Regulatory Role of Hypoxia Inducible Factor in the Biological Behavior of Nucleus Pulposus Cells

Hao Li, Cheng Zhen Liang, and Qi Xin Chen
Department of Orthopedics Surgery, The Second Hospital of Medical College, Zhejiang University, Hangzhou, China.

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Corresponding author: Dr. Qi Xin Chen, Department of Orthopedics Surgery, The Second Hospital of Medical College, Zhejiang University, Jie fang Road 88#, Hangzhou 310009, China. Tel: 86-57187767023, Fax: 86-57187022776 E-mail: zrcqx@zju.edu.cn

INTRODUCTION

Low back pain is a major public health problem that leads to intense discomfort and loss of function, resulting in considerable economic loss and health care expenditures.1-3 It is reported that the lifetime prevalence of low back pain ranges from 30% to 85%, with a point prevalence of 20% to 40% in Western developed countries.4 The etiology of low back pain remains unclear, but intervertebral disc (IVD) degeneration has been implicated as one of the major causes.5,6 IVD is a specialized complex that separates the vertebra and functions to provide load bearing and allow for flexibility of the spinal column.7,8 Healthy human IVDs are composed of the peripheral annulus fibrosus (AF), the central nucleus pulposus (NP), and the superior and inferior cartilaginous endplates. The NP is populated by NP cells which play a critical role in the generation and maintenance of the IVD matrix.9 Histopathological observations have indicated that significant degenerative changes occur in the NP during the degeneration process,10,11 alternated phenotypes, reduced cell survival, decreased metabolic activity, loss of matrix produc-
tion and dystrophic mineralization of NP cells may be key contributors to progressive IVD degeneration.

Current treatment modalities for symptomatic IVD degeneration are mainly targeted at the symptoms of pain and neurological deficits, without treating the degenerative problems or repairing the biomechanical function of the degenerative disc. Thus, a better understanding of the regulation of the phenotypes, survival, metabolism, matrix production and dystrophic mineralization of NP cells may help to develop prevention and treatment strategies for IVD degeneration.

The IVD is the largest avascular structure in the body. A limited number of blood vessels infiltrate the superficial region of the cartilaginous endplate and the outer third of the AF, but none of these vessels infiltrate the NP. Necessary nutrients, including oxygen and glucose, reach the NP predominantly through diffusion which imposes a hypoxic state on the NP cells. In addition, this hypoxic state is enhanced by the loss of cartilaginous endplate permeability during IVD degeneration. Hypoxia is an important cellular stress with significant pathological implications in many disease processes, such as cerebral ischemia, cancer and chronic degenerative disorders. Hypoxia-inducible factor (HIF) is a transcription factor that initiates a coordinated cellular cascade in response to a low oxygen tension environment, including the regulation of numerous enzymes in response to hypoxia. Recently, the expression of HIF in NP cells has been reported by many groups.

Based on these previous studies, this review will discuss the regulatory role of HIF in the biological behaviors of NP cells. Additionally, we will summarize current data on the expression of HIF in NP cells and further discuss the various roles HIF plays in the regulation of the phenotypes, survival, metabolism, matrix production and dystrophic mineralization of NP cells.

HIF IN NP CELLS

In response to hypoxic conditions, cells respond to low oxygen tension by up-regulating the synthesis of HIF proteins. The HIF family of proteins comprises several distinct HIF proteins, HIF-1, HIF-2, and HIF-3, each of which consist of an α-subunit and a constitutively expressed β-subunit known as aryl hydrocarbon receptor nuclear translocator. Trans-activation of HIF target genes involves the dimerization of the two subunits and binding to an enhancer element, the hypoxia response element (HRE) in the target genes.

Under normoxic conditions, α-subunits cannot be detected as they undergo rapid degradation, but under hypoxic conditions they are stabilized, accumulated and translocated to the nucleus where they dimerize with β-subunits to bind to HREs and activate the expression of numerous hypoxia response genes.

Among the three HIF isoforms, HIF-1 and HIF-2 play an important role in the regulation of the biological behaviors of NP cells. Risbud, et al. examined the expression of HIF-1α in rat, human, and sheep NP cells under both hypoxia and normoxia (2% and 21% oxygen) and found that NP cells consistently expressed functionally active HIF-1α protein under hypoxia; Agrawal, et al. observed HIF-2α expression in rat NP cells. Furthermore, they found that the protein and mRNA levels of HIF-2α were similar under both normoxic and hypoxic conditions, though there was a significant increase in HIF-2α transactivation under hypoxic conditions.

Prolyl-4-hydroxylase domain (PHD) proteins are members of the 2-oxoglutarate/iron dependent dioxygenase superfamily and include PHD1, PHD2 and PHD3. Previous studies have shown that in many cell types the degradation of HIF-1α and HIF-2α is primarily mediated by oxygen-dependent proteasome and catalyzed by PHDs. However, in NP cells, the degradation of HIF-1α and HIF-2α is mainly mediated through 26S proteasome, irrespective of oxygen tension. Moreover, among all PHDs, only PHD2 controls limited HIF-1α degradation in an oxygen-dependent manner, while the degradation of HIF-2α is largely independent of PHD activity.

HIF-1 AS A PHENOTYPIC MARKER OF NP CELLS

Currently, the phenotype characteristics of NP cells have not been clearly defined. Using western blot and immunohistochemistry analysis, Rajpurkar, et al. demonstrated that the expression of HIF-1α was only found in NP cells, but not in AF and cartilaginous endplate cells, while HIF-1β expression levels were significant higher in NP cells than that in AF cells and cartilaginous endplate cells. Based on these data they suggested that the difference in the expression of the two HIF-1 isoforms provides a phenotypic signature that could be used to distinguish NP cells from neighboring AF cells and cartilaginous endplate cells.

In another study, Richardson, et al. detected the expres-
NP cell survival is crucial for the homeostasis and function of the IVD. The chemical microenvironment of IVD is harsh and is characterized by low oxygen tension. However, in vitro studies indicated that NP cells could survive at low oxygen tension without a significant loss of cell viability. Thus, NP cells may develop some mechanisms to ensure their survival in the hypoxic environment of the IVD.

It is known that the Fas and Fas ligand (FasL) system delivers a death signal that rapidly commits cells to apoptosis. Notably, Fas and FasL are coexpressed in the disc cells of herniated lumbar IVD tissues, a member of a growing family of β-galactoside-binding animal lectins, is involved in the regulation of cell adhesion and apoptosis. Zeng, et al. analyzed the interaction of HIF-1α with the gal-3 promoter in rat NP cells and found that the inhibition of HIF-1α down-regulated the promoter activity of gal-3, sequentially enhancing Fas/FasL-mediated NP cell apoptosis. They further confirmed that HIF-1α induces the expression of gal-3 and sequentially inhibits Fas/FasL-mediated apoptosis of NP cells.

Vascular endothelial growth factor (VEGF) and its receptors (VEGFRs) play crucial roles in both physiological and pathological angiogenesis. Several lines of evidence suggest that VEGF serves as a survival factor in normal IVD tissue. Fujita, et al. found that NP cells expressed both VEGF-A and mbVEGFR-1 (a membrane-bound form of VEGF-A receptor) and treatment of NP cells with the VEGF-A antagonist VEGFR-1-Fc led to NP cell apoptosis, suggesting that VEGF-A/VEGFR-1 cascade mediates an anti-apoptotic function in NP cells. Agrawal, et al. found that under hypoxic conditions, HIF-1α and HIF-2α regulated the promoter activity and expression level of cited2, a p300 binding protein. The forced expression or suppression of cited2 would result in corresponding changes in the expression of VEGF in NP cells. Based on these studies, we concluded that HIF-1 and HIF-2 may also serve to enhance NP cell survival in the specialized microenvironment of the IVD via HIF-1 and HIF-2 mediated VEGF expression.

In the presence of low oxygen tension, the energy metabolism of NP cells almost completely relies on anaerobic glycolysis. HIF plays an important role in the maintenance of the metabolic activities of NP cells. One of the important genes involved in promoting anaerobic glycolysis of NP cells is hypoxia responsive glucose transporter (GLUT). In all mammalian cells, GLUT is an integral membrane protein that presents on the cell surface and serves to transport glucose down its concentration gradient by diffusion.

Richardson, et al. analyzed the expression of GLUT-1, GLUT-3, GLUT-9 and HIF-1α in normal and degenerate human IVDs. The results indicated that HIF-1α, GLUT-1, GLUT-3 and GLUT-9 were coexpressed in normal human IVDs and an increase in HIF-1α expression was associated with increases in GLUT-1, GLUT-3 and GLUT-9 expression in NP cells. However, there was no correlation between the expression of HIF-1α and GLUT-1, 3 or 9 in AF cells. Interestingly, they also observed that the expression of GLUTs increased as IVD degeneration progressed.

Taken together, HIF-1 maintains the metabolic activities of NP cells under a hypoxic environment in IVDs, mainly via the regulation of GLUT-1, GLUT-3 and GLUT-9 expression.

Although disc cells constitute only 1% of the adult disc tissue by volume, these cells are responsible for maintaining the extracellular matrix of the disc, which is key to the function of IVDs. The homeostasis of extracellular matrix in IVDs is biologically regulated by the active maintenance of a balance between the anabolism and catabolism of disc cells. Degenerative disorders of the IVD are characterized by disequilibrium between extracellular matrix repair and degen-
The disc extracellular matrix is predominantly composed of proteoglycans and collagens, and extracellular matrix compositions of AF and NP are distinct.58 Proteoglycans are abundant in the NP, which permits the IVD to withstand compressive loads.59 The collagen network provides the tensile properties for the spine to bend and flex. Agrawal, et al.29 reported that HIF-1α promotes aggrecan (the major proteoglycan) synthesis directly by inducing its mRNA and protein expression and, possibly, indirectly, through lineage specification as well as the promotion of sulfation reactions.

Another component of the disc extracellular matrix is glycosaminoglycan, which is critical to preservation of the gelatinous nature of the NP.57 Glucose is known as an important factor for the synthesis of this large molecule.58 As mentioned above, HIF-1 regulates the expression of a number of GLUTs, which could promote NP cells to uptake glucose across the plasma membrane. In this aspect, HIF-1 reportedly promotes the synthesis of glycosaminoglycans. On the contrary, there are some disagreements about the role of HIFs in glycosaminoglycan synthesis. Gogate, et al.59 measured the effects of HIF-1α and HIF-2α on the promoter activity of β-1, 3-glucuronyltransferase-1 (GlcAT-1), a key enzyme in chondroitin sulfate (the major glycosaminoglycan) synthesis in NP cells. They found that HIF-1α and HIF-2α suppressed the promoter activity of GlcAT-1 through interactions with one or more HREs. These data indicate that HIFs serve as transcriptional repressors of GlcAT-1 in NP cells.

### CONCLUSION

In summary, a better understanding of the regulatory roles of HIF in the biological behavior of NP cells would shed new light on the prevention and biological repair of IVD degeneration. Based on the above discussion, it is reasonable to predict that HIF may be a potential target for the prevention and treatment of IVD degeneration, given that HIF could modulate the biological behavior of NP cells in regards to degeneration. Development of a HIF-targeted drug to enhance cell survival, maintain metabolic activities, stimulate extracellular matrix synthesis and control dystrophic mineralization in NP cells may lead to better management of IVD degeneration.
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REFERENCES

1. Apeltdoor AT, Bosmans JE, Ostelo RW, de Vet HC, van Tulder MW. Cost-effectiveness of a classification-based system for subacute and chronic low back pain. Eur Spine J 2012;21:1290-300.
2. Lin CW, Haas M, Maher CG, Machado LA, van Tulder MW. Cost-effectiveness of guideline-endorsed treatments for low back pain: a systematic review. Eur Spine J 2011;20:1024-38.
3. Ohtori S, Inoue G, Orita S, Eguchi Y, Ochiai N, Kishida S, et al. Transdermal fentanyl for chronic low back pain. Yonsei Med J 2012;53:788-93.
4. Oksuz E. Prevalence, risk factors, and preference-based health states of low back pain in a Turkish population. Spine (Phila Pa 1976) 2006;31:E968-72.
5. Ciapetti G, Granchi D, Devescovi V, Leonardi E, Greggi T, Di Silvestre M, et al. Ex vivo observation of human intervertebral disc tissue and cells isolated from degenerated intervertebral discs. Eur Spine J 2012;21 Suppl 1:S10-9.
6. Park MS, Lee HM, Hahn SB, Moon SH, Kim YT, Lee CS, et al. The association of the activation-inducible tumor necrosis factor receptor and ligand with lumbar disc herniation. Yonsei Med J 2007;48:839-46.
7. Roberts S. Disc morphology in health and disease. Biochem Soc Trans 2002;30(Pt 6):S864-9.
8. Chen CS, Cheng CK, Liu CL, Lo WH. Stress analysis of the disc adjacent to interbody fusion in lumbar spine. Med Eng Phys 2001;23:483-91.
9. Stemple DL. Structure and function of the notochord: an essential organ for chordate development. Development 2005;132:2503-12.
10. Rajparohit R, Risbud MV, Ducheyne P, Vresilovic EJ, Shapiro IM. Phenotypic characteristics of the nucleus pulposus: expression of hypoxia inducing factor-1, glucose transporter-1 and MMP-2. Cell Tissue Res 2002;308:401-7.
11. Yang X, Li X. Nucleus pulposus tissue engineering: a brief review. Eur Spine J 2009;18:1564-72.
12. Costa F, Sassi M, Ortolina A, Cardia A, Assietti R, Zerbi A, et al. Stand-alone cage for posterior lumbar interbody fusion in the treatment of high-degree degenerative disc disease: design of a new device for an "old" technique. A prospective study on a series of 116 patients. Eur Spine J 2011;20 Suppl 1:S46-56.
13. Taylor JR. Growth of human intervertebral discs and vertebral bodies. J Anat 1975;120(Pt 1):49-68.
14. Rudert M, Tillmann B. Lymph and blood supply of the human intervertebral disc. Cadaver study of correlations to discitis. Acta Orthop Scand 1993;64:37-40.
15. Fujita N, Markova D, Anderson DG, Chiba K, Toyama Y, Shapiro IM, et al. Expression of prolyl hydroxylases (PHDs) is selectively controlled by HIF-1 and HIF-2 proteins in nucleus pulposus cells of the intervertebral disc: distinct roles of PHD2 and PHD3 proteins in controlling HIF-1α activity in hypoxia. J Biol Chem 2012;287:16975-86.
16. Urban JP. The role of the physicochemical environment in determining disc cell behaviour. Biochem Soc Trans 2002;30(Pt 6):858-64.
17. Urban JP, Smith S, Fairbank JC. Nutrition of the intervertebral disc. Spine (Phila Pa 1976) 2004;29:2700-9.
18. Richardson SM, Knowles R, Tyler J, Mobasher A, Hoyaland JA. Expression of glucose transporters GLUT-1, GLUT-3, GLUT-9 and HIF-1α in normal and degenerate human intervertebral disc. Histochem Cell Biol 2008;129:503-11.
19. Carmeliet P, Dor Y, Herbert JM, Fukumura D, Braschelmann K, Dewerchin M, et al. Role of HIF-1α in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. Nature 1998;394:485-90.
20. Semenza GL. Hypoxia-inducible factor 1: oxygen homeostasis and disease pathophysiology. Trends Mol Med 2001;7:345-50.
21. Semenza GL. HIF-1: mediator of physiological and pathophysiological responses to hypoxia. J Appl Physiol 2000;88:1474-80.
22. Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Mol Cell Biol 1992;12:5447-54.
23. Zhao J, Zhang P, Qin L, Pan XH. Hypoxia is essential for bone-tendon junction healing: the molecular biological evidence. Int Orthop 2011;35:925-8.
24. Risbud MV, Guttapalli A, Stokes DG, Hawkins D, Danielson KG, Schaer TP, et al. Nucleus pulposus cells express HIF-1α under normoxic culture conditions: a metabolic adaptation to the intervertebral disc microenvironment. J Cell Biochem 2006;98:152-9.
25. Agrawal A, Gaigghate S, Smith H, Anderson DG, Albert TJ, Shapiro IM, et al. Cited2 modulates hypoxia-inducible factor-dependent expression of vascular endothelial growth factor in nucleus pulposus cells of the rat intervertebral disc. Arthritis Rheum 2008;58:3798-808.
26. Fujita N, Chiba K, Shapiro IM, Risbud MV. HIF-1α and HIF-2α degradation is differentially regulated in nucleus pulposus cells of the intervertebral disc. J Bone Miner Res 2012;27:401-12.
27. Zeng Y, Danielson KG, Albert TJ, Shapiro IM, Risbud MV. HIF-1 alpha is a regulator of galectin-3 expression in the intervertebral disc. J Bone Miner Res 2007;22:1851-61.
28. Ha KY, Koh IJ, Kirpalani PA, Kim YY, Cho YK, Kagah GS, et al. The expression of hypoxia inducible factor-1alpha and apoptosis in herniated discs. Spine (Phila Pa 1976) 2006;31:1309-13.
29. Agrawal A, Guttapalli A, Narayan S, Albert TJ, Shapiro IM, Risbud MV. Normoxic stabilization of HIF-1alpha drives glycolytic metabolism and regulates aggrecan gene expression in nucleus pulposus cells of the rat intervertebral disc. Am J Physiol Cell Physiol 2007;293:C621-31.
30. Gogate SS, Nasser R, Shapiro IM, Risbud MV. Hypoxic regulation of β-1,3-glucuronyltransferase 1 expression in nucleus pulposus cells of the rat intervertebral disc: role of hypoxia-inducible factor proteins. Arthritis Rheum 2011;63:1950-60.
31. Skubute R, Markova D, Freeman TA, Anderson DG, Dion AS, Williams CJ, et al. Hypoxia-inducible factor regulation of ANK expression in nucleus pulposus cells: possible implications in controlling dystrophic mineralization in the intervertebral disc. Arthritis Rheum 2010;62:2707-15.
32. Obach M, Navarro-Sabaté A, Caro J, Kong X, Duran J, Gómez M, et al. 6-Phosphofructo-2-kinase (pflhβ3) promoter contains hypoxia-inducible factor-1 binding sites necessary for transactivation in response to hypoxia. J Biol Chem 2004;279:53562-70.

33. Brahimı-Horn C, Berra E, Pouysségur J. Hypoxia: the tumor’s gateway to progression along the angiogenic pathway. Trends Cell Biol 2001;11:S32-6.

34. Boskkey AL. Signaling in response to hypoxia and normoxia in the intervertebral disc. Arthritis Rheum 2008;58:3637-9.

35. Semenza G. Signal transduction to hypoxia-inducible factor 1. Biochem Pharmacol 2002;64:993-8.

36. Kaufman B, Scharf O, Arbeit J, Ashcroft M, Brown JM, Bruck RK, et al. Proceedings of the Oxygen Homeostasis/Hypoxia Meeting. Cancer Res 2004;64:3350-6.

37. Risbud MV, Schipani E, Shapiro JM. Hypoxic regulation of nucleus pulposus cell survival: from niche to notch. Am J Pathol 2010;176:1577-83.

38. Takeda K, Fong GH. Prolyl hydroxylase domain 2 protein suppresses hypoxia-induced endothelial cell proliferation. Hypertension 2007;49:178-84.

39. Appelhoff RJ, Tian YM, Raval RR, Turley H, Harris AL, Pugh CW, et al. Differential function of the prolyl hydroxylases PHD1, PHD2, and PHD3 in the regulation of hypoxia-inducible factor. J Biol Chem 2004;279:38458-65.

40. Fujita N, Imai J, Suzuki T, Yamada M, Ninomiya K, Miyamoto K, et al. Vascular endothelial growth factor-A is a survival factor for nucleus pulposus cells in the intervertebral disc. Biochem Biophys Res Commun 2008;372:367-72.

41. Bibby SR, Urban JP. Effect of nutrient deprivation on the viability of intervertebral disc cells. Eur Spine J 2004;13:695-701.

42. Horner HA, Urban JP. 2001 Volvo Award Winner in Basic Science Studies: effect of nutrient supply on the viability of cells from the nucleus pulposus of the intervertebral disc. Spine (Phila Pa 1976) 2001;26:2543-9.

43. Park JB, Chang H, Kim KW. Expression of Fas ligand and apoptosis of disc cells in herniated lumbar disc tissue. Spine (Phila Pa 1976) 2001;26:618-21.

44. Hsu DK, Yang RY, Pan Z, Yu L, Salomon DR, Fung-Leung WP, et al. Targeted disruption of the galectin-3 gene results in attenuated disc cell death in a murine model of degenerative disc disease. J Bone Miner Res 2001;16:1073-83.

45. Takahashi S. Vascular endothelial growth factor (VEGF), VEGF receptors and their inhibitors for antiangiogenic tumor therapy. Biol Pharm Bull 2011;34:1785-8.

46. Malandrino A, Noailly J, Lacroix D. The effect of sustained compression on oxygen metabolic transport in the intervertebral disc decreases with degenerative changes. PLoS Comput Biol 2011;7:e1002112.

47. Holm S, Maroudas A, Urban JP, Selstam G, Nachemson A. Nutrition of the intervertebral disc: solute transport and metabolism. Connect Tissue Res 1981;8:101-19.

48. Semenza GL, Roth PH, Fang HM, Wang GL. Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. J Biol Chem 1994;269:23757-63.

49. Papandreou I, Cairns RA, Fontana L, Lim AL, Denko NC. HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. Cell Metab 2006;3:187-97.

50. Vannucci SI, Reinhart R, Maher F, Bondy CA, Lee WH, Vannucci RC, et al. Alterations in GLUT1 and GLUT3 glucose transporter gene expression following unilateral hypoxia-ischemia in the immature rat brain. Brain Res Dev Brain Res 1998;107:255-64.

51. Bell GI, Kayano T, Buse JB, Burant CF, Takeda J, Lin D, et al. Molecular biology of mammalian glucose transporters. Diabetes Care 1990;13:198-208.

52. Joost HG, Bell GI, Best JD, Birnbaum MJ, Charron MJ, Chen YT, et al. Nomenclature of the GLUT/SLC2A family of sugar/polyol transport facilitators. Am J Physiol Endocrinol Metab 2002;282:E974-6.

53. Bibby SR, Jones DA, Lee RB, Yu J, Urban JPG. The pathophysiology of the intervertebral disc. Joint Bone Spine 2001;68:537-42.

54. Bruehlmann SB, Rattner JB, Matyas JR, Duncan NA. Regional variations in the cellular matrix of the annulus fibrosus of the intervertebral disc. J Anat 2002;201:159-71.

55. Le Maitre CL, Freemont AJ, Hoyland JA. Localization of degenerative enzymes and their inhibitors in the degenerate human intervertebral disc. J Pathol 2004;204:47-54.

56. Le Maitre CL, Pockert A, Buttle DJ, Freemont AJ, Hoyland JA. Matrix synthesis and degradation in human intervertebral disc degeneration. Biochem Soc Trans 2007;35(Pt 4):652-5.

57. Boxberger JL, Sen S, Yerramalli CS, Elliott DM. Nucleus pulposus glycosaminoglycan content is correlated with axial mechanics in rat lumbar motion segments. J Orthop Res 2006;24:1906-15.

58. Kirsch T. Determinants of pathological mineralization. Curr Opin Rheumatol 2006;18:174-80.

59. Golub EE. Biominerallization and matrix vesicles in biology and pathology. Semin Immunopathol 2011;33:409-17.

60. Melrose J, Burkhard D, Taylor TK, Dillon CT, Read R, Cakm, et al. Calcification in the ovine intervertebral disc: a model of hydroxyapatite deposition disease. Eur Spine J 2009;18:479-89.

61. Giachelli CM. Ectopic calcification: gathering hard facts about soft tissue mineralization. Am J Pathol 1999;154:671-5.

62. Gavrilo J, Funk W, Bechtold PM, Pfeifer M, Feidler M, et al. Alterations in GLUT1 and GLUT3 glucose transporter gene expression in human intervertebral discs. Eur Spine J 2007;16(Suppl):1238-47.

63. Zaka R, Williams CJ. Role of the progressive ankylosis gene in cartilage mineralization. Curr Opin Rheumatol 2006;18:181-6.

64. Pendleton A, Johnson MD, Hughes A, Gavilo J, Ho AM, Doherty M, et al. Mutations in ANKH cause chondrocalcinosis. J Bone Miner Res 2007;22:E974-6.

65. Pearce SI, Pendleton A, Johnson MD, Hughes A, Gurley KA, Ho AM, et al. Mutations in ANKH cause chondrocalcinosis. J Bone Miner Res 2007;22:E974-6.