doi: 10.3978/j.issn.2095-6959.2018.12.033
View this article at: http://dx.doi.org/10.3978/j.issn.2095-6959.2018.12.033

甲状旁腺激素相关蛋白对骨关节炎软骨细胞修复的作用机制及应用进展

李啸宇[1] 综述 黎润光[2] 审校

(南方医科大学 1. 第一临床医学院；2. 南方医院创伤骨科，广州 510515)

[摘 要] 甲状旁腺激素相关蛋白(parathyroid hormone-related peptide，PTHrP)与关节软骨细胞有关的作用机制，包括在生长发育过程中与调节软骨内成骨过程相关的作用机制，在生理状态下与维持关节软骨正常形态相关的作用机制，及其与骨关节炎(osteoarthritis，OA)关节软骨发病过程中的相关的作用机制。探究PTHrP在OA治疗中的应用，包括其中央区及C端肽段的独特作用及影响其效果的不同因素，如施加时间的间隔、每次施加的时间长度及施加的时间点。

[关键词] 甲状旁腺激素相关蛋白；骨关节炎；软骨内成骨；关节软骨；治疗

Mechanism and application of parathyroid hormone-related proteins on the repair of osteoarthritis cartilage cells

LI Xiaoyu[1], LI Runguang[2]

(1. First Clinical Medical College; 2. Department of Orthopaedics and Traumatology, Nanfang Hospital, Southern Medical University, Guangzhou 510515, China)

Abstract The mechanisms of parathyroid hormone-related protein (PTHrP) concerning about articular cartilage cells included regulating endochondral ossification during the process of growth and development, keeping the normal articular cartilage form in a physiological manner, and preventing osteoarthritis (OA). Then we introduced the application of PTHrP in treatment of OA, including the unique functions of the central region and the C-terminal peptides of PTHrP and the factors influencing its effects, such as the interval of time, length of time and the time point of applying.

Keywords parathyroid hormone-related protein; osteoarthritis; endochondral ossification; articular cartilage; treatment

收稿日期 (Date of reception)：2018–10–09
通信作者 (Corresponding author)：黎润光，Email: lirunguang79@163.com
基金项目 (Foundation item)：广东省自然科学基金 (2018A03031364)；南方医科大学省级大学生创新训练计划项目 (201712121134)。
This work was supported by the Natural Science Foundation of Guangdong Province (2018A03031364) and Provincial Student's Platform for Innovation and Entrepreneurship Training Program of Southern Medical University (201712121134), China.

骨关节炎(osteoarthritis，OA)是一种由关节软骨及其软骨下骨破坏导致的关节疾病。关节软骨细胞异常的增殖以及肥大化的分化对OA的启动及进展至关重要。软骨细胞肥大的特征包括细胞体积增加、细胞外基质重构和末端分化标志物的表达，其特征在于SOX9(sry-box 9)的表达降低和软骨降解酶的产生增加，例如基质金属蛋白酶13(matrix metalloproteinase 13，MMP13)和具有血小板反应蛋白基序的解整合素和金属蛋白酶5(a disintegrin and metalloproteinase with thrombospondin motifs 5，ADAMTS5)[1]。在生长板中，甲状旁腺激素相关蛋白(parathyroid hormone-related peptide，PTHrP)阳性软骨细胞中表达一组骨骼干细胞和祖细胞标志物，并具有骨骼干细胞的特性，可以分化为柱状软骨细胞、成骨细胞和骨髓基质细胞[2]。它的类似物，甲状旁腺素(parathyroid hormone，PTH)，与其有相同的N端34个残基的肽段，因此它们共享有相同的受体甲状旁腺素1受体(PTHrP receptor 1，PTHR1)[3]。在功能区的高度相似性使得PTH及PTHrP在阻止软骨细胞肥大化上具有相似的效应，如PTH/PTHrP-盐诱导激酶3(salt-inducible kinase 3，SIK3)途径通过改变哺乳动物(或机械)雷帕霉素靶点[mammalian (or mechanistic) target of rapamycin，mTOR]信号影响骨骼发育[4]。PTHrP因其抑制软骨细胞肥大化的生理作用日益引起人们的关注。

1 软骨内成骨过程、正常关节软骨及OA关节软骨中的PTHrP

1.1 软骨内成骨过程中PTHrP的调节因素及作用机制

1.1.1 软骨内成骨过程中PTHrP的调节因素

在软骨内骨化中，软骨细胞需经过一个时间及空间上有序的分化过程：关节周围增殖软骨细胞，柱状增殖软骨细胞，肥大前软骨细胞及肥大软骨细胞。与PTHrP在软骨内骨化过程中的调节作用高度相关的一个重要因子是印度豪猪蛋白(Indian Hedgehog，Ihh)。由肥大前软骨细胞表达的Ihh刺激增殖软骨细胞产生PTHrP，加速了周围软骨细胞的增殖，并预防了软骨细胞肥大化的开始，最终使得软骨细胞保持增殖状态[5]。这一负反馈环调节着软骨细胞增殖与成熟间的平衡，保证了有序的骨形成。Ihh影响PTHrP表达及信号通路的具体机制尚不完全清楚。已有研究[6]表明：Ihh下游作用因子Gli2与Foxc1(forkhead box C1)相联合，上调PTHrP的表达。BMP-6和Bcl-2可能是Ihh信号转导的下游调节因子[7]。Ihh可触发PTHrP信号通路下游因子Nkx3.2(NK3 homeobox 2)蛋白降解[8]。除了Ihh，在软骨内成骨过程中，PTHrP也可以被其他因子调节。胰岛素样生长因子1受体(insulin-like growth factor 1 receptor，IGF-1R)抑制PTHrP表达[9]。转录激活因子6(activating transcription factor6，ATF6)裂解的N端细胞质结构域ATF6a抑制PTHrP表达[10]。周期性应力在软骨细胞增殖及基质形成阶段显著增加软骨细胞中PTHrP mRNA水平，而且PTHrP的机械诱导效应可以被Ca^{2+}通道阻滞剂抑制[11]。不同量级的应力对PTHrP表达的影响也不同，在一定范围内的应力可以增加骺板软骨细胞PTHrP的表达，而过大的应力使得PTHrP表达显著减少[12]。

1.1.2 软骨内成骨过程中PTHrP的作用机制

PTHrP由关节周围增生软骨细胞分泌，而PTHrP的受体PTH1R主要表达在肥大前软骨细胞中[13]。PTHrP对肥大化的抑制作用依赖于与PTH1R的结合。PTH1R是一种经典的G蛋白偶联受体，它通过激发Gs蛋白，产生cAMP，进而加强蛋白激酶A(protein kinase A，PKA)活性或者通过Gq/11蛋白激活磷脂酶C(phospholipase C，PLC)加强蛋白激酶C(protein kinase C，PKC)活性[14]。

PTHrP阻止软骨细胞肥大化的抑制效应主要是通过激活PKA信号通路实现的。磷酸化的Sox9可以通过上调Nkx3.2的表达抑制Runx2表达[15]。激活的蛋白磷脂酶2A(PP2A)，促进组蛋白脱乙酰酶4(HDAC4)的脱磷酸化，HDAC4易位到细胞核，抑制MEF2C转录因子对Col10a1(collagen type X，α1)基因表达的促进作用，最终减缓软骨细胞肥大化的速率[16]。PKA通路下游，细胞周期蛋白D1(cyclin D1)-细胞周期蛋白依赖性激酶4(cyclin dependent kinase 4，CDK4)复合物诱导Runx2和Runx3的磷酸化和降解[17]。

PLC/PKC通路对软骨细胞肥大化起抑制作用。p38丝裂原激活的蛋白激酶(p38 MAPK)诱导了Bcl-2及X型胶原的表达，激活PTH1R可通过PKC途径阻断p38 MAPK的活性，减少MEF2C磷酸化，最终减少肥大化基因表达[18]。

一些暂未明确信号通路的PTHrP下游信号因子也被发现。PTHrP可能通过p27调控骨骼生长和发育，p27基因的敲除可以抵消敲入PTHrP基因造成的骨骼生长迟缓和成骨细胞骨形成缺陷效应[19]。细胞外基质蛋白1(extracellular matrix protein 1，ECM1)似乎对PTHrP在软骨形成中起至关重要

的作用，ECM1的阻滞几乎消除了PTHrP对软骨细胞肥大化的调控作用，而ECM1的过度表达则消除了PTHrP基因敲除导致的小鼠生长板发育的紊乱[20]。在生长板上初始纤毛可以感知化学和机械刺激，纤毛直接受到PTHrP的影响，但其具体影响尚不明确[21]。细胞周期素依赖性激酶1(cyclin-dependent kinase 1，Cdk1)在柱状增生性软骨细胞中高度表达，在分化为肥大软骨细胞的过程中明显下调。研究[22]显示Cdk1具有介导PTHrP抑制软骨细胞分化的功能。

1.2 正常关节软骨中的 PTHrP 的表达及影响因素

生长板软骨和关节软骨产生PTHrP的软骨细胞，都具有相同的来源——软骨骺，只是被次级骨化中心分为了2个亚群——关节表面软骨细胞及生长板的关节周围增殖软骨细胞。来源的一致性导致了二者在功能上的相似性。PTHrP在关节软骨维持的生理调节中起重要作用。在健康关节软骨细胞中，由表面层软骨细胞分泌的PTHrP抑制深层软骨细胞的肥大化以保持关节软骨的稳态[23]。

机械负荷可诱导PTHrP在关节软骨软骨细胞中的表达。在表面层软骨细胞中，流体剪切应力增加PTHrP的表达，进而驱动PKA和Ca^{2+}调控的信号通路，促进依赖cAMP反应元件结合蛋白(cAMP response element-binding protein，CREB)的Prg4表达，其表达产物分泌性蛋白多糖lubricin对机械负荷敏感，且可以防止OA的发生[24]。关节腔内的低氧环境对PTHrP的表达亦十分重要，正常人类关节软骨细胞所处的低氧环境诱导低氧诱导因子(hypoxia-inducible factor，HIF)-1α和HIF-2α表达，从而上调PTHrP表达，随后PTHrP诱导SOX9表达，导致COL2A1(collagen type II，α1)的表达增加[25]。

1.3 OA 关节软骨中的 PTHrP 作用变化

生理状态下机械应力可以调节PTHrP表达。OA关节软骨表面层抗拉刚度显著下降，结构的不稳定极可能造成软骨细胞受力的不均衡，影响PTHrP的表达，最终影响下游调节系统。此外，关节软骨的维持需要转录因子Erg，Erg在人类OA关节软骨受严重影响的区域上调，但在受影响较少的软骨区域几乎不受影响，PTHrP是Erg可能的下游效应物[26]。骨唾液蛋白(bone sialoprotein，BSP)是肥大化软骨细胞诱导血管生成中一个关键的中介因子，在OA软骨细胞中，外加的PTHrP显著下调肥大化软骨细胞BSP的表达[27]。激活的钙/钙调蛋白依赖性蛋白激酶II(calcium/calmodulin dependent protein kinase II，CaMKII)对促进增生性软骨细胞从增殖转变到肥大前状态起十分重要的作用，CaMKII通过调节Ihh和PTHrP途径，在促进软骨细胞分化过程中发挥作用[28]。MMP降解PTHrP1-36产生PTHrP1-17，影响了PTHrP的生理功能[29]。

虽然PTHrP对OA具有修复作用，但在一些对OA有治疗作用的通路中，PTHrP的表达被抑制。mTORC1导致PTHrP转录，打断mTORC1促进软骨细胞自噬及存活，并减轻OA的严重程度[30]。mTORC1上游抑制因子——结节性硬化症复合体1(tuberous sclerosis complex 1，Tsc1)敲除诱发出自发性OA，这与mTORC1的过度激活及PTR1的下调有关[31]。将TGF-β基因转染入OA软骨细胞虽然显著减低了软骨细胞增生和晚期分化的相关标志物的表达，但是也降低了PTHrP的表达[32]。

2 PTHrP 在 OA 治疗上的应用

PTHrP在正常关节软骨中比在骨赘中表达增加[33]。很可能是由于修复组织中PTHrP表达减少，而导致软骨细胞异常的肥大。因此，为病变软骨补充外源性PTHrP具有重要意义。

PTHrP在人体内具有多种肽段，不同肽段有不同的功能。PTHrP1~36及更长的N端肽段主要以分泌蛋白的形式在细胞外与PTHR1结合引发抑制软骨细胞肥大的作用[3]。位于氨基酸残基84和93之间的核定位序列的中央区PTHrP肽段具有促进细胞增殖的作用[34]，核PTHrP通过诱导c-myc和skp2的表达降低细胞周期抑制剂p27的水平，进而刺激血管平滑肌细胞体外和体内的增殖[35]。由PTHrP107~138和109~138等组成的C端片段具有抑制破骨细胞功能并刺激成骨细胞增殖的功能[36]。中央区和C端PTHrP的丢失与p21，p16INK4a等衰老标志物表达的增加及维持干/祖细胞的Bmi-1的表达减少有关[37]。

在探索外源性PTHrP在OA治疗中的应用时，不同的肽段也被应用。涉及PTHrP C端肽段的研究[38]显示了其独特的作用：PTHrP107~111及PTHrP107-139显著减少了人类OA成骨细胞衰老标志物表的表达；PTHrP1~37，PTHrP107~111及PTHrP107~139三种多肽均支持成骨功能，然而C端结构域比N端结构域更加有效。

PTHrP的施加方式也显著影响PTHrP的治疗效果。在体外实验[39]中，脉冲式施加PTHrP的方式中，施加时间的间隔及每次施加的时间长度对MSCs

的成软骨分化有重要影响，而这可能与IGF和SOX9相关的机制有关。在体内，形成OA后不同的不同时间点施加PTHrP对治疗效果同样十分重要[40]。

3 结语

PTHrP在骨软骨代谢过程中的生理作用日益引起关注，其独特的生理作用使其在多种骨软骨疾病中有极大的研究价值。虽然目前对PTHrP有了一定的研究成果，但一些与PTHrP有关的作用因子尚未明确其完整的上下游通路，更多的与PTHrP作用有关的因子有待发现。在具体的应用过程中，PTHrP肽段的选取及具体施加的方式也有待进一步探索。

参考文献

1. Rong J, Zhu M, Munro J, et al. Altered expression of the core circadian clock component PERIOD2 contributes to osteoarthritis-like changes in chondrocyte activity[J]. Chronobiol Int, 2018, [Epub ahead of print].
2. Mizuhashi K, Ono W, Matsushita Y, et al. Resting zone of the growth plate houses a unique class of skeletal stem cells[J]. Nature, 2018, 563(7730): 254-258.
3. Pioszak AA, Parker NR, Gardella TJ, et al. Structural basis for parathyroid hormone-related protein binding to the parathyroid hormone receptor and design of conformation-selective peptides[J]. J Biol Chem, 2009, 284(41): 28382-28391.
4. Csukasi F, Duran I, Barad M, et al. The PTH/PTHrP-SIK3 pathway affects skeletogenesis through altered mTOR signaling[J]. Sci Transl Med, 2018, 10(459).
5. Vortkamp A, Lee K, Lanske B, et al. Regulation of rate of cartilage differentiation by Indian hedgehog and PTH-related protein[J]. Science, 1996, 273(5275): 613-622.
6. Yoshida M, Hata K, Takashima R, et al. The transcription factor Foxc1 is necessary for Ihh-Gli2-regulated endochondral ossification[J]. Nat Commun, 2015, 6: 6653.
7. Ma RS, Zhou ZL, Luo JW, et al. The Ihh signal is essential for regulating proliferation and hypertrophy of cultured chicken chondrocytes[J]. Comp Biochem Physiol B Biochem Mol Biol, 2013, 166(2): 117-122.
8. Choi SW, Jeong DU, Kim JA, et al. Indian Hedgehog signalling triggers Nkx3.2 protein degradation during chondrocyte maturation[J]. Biochem J, 2012, 443(3): 789-798.
9. Wang Y, Cheng Z, Elalieh H , et al. IGF-1R signaling in chondrocytes modulates growth plate development by interacting with the PTHrP/Ihh pathway[J]. J Bone Miner Res, 2011, 26(7): 1437-1446.
10. Guo F, Han X, Wu Z, et al. ATF6a, a Runx2-activable transcription factor, is a new regulator of chondrocyte hypertrophy[J]. J Cell Sci, 2016, 129(4): 717-728.
11. Tanaka N, Ohno S, Honda K, et al. Cyclic mechanical strain regulates the PTHrP expression in cultured chondrocytes via activation of the Ca^{2+} channel[J]. J Dent Res, 2005, 84(1): 64-68.
12. Sun K, Liu F, Wang J, et al. The effect of mechanical stretch stress on the differentiation and apoptosis of human growth plate chondrocytes[J]. In Vitro Cell Dev Biol Anim, 2017, 53(2): 141-148.
13. Lee K, Deeds JD, Segre GV. Expression of parathyroid hormone-related peptide and its receptor messenger ribonucleic acids during fetal development of rats[J]. Endocrinology, 1995, 136(2): 453-463.
14. Yang D, Singh R, Divieti P, et al. Contributions of parathyroid hormone (PTH)/PTH-related peptide receptor signaling pathways to the anabolic effect of PTH on bone[J]. Bone, 2007, 40(6): 1453-1461.
15. Yamashita S, Andoh M, Ueno-Kudoh H, et al. Sox9 directly promotes Bapx1 gene expression to repress Runx2 in chondrocytes[J]. Exp Cell Res, 2009, 315(13): 2231-2240.
16. Arnold MA, Kim Y, Czubryt MP, et al. MEF2C transcription factor controls chondrocyte hypertrophy and bone development[J]. Dev Cell, 2007, 12(3): 377-389.
17. Zhang M, Xie R, Hou W, et al. PTHrP prevents chondrocyte premature hypertrophy by inducing cyclin-D1-dependent Runx2 and Runx3 phosphorylation, ubiquitylation and proteasomal degradation[J]. J Cell Sci, 2009, 122(Pt 9): 1382-1389.
18. Zhen X, Wei L, Wu Q, et al. Mitogen-activated protein kinase p38 mediates regulation of chondrocyte differentiation by parathyroid hormone[J]. J Biol Chem, 2001, 276(7): 4879-4885.
19. Zhu M, Zhang J, Dong Z, et al. The p27 pathway modulates the regulation of skeletal growth and osteoblastic bone formation by parathyroid hormone-related peptide[J]. J Bone Miner Res, 2015, 30(11): 1969-1979.
20. Kong L, Zhao YP, Tian QY, et al. Extracellular matrix protein 1, a direct targeting molecule of parathyroid hormone-related peptide, negatively regulates chondrogenesis and endochondral ossification via associating with progranulin growth factor[J]. FASEB J, 2016, 30(8): 2741-2754.
21. Moore ER, Jacobs CR. The primary cilium as a signaling nexus for growth plate function and subsequent skeletal development[J]. J Orthop Res, 2018, 36(2): 533-545.
22. Saito M, Mulati M, Talib SZ, et al. The indispensable role of cyclin-dependent kinase 1 in skeletal development[J]. Sci Rep, 2016, 6: 20622.
23. Jiang J, Leong NL, Mung JC, et al. Interaction between zonal populations of articular chondrocytes suppresses chondrocyte mineralization and this process is mediated by PTHrP[J].

Osteoarthritis Cartilage, 2008, 16(1): 70-82.

24. Ogawa H, Kozhemyakina E, Hung HH, et al. Mechanical motion promotes expression of Prg4 in articular cartilage via multiple CREB-dependent, fluid flow shear stress-induced signaling pathways[J]. Genes Dev, 2014, 28(2): 127-139.
25. Pelosi M, Lazzarano S, Thoms B L, et al. Parathyroid hormone-related protein is induced by hypoxia and promotes expression of the differentiated phenotype of human articular chondrocytes[J]. Clin Sci (Lond), 2013, 125(10): 461-470.
26. Ohta Y, Okabe T, Larmour C, et al. Articular cartilage endurance and resistance to osteoarthritic changes require transcription factor Erg[J]. Arthritis Rheumatol, 2015, 67(10): 2679-2690.
27. Pesesse L, Sanchez C, Walsh DA, et al. Bone sialoprotein as a potential key factor implicated in the pathophysiology of osteoarthritis[J]. Osteoarthritis Cartilage, 2014, 22(4): 547-556.
28. Liu Q, Yang HX, Wan XH, et al. Calcium/calmodulin dependent protein kinaseII in occlusion induced degenerative cartilage of rat mandibular condyle[J]. J Oral Rehabil, 2018, 45(6): 442-451.
29. Frieling JS, Shay G, Izumi V, et al. Matrix metalloproteinase processing of PTHrP yields a selective regulator of osteogenesis, PTHrP1-17[J]. Oncogene, 2017, 36(31): 4498-4507.
30. Yan B, Zhang Z, Jin D, et al. mTORC1 regulates PTHrP to coordinate chondrocyte growth, proliferation and differentiation[J]. Nat Commun, 2016, 7: 11151.
31. Zhang H, Wang H, Zeng C, et al. mTORC1 activation downregulates FGFR3 and PTH/PTHrP receptor in articular chondrocytes to initiate osteoarthritis[J]. Osteoarthritis Cartilage, 2017, 25(6): 952-963.
32. Venkatesan JK, Rey-Rico A, Schmitt G, et al. rAAV-mediated overexpression of TGF-beta stably restructures human osteoarthritic articular cartilage in situ[J]. J Transl Med, 2013, 11: 211.
33. Gelse K, Ekici AB, Cipa F, et al. Molecular differentiation between osteophytic and articular cartilage—clues for a transient and permanent chondrocyte phenotype[J]. Osteoarthritis Cartilage, 2012, 20(2): 162-171.
34. Park SI, Mccauley LK. Nuclear localization of parathyroid hormone-related peptide confers resistance to anoikis in prostate cancer cells[J]. Endocr Relat Cancer, 2012, 19(3): 243-254.
35. Sicari BM, Troxell R, Salim F, et al. c-myc and skp2 coordinate p27 degradation, vascular smooth muscle proliferation, and neointima formation induced by the parathyroid hormone-related protein[J]. Endocrinology, 2012, 153(2): 861-872.
36. Horwitz MJ, Tedesco MB, Garcia-Ocana A, et al. Parathyroid hormone-related protein for the treatment of postmenopausal osteoporosis: defining the maximal tolerable dose[J]. J Clin Endocrinol Metab, 2010, 95(3): 1279-1287.
37. Toribio RE, Brown HA, Novince CM, et al. The midregion, nuclear localization sequence, and C terminus of PTHrP regulate skeletal development, hematopoiesis, and survival in mice[J]. FASEB J, 2010, 24(6): 1947-1957.
38. Platas J, Guillen MI, Gomar F, et al. Anti-senescence and anti-inflammatory effects of the C-terminal moiety of PTHrP peptides in OA osteoblasts[J]. J Gerontol A Biol Sci Med Sci, 2017, 72(5): 624-631.
39. Fischer J, Ortel M, Hagmann S, et al. Role of PTHrP(1-34) pulse frequency versus pulse duration to enhance mesenchymal stromal cell chondrogenesis[J]. J Cell Physiol, 2016, 231(12): 2673-2681.
40. Zhang W, Chen J, Tao J, et al. The promotion of osteochondral repair by combined intra-articular injection of parathyroid hormone-related protein and implantation of a bi-layer collagen-silk scaffold[J]. Biomaterials, 2013, 34(25): 6046-6057.

本文引用：李啸宇, 黎润光. 甲状旁腺激素相关蛋白对骨关节炎软骨细胞修复的作用机制及应用进展[J]. 临床与病理杂志, 2018, 38(12): 2712-2716. doi: 10.3978/j.issn.2095-6959.2018.12.033

Cite this article as: LI Xiaoyu, LI Runguang. Mechanism and application of parathyroid hormone-related proteins on the repair of osteoarthritis cartilage cells[J]. Journal of Clinical and Pathological Research, 2018, 38(12): 2712-2716. doi: 10.3978/j.issn.2095-6959.2018.12.033