Deciphering the Signaling Mechanisms of Osteosarcoma Tumorigenesis

Subjects: Oncology

Contributor: Bikesh K. Nirala , Taku Yamamichi , Jason T. Yustein

Osteosarcoma (OS) is the predominant primary bone tumor in the pediatric and adolescent populations. It has high metastatic potential, with the lungs being the most common site of metastasis. In contrast to many other sarcomas, OS lacks conserved translocations or genetic mutations; instead, it has heterogeneous abnormalities, including somatic DNA copy number alteration, ploidy, chromosomal amplification, and chromosomal loss and gain.

osteosarcoma signaling pathways oncogenes tumor suppressors

1. Introduction

Osteosarcoma (OS) is a rare cancer arising from the mesenchymal cells forming the bone. It is the most common and highly metastatic bone tumor in children and adolescents [1]. Its incidence is higher in adolescents (0.8–1.1/100,000/year in the age group of 15–19 years), with a second peak in older adults [2][3]. Nearly two-thirds of primary tumors occur near the knee joint, with the most common sites being the distal femur, proximal humerus and proximal tibia [4]. Despite the extensive genomic aberrations, OS has no pathognomonic DNA translocation or targetable mutations [5]. Thus, no effective molecularly targeted therapies for OS are currently available. The diagnosis of OS is based on morphological characteristics since no specific molecular markers or testing are available in clinical practice. The management of OS is challenging and requires a multidisciplinary approach. Surgical excision and systematic multiagent therapy are standard clinical practices for OS treatment. However, there is a pressing need to identify novel therapeutic approaches and biomarkers to manage the disease better, given the high relapse rate and poor prognosis of metastatic disease. One of the critical factors in OS development is chromosomal instability and genetic changes [6]. Oncogenes and tumor suppressor genes are often affected in OS. The immune system also plays a role in regulating tumor growth and propagation, and it is evident that the tumor-infiltrating immune cells contribute to the metastatic cascade [7]. Tumor metastasis is the primary challenge for OS therapy [8]. The five-year survival rate of OS has increased to about 70% since the 1970s, although it is only 20–30% for patients with metastasis [9]. Most OSs infiltrate the surrounding tissue and metastasize to the lung.

A better understanding of the bone microenvironment, the interaction between the tumor and non-tumor cells, and the mechanism of OS metastasis will help find a therapeutic target for OS. Several major signaling pathways have been identified in OS tumor development and metastasis, including the PIK3, JAK/STAT, Wnt/β-catenin, NOTCH, Hedgehog, Ras, TGF-β, MAPK/AKT/mTOR, RANK/RANKL, and NF-κB signaling pathways [10][11].

2. Molecular Abnormalities in Osteosarcoma

2.1. Chromosomal Abnormalities

OS is a genetically complex and heterogeneous tumor characterized by chromosomal instability and genetic alterations that lead to aneuploidy and increased tumor aggressiveness. The high rates of chromosomal rearrangements in OS include structural chromosomal abnormalities, such as translocations, deletions, amplifications, and chromothripsis, an extreme form of chromosomal instability [12]. Chromothripsis is associated with increased genomic instability and tumor progression, and it frequently occurs in highly aggressive tumors, including OS. Although the exact cause of chromothripsis and its role in tumorigenesis remain unclear, recent genomic studies have revealed that it is context-dependent and occurs at an overall incidence of between 2% and 3% in pan-cancer samples but over 77% in OS and 100% in liposarcoma [13]. Some potential mechanisms underlying chromothripsis are emerging, including the generation of DNA breaks and rejoining of the DNA fragments, generation of micronuclei, premature chromosome condensation, breakage–fusion–bridge cycle and telomere dysfunction, and ionizing radiation [14]. While the exact mechanistic cause of chromothripsis is still undefined, Crasta et al. identified the micronuclei, having many features of primary nuclei, formed from the acentric fragments of chromosomes, which produce DNA damage behind the chromothripsis [15]. Zhang et al., by using a combination of live cell imaging and single-cell genome sequencing, demonstrated that micronucleus formation could indeed generate a spectrum of genomic rearrangements, which recapitulate the features of chromothripsis [16]. Gong et al. described how Ran GTPase-activating protein 1 (RanGAP1) is commonly reduced or inactivated in human OS, leading to a high probability of chromothripsis, which drives tumorigenesis through its direct effects on the spindle-assembly checkpoint and decatenation and secondary effects on DNA damage surveillance [17].

2.2. Inactivation of Tumor Suppressor Genes and Amplification of Oncogenes

Inactivation of tumor suppressor genes such as *TP53*, *RB1*, *ATRX*, and *DLG2* is frequently observed in OS, which is thought to be involved in OS tumorigenesis [18]. The *TP53* gene is OS's most frequently dysregulated gene [18]. Whole-genome DNA sequencing from OS tumor samples demonstrates multiple somatic chromosomal lesions, including structural variations (SVs) and copy number alterations (CNAs). Kataegis is a single nucleotide variation (SNV) detected in 50% of OS tumors. Chen et al. identified p53 pathway lesions in all OS patients, while it was translocated in around 50% of the patients to the first intron of the *TP53* gene, leading to gene inactivation. This mechanism of *TP53* gene inactivation is unique to OS among pediatric cancers [18]. The p53 protein is a tumor suppressor protein involved in DNA damage recognition that induces apoptosis, cellular quiescence, or senescence. Another tumor suppressor gene frequently inactivated in OS is *RB1*, located at chromosome *13q14.2* [19]. *RB1* encodes the tumor suppressor protein pRB, which is vital for preventing cell cycle progression. *ATRX* is an important tumor suppressor in OS, and it is a part of a multiprotein complex that regulates chromatin remodeling, nucleosome assembly, and telomere maintenance. Furthermore, a recent report noted that loss of ATRX promotes OS tumor through increased NF-κB signaling and integrin binding [20]. *DLG2* is a tumor suppressor gene, and its copy number loss occurs in 42% of human and 56% of canine OS [21]. Deletion of *Dlg2* in a mouse model led to the acceleration of OS development [21].

Hyperactivation of tumor-promoting genes such as *MYC* and *MDM2* is associated with OS tumorigenesis. The gain of the *8q24* chromosomal locus, which harbors the oncogene *MYC*, has been reported in several OS patients [22][23]. MYC is involved in cell cycle regulation, protein biogenesis, metabolism, signal transduction, transcription, and translation [24][25]. A recently generated *Myc* knock-in genetically engineered mouse model of an OS tumor not only identified intrinsic Myc-mediated mechanisms of OS tumorigenesis but also identified a novel molecular mechanism through which Myc regulates the profile and function of the OS immune landscape [26][27][28]. The oncoprotein MDM2 is a p53 inhibitor, which promotes p53 degradation and downregulates its transcription. Amplification of *MDM2* (chromosome *12q15*) is more frequent in OS metastasis and recurrence.

2.3. Epigenetic Modification in OS Progression

Epigenetic modifications, including DNA methylation, histone acetylation, and methylation, are critical in the pathogenesis of several cancers, including OS [29]. The level of histone H3 lysine trimethylation was reported to be lower in human OS tissue and cell lines compared with normal bone tissue and osteoblast cells. Enhancement of H3 methylation after treatment with the histone lysine demethylase inhibitor 5-carboxy-8-hydroxyquinoline (IOX-1) showed inhibition of OS migratory and invasive capabilities. Enhanced histone H3 lysine trimethylation levels sensitized cisplatin against the cisplatin-resistant (MG63-CR) cells [30]. Previous studies showed enhancement of the expression of the lysine-specific demethylases KDM1A, KDM2B, KDM4A, KDM6A, KDM6B in OS progression [31][32][33][34]. Recently, Twenhafel et al. provided a comprehensive review of recent advances in the epigenetics of OS and highlighted the clinical benefits in the field of OS research [35]. Morrow et al. highlighted the genetic and epigenetic defects in OS and emphasized the role of epigenetic dysregulation in tumor suppression and oncogene regulation [6].

References

1. Lindsey, B.A.; Markel, J.E.; Kleinerman, E.S. Osteosarcoma Overview. Rheumatol. Ther. 2017, 4, 25–43.
2. Strauss, S.J.; Frezza, A.M.; Abecassis, N.; Bajpai, J.; Bauer, S.; Biagini, R.; Bielack, S.; Blay, J.Y.; Bolle, S.; Bonvalot, S.; et al. Bone sarcomas: ESMO-EURACAN-GENTURIS-ERN PaedCan Clinical Practice Guideline for diagnosis, treatment and follow-up. Ann. Oncol. 2021, 32, 1520–1536.
3. Cole, S.; Gianferante, D.M.; Zhu, B.; Mirabello, L. Osteosarcoma: A Surveillance, Epidemiology, and End Results program-based analysis from 1975 to 2017. Cancer 2022, 128, 2107–2118.
4. Heare, T.; Hensley, M.A.; Dell'Orfano, S. Bone tumors: Osteosarcoma and Ewing's sarcoma. Curr. Opin. Pediatr. 2009, 21, 365–372.
5. Martin, J.W.; Squire, J.A.; Zielenska, M. The Genetics of Osteosarcoma. Sarcoma 2012, 2012, 627254.

6. Morrow, J.J.; Khanna, C. Osteosarcoma Genetics and Epigenetics: Emerging Biology and Candidate Therapies. Crit. Rev. Oncog. 2015, 20, 173–197.
7. Louise, M.E.J.; Emma, E.R.; Craig, D.L.; Willem, W.O. The immune system in cancer metastasis: Friend or foe? J. ImmunoTherapy Cancer 2017, 5, 79.
8. Bielack, S.S.; Kempf-Bielack, B.; Delling, G.; Exner, G.U.; Flege, S.; Helmke, K.; Kotz, R.; Salzer-Kuntschik, M.; Werner, M.; Winkelmann, W.; et al. Prognostic factors in high-grade osteosarcoma of the extremities or trunk: An analysis of 1702 patients treated on neoadjuvant cooperative osteosarcoma study group protocols. J. Clin. Oncol. 2002, 20, 776–790.
9. Mirabello, L.; Troisi, R.J.; Savage, S.A. Osteosarcoma incidence and survival rates from 1973 to 2004. Cancer 2009, 115, 1531–1543.
10. Adamopoulos, C.; Gargalionis, A.N.; Basdra, E.K.; Papavassiliou, A.G. Deciphering signaling networks in osteosarcoma pathobiology. Exp. Biol. Med. 2016, 241, 1296–1305.
11. Li, S.; Zhang, H.; Liu, J.; Shang, G. Targeted therapy for osteosarcoma: A review. J. Cancer Res. Clin. Oncol. 2023.
12. Leibowitz, M.L.; Zhang, C.Z.; Pellman, D. Chromothripsis: A New Mechanism for Rapid Karyotype Evolution. Annu. Rev. Genet. 2015, 49, 183–211.
13. Isidro, C.-C.; June-Koo, L.; Ruibin, X.; Dhawal, J.; Youngsook, L.J.; Lixing, Y.; Dmitry, G.; Leszek, J.K.; Cheng-Zhong, Z.; David, S.P.; et al. Comprehensive analysis of chromothripsis in 2658 human cancers using whole-genome sequencing. Nat. Genet. 2020, 52, 331–341.
14. Rode, A.; Maass, K.K.; Willmund, K.V.; Lichter, P.; Ernst, A. Chromothripsis in cancer cells: An update. Int. J. Cancer 2016, 138, 2322–2333.
15. Crasta, K.; Ganem, N.J.; Dagher, R.; Lantermann, A.B.; Ivanova, E.V.; Pan, Y.; Nezi, L.; Protopopov, A.; Chowdhury, D.; Pellman, D. DNA breaks and chromosome pulverization from errors in mitosis. Nature 2012, 482, 53–58.
16. Zhang, C.Z.; Spektor, A.; Cornils, H.; Francis, J.M.; Jackson, E.K.; Liu, S.; Meyerson, M.; Pellman, D. Chromothripsis from DNA damage in micronuclei. Nature 2015, 522, 179–184.
17. Gong, Y.; Zou, S.; Deng, D.; Wang, L.; Hu, H.; Qiu, Z.; Wei, T.; Yang, P.; Zhou, J.; Zhang, Y.; et al. Loss of RanGAP1 drives chromosome instability and rapid tumorigenesis of osteosarcoma. Dev. Cell 2023, 58, 192–210.e11.
18. Chen, X.; Bahrami, A.; Pappo, A.; Easton, J.; Dalton, J.; Hedlund, E.; Ellison, D.; Shurtleff, S.; Wu, G.; Wei, L.; et al. Recurrent somatic structural variations contribute to tumorigenesis in pediatric osteosarcoma. Cell Rep. 2014, 7, 104–112.
19. Wong, F.L.; Boice, J.D., Jr.; Abramson, D.H.; Tarone, R.E.; Kleinerman, R.A.; Stovall, M.; Goldman, M.B.; Seddon, J.M.; Tarbell, N.; Fraumeni, J.F., Jr.; et al. Cancer incidence after

retinoblastoma. Radiation dose and sarcoma risk. JAMA 1997, 278, 1262–1267.

20. Bartholf DeWitt, S.; Hoskinson Plumlee, S.; Brighton, H.E.; Sivaraj, D.; Martz, E.J.; Zand, M.; Kumar, V.; Sheth, M.U.; Floyd, W.; Spruance, J.V.; et al. Loss of ATRX promotes aggressive features of osteosarcoma with increased NF-κB signaling and integrin binding. JCI Insight 2022, 7, e151583.
21. Shao, Y.W.; Wood, G.A.; Lu, J.; Tang, Q.-L.; Liu, J.; Molyneux, S.; Chen, Y.; Fang, H.; Adissu, H.; McKee, T.; et al. Cross-species genomics identifies DLG2 as a tumor suppressor in osteosarcoma. Oncogene 2019, 38, 291–298.
22. Smida, J.; Baumhoer, D.; Rosemann, M.; Walch, A.; Bielack, S.; Poremba, C.; Remberger, K.; Korsching, E.; Scheurlen, W.; Dierkes, C.; et al. Genomic Alterations and Allelic Imbalances Are Strong Prognostic Predictors in Osteosarcoma. Clin. Cancer Res. 2010, 16, 4256.
23. Rao, P.H.; Zhao, S.; Zhao, Y.J.; Yu, A.; Rainusso, N.; Trucco, M.; Allen-Rhoades, W.; Satterfield, L.; Fuja, D.; Borra, V.J.; et al. Coamplification of Myc/Pvt1 and homozygous deletion of Nlrp1 locus are frequent genetics changes in mouse osteosarcoma. Genes Chromosomes Cancer 2015, 54, 796–808.
24. Sayles, L.C.; Breese, M.R.; Koehne, A.L.; Leung, S.G.; Lee, A.G.; Liu, H.-Y.; Spillinger, A.; Shah, A.T.; Tanasa, B.; Straessler, K.; et al. Genome-Informed Targeted Therapy for Osteosarcoma. Cancer Discov. 2019, 9, 46.
25. Grisanzio, C.; Freedman, M.L. Chromosome 8q24-Associated Cancers and MYC. Genes Cancer 2010, 1, 555–559.
26. Nirala, B.K.; Patel, T.D.; Kurenbekova, L.; Shuck, R.; Dasgupta, A.; Rainusso, N.; Coarfa, C.; Yustein, J.T. MYC regulates CSF-1 expression via microRNA 17/20a to modulate tumor-associated macrophages in osteosarcoma. JCI Insight 2023, 8, e164947.
27. Nirala, B.K.; Kurenbekova, L.; Shuck, R.L.; Patel, T.; Rajapakshe, K.; Yustein, J.T. Development and characterization of a c-Myc-driven preclinical mouse model of osteosarcoma to investigate the tumor immune microenvironment. Cancer Res. 2022, 82 (Suppl. 12), 1668.
28. Nirala, B.K.; Kurenbekova, L.; Patel, T.; Shuck, R.L.; Dasgupta, A.; Rainusso, N.C.; Yustein, J.T. Abstract 6713: Myc-regulated miR17, 20a modulate RANK expression in osteosarcoma. Cancer Res. 2023, 83 (Suppl. 7), 6713.
29. Lu, Y.; Chan, Y.-T.; Tan, H.-Y.; Li, S.; Wang, N.; Feng, Y. Epigenetic regulation in human cancer: The potential role of epi-drug in cancer therapy. Mol. Cancer 2020, 19, 79.
30. Chang, S.L.; Lee, C.W.; Yang, C.Y.; Lin, Z.C.; Peng, K.T.; Liu, S.C.; Wang, S.W.; Tsai, H.C.; Fong, Y.C.; Lai, C.Y.; et al. IOX-1 suppresses metastasis of osteosarcoma by upregulating histone H3 lysine trimethylation. Biochem. Pharmacol. 2023, 210, 115472.

31. Kurmasheva, R.T.; Erickson, S.W.; Han, R.; Teicher, B.A.; Smith, M.A.; Roth, M.; Gorlick, R.; Houghton, P.J. In vivo evaluation of the lysine-specific demethylase (KDM1A/LSD1) inhibitor SP-2577 (Seclidemstat) against pediatric sarcoma preclinical models: A report from the Pediatric Preclinical Testing Consortium (PPTC). Pediatr. Blood Cancer 2021, 68, e29304.
32. Shi, X.; Fan, M. Tip60-dependent acetylation of KDM2B promotes osteosarcoma carcinogenesis. J. Cell. Mol. Med. 2019, 23, 6154–6163.
33. He, C.; Sun, J.; Liu, C.; Jiang, Y.; Hao, Y. Elevated H3K27me3 levels sensitize osteosarcoma to cisplatin. Clin. Epigenetics 2019, 11, 8.
34. Chen, M.; Jiang, Y.; Sun, Y. KDM4A-mediated histone demethylation of SLC7A11 inhibits cell ferroptosis in osteosarcoma. Biochem. Biophys. Res. Commun. 2021, 550, 77–83.
35. Twenhafel, L.; Moreno, D.; Punt, T.; Kinney, M.; Ryznar, R. Epigenetic Changes Associated with Osteosarcoma: A Comprehensive Review. Cells 2023, 12, 1595.