J Musculoskel Neuron Interact 2002; 2(6):554-560

Mini-Review Article

Hylonome

Genetic and molecular aspects of osteosarcoma

M.F. Hansen

Center for Molecular Medicine, University of Connecticut Health Center, Farmington, CT, USA

Keywords: Osteosarcoma, Tumor Suppressor Genes, Inherited Cancer, Cancer Predisposition, Bone Cancer

Osteosarcoma is the most common primary tumor of bone. It accounts for approximately 19% of all malignant tumors of bone. Osteosarcoma affects individuals in the general population at a frequency of approximately 1 in 100,000 with males slightly more commonly affected than females.

Molecular genetics of osteosarcoma

Osteosarcoma tumorigenesis has been linked to alterations in several genes. The first association of osteosarcoma with an inherited predisposition was the observation by Kitchin and Ellsworth that patients with bilateral retinoblastoma had an unusually high incidence of osteosarcomas regardless of whether the patient had been treated with radiation[1]. They concluded that as patients with bilateral disease had the inherited form of retinoblastoma that there must be a pleiotropic effect of the gene for retinoblastoma that resulted in an increased predisposition for secondary osteosarcomas. This predisposition was further demonstrated by the observation that osteosarcoma tumors from patients with bilateral retinoblastoma underwent tumor-specific loss of constitutional heterozygosity (LoH) for the same region of chromosome 13 that occurred in the retinoblastoma tumors[2]. This association was confirmed by the identification of the retinoblastoma susceptibility gene (RB1) on human chromosome 13 which permitted several groups to demonstrate that mutations in the RB1 gene occurred in a high percentage of osteosarcomas[3-12].

The second gene associated with osteosarcoma was the p53 gene. Li and Fraumeni had identified a familial cancer syndrome based on rhabdomyosarcoma and associated with breast cancer and other neoplasms[13]. Osteosarcoma was later confirmed as a part of the constellation of tumors within the Li-Fraumeni Syndrome[14-16]. Mutations in the p53 gene were first observed in sporadic osteosarcoma[17], and then followed by the discovery of germline mutations in Li-Fraumeni Syndrome families[18]. Subsequently it was shown that p53 is commonly mutated in sporadic osteosarcoma as well[9,19-23].

Both RB1 and p53 are considered prototypic tumor suppressor genes in that complete loss of function is required before tumorigenesis can occur. Both genes are involved in cell cycle regulation. The RB1 gene functions as a tumor suppressor by acting as the major regulator of the G1 to S phase progression in the cell cycle. The RB1 protein accomplishes this by binding to and suppressing the function of the E2F transcription factor[24]. The ability of the RB1 protein to bind E2F is controlled by phosphorylation, which is mediated primarily by the cyclin D1/CDK4 complex. The p16 protein product of the INK4A gene in turn inhibits CDK4. Thus, RB1 and p16 suppress cell proliferation while cyclin D1 and CDK4 promote proliferation. Several studies have suggested that the gene encoding the p16 protein (CDKN2A) is inactivated in osteosarcomas that lack RB mutations and that the p16-pRb cell-cycle control pathway is deregulated in the majority of osteosarcomas[25-27].

p53 also plays a role in cell cycle control by regulating DNA repair. p53 is believed to function as a cell cycle checkpoint after DNA damage following irradiation with cells appearing to enter a sustained arrest in the G2 phase of the cell cycle. p53 also has a critical role in regulating apoptosis[28]. Thus, expression of mutant forms of p53 likely alter cellular resistance to the DNA damage.

As with RB1, mutations in genes that regulate p53 have been identified in osteosarcoma. The MDM2 gene, located on chromosome 12q13 along with CDK4, encodes a protein that binds p53 and blocks the activity of the p53 by directing it to the ubiquitination pathway. Overexpression of MDM2 in osteosarcomas provides an alternative means to disrupt the normal p53 pathway[29-38]. Another protein involved in this pathway is the p14 product of the INK4A gene, which is transcribed from the same gene producing the p16 protein involved in the RB1 pathway. The p14 protein exerts a protective effect on p53 by binding to the MDM2 gene product. Alterations consistent with inactivation of p14 have been

Corresponding author: Marc F. Hansen, Ph.D., Center for Molecular Medicine, University of Connecticut Health Center, 263 Farmington, Avenue, Farmington CT 06030-0002, USA
E-mail: mhansen@nso2.uchc.edu

Accepted 1 August 2002

found in osteosarcoma tumors and cell lines[39,40].

Genes other than p53 and RB1 have also been associated with osteosarcoma. High frequencies of allelic loss have been detected at 3q and 18q, suggesting that at least two other tumor suppressor genes important in osteosarcoma may exist[41-43]. HER2/neu (c-erbB-2) overexpression has been observed in approximately 40% of cases and has been associated with early pulmonary metastases and decreased survival[44,45]. Bone morphogenetic proteins are important in the induction of cartilage and bone formation and patterning of skeletal elements[46]. Expression of bone morphogenetic protein type II receptor was found to correlate with metastasis in osteosarcomas[47].

Rothmund-Thomson Syndrome has also been linked to osteosarcoma[48-52]. Mutations in the RECQL4 gene have been implicated in at least a subset of Rothmund-Thomson Syndrome patients[51,53]. The RECQL4 gene product has homology to the E. coli DNA helicase RecQ, which has been implicated in double-strand break repair and suppression of illegitimate recombination[53,54]. DNA helicases function in all processes in which access to single-stranded DNA is required, including DNA replication, DNA repair and recombination, and transcription of RNA. The functions of the RecQ-like genes are unknown; however, a growing body of evidence points to a function in restarting DNA replication after the replication fork has become stalled and in suppressing genetic recombination and in ensuring accurate chromosome segregation[55-58].

Paget's Disease of bone has been linked to osteosarcoma as well. As early as 1889, Sir James Paget observed sarcomas arising in five of his 23 patients with osteitis deformans[59]. Various reports have placed the incidence of osteosarcoma in Paget's Disease between 0.7-5% of Paget's patients[60-63]. Osteosarcoma secondary to Paget's Disease is uncommon in patients with monostotic disease but may occur in up to 10 percent of patients with severe, polyostotic involvement[64]. Although the incidence of osteosarcoma in Paget's Disease is relatively low, it contributes significantly to the mortality and morbidity because of the high incidence of Paget's Disease in the population[65]; osteosarcoma related to Paget's Disease account for about 3% of all osteosarcomas[66], 20% of the patients with osteosarcoma who are older than 40 years of age[61] and as high as 50% of the patients with osteosarcoma over the age of 60[67]. Analysis of LoH in 96 sporadic osteosarcomas identified a putative tumor suppressor locus that mapped to chromosome 18q[43]. Analysis of osteosarcomas from patients with Paget's Disease revealed that these tumors also underwent LoH in this region[43]. This region has also been implicated in predisposition to familial Paget's Disease of bone[68,69], suggesting that the association between Paget's Disease and osteosarcoma may be due to common underlying genetic origins. Of interest, within this region lies the gene for the Receptor Activator of NF-kB (RANK), one of the major genes involved in regulation of bone remodeling[70-87]. However, although germline mutations in RANK have been discovered in Familial Expansile Osteolysis[88,89] and Expansile Skeletal Hyperphosphatasia[90], two skeletal hyperplasia syndromes similar to Paget's Disease of bone, and although osteoclasts in pagetic patients have been shown to be hypersensitive to RANKL suggesting that there may be an alteration in RANK signaling[91-93], examination of the TNFRSF11A locus for mutations in both Paget's patients and osteosarcoma tumor cell lines, has thus far failed to reveal mutations in the coding region of the gene[94, 95].

Other genetic questions in osteosarcoma

Osteosarcoma is a highly variable disease which likely reflects the cell of origin of the tumor in the mesenchymal-osteoblastic lineage. Osteosarcomas have traditionally been divided into two broad groups based on cell morphology: conventional histology comprising the osteoblastic, chondroblastic, fibroblastic and small cell forms of osteosarcoma and the atypical histology osteosarcoma including parosteal, periosteal, telangiectatic, high-grade surface, giant cell and well-differentiated intraosseus osteosarcomas[96,97]. The molecular genetic bases of these variations in histology have yet to be systematically explored.

The age of onset of osteosarcoma can be divided into three distinct peaks. The first peak is from 10 to 25 years of age with a peak occurrence between ages 10 and 18 coinciding with active skeletal growth during the post-pubescent growth spurt and primarily occurs at appendicular skeletal locations with the majority of tumors occurring at the distal femur/proximal tibia[98]. The second peak occurs between 30 and 40 years old and affects primarily the head and neck, most commonly the mandible[99-106]. The third peak takes place after the sixth decade of life and primarily affects the axial skeleton and is almost exclusively related to Paget's disease of bone.

Six to 13% of osteosarcomas occur in the head and neck with the most common site being the mandible, followed by the maxilla and the other bones of the skull[107-110]. Based on degree of cellular atypia, frequency of local versus distant metastases, time until metastases, and median age of onset, there is strong evidence that osteosarcoma of the long bones and osteosarcomas of the head and neck represent separate diseases[100,101,104,107-116]. One of the possible reasons for this difference in phenotype may be that the bones of the head and neck undergo a different program of development from those of the long bones of the skeleton[117]. In the precursors of the bones of the head and neck (the calvaria of the skull, the maxilla and the mandible), mesenchymal cells differentiate directly into osteoblasts in areas of membranous ossification in a process known as intramembranous ossification. In the remaining portions of the skeleton, mesenchymal cells differentiate into chondrocytes which secrete the characteristic extracellular matrix of hyaline cartilage. Cartilage models of bones (anlagen) are formed and subsequently replaced by bone in a process called endochondral ossification. Examination of the process of endochondral and intramembranous ossification has demonstrated that there are genes

which are expressed in common to both pathways as well as genes that appear to be unique to each of the pathways.

Telomere maintenance is regarded as a key mechanism in overcoming cellular senescence in tumor cells and is frequently achieved by the activation of telomerase. However, there is an alternative mechanism of telomere lengthening which is characterized by an absence of telomerase activity. In osteosarcomas, the majority of tumors appear to maintain their telomeres through this ALT pathway[118,119]. This is interesting in light of the discovery that osteosarcomas, unlike most childhood tumors, show a significant degree of aneuploidy[120-128]. Osteosarcoma tumor karyotypes range from near-diploid to near-hexaploid with many specimens showing multiple clones with different degrees of ploidy. The underlying basis for this variation is also unclear at present.

Conclusion - the future of osteosarcoma research

Thus the genetics of osteosarcoma has many opportunities for new discoveries. Microarray analysis, comparative genome hybridization, spectral karyotyping, mass spectroscopy proteonomics and transgenic mouse models all hold promise for new discoveries in the molecular genetics of this fascinating disease. All could lead to significant expansions of biomedical research horizons in this understudied disease, precipitate a paradigm shift in research, and lead to substantial improvements in the treatment of this serious disease.

Acknowledgments

This work is supported by NIH grants CA74802, AR44904 and AR47684.

References

1. Kitchin FD, Ellsworth RM. Pleiotropic effects of the gene for retinoblastoma. J Med Genet 1974; 11:244-246.
2. Hansen MF, Koufos A, Gallie BL, Phillips RA, Fodstad O, Brogger A. Gedde-Dahl T, Cavenee WK. Osteosarcoma and retinoblastoma: a shared chromosomal mechanism revealing recessive predisposition. Proc Natl Acad Sci USA 1985; 82:6216-6220.
3. Friend SH, Bernards R, Rogelj S, Weinberg RA, Rapaport JM, Albert DM, Dryja TP. A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature 1986; 323:643-646.
4. Fung YK, Murphree AL, T'Ang A, Qian J, Hinrichs SH, Benedict WF. Structural evidence for the authenticity of the human retinoblastoma gene. Science 1987; 236:1657-1661.
5. Lee WH, Bookstein R, Hong F, Young LJ, Shew JY, Lee EY. Human retinoblastoma susceptibility gene: cloning, identification, and sequence. Science 1987; 235:1394-1399.
6. Weichselbaum RR, Beckett M, Diamond A. Some retinoblastomas, osteosarcomas, and soft tissue sarcomas may share a common etiology. Proc Natl Acad Sci USA 1988; 85:2106-2109.
7. Araki N, Uchida A, Kimura T, Yoshikawa H, Aoki Y, Ueda T, Takai S, Miki T, Ono K. Involvement of the retinoblastoma gene in primary osteosarcomas and other bone and soft-tissue tumors. Clin Orthop 1991; 270:271-277.
8. Hansen MF. Molecular genetic considerations in osteosarcoma. Clin Orthop 1991; 270:237-246.
9. Scholz RB, Kabisch H, Weber B. Roser K, Delling G, Winkler K. Studies of the RB1 gene and the p53 gene in human osteosarcomas. Pediatr Hematol Oncol 1992; 9:125-137.
10. Schreck RR. Tumor suppressor gene (Rb and p53) mutations in osteosarcoma. Pediatr Hematol Oncol 1992; 9:ix-x.
11. Hansen MF, Xu HJ, Zhao J, Hu SX, Raymong AK, Benedict WF. Expression patterns of the retinoblastoma susceptibility gene in conventional and variant histology osteosarcoma. In: Novak JF, McMaster JH (eds) Frontiers of osteosarcoma research. Hogrefe and Huber, Seattle, WA; 1993:347-357.
12. Wadayama B, Toguchida J, Shimizu T, Ishizaki K, Sasaki MS, Kotoura Y, Yamamuro T. Mutation spectrum of the retinoblastoma gene in osteosarcomas. Cancer Res 1994; 54:3042-3048.
13. Li FP, Fraumeni JF Jr. Soft-tissue sarcomas, breast cancer, and other neoplasms: A familial syndrome? Ann Intern Med 1969; 71:747-752.
14. Hartley AL, Birch JM, Marsden HB, Harris M. Breast cancer risk in mothers of children with osteosarcoma and chondrosarcoma. Br J Cancer 1986; 54:819-823.
15. Li FP, Fraumeni JF Jr, Mulvihill JJ, Blattner WA, Dreyfus MG, Tucker MA, Miller RW. A cancer family syndrome in twenty-four kindreds. Cancer Res 1988; 48:5358-5362.
16. Porter DE, Holden ST, Steel CM, Cohen BB, Wallace MR, Reid R. A significant proportion of patients with osteosarcoma may belong to Li-Fraumeni cancer families. J Bone Joint Surg Br 1992; 74:883-886.
17. Masuda H, Miller C, Koeffler HP, Barrifora H, Cline MJ. Rearrangement of the p53 gene in human osteogenic sarcomas. Proc Natl Acad Sci USA 1987; 84:7716-7719.
18. Malkin D, Li FP, Strong LC, Fraumeni JFJ, Nelson CE, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky MA, Friend SF. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 1990; 250:1233-1238.
19. Diller L, Kassel J, Nelson CE, Gryka MA, Litwak G, Gebhardt M, Bressac B, Ozturk M, Baker SJ, Vogelstein B. p53 functions as a cell cycle control protein in osteosarcomas. Mol Cell Biol 1990; 10:5772-5781.
20. Miller CW, Aslo A, Tsay C, Slamon D, Ishizaki K, Toguchida J, Yamamuro T, Lampklin B, Koeffler HP. Frequency and structure of p53 rearrangements in human osteosarcoma. Cancer Res 1990; 50:7950-7954.
21. Mulligan LM, Matlashewski GJ, Scable HJ, Cavenee WK. Mechanisms of p53 loss in human sarcomas. Proc Natl Acad Sci USA 1990; 87:5863-5867.
22. Iavarone A, Matthay KK, Steinkirchner TM, Israel MA. Germ-line and somatic p53 gene mutations in multifocal osteogenic sarcoma. Proc Natl Acad Sci USA 1992; 89:4207-4209.
23. Toguchida J, Yamaguchi T, Ritchie B, Beauchamp RKL, Dayton SH, Herrera GE, Yamamuro T, Kotoura Y, Sasaki MS, Little JB. Mutation spectrum of the p53 gene in bone and soft tissue sarcomas. Cancer Res 1992; 52:6194-6199.
24. Nevins JR. The Rb/E2F pathway and cancer. Hum Mol Genet 2001; 10:699-703.
25. Miller CW, Aslo A, Campbell MJ, Kawamata N, Lampkin

BC, Koeffler HP. Alterations of the p15, p16,and p18 genes in osteosarcoma. Cancer Genet Cytogenet 1996; 86:136-142.
26. Nielsen GP, Burns KL, Rosenberg AE, Louis DN. CDKN2A gene deletions and loss of p16 expression occur in osteosarcomas that lack RB alterations. Am J Pathol 1998; 153:159-163.
27. Benassi MS, Molendini L, Gamberi G, Ragazzini P, Sollazzo MR, Merli M, Asp J, Magagnoli G, Balladelli A, Bertoni F, Picci P. Alteration of pRb/p16/cdk4 regulation in human osteosarcoma. Int J Cancer 1999; 84:489-493.
28. Levine A. p53, the cellular gatekeeper for growth and division. Cell 1997; 88:323-331.
29. Khatib ZA, Matsushime H, Valentine M, Shapiro DN, Sherr CJ, Look AT. Coamplification of the CDK4 gene with MDM2 and GLI in human sarcomas. Cancer Res 1993; 53:5535-5541.
30. Ladanyi M, Cha C, Lewis R, Jhanwar SC, Huvos AG, Healey JH. MDM2 gene amplification in metastatic osteosarcoma. Cancer Res 1993; 53:16-18.
31. Florenes VA, Maelandsmo GM, Forus A, Andreassen A, Myklebost O, Fodstad O. MDM2 gene amplification and transcript levels in human sarcomas: relationship to TP53 gene status. J Natl Cancer Inst 1994; 86:1297-1302.
32. Nakayama T, Toguchida J, Wadayama B, Kanoe H, Kotoura Y, Sasaki MS. MDM2 gene amplification in bone and soft-tissue tumors: association with tumor progression in differentiated adipose-tissue tumors. Int J Cancer 1995; 64:342-346.
33. Tarkkanen M, Karhu R, Kallioniemi A, Elomaa I, Kivioja AH, Nevalainen J, Bohling T, Karaharju E, Hyytinen E, Knuutila S. Gains and losses of DNA sequences in osteosarcomas by comparative genomic hybridization. Cancer Res 1995; 55:1334-1338.
34. Miller CW, Aslo A, Won A, Tan M, Lampkin B, Koeffler HP. Alterations of the p53, Rb and MDM2 genes in osteosarcoma. J Cancer Res Clin Oncol 1996; 122: 559-565.
35. Lonardo F, Ueda T, Huvos AG, Healey J, Ladanyi M. p53 and MDM2 alterations in osteosarcomas: correlation with clinicopathologic features and proliferative rate. Cancer 1997; 79:1541-1547.
36. Pellin A, Boix-Ferrero J, Carpio D, Lopez-Terrada D, Carda C, Navarro S, Peydro-Olaya A, Triche TJ, Llombart-Bosch A. Molecular alterations of the RB1, TP53, and MDM2 genes in primary and xenografted human osteosarcomas. Diagn Mol Pathol 1997; 6:333-341.
37. Ragazzini P, Gamberi G, Benassi MS, Orlando C, Sestini R, Ferrari C, Molendini L, Sollazzo MR, Merli M, Magagnoli G, Bertoni F, Bohling T, Pazzagli M, Ricci P. Analysis of SAS gene and CDK4 and MDM2 proteins in low-grade osteosarcoma. Cancer Detect Prev 1999; 23:129-136.
38. Wunder JS, Eppert K, Burrow SR, Gokgoz N, Bell RS, Andrulis IL, Gogkoz N. Co-amplification and overexpression of CDK4, SAS and MDM2 occurs frequently in human parosteal osteosarcomas. Oncogene 1999; 18:783-788.
39. Benassi MS, Molendini L, Gamberi G, Magagnoli G, Ragazzini R, Gobbi GA, Sagiorgi L, Pazzaglia L, Asp J, Brantsing C, Picci P. Involvement of INK4A gene products in the pathogenesis and development of human osteosarcoma. Cancer 2001; 92:3062-3067.
40. Park YB, Park MJ, Kimura K, Shimizu K, Lee SH, Yokota J. Alterations in the INK4a/ARF locus and their effects on the growth of human osteosarcoma cell lines. Cancer Genet Cytogenet 2002; 133:105-111.
41. Yamaguchi T, Toguchida J, Yamamuro T, Kotoura Y, Takada N, Kawaguchi N, Kaneko Y, Nakamura Y, Sasaki MS, Ishizaki K. Allelotype analysis in osteosarcomas: frequent allele loss on 3q, 13q, 17p, and 18q. Cancer Res 1992; 52:2419-2423.
42. Kruzelock RP, Murphy EC, Stron LC, Hansen MF. Localization of a novel tumor suppressor locus on human chromosome 3q important in osteosarcoma tumorigenesis. Cancer Res 1997; 57:106-109.
43. Nellissery MJ, Padalecki SS, Brkanac Z. Singer FR, Roodman GD, Unni KK, Leach RJ, Hansen MF. Evidence for a novel osteosarcoma tumor suppressor gene in the chromosome 18 region genetically linked with Paget disease of bone. Am J Hum Genet 1998; 63:817-824.
44. Onda M, Matsuda S, Higaki S, Iijima T, Fukushima J, Yokokura A, Kojima T, Horiuchi H, Kurokawa T, Yamamoto T. ErbB-2 expression is correlated with poor prognosis for patients with osteosarcoma. Cancer 1996; 77:71-78.
45. Gorlick R, Huvos AG, Heller G, Aledo A, Beardsley GP, Healey JH, Meyers PA. Expression of HER2/erbB-2 correlates with survival in osteosarcoma. J Clin Oncol 1999; 17:2781-2788.
46. Hoffmann A, Gross G. BMP signaling pathways in cartilage and bone formation. Crit Rev Eukaryot Gene Expr 2001; 11:23-45.
47. Guo W, Gorlick R, Lladanyi M, Meyers PA, Huvos AG, Berino JR, Healey JH. Expression of bone morphogenetic proteins and receptors in sarcomas. Clin Orthop 1999; 365:175-183.
48. Drouin CA, Mongrain E, Sasseville E, Bouchard HL, Drouin M. Rothmund-Thomson syndrome with osteosarcoma. J Am Acad Dermatol 1993; 28:301-305.
49. Leonard A, Craft AW, Moss C, Malcolm AJ. Osteogenic sarcoma in the Rothmund-Thomson syndrome. Med Pediatr Oncol 1996; 26:249-253.
50. Spurney C, Gorlick R, Meyers PA, Healey JH, Huvos AG. Multicentric osteosarcoma, Rothmund-Thomson syndrome, and secondary nasopharyngeal non-Hodgkin's lymphoma: a case report and review of the literature. J Pediatr Hematol Oncol 1998; 20:494-497.
51. Lindor NM, Furuichi Y, Kitao S, Shimamoto A, Arndt C, Jalal S. Rothmund-Thomson syndrome due to RECQ4 helicase mutations: report and clinical and molecular comparisons with Bloom syndrome and Werner syndrome. Am J Med Genet 2000; 90:223-228.
52. Wang LL, Levy ML, Lewis RA, Chintagumpala MM, Lev D, Rogers M, Plon SE. Clinical manifestations in a cohort of 41 Rothmund-Thomson syndrome patients. Am J Med Genet 2001; 102:11-17.
53. Kitao S, Shimamoto A, Goto M, Miller RW, Smithson WA, Lindor NM, Furuichi Y. Mutations in RECQL4 cause a subset of cases of Rothmund-Thomson syndrome. Nat Genet 1999; 22:82-84.
54. Kitao S, Lindor NM, Shiratori M, Furuichi Y, Shimamoto A. Rothmund-Thomson syndrome responsible gene, RECQL4: genomic structure and products. Genomics 1999; 61:268-276.
55. Frei C, Gasser SM. RecQ-like helicases: the DNA replication checkpoint connection. J Cell Sci 2000; 113(Pt 15):2641-2646.
56. Karow JK, Wu L, Hickson ID. RecQ family helicases: roles in cancer and aging. Curr Opin Genet Dev 2000; 10:32-38.
57. van Brabant AJ, Stan R, Ellis NA. DNA helicases, genomic instability, and human genetic disease. Annu Rev Genomics Hum Genet 2000; 1:409-459.
58. Wu L, Hickson ID. RecQ helicases and topoisomerases: components of a conserved complex for the regulation of genetic recombination. Cell Mol Life Sci 2001; 58:894-901.

59. Paget J. Remarks on osteitis deformans. Illus Med News 1889; 2:181-182.
60. Freydinger JE, Duhig JT, McDonald LW. Sarcoma complicating Paget's disease of bone: A study of seven cases with report of one long survival after surgery. Arch Pathol 1963; 75:496-500.
61. Wick MR, Siegal GP, Unni KK, McLeod RA, Greditzer HGD. Sarcomas of bone complicating osteitis deformans (Paget's disease): fifty years' experience. Am J Surg Pathol 1981; 5:47-59.
62. Greditzer HG, McLeod RA, Unni KK, Beabout JW. Bone sarcomas in Paget disease. Radiol 1983; 146:327-333.
63. Hadjipavlou A, Lander P, Srolovitz H, Enker IP. Malignant transformation in Paget disease of bone. Cancer 1992; 70:2802-2808.
64. Fechner F, Se M. Tumors of the bones and joints. Atlas of tumor pathology, 3rd Series, Fascicle 8. Armed Forces Institute of Pathology; Washington D.C.; 1992.
65. Klein RM, Norman A. Diagnostic procedures for Paget disease Radiologic, pathologic and laboratory testing. Endocrinol Metab Clin North Am 1995; 24:437-450.
66. Unni KK, Dahlin DC. Premalignant tumors and conditions of bone. Am J Surg Pathol 1979; 3:47-60.
67. Huvos AG. Osteogenic sarcoma of bones and soft tissues in older persons. A clinicopathologic analysis of 117 patients older than 60 years. Cancer 1986; 57:1442-1449.
68. Cody JD, Singer FR, Roodman GD, Otterund B, Lewis TB, Lepert M, Leach RJ. Genetic linkage of Paget's disease of the bone to chromosome 18q. Am J Hum Genet 1997; 61:1117-1122.
69. Haslam SI, van Hul W, Morales-Piga A, Balemans W, San-Millan JL, Nakatsuka K, Willems P, Haites NE, Ralson SH. Paget's disease of bone: evidence for a susceptibility locus on chromosome 18q and for genetic heterogeneity. J Bone Miner Res 1998; 13: 911-917.
70. Anderson DM, Maraskovsky E, Billingsley WL, Dougall WC, Tometski ME, Roux ER, Teepe MC, DuBose RF, Cosman D, Galibert L. A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. Nature 1997; 390:175-179.
71. Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Sander S, Van G, Tarpley J, Derby P, Lee R, Boyle WJ. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 1997; 89:309-319.
72. Fuller K, Wong B, Fox S, Choi Y, Chambers TJ. TRANCE is necessary and sufficient for osteoblast-mediated activation of bone resorption in osteoclasts. J Exp Med 1998; 188:997-1001.
73. Nakagawa N, Kinosaki M, Yamaguchi K, Shima N, Yasuda H, Yano K, Morinaga T, Higashio K. RANK is the essential signaling receptor for osteoclast differentiation factor in osteoclastogenesis. Biochem Biophys Res Commun 1998; 253:395-400.
74. Yasuda H, Shima N, Nakagawa N, Mochizuki SI, Yano K, Fujise N, Sato Y, Goto M, Yamaguchi K, Kuyiyama M, Kanno T, Murakami A, Tsuda E, Morinaga T, Higashio K. Identity of osteoclastogenesis inhibitory factor (OCIF) and osteoprotegerin (OPG): a mechanism by which OPG/OCIF inhibits osteoclastogenesis *in vitro*. Endocrinology 1998; 139:1329-1337.
75. Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Moorinaga T, Higashio K, Udagawa N, Takahashi N, Suda T. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci USA 1998; 95:3597-3602.
76. Burgess TL, Qian Y, Kaufman S, Ring BD, Van G, Capparelli C. Kelley M, Hsu H, Boyle WJ, Dunstan CR, Hu S, Lacey DL. The ligand for osteoprotegerin (OPGL) directly activates mature osteoclasts. J Cell Biol 1999; 145:527-538.
77. Dougall WC, Glaccum M, Charrier K, Rohrbach K, Brasel K, De Smedt T, Daro E, Smith J, Tometsko ME, Maliszewski CR, Armstron A, Shen V, Bain S, Cosman D, Anderson D, Morrissey PJ, Peschon JJ, Schuh J. RANK is essential for osteoclast and lymph node development. Genes Dev 1999; 13:2412-2424.
78. Hsu H, Lacey DL, Dunstan CR, Solovyev I, Colombero A, Timms E, Tan HLL, Elliot G, Kelley MJ, Sarosi I, Wang L, Xia XZ, Elliott R, Chiu L, Black, T, Scully S, Capparelli C, Morony S, Shimamoto G, Bass MB, Boyle WJ. Tumor necrosis factor receptor family member RANK mediates osteoclast differentiation and activation induced by osteoprotegerin ligand. Proc Natl Acad Sci USA 1999; 96:3540-3545.
79. Kong YY, Yoshida H, Sarosi I, Tan HL, Eimms E, Capparelli C, Morony S, Oliveira dos-Santos AJ, Van G, Itie A, Khoo W. Wakeham A, Dunstan CR, Lacey DL, Mak TW, Boyle WJ, Penninger JM. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature 1999; 397:315-323.
80. Suda T, Takahashi N, Udagawa N, Jimi E, Gillespie MT, Martin TJ. Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Endocr Rev 1999; 20:345-357.
81. Udagawa N, Takahashi N, Jimi E, Matsuzaki K, Tsurukai T, Itoh K, Nakagawa N, Yasuda H, Goto M. Tsuda E, Higashio K, Gillespie MT, Martin TJ, Suda T. Osteoblasts/stromal cells stimulate osteoclast activation through expression of osteoclast differentiation factor/RANKL but not macrophage colony-stimulating factor: receptor activator of NF-kappa B ligand. Bone 1999; 25:517-523.
82. Wong BR, Besser D, Kim N, Arron JR, Vologodskaia M, Hanafusa H, Choi Y. TRANCE, a TNF family member, activates Akt/PKB through a signaling complex involving TRAF6 and c-Src. Mol Cell 1999; 4:1041-1049.
83. Fazzalari NL, Kuliwaba JS, Atkins GJ, Forwood MR, Findlay DM. The ratio of messenger RNA levels of receptor activator of nuclear factor kappaB ligand to osteoprotegerin correlates with bone remodeling indices in normal human cancellous bone but not in osteoarthritis. J Bone Miner Res 2001; 16:1015-1027.
84. Hofbauer LC, Heufelder AE. Role of receptor activator of nuclear factor kappaB ligand and osteoprotegerin in bone cell biology. J Mol Med 2001; 79:243-253.
85. Horowitz MC, Xi Y, Wilson K, Kacena MA. Control of osteoclastogenesis and bone resorption by members of the TNF family of receptors and ligands. Cytokine Growth Factor Rev 2001; 12:9-18.
86. Kostenuik PJ, Shalhoub V. Osteoprotegerin: a physiological and pharmacological inhibitor of bone resorption. Curr Pharm Des 2001; 7:613-635.

87. Thomas GP, Baker SU, Eisman JA, Gardiner EM. Changing RANKL/OPG mRNA expression in differentiating murine primary osteoblasts. J Endocrinol 2001; 170:451-460.
88. Hughes AE, Ralston SH, Marken J, Bell C, MacPherson H, Wallace RG, van Hul W, Whyte MP, Nakatsuka K, Hovy L, Anderson DM. Mutations in TNFRSF11A, affecting the signal peptide of RANK, cause familial expansile osteolysis. Nat Genet 2000; 24:45-48.
89. Whyte MP, Reinus WR, Podgornik MN, Mills BG. Familial expansile osteolysis (excessive RANK effect) in a 5-generation American kindred. Medicine (Baltimore) 2002; 81:101-121.
90. Whyte MP, Hughes AE. Expansile skeletal hyperphosphatasia is caused by a 15 base pair tandem duplication in TNFRSF11A encoding RANK and is allelic to familial expansile osteolysis. J Bone Miner Res 2002; 17:26-29.
91. Menaa C, Barsony J, Reddy SV, Cornish J, Cundy T, Roodman GD. 1,25-Dihydroxyvitamin D3 hypersensitivity of osteoclast precursors from patients with Paget's disease. J Bone Miner Res 2000; 15:228-236.
92. Menaa C, Reddy SV, Kurihara N, Maeda H, Anderson D, Cundy T, Cornish J, Singer FR, Bruder JM, Roodman GD. Enhanced RANK ligand expression and responsivity of bone marrow cells in Paget's disease of bone. J Clin Invest 2000; 105:1833-1838.
93. Neale SD, Smith R, Wass JA, Athanasou NA. Osteoclast differentiation from circulating mononuclear precursors in Paget's disease is hypersensitive to 1,25-dihydroxyvitamin D(3) and RANKL. Bone 2000; 27:409-416.
94. Sparks AB, Peterson SN, Bell C, Loftus BJ, Hocking L, Cahil DP, Frassica FJ, Streeten EA, Levine MA, Fraser CM, Adams MD, Broder S, Venter JC, Kinzler KW, Bogelstein B, Ralston SH. Mutation screening of the TNFRSF11A gene encoding receptor activator of NF kappa B (RANK) in familial and sporadic Paget's disease of bone and osteosarcoma. Calcif Tissue Int 2001; 68:151-155.
95. Wuyts W, Van Wesenbeeck L, Morales-Piga A, Ralston S, Hocking L, Vanhoenacker F, Westhovens R, Verbruggen L, Anderson D, Hughes A, Van Hul W. Evaluation of the role of RANK and OPG genes in Paget's disease of bone. Bone 2001; 28:104-107.
96. Dahlin DC, Unni KK. Bone tumors: general aspects and data on 8,542 cases. CC Thomas, Springfield; 1986.
97. Huvos AG. Bone tumors: diagnosis, treatment, and prognosis, WB Saunders; 1991.
98. Parkin DM, Stiller CA, Nectoux J. International variations in the incidence of childhood bone tumors. Int J Cancer 1993; 53:371-376.
99. Briant TD, Bird R. Osteogenic sarcoma of the mandible. J Otolaryngol 1981; 10:149-161.
100. Vener J, Rice DH, Newman AN. Osteosarcoma and chondrosarcoma of the head and neck. Laryngoscope 1984; 94:240-242.
101. Slootweg PJ, Muller H. Osteosarcoma of the jaw bones. Analysis of 18 cases. J Maxillofac Surg 1985; 13:158-66.
102. Tanzawa H, Uchiyama S, Sato K. Statistical observation of osteosarcoma of the maxillofacial region in Japan. Analysis of 114 Japanese cases reported between 1930 and 1989. Oral Surg Oral Med Oral Pathol 1991; 72:444-448.
103. Oda D, Bavisotto LM, Schmidt RA, McNutt M, Bruckner JD, Conrad EU III, Weymuller EA Jr. Head and neck osteosarcoma at the University of Washington. Head Neck 1997; 19:513-523.
104. Bennett JH, Thomas G, Evans AW, Speight PM. Osteosarcoma of the jaws: a 30-year retrospective review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000; 90:323-332.
105. Piattelli A, Favia GF. Periosteal osteosarcoma of the jaws: report of 2 cases. J Periodontol 2000; 71:325-329.
106. Mardinger O, Givol N, Talmi YP, Taicher S. Osteosarcoma of the jaw: The Chaim Sheba Medical Center experience. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001; 91:445-451.
107. Garrington GE, Scofield HH, Cornyn J, Hooker SP. Osteosarcoma of the jaws. Analysis of 56 cases. Cancer 1967; 20:377-391.
108. Caron AS, Hajdu SI, Strong EW. Osteogenic sarcoma of the facial and cranial bones. A review of forty- three cases. Am J Surg 1971; 122:719-725.
109. Clark JL, Unni KK, Dahlin DC, Devine KD. Osteosarcoma of the jaw. Cancer 1983; 51:2311-2316.
110. Delgado R, Maafs E, Alfeiran A, Mohar A, Barrera JL, Zinser J, Beltran A. Osteosarcoma of the jaw. Head Neck 1994; 16:246-252.
111. Bertoni F, Dallera P, Bacchini P, Marchetti C, Campobassi A. The Istituto Rizzoli-Beretta experience with osteosarcoma of the jaw. Cancer 1991; 68:1555-1563.
112. Mark RJ, Sercarz JA, Tran L, Dodd LG, Selch M, Calcaterra TC. Osteogenic sarcoma of the head and neck. The UCLA experience. Arch Otolaryngol Head Neck Surg 1991; 117:761-766.
113. Vege DS, Borges AM, Aggrawal K, Balasubramaniam G, Parikh DM, Bhaser B. Osteosarcoma of the craniofacial bones. A clinico pathological study. J Craniomaxillofac Surg 1991; 19:90-93.
114. Lewis M, Perl A, Som PM, Urken ML, Brandwein MS. Osteogenic sarcoma of the jaw. A clinicopathologic review of 12 patients. Arch Otolaryngol Head Neck Surg 1997; 123:169-174.
115. Ha PK, Eisele DW, Frassica FJ, Zahurak ML, McCarthy EF. Osteosarcoma of the head and neck: a review of the Johns Hopkins experience. Laryngoscope 1999; 109:964-969.
116. Daw NC, Mahmoud HH, Meyer WH, Jenkins JJ, Kaste SC, Poquette CA, Kun LE, Pratt CB, Rao BN. Bone sarcomas of the head and neck in children: the St Jude Children's Research Hospital experience. Cancer 2000; 88:2172-2180.
117. Olsen BR. Bone morphogenesis and embryologic development. In: Favus MJ (ed) Primer on the metabolic bone diseases and disorders of mineral metabolism. Lippincott William &Wilkins, Philadelphia; 1999:11-14.
118. Aue G, Muralidhar B, Schwartz HS, Butler MG. Telomerase activity in skeletal sarcomas. Ann Surg Oncol 1998; 5:627-634.
119. Scheel C, Schaefer KL, Jauch A, Keller M, Wai D. Brinkschmidt C. van Valen F, Boecker W. Dockhorn-Dworniczak B, Poremba C. Alternative lengthening of telomeres is associated with chromosomal instability in osteosarcomas. Oncogene 2001; 20:3835-3844.
120. Hiddemann W, Roessner A, Wormann B, Mellin W, Klockenkemper B, Bosing T, Buchner T, Grundmann E. Tumor heterogeneity in osteosarcoma as identified by flow cytometry. Cancer 1987; 59:324-328.
121. Bauer HC, Kreicbergs A, Silfversward C, Tribukait B. DNA analysis in the differential diagnosis of osteosarcoma. Cancer 1988; 61:2532-2540.
122. Fletcher JA, Gebhardt MC, Kozakewich HP. Cytogenetic

aberrations in osteosarcomas. Nonrandom deletions, rings, and double-minute chromosomes. Cancer Genet Cytogenet 1994; 77: 81-88.
123. Hoogerwerf WA, Hawkins AL, Perlman EJ, Griffin CA. Chromosome analysis of nine osteosarcomas. Genes Chromosomes Cancer 1994; 9:88-92.
124. Lopez-Gines C, Carda-Batalla C, Lopez-Terrada L, Llombart-Bosch A. Presence of double minutes and monosomy 17p in xenografted human osteosarcomas. Cancer Genet Cytogenet 1996; 90:57-62.
125. Tarkkanen M, Nordling S, Bohling T, Kivioja A, Karaharju ESJ, Elomaa I, Knuutila S. Comparison of cytogenetics, interphase cytogenetics, and DNA flow cytometry in bone tumors. Cytometry 1996; 26:185-191.
126. Bridge J, Nelson M, McComb E, McGuire MH, Rosenthal H, Vergara G, Maale GE, Spanier S, Neff JR. Cytogenetic findings in 73 osteosarcoma specimens and a review of the literature. Cancer Genet Cytogenet 1997; 95:74-87.
127. Boehm AK, Squire JA, Bayani J, Nelson M, Neff JR, Bridge JA. Cytogenetic findings in 35 osteosarcoma specimens and a review of the literature. Pediatr Pathol Mol Med 2000; 19:359-376.
128. Zielenska M, Bayani J, Pandita A, Toledo S, Marrano P, Andrade J, Petrilli A, Thorner P, Sorensen P, Squire JA. Comparative genomic hybridization analysis identifies gains of 1p35 approximately p36 and chromosome 19 in osteosarcoma. Cancer Genet Cytogenet 2001; 130:14-21.