Strong Opponents: Overcoming Obstacles of Osteosarcoma Metastatic Adaptation in The Lungs

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Abstract

Osteosarcoma (OS) five-year survival rates drop from approximately 70% to 20% when it has metastasized to the lungs. Thus, there is an urgent need to understand the processes that allow these cancer cells to metastasize. In particular, it is critical to identify how the premetastatic niche is formed and how the cancer cells adapt to the lung microenvironment. This review paper surveys the current literature on the mechanisms of this. Here we describe the role of driver mutations, cancer-associated fibroblasts, extracellular matrix stiffness, extracellular signaling, and other mechanisms contributing to metastasis. OS tumor cells secrete the molecules CXCL14, TRIM66, and Tim-3 to generate alterations to the tumor microenvironment that facilitate lung metastasis through epithelial-to-mesenchymal transition and actomyosin contractility. The activity of the MAPK and Wnt signaling pathways have been shown to correlate with the dysregulation of necessary normal processes including cell apoptosis, proliferation, and tissue homeostasis. These mechanisms can help inform future therapies targeting these essential components of OS lung metastasis.

Introduction

Osteosarcoma (OS) is a primary malignant bone tumor known for its high incidence among children and young adults. Survival rates for this cancer fall from approximately 70% to 20% when it metastasizes, with the lungs as its most common location of metastasis.[1] Cancer metastasis is a daunting diagnosis due to its implications of the cancer having spread past the primary tumor site: the area in which the cancer originated. The low survival rates of patients who exhibit secondary lesions of osteosarcoma place an urgency to find ways to prevent the spread of malignant cancer cells in the first place. To metastasize to the lungs, OS tumor cells must overcome many obstacles presented by the hostile environment of the lung. During the metastatic cascade, cancer cells travel from the primary tumor to other body parts, starting with detachment, where cancer cells separate from the primary tumor. Then, intravasation takes the cells into the circulatory system, where they travel to form a secondary lesion in the body. This leads them to many instances of a "metastatic bottleneck," which results in the thinning of disseminated tumor cell populations, most notably at the early stages of dissemination.[2] Therefore, one must refer to the OS tumor cell adaptation methods to discover any causes of their successful proliferation. Studying the metastatic cascade may assist researchers in finding key vulnerabilities for cancer therapies in the future.

Next, one could examine key features of OS metastasis colonization in the lung. Many attributes of the tumor microenvironment that facilitate the entry of OS tumor cells in forming secondary lesions can be targeted by researchers to reduce cases of metastatic fulfillment. For example, this may range from the intrinsic ability of OS tumor cells to metastasize to the activities of fibroblasts residing in the tumor microenvironment. Extracellular matrix (ECM) stiffness is a process that requires the involvement of cancer-associated fibroblasts (CAFs) to harden the proteins of the ECM in the tumor microenvironment. Changes to the microenvironment, such as ECM stiffness, can also be delved into to further clarify the processes that allow for the successful proliferation of OS tumor cells. Research into epigenetics, which are heritable fluctuations in gene expression that do not result from alterations in the DNA sequence, may yield answers regarding the intrinsic potential for metastasis in OS tumor cells.[3] This review will provide an analysis regarding many studies of OS metastatic processes in the lung.

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[1] Jéssica Albuquerque M. Silva et al., "CT Features of Osteosarcoma Lung Metastasis: A Retrospective Study of 127 Patients," *Jornal Brasileiro De Pneumologia*, March 29, 2023, e20220433, https://doi.org/10.36416/1806-3756/e20220433.

[2] Camille A. McAloney et al., "Host-derived Growth Factors Drive ERK Phosphorylation and MCL1 Expression to Promote Osteosarcoma Cell Survival During Metastatic Lung Colonization," *Cellular Oncology* 47, no. 1 (September 7, 2023): 259–82, https://doi.org/10.1007/s13402-023-00867-w.

[3] James P. Hamilton, "Epigenetics: Principles and Practice," *Digestive Diseases* 29, no. 2 (January 1, 2011): 130–35, https://doi.org/10.1159/000323874.

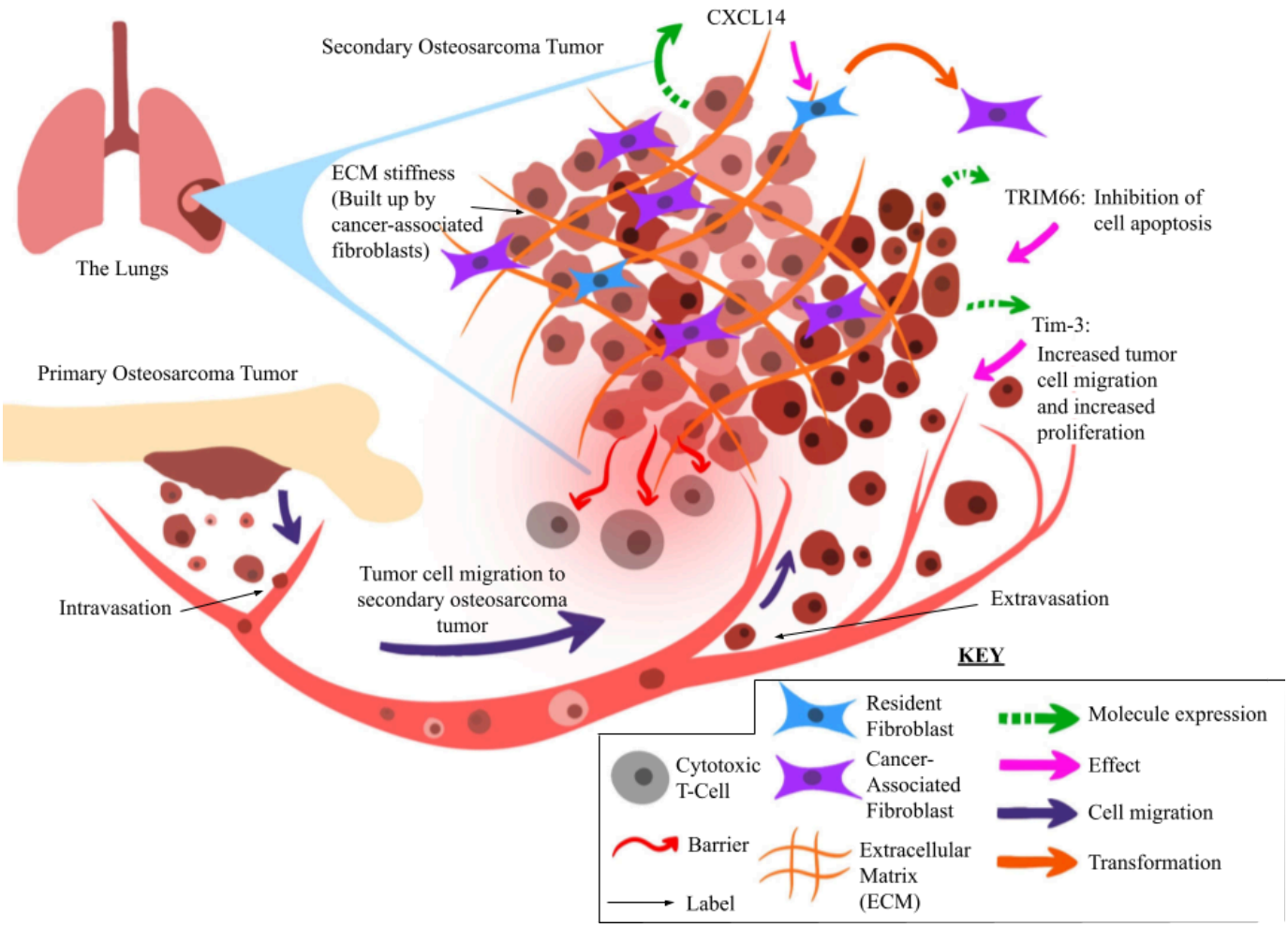

Figure 1. Overview of key features of osteosarcoma metastasis in the lung.

Intrinsic Metastatic Potential of Osteosarcoma

The molecular nature of OS cells should be considered when studying their metastatic process. This can be analyzed by exploring the epigenetics involved with the primary OS tumor and specific genes called oncogenes. Oncogenes can be defined as cellular genes that have lost their function due to fusion with another gene, driver mutations, or overexpression and can cause cells to grow and divide uncontrollably.[4] Driver mutations are mutations in oncogenes that act as a necessary step in cancer growth and dissemination, playing a key role in the development of ordinary cells into cancer cells.[5] Cancer cells then often rely on the overexpression of such oncogenes to boost survival of the metastatic process.[6] Paired with the

[4] Geoffrey Brown, "Oncogenes, Proto-Oncogenes, and Lineage Restriction of Cancer Stem Cells," *International Journal of Molecular Sciences* 22, no. 18 (September 7, 2021): 9667, https://doi.org/10.3390/ijms22189667.

[5] *NCI Dictionary of Cancer Terms*, s.v. "driver mutation."

[6] R Kumar et al., "Epigenomic Regulation of Oncogenesis by Chromatin Remodeling," Oncogene 35, no. 34 (January 25, 2016): 4423–36, https://doi.org/10.1038/onc.2015.513.

dysregulation of chromatin remodeling–an inadequate expression of regulatory genes–culminates in oncogenesis. In oncogenesis, chromatin remodeling can perform epigenomic regulation of oncogene expression. By identifying novel candidate genes showing increased levels of chromatin activity, such as PPP1R1B, PREX1, and IGF2BP1, Singh et al. revealed that among the others, the loss of phosphatidylinositol-3,4,5-trisphosphate dependent Rac exchange factor 1 (PREX1) resulted in a significant negative impact on OS proliferation, migration, and capacity to form colonies.[7] They also suggested that notable Wnt signaling played a prominent role in OS growth, colony formation, invasion, and metastasis. In normal circumstances, the Wnt signaling pathway regulates critical cellular functions such as proliferation, tissue homeostasis, cell death, cell differentiation, migration, and invasion.[8] Thus, the avid expression of this mechanism in OS processes implies that it is involved in OS expansion and metastasis. In a study done by McAloney, Makkawi, et al., they analyzed single-cell RNA sequencing data from primary OS tumors at different points of metastasis development and revealed that the microenvironment of OS tumor cells led them to evolve from being phenotypically homogenous to a state more similar to primary lesions in tumor heterogeneity.[9] Through the usage of an orthotopic cell line and PDX models of OS, Rajan et al. displayed that OS tumor cells maintain transcriptional heterogeneity as they respond to the changing conditions of their microenvironment.[10] And so, tumor heterogeneity can be considered a mechanism through which OS tumor cells adapt to their microenvironment. Moreover, the MAPK pathway, activated by the binding of growth factors to tyrosine kinase receptors, was demonstrated to increase the expression of the MCL1 gene, which inhibits cell apoptosis. MCL1 was found to occur in higher numbers in metastatic OS tumors compared to primary tumors. It can, therefore, be a potential target for therapies as an essential component of OS metastasis. Overall, the intrinsic ability of OS tumor cells to metastasize can be attributed to the overexpression of specific oncogenes and increased levels of chromatin activity.

Extracellular Signaling Molecules Facilitate Metastasis

In cell-to-cell communication, signaling molecules are present to mediate cellular function. CXCL14, or C-X-C motif chemokine ligand 14, is a signaling molecule that plays a notable role in OS metastasis. To begin, chemokines like CXCL14 are small proteins that are

[7] Irtisha Singh et al., “Intrinsic Epigenetic State of Primary Osteosarcoma Drives Metastasis,” *Molecular Cancer Research* 22, no. 9 (June 6, 2024): 864–78, https://doi.org/10.1158/1541-7786.mcr-23-0055.

[8] Ya Zhang and Xin Wang, “Targeting the Wnt/B-catenin Signaling Pathway in Cancer,” *Journal of Hematology & Oncology* 13, no. 1 (December 1, 2020), https://doi.org/10.1186/s13045-020-00990-3.

[9] McAloney et al., “Host-Derived Growth Factors Drive ERK Phosphorylation and MCL1 Expression to Promote Osteosarcoma Cell Survival During Metastatic Lung Colonization,” September 7, 2023.

[10] Sanjana Rajan et al., “Osteosarcoma Tumors Maintain Intra-tumoral Transcriptional Heterogeneity During Bone and Lung Colonization,” *BMC Biology* 21, no. 1 (April 27, 2023), https://doi.org/10.1186/s12915-023-01593-3.

part of the secreted elements involved in tumor communication.[11] According to the study done by Xu, Deng, et al., CXCL14 supports lung metastatic formation by binding a candidate receptor integrin α11β1 axis and activating actomyosin contractility and matrix remodeling abilities.[12] Actomyosin contractility is a process needed by all types of cell migration to some degree and is thus high around the boundaries of invading cancer cells.[13] Additionally, this chemokine was positively associated with stromal cluster-related pathways involving collagen. From this, they observed that normal tissue-associated fibroblasts turned into metastasis tissue-associated fibroblasts that create an immunosuppressive pulmonary metastatic niche by preventing the entrance of CD8+ T-cells. Thus, its inability to enter the metastatic niche to rid it of metastasis-associated cells ultimately generates a problematic situation for the body's immune system.

Furthermore, higher expression of transcription intermediate factor 1δ, TIF1δ (TRIM66) has been correlated to higher rates of local recurrence and lung metastasis and a shorter survival time for patients afflicted with OS. In this study, Chen, Guo, et al. found that through the TGF-β signaling pathway, TRIM66 was able to act as an oncogene, promoting OS proliferation and metastasis.[14] TRIM66 also inhibited cell apoptosis via the down-regulation of p53 expression in OS cells. Additionally, the altered expression of TRIM66 and other TRIM molecules, such as TRIM24, has revealed that the family of molecules is associated with cancers and cancer-related diseases.

It has been shown that T-cell immunoglobulin mucin domain molecule-3 (Tim-3) expression facilitates epithelial-mesenchymal transition (EMT), a process that reduces cell-cell interactions and increases cell migration through activating the NF-kB/Snail signaling pathway.[15] Thus, various signaling pathways activated by signaling molecules facilitate metastasis due to their activity in activating fibroblasts and association with environments that facilitate OS metastasis.

The Impact of Cancer-Associated Fibroblasts

[11] Elin Sjöberg et al., "Expression of the Chemokine CXCL14 in the Tumour Stroma Is an Independent Marker of Survival in Breast Cancer," *British Journal of Cancer* 114, no. 10 (April 26, 2016): 1117–24, https://doi.org/10.1038/bjc.2016.104.

[12] Yanyang Xu et al., "Osteosarcoma Cells Secrete CXCL14 That Activates Integrin A11β1 on Fibroblasts to Form a Lung Metastatic Niche," *Cancer Research* 84, no. 7 (January 31, 2024): 994–1012, https://doi.org/10.1158/0008-5472.can-23-1307.

[13] Irene Rodriguez-Hernandez et al., "Rho, ROCK and Actomyosin Contractility in Metastasis as Drug Targets," *F1000Research* 5 (April 29, 2016): 783, https://doi.org/10.12688/f1000research.7909.1.

[14] Yu Chen et al., "TRIM66 Overexpresssion Contributes to Osteosarcoma Carcinogenesis and Indicates Poor Survival Outcome," *Oncotarget* 6, no. 27 (June 17, 2015): 23708–19, https://doi.org/10.18632/oncotarget.4291.

[15] Z.M. Feng and S.M. Guo, "Tim-3 Facilitates Osteosarcoma Proliferation and Metastasis Through the NF-κB Pathway and Epithelial-mesenchymal Transition," *Genetics and Molecular Research* 15, no. 3 (January 1, 2016), https://doi.org/10.4238/gmr.15037844; Giuseppina Sannino et al., "Epithelial-to-Mesenchymal and Mesenchymal-to-Epithelial Transition in Mesenchymal Tumors: A Paradox in Sarcomas?," Cancer Research 77, no. 17 (August 16, 2017): 4556–61, https://doi.org/10.1158/0008-5472.can-17-0032.

CAFs, frequently identified by fibroblast activation proteins (FAPs) and α-smooth muscle actin (α-SMA) proteins, are a prevalent component of the tumor microenvironment. These cells play a prominent role in tumor progression and sustaining metastasis.[16] Additionally, they facilitate increased extracellular matrix (ECM) stiffness. Depending on context, normal fibroblasts are resting mesenchymal cells embedded within the interstitial fibrillar ECM.[17] Active fibroblasts associated with cancer are termed so due to their nature in co-evolving with cancer by acquiring a tumor-supporting phenotype. In a study done by Wang et al., bone marrow stem cells (BMSCs) were exposed to OS cells and displayed a higher expression of the α-SMA protein, possibly indicating the presence of CAFs, smooth muscle cells, or pericytes.[18] Additionally, they found that human bone marrow stem cells' proliferation and invasion ability (HBMSCs) significantly improved in samples exposed to various OS cells. This reveals that exposure to OS cells leads to a differentiation of HBMSC characteristics and the aforementioned CAF differentiation. Moreover, CAFs gain more metabolic activity than normal fibroblasts and have higher production rates of a more rigid collagen disposition.[19] In a study done by Zhihao et al., it was shown that CAFs play a prominent role in inducing a phenotypic transformation of the tumor and forming the immune microenvironment during OS progression.[20]

Myofibroblasts are differentiated from the previously mentioned fibroblasts and specialize in tissue repair. Inside the cell, fibroblasts synthesize and lay down fibril matrices in a controlled manner for standard processes. That being said, in OS conditions, tissue fibrosis may eliminate fibril matrix degradation, leading to the buildup of such structures. Myofibroblast reprogramming of non-fibrogenic cells creates an over-prolonged cycle of myofibroblast activation. In a study done by Zhang et al., adding fibronectin (FN) to tumorsphere formation-incapable OS cells assisted them in gaining tumor sphere-forming ability.[21] This was done considering that the accumulation of FN is an integral part of facilitating the proliferation and fibrotic activity of myofibroblasts through integrins. By analyzing the prevalence of these various cell markers, the presence of CAFs may give us further insight on OS cell occurrence.

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[16] Raisa A. Glabman, Peter L. Choyke, and Noriko Sato, “Cancer-Associated Fibroblasts: Tumorigenicity and Targeting for Cancer Therapy,” Cancers 14, no. 16 (August 12, 2022): 3906, https://doi.org/10.3390/cancers14163906.

[17] Xueman Chen and Erwei Song, “Turning Foes to Friends: Targeting Cancer-associated Fibroblasts,” *Nature Reviews Drug Discovery* 18, no. 2 (November 23, 2018): 99–115, https://doi.org/10.1038/s41573-018-0004-1.

[18] Yu-Ming Wang, Wei Wang, and En-Duo Qiu, “Osteosarcoma Cells Induce Differentiation of Mesenchymal Stem Cells Into Cancer Associated Fibroblasts Through Notch and Akt Signaling Pathway,” August 1, 2017, https://pmc.ncbi.nlm.nih.gov/articles/PMC6965449/.

[19] Glabman, Choyke, and Sato, “Cancer-Associated Fibroblasts: Tumorigenicity and Targeting for Cancer Therapy.”

[20] Zhang Zhihao et al., “Cancer-associated Fibroblast Infiltration in Osteosarcoma: The Discrepancy in Subtypes Pathways and Immunosuppression,” *Frontiers in Pharmacology* 14 (June 27, 2023), https://doi.org/10.3389/fphar.2023.1136960.

[21] Wu Zhang et al., “Adaptive Fibrogenic Reprogramming of Osteosarcoma Stem Cells Promotes Metastatic Growth,” *Cell Reports* 24, no. 5 (July 1, 2018): 1266-1277.e5, https://doi.org/10.1016/j.celrep.2018.06.103.

Extracellular Matrix Stiffness and OS Lung Metastasis

The extracellular matrix (ECM) is a network of proteins responsible for supporting the cells and tissues of the body and providing cells with regulatory environmental cues.[22] That being said, they exist within the tumor microenvironment, where they form a significant physical barrier that presents challenges to immunotherapy by preventing the entrance of immune cells and blocking the proper delivery of immunotherapeutic agents.[23]

This is done by a process known as ECM stiffness, a hardening of ECM proteins facilitated by CAFs. Lung fibrosis, which is achieved by ECM stiffening, is one of the modifications made by metastatic tumor cells to prepare the environment for colonization.[24] A study on high lung metastasis mice injected with OS LM8 cells displayed higher amounts of activated fibroblasts and fine collagen fibers in OS lung metastasis. In most solid cancers, fine collagen fiber remodeling plays a notable part in tumor progression and altering the tumor microenvironment to be more facilitative for cancer, such as with ECM stiffness.[25] It was also shown that lung fibrosis began after metastatic colonization in the lungs and significantly increased the rates of lung colonization of the OS cells. Note that LM8 cells are a type of OS mouse cell line. Furthermore, an intracellular signaling molecule that has been shown to facilitate metastasis is the YAP molecule. By comparing the expression levels of the YAP molecule in primary and metastatic regions, Yui et al., observed that there was an increased presence of YAP molecules in metastatic areas compared to the primary tumor.[26] Moreover, they found that YAP molecule expression decreased in a softer environment. This suggests that a stiffer environment through mechanisms such as ECM stiffness would be needed to facilitate metastatic adaptation processes.

Conclusion

Although many OS processes seem to have unsolvable characteristics, targeting crucial points of the disease to look for potential therapies in the future may ultimately lead researchers on a path of discovery. Through more research with clinical trials with patients in the most precarious stages of OS–when metastasis has already taken place–more solutions can be brought up regarding the treatment of latter-stage OS. A study by Silva et al. revealed that out of seventy-five cases diagnosed with lung metastases, calcification was observed in 47%. The role of lung calcification in facilitating OS lung metastasis remains unclear and requires further research. There is a current lack of research incorporating lung calcification in mice, which may

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[22] Ting Guo et al., “Extracellular Matrix Stiffness in Lung Health and Disease,” *Comprehensive Physiology*, June 29, 2022, 3523–58, https://doi.org/10.1002/cphy.c210032.

[23] Zizhao Mai et al., “Modulating Extracellular Matrix Stiffness: A Strategic Approach to Boost Cancer Immunotherapy,” *Cell Death and Disease* 15, no. 5 (May 1, 2024), https://doi.org/10.1038/s41419-024-06697-4.

[24] Yoshihiro Yui et al., “Lung Fibrosis Is a Novel Therapeutic Target to Suppress Lung Metastasis of Osteosarcoma,” *International Journal of Cancer* 151, no. 5 (March 28, 2022): 739–51, https://doi.org/10.1002/ijc.34008.

[25] Sanja Z. Despotović et al., “Altered Organization of Collagen Fibers in the Uninvolved Human Colon Mucosa 10 Cm and 20 Cm Away From the Malignant Tumor,” *Scientific Reports* 10, no. 1 (April 14, 2020), https://doi.org/10.1038/s41598-020-63368-y.

[26] Yui et al., “Lung Fibrosis Is a Novel Therapeutic Target to Suppress Lung Metastasis of Osteosarcoma.”

hold explanations regarding any possible adverse effects of lung calcification in human patients. The role of CAFs in facilitating the creation of a tough lung calcification environment can be targeted for inhibition. Lung calcification may play a similar role to ECM stiffness, creating an immunosuppressive environment to block out the body's defense mechanisms. Similarly, it directly alters the cellular phenotype in which the sarcoma resides in the lungs. A crucial part of OS lung metastasis is the alteration of the tumor microenvironment with processes such as ECM stiffness. These changes play a large part in facilitating further expansion of OS metastasis. PREX1, which was mentioned before to play an important role in OS sustenance, may be a novel target for future OS therapies. Further trials focusing on inhibiting the PREX1 gene as a potential therapy may yield results on its true contributions to further OS metastasis, as the lack of PREX1 may deter some aspects of OS growth in the body. Cell-to-cell communication between the components of OS through the overexpression of signaling molecules such as CXCL14, TRIM66, and Tim-3 allows for the activation of CAFs and, thus, the generation of a malignant environment.

Many characteristics of the OS metastasis colonization process can be studied further in order to reveal potential therapies for cancer treatment. For example, the results of the study done by Yui et al. explain the increased stiffness of the microenvironment, which can be seen through the processes of ECM stiffness during OS metastasis in the lung.[27] Increasing the softness of the tumor microenvironment and inhibiting the formation of a scaffold may then limit tumor cells in metastasizing. Furthermore, treatments such as AZD5991, a macrocyclic molecular inhibitor with a high affinity for MCL1, have been shown to reduce and even eliminate detectable metastatic cancer in mice.[28] By targeting MCL1 expression in OS metastases, they observed a significant decrease in metastatic colonization of OS cells, even more so when paired with other chemotherapeutic agents. They then suggested using MCL1 as a target in future human and canine clinical trials, as it showed promising therapeutic potential. AZD5991 and other MCL1 inhibitors have already been undergoing human trials for hematogenous malignancies and continue to be a viable option for more expanded use in the future.[29] Moreover, the FDA-approved antifibrotic agents nintedanib and pirfenidone were shown to suppress OS lung metastasis by targeting lung fibrosis.[30] In severe combined immunodeficiency (SCID) mice, the utilization of an FDA-approved multi-kinase inhibitor named sorafenib that inhibited OS cell line proliferation by targeting Raf-kinases such as RAF-1 and B-RAF led to cell apoptosis and the downregulation of oncogenes such as MCL1 that reduced the tumor volume

[27] Yui et al., "Lung Fibrosis Is a Novel Therapeutic Target to Suppress Lung Metastasis of Osteosarcoma."

[28] McAloney et al., "Host-Derived Growth Factors Drive ERK Phosphorylation and MCL1 Expression to Promote Osteosarcoma Cell Survival During Metastatic Lung Colonization."

[29] Haolan Wang et al., "Targeting MCL-1 in Cancer: Current Status and Perspectives," *Journal of Hematology & Oncology* 14, no. 1 (April 21, 2021), https://doi.org/10.1186/s13045-021-01079-1.

[30] Yui et al., "Lung Fibrosis Is a Novel Therapeutic Target to Suppress Lung Metastasis of Osteosarcoma."

of OS xenografts.[31] The targeting of signaling molecules that have been shown to facilitate OS metastasis, such as CXCL14, TRIM66, or Tim-3, may also present therapeutic opportunities for repromoting cell apoptosis and inhibiting increased proliferation of OS cancer cells. Additionally, the increased expression of these molecules in OS tissue may make them accurate indicators of the sarcoma. Overall, the process of OS adaptation and metastasis in the lungs heavily emphasizes altering the tumor microenvironment through interactions between already existing components of the lung, such as fibroblasts, specific pathways, and the ECM.

[31] Ymera Pignochino et al., “Sorafenib Blocks Tumour Growth, Angiogenesis and Metastatic Potential in Preclinical Models of Osteosarcoma Through a Mechanism Potentially Involving the Inhibition of ERK1/2, MCL-1 and Ezrin Pathways,” Molecular Cancer 8, no. 1 (January 1, 2009): 118, https://doi.org/10.1186/1476-4598-8-118.

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