脂肪组织, 同时抑制BMSC的氧化应激反应[33]。天麻多糖[*Gastrodia elata* Bl)和防风(*Saposhnikovia divaricata* (Turcz.) Schischk]多糖可分别提高去卵巢小鼠、大鼠的骨密度以及血清中的Ca^{2+}、Mg^{2+}和P^{2-}的水平[31-32,71]; 仙鹤草(*Hairyvein Agrimonia* Herb and Bud)多糖可以抑制地塞米松诱导的成骨细胞凋亡[72]; 山药(*Rhizoma dioscoreae*)多糖可抑制骨退行性变[36]; 罗望子(*Tamarindus indica* L.)多糖可诱导成骨细胞分化[37]; 枸杞(*Lycium chinense Miller*)多糖[30]、锁阳(*Cynomorium songaricum* Rupr.)多糖[15]已被证实对动物的骨骼健康状况有改善作用; 红芪(*Hedysarum polybotrys Hand*.-Mazz.)多糖[73-74]、淫羊藿(*Epimedium brevicornum* Maxim)多糖[34]、红花

(*Carthami Flos*)多糖[35]等则被证明能促进成骨细胞矿化等。

3.2 微生物中的多糖在骨质疏松中的应用

3.2.1 酿酒酵母(*Saccharomyces cerevisiae*) β-葡聚糖 酿酒酵母, 又称面包酵母或芽殖酵母, 因其与同为真核生物的动物细胞和植物细胞具有许多相同的结构, 且具有繁殖速度快、易于培养等优点, 被作为研究真核生物的模式生物[75]。β-葡聚糖主要由β-1,3糖苷键连接*D*-吡喃葡萄糖的主链以及长短不一的β-1,6支链结构组成, 广泛存在于自然界中, 是许多微生物、植物、真菌细胞壁的组成成分[76]。在糖尿病诱发牙周炎的大鼠模型中, 酿酒酵母β-葡聚糖通过下调RANKL和上调骨保护素(osteoprotegerin, OPG)的表达水平, 从而减少牙周炎大鼠的牙槽骨骨质流失[39,77]。此外, 在丝线结扎和脂多糖(lipopolysaccharide, LPS)诱导双重作用下的牙周炎大鼠中, 酿酒酵母β-葡聚糖能够有效减少其牙周骨质的流失[40]。

3.2.2 黑酵母(*Aureobasidium pullulans*) β-葡聚糖 黑酵母菌, 又名出芽短梗霉、暗金黄担子菌, 是一种类似酵母的真菌。黑酵母菌细胞壁的周边会产生多糖物质, 其中最重要的是β-葡聚糖。黑酵母β-葡聚糖抑制骨吸收的作用已在动物中得到证实。例如, 在口腔健康方面, 黑酵母β-葡聚糖被认为具有预防和治疗牙周炎的作用。实验证明, 在丝线结扎诱导的大鼠牙周炎模型中, 口服黑酵母β-葡聚糖可降低模型大鼠牙槽骨骨流失的程度、减少破骨细胞数量和血清中炎症因子如白介素1(interleukin-1, IL-1)和肿瘤坏死因子α(tumor necrosis factor-α, TNF-α)的浓度[41]。还有研究表明, 黑酵母β-葡聚糖和葡萄糖酸钙的混合物通过局部给药方式可显著抑制大鼠牙周因结扎引起的细菌增殖和牙槽骨丢失[42]。此外, 在卵巢摘除的小鼠中, 黑酵母提取的含有多聚糖的胞外聚合物, 能够显著减轻小鼠因卵巢摘除而导致的骨质疏松症状[43,67]。

3.2.3 可德胶(Curdlan)多糖 可德胶多糖是由产碱杆菌(*Alcaligenes faecalis var. myxogenes*)发酵而来的, 是一种由β-1,3-*D*糖苷键构成的直链型葡聚糖。dectin-1是β-葡聚糖的受体, 可德胶多糖是dectin-1的激动剂。在小鼠骨髓细胞和dectin-1过表达的RAW264.7细胞(d-RAWs)中, 可德胶多糖以剂量依赖性方式抑制RANKL诱导的破骨细胞分化、骨吸收和肌动蛋白的形成[44]。此外, 可德胶通过降解脾酪氨酸激酶(spleen tyrosine kinase, Syk), 抑制RANKL诱导的活化T细胞核因子(nuclear factor of activated T-cells, NFATc1)表达, 从而降低破骨细胞相关标记基因, 包括*TRAP*、破骨细胞刺激性跨膜蛋白(*osteoclast stimulatory transmembrane protein*, *OC-STAMP*)、*CTSK*、*MMP-9*的表达水平[44]。

3.2.4 其他微生物多糖 茯苓(*Poria cocos* (*Schw.*) *Wolf.*)属于多孔菌科的一种真菌, 其化学成分主要包括三萜、类固醇、氨基酸多糖等。最新的研究表明, 茯苓多糖抑制RANKL诱导的RAW264.7和BMMS细胞朝破骨细胞方向分化, 同时减弱了破骨细胞的骨吸收[48]; 云芝[*Polystictus versicolor* (L.) Fr.], 与茯苓同属于多孔菌科真菌, 是目前最受欢迎的药用蘑菇之一。云芝多糖肽能显著改善糖尿病大鼠的骨骼健康状况, 提高糖尿病大鼠的骨体积、骨小梁数量、股骨生物力学强度以及降低股骨皮质孔隙率[49]; 从冰岛地衣(Cetraria islandica)中提取的地衣多糖(Lichenan), 其结构也属于β-葡聚糖, 有文章指出, 地衣多糖能显著抑制骨髓干细胞向破骨细胞分化[50]。

3.3 藻类中的多糖在骨质疏松中的应用

3.3.1 岩藻依聚糖(fucoidan) 岩藻依聚糖是一种含有大量*L*-岩藻糖和硫酸酯基团的多糖, 是褐藻类如墨角藻(*bladderwrack*)、裙带菜(*wakame*)等和一些海洋无脊椎动物(如海胆和海参)的主要组成成分[78]。低分子量的岩藻依聚糖可诱导骨细胞增殖, 促进ALP、I型胶原蛋白(collagen I)等成骨标志物的表达和矿物沉积[51-52]。有研究表明, 从裙带菜中提取的岩藻依聚糖能够显著诱导参与成骨细胞分化的ALP、OCN、骨形成蛋白-2(bone morphogenetic protein type 2, BMP-2)等细胞因子的表达, 且对成骨细胞没有任何细胞毒性作用[53]。岩藻依聚糖除了能够促进成骨细胞分化外, 还有抑制破骨细胞的功能。在去卵巢大鼠中, 岩藻依聚糖通过抑制大鼠体内破骨细胞的分化进而减轻大鼠骨质疏松程度[54]。

3.3.2 海藻酸盐(Alginate) 骨组织工程是一个新兴领域, 为治疗骨损伤提供了一个全新的治疗思路。海藻酸盐是一种海洋藻类衍生多糖, 通常通过用碱水溶液(NaOH)从海带(*Laminaria japonica*)、泡叶藻(*Ascophyllum nodosum*)、巨藻(*Macrocystis pyrifera*)等褐藻科藻类中提取[79]。海藻酸盐是一类由(1,4)-连接的β-*D*-甘露糖醛酸(M)和α-*L*-古洛糖醛酸(G)残基组成的直线型共聚物, 在组织工程、药

物输送、伤口愈合和细胞培养等领域具有潜在的生物材料应用价值[55]。与其他软骨和骨骼再生材料相比, 海藻酸盐具有得天独厚的优势, 因为它可以以微创的方式被引入体内, 而且易于通过黏附配体进行化学修饰, 并且可调控例如骨形成蛋白、转化生长因子等成骨诱导因子的释放[55]。近年来, 干细胞疗法一直被认为是解决骨修复和骨损伤最为有效的方法之一。研究表明, 将成骨组织的干细胞如间充质干细胞(mesenchymal stem cells, MSCs)接种到可注射的海藻酸钠支架上, 可以观察到骨再生, 从而形成新的骨组织[56,80]。除了在海藻酸盐支架上接种干细胞疗法外, 也有文献报道, 将锶交联到海藻酸盐支架上, 也可促进成骨标志物如ALP的表达, 进而维持成骨细胞的生长和分化[57]。动物实验中, 羟基磷灰石与海藻酸盐生物复合材料能够促进大鼠体内骨骼矿化[58]。

3.3.3 *其他藻类中的多糖* 从铜藻(*Sargassum horneri*)中提取的多糖代谢物, 不但能够促进骨形成, 还具有抗骨吸收的作用。实验证明, 铜藻多糖提取物对去卵巢大鼠和健康人的骨质流失具有预防作用。同时, 还可以防止与衰老和疾病相关的骨质流失情况[59-60]。来源于海洋藻类衍生多糖的甲壳素(chitin)、壳聚糖(chitosan)和卡拉胶(carrageenan)被广泛用于骨组织工程。研究表明, 壳聚糖和甲壳素纳米复合支架具有优异的生物相容性、生物降解能力、对细胞无毒性以及良好的促进骨细胞黏附增殖等能力[61]; 以卡拉胶为原料制成的卡拉胶纳米复合水凝胶能够促进大鼠脂肪来源的间充质干细胞成骨分化, 提高其*RUNX2*、*COL*和*OPN*等成骨相关基因的表达[62]; 从褐藻(*Laminaria* sp.)中提取的昆布多糖(Laminarin), 同样被证明具有抑制破骨细胞分化的能力[50]。

4 多糖治疗骨质疏松分子机制研究进展

骨质疏松的发生主要是由骨重塑的失衡引起的。成年人的整个骨骼大概每10年更换一次, 这个过程便被称为骨重塑。骨重塑包括骨吸收和骨形成两方面, 主要由成骨细胞和破骨细胞参与调节。在此过程中, 骨骼表面衰老或受损的骨被来源于造血干细胞的破骨细胞吸收, 随后, 来源于间充质细胞的成骨细胞被源源不断产生的新骨所取代[81]。破骨细胞的分化起始受破骨细胞生成因子, 即巨噬细胞集落刺激因子(macrophage colony stimulating factor, M-CSF)和核因子κB(nuclear factor kappa-B, NF-κB)的受体激活剂RANKL调控。RANKL受其受体OPG的调节, OPG的表达则抑制破骨细胞形成。因此, RANKL/OPG通路是破骨细胞增殖和活化的主导调控因子, RANKL/OPG值是决定骨量和骨骼完整性的重要因素[82]。成骨细胞由多能间充质干细胞分化而来, 迁移到损伤部位, 增殖并分化。这些骨髓来源的间充质干细胞的成骨潜能对于骨的重塑和愈合至关重要。若成骨细胞的功能失调或者破骨细胞数量和活性发生异常, 骨吸收量超过骨形成量, 则会导致最终的净骨量减少、骨质疏松发生[83]。

研究表明, 多糖主要通过调节成骨细胞和破骨细胞的活性, 维持骨形成和骨吸收的平衡, 从而达到改善骨骼健康状况的作用。本文总结了多糖调控骨细胞的发生和分化等过程涉及的众多信号通路, 主要包括Wnt/β-catennin信号通路、BMP/Smads信号通路和OPG/RANKL/RANK信号通路以及其他信号通路。

4.1 Wnt/β-catenin信号通路

Wnt/β-catenin信号通路已被证实在BMSC的自我更新、分化, 成骨前体细胞增殖, 成骨细胞形成和凋亡中扮演着重要的角色。当Wnt通路被激活时, Wnt蛋白与受体Frizzled及LRP5/6结合成复合体, 促进糖原合成酶激酶(Gsk3β)磷酸化, 阻断β-catenin磷酸化, 抑制其降解。聚集在细胞质中的β-catenin随后被转入细胞核中, 发挥其生物学功能, 激活相关靶基因。已有文章报道, 在成骨细胞中特异性敲除*LRP5/6*基因会导致骨量明显下降。β-catenin蛋白的缺失导致成骨前体细胞软骨向分化, 小鼠出现骨丢失、异位软骨形成等表型。DKK-1(Dickkopf-1)是Wnt信号通路的抑制剂, *DKK-1*敲除小鼠表现为骨量增加[5]。

实验表明, 巴戟天多糖通过下调成骨细胞中的DKK-1蛋白的表达, 提高成骨细胞体外增殖率, 增强ALP的活性[84]。黄精多糖则可通过诱导BMSC中的β-catenin蛋白表达, 激活Wnt/β-catenin信号通路, 促进BMSCs的骨向分化, 同时抑制破骨细胞生成和骨溶解, 从而达到预防和治疗骨质疏松症的目的[16-17,85]。黄芪多糖可激活BMSC中的PI3K/AKT和Wnt/β-catenin通路信号转导[86], 通过调节FoxO3a/Wnt/β-catenin信号通路来缓解由于氧化应激导致的骨质疏松[87]。肉苁

蓉多糖抗骨质疏松作用的分子机制可能与激活Wnt/β-catenin信号通路有关[27]。枸杞多糖通过激活Wnt信号通路相关蛋白β-catenin和Wnt10b的表达, 促进BMSC分化为成骨细胞, 并增加矿化结节[88]。仙鹤草多糖被证明可以通过激活MC3T3-E1细胞中的Wnt/β-catenin信号通路, 保护MC3T3-E1细胞免受地塞米松诱导的细胞损伤, 从而有望成为治疗类固醇诱导的股骨头缺血性坏死的新型替代药物[29]。

4.2 BMP/Smads信号通路

骨形态发生蛋白家族(bone morphogenetic proteins, BMPs)是器官发育的重要调节因子, 其中, 许多家族成员在MSCs的成骨过程中具有重要作用。在BMP/Smads信号通路中, BMP配体激活II型受体BMPRII, 使其磷酸化, 磷酸化的BMPRII继续激活I型受体BMPRI, 磷酸化的BMPRI进而再使下游的Smad1/5/8磷酸化, 最终将胞外信号传递到细胞核内, 从而发挥其调控作用[89]。

研究表明, 从金毛狗中提取的新型多糖CBP70-1-2能够提高BMP2的表达和转录活性; BMP2表达量的提高, 诱导下游的Smad1磷酸化的增加, 进一步刺激成骨标记基因*Runx2*的表达, 促进骨形成[90]。在黄精多糖干预的去卵巢大鼠体内, BMP2蛋白表达量提高, 成骨相关标志物如ALP、OPG和BGP活性增强, 最终使去卵巢大鼠骨质疏松症状得到缓解[91]; 黄芪多糖可能通过激活BMP2/smads相关通路, 调节去卵巢大鼠的骨代谢功能, 改善其骨骼健康状况[25]; 天麻多糖通过阻断BMP2/Smad/Id1信号通路来抑制RANKL诱导的小鼠破骨细胞形成[71]; 上文提到的仙鹤草多糖, 除了激活Wnt/β-catenin通路外, 也同时激活BMP2蛋白, 从而促进成骨细胞的增殖和分化[29]; 岩藻依聚糖则是通过JNK和ERK依赖性的BMP2-Smad1/5/8信号通路促进成骨细胞的分化[92]; 同样, 海藻酸盐也是通过刺激BMP的表达, 从而诱导骨的形成[55]。

4.3 OPG/RANKL/RANK信号通路

与上述两条信号通路主要调控成骨细胞的分化不同, OPG/RANKL/RANK是调节破骨细胞分化和骨吸收的关键信号通路。RANKL是TNF超家族成员, 能与破骨细胞表面的RANK受体结合, 招募TNFR相关因子, 启动下游信号, 包括p38、JNK和ERK, 从而激活破骨细胞转录因子, 如NF-κB、激活蛋白1(activating protein-1, AP-1)、环磷酸腺苷反应元件结合蛋白(cAMP-response element binding protein, CREB)、NFATc1的表达。OPG是TNF受体家族成员, 能与RANKL竞争受体结合位点, 从而抑制破骨细胞的分化[93]。

许多植物多糖不仅能够调控成骨细胞的分化, 促进骨形成, 同时也能调节破骨细胞的活性, 抑制骨吸收。例如, 黄精多糖和天麻多糖都能够通过抑制RANKL诱导的RAW246.7破骨细胞形成特异性基因*TARP*、*NFATc1*、*MMP-9*和*CTSK*的表达, 从而抑制破骨细胞分化[17,71]; 牛膝多糖可能通过提高OPG和RANK蛋白表达量, 抑制RANKL表达, 从而降低去卵巢大鼠血清中骨吸收标志物如TPACP5b、NTX和CTX的水平, 进而恢复去卵巢大鼠的骨密度[94], 此外, 牛膝多糖还被证实可抑制RANKL诱导的MAPK磷酸化[95]; 黄芪多糖和锁阳多糖则通过提高去卵巢模型动物血清中OPG的水平, 同时降低RANKL的表达量, 进而导致RANKL/OPG值降低, 从而抑制破骨细胞的活性[70,96-97]。肉苁蓉多糖能够抑制RANKL诱导的破骨细胞与骨髓来源巨噬细胞(bone marrow-derived macrophage, BMM)的分化[98]。

与植物来源的多糖不同, 微生物来源的多糖则主要通过调控破骨细胞的活性来调节骨骼健康状况。实验证明, 酿酒酵母β-葡聚糖通过抑制NF-κB信号, 下调c-fos蛋白的表达水平, 以及刺激破骨细胞生成负调节因子[干扰素调节因子-8(interferon regulatory factor-8, IRF-8)]的表达量, 达到抑制破骨细胞分化的目的[38]。口服酿酒酵母β-葡聚糖的大鼠血清中调节成骨细胞的数量及活性的转录生长因子-β1(transforming growth factor-β1, TGF-β1)和抗破骨细胞生成因子白介素-10(interleukin-10, IL-10)的水平显著提高[40]。可德胶多糖提高dectin-1活性后, 高活性的dectin-1能够提高白介素-33(interleukin-33, IL-33)的分泌。IL-33提高破骨细胞前体中NFATc1的抑制剂V-maf肌肉腱膜纤维瘤同源基因B(musculoaponeurotic fibrosarcoma oncogene homolog B, MafB)的表达水平, 进而抑制破骨细胞前体分化为成熟的破骨细胞[45]。低分子量可德胶(MW 3 000 kDa)则是通过与另一种受体TLR2/TLR6结合影响骨生成活动。TLRs是Toll样受体(Toll-like receptors), TLR2信号转导可诱导破骨细胞形成和炎症性骨吸收[46]。低分子量可德胶可有效抑制TLR2/6配体诱导的破骨细胞形成, 并减弱器官培养实验中小鼠颅骨的骨吸收。同时, 低分子

量可德胶抑制RANKL的表达, 导致小鼠骨髓巨噬细胞向破骨细胞分化的能力减弱[47]。

4.4 其他信号通路

除以上提到的三个主要的信号通路外, 还有一些信号通路也参与到了多糖调节骨细胞发生分化的过程中。例如, 淫羊藿多糖通过PI3K/Akt/mTOR信号通路降低地塞米松诱导的成骨细胞中Bax和caspase-3的表达水平, 从而达到抑制成骨细胞凋亡的目的[34]; 李波等[99]的文章认为, 黄精多糖可能是通过基于内源性非编码小分子RNA-1224(miR-1224)的Hippo信号通路抑制破骨细胞生成的; 茯苓多糖通过抑制NFATc1活性以及ERK和STAT3磷酸化, 减弱RNAKL诱导的破骨细胞生成能力, 以此抑制破骨细胞的数量及活性[48]。岩藻依聚糖通过调节Akt/GSK3β/PTEN/NFATc1信号通路和钙调磷酸酶活性来阻止RANKL刺激的破骨细胞生成和LPS诱导的炎症性骨质流失[100]。

5 问题与展望

随着世界人口老龄化进程的加快, 骨质疏松、骨质流失等骨科疾病已被公认为严重的社会健康问题。而自然界分布广泛的多糖, 其抗骨质疏松症疗效正逐步被发掘。以上众多研究表明, 多糖主要通过促进成骨细胞的分化和活性, 调节破骨细胞活性、抑制骨吸收两方面, 进而达到治疗绝经后骨质疏松症、老年性骨质疏松症和糖皮质激素诱导的继发性骨质疏松症等的目的。多糖改善骨骼健康状态的机制通路主要包括Wnt/β-catennin信号通路、BMP/Smads信号通路和OPG/RANKL/RANK信号通路等。本文将自然界的具有治疗骨质疏松作用, 调节骨骼健康状态的众多多糖进行了梳理和总结, 将帮助后来研究者更好地理解多糖的抗骨质疏松作用和所涉及的信号通路调控, 为多糖临床研究和应用提供充实的理论依据。

虽然, 多项体内和体外研究实验都已经明确证明了多糖能够有效改善骨骼健康状况。然而, 由于多糖的结构复杂多样, 不同来源的多糖结构不尽相同, 甚至差别巨大, 进而导致其功能的极大差异, 这对深入研究多糖是如何调节成骨细胞和破骨细胞的活性, 改善骨骼健康的分子机制造成了一定的困难。因此, 在今后从天然产物中开发多糖的过程中, 应结合包括转录组学、蛋白质组学以及微生物组学等在内的多组学联合分析, 进一步明确多糖的生物活性与化学结构的关系, 进而阐明多糖在成骨细胞和破骨细胞上的作用靶点和具体的信号通路。

综上所述, 天然产物多糖在骨质疏松症中具有巨大的潜在应用价值, 目前科研工作者所做的这些探索, 不仅有助于多糖从天然产物到功能性食品的转变, 也为开发天然无毒副作用的抗骨质疏松症药物提供坚实的理论依据, 对今后骨骼健康疾病的治疗具有重要意义。

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