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NON-THEMATIC REVIEW

Cross-talk between lung cancer and bones results in neutrophils that promote tumor progression

Patrick O. Azevedo[1] · Ana E. Paiva[1] · Gabryella S. P. Santos[1] · Luiza Lousado[1] · Julia P. Andreotti[1] · Isadora F. G. Sena[1] · Carlos A. Tagliati[2] · Akiva Mintz[3] · Alexander Birbrair[1,3]

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Abstract
Lung cancer is the leading cause of cancer mortality around the world. The lack of detailed understanding of the cellular and molecular mechanisms participating in the lung tumor progression restrains the development of efficient treatments. Recently, by using state-of-the-art technologies, including *in vivo* sophisticated Cre/loxP technologies in combination with lung tumor models, it was revealed that osteoblasts activate neutrophils that promote tumor growth in the lung. Strikingly, genetic ablation of osteoblasts abolished lung tumor progression *via* interruption of SiglecF^{high}–expressing neutrophils supply to the tumor microenvironment. Interestingly, SiglecF^{high} neutrophil signature was associated with worse lung adenocarcinoma patients outcome. This study identifies novel cellular targets for lung cancer treatment. Here, we summarize and evaluate recent advances in our understanding of lung tumor microenvironment.

Keywords Osteoblasts · Neutrophils · Lung · Tumor microenvironment

1 Introduction

Lung cancer is the major cause of death worldwide [1]. Although meaningful increment has been made in our knowledge of disease pathogenesis, lung cancer is still a fatal disease [2]. The initiation and progression of lung cancer are attributed to genetic and certain environmental factors, such as air pollution and exposure to tobacco smoke. Despite several improvements, treatments like surgery, radiotherapy, and chemotherapy can rarely control completely this disease. Patients with lung cancer have a limited long-term survival, mainly due to the escape of tumor initiating cells of the initial treatment [3]. Since these escaped cells are more resistant to treatments, adjuvant therapies that could effectively destroy these remaining cancer cells would have a considerable impact on tumor treatment.

In the past few decades, research groups have focused their concentration mostly on cancer cells [4]. Nevertheless, emerging evidence demonstrates that the surroundings where these malignant cells are located play key roles in tumor development [5]. Tumor microenvironment is the local environment where tumorigenesis and tumor growth occur, composed of blood vessels, innervations, extracellular matrix, signaling molecules, growth factors, and other non-malignant cells, including immune cells, mesenchymal stem cells, fibroblasts, adipocytes, and pericytes [6–35]. The tumor microenvironment plays important roles in tumor initiation, development, invasion, and metastasis [36]. The constituents of the tumor microenvironment may cross-talk with cancer cells as well as between them.

Lung tumors are also comprised of neoplastic cells and their surrounding microenvironment, which affects the proliferation, survival, migration, and drug resistance of lung cancer cells [37]. Although enormous advancement has been accomplished in our understanding of the importance of the tumor microenvironment, each new discovery informs us how complex is the control of tumor growth. The relationship between the lung cancer cells and the microenvironment in which they reside plays a pivotal role in determining whether and how

✉ Alexander Birbrair
birbrair@icb.ufmg.br

[1] Department of Pathology, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

[2] Department of Clinical and Toxicological Analysis, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

[3] Department of Radiology, Columbia University Medical Center, New York, NY, USA

these malignant cells grow. The capacity to prevent, limit, or reverse lung tumor growth in cancer patients unfortunately has not progressed significantly, due to the complexity of the mechanisms underlying this process. Uncovering the cellular and molecular mechanisms by which the tumor growth is controlled is crucial for the success of clinical applications.

Neutrophils are the most abundant circulating cells in the blood, being constantly produced in the bone marrow due to their short lifespan [38]. After inflammatory stimuli, they migrate to tissues where they perform their functions [39]. Interestingly, growing evidence suggests that neutrophils may regulate tumor development [40]. Although neutrophils may present pro- or anti-tumoral activity, depending on specific tumor microenvironments [41], we are still far from fully understanding their function in the lung tumor microenvironment, and how this role can be regulated. Now, in an article in *Science*, Engblom and colleagues reveal the heterogeneity of neutrophils in the lung cancer microenvironment, and that only SiglecF^{high}–expressing neutrophils promote tumor growth [42]. The authors investigated the systemic cross-talk between lung tumor and the bone that regulates the recruitment of pro-tumoral neutrophils by using elegant state-of-the-art techniques, including *in vivo* sophisticated Cre/loxP technologies in combination with several lung tumor models. Engblom and colleagues revealed, by using fluorescence-molecular tomography and Ocn-YFP mice, that lung tumors disrupt bone homeostatic activity, by increasing the number of osteocalcin (Ocn)-expressing osteoblasts through tumor-secreted factors [42]. Strikingly, these osteoblasts, in turn, promote lung tumor growth through the priming of pro-tumoral neutrophils (Fig. 1). The authors demonstrated that genetic ablation of osteoblasts, by using Ocn-Cre/iDTR mice, abolished lung tumor progression, *via* the interruption of SiglecF^{high}–expressing neutrophils supply to the tumor microenvironment [42]. Moreover, Engblom and colleagues showed that SiglecF^{high}–expressing neutrophils expand tumor growth *in vivo*. Importantly, for translation into clinics, the authors found an association of SiglecF^{high} neutrophil signature with worse lung adenocarcinoma patients outcome. This study identifies a systemic communication between lung tumor and bone, even in the absence of metastasis. These results also offer novel therapeutic targets for lung tumor treatments.

Here, we discuss the findings from this work and evaluate recent advances in our understanding of the lung tumor microenvironment.

2 Perspectives/future directions

2.1 Tumor regulation of the immune system

Deciphering how exactly malignant cancer cells regulate the immune system promises to bring improvements in the way we treat cancer [43]. Immune cells within the tumor may

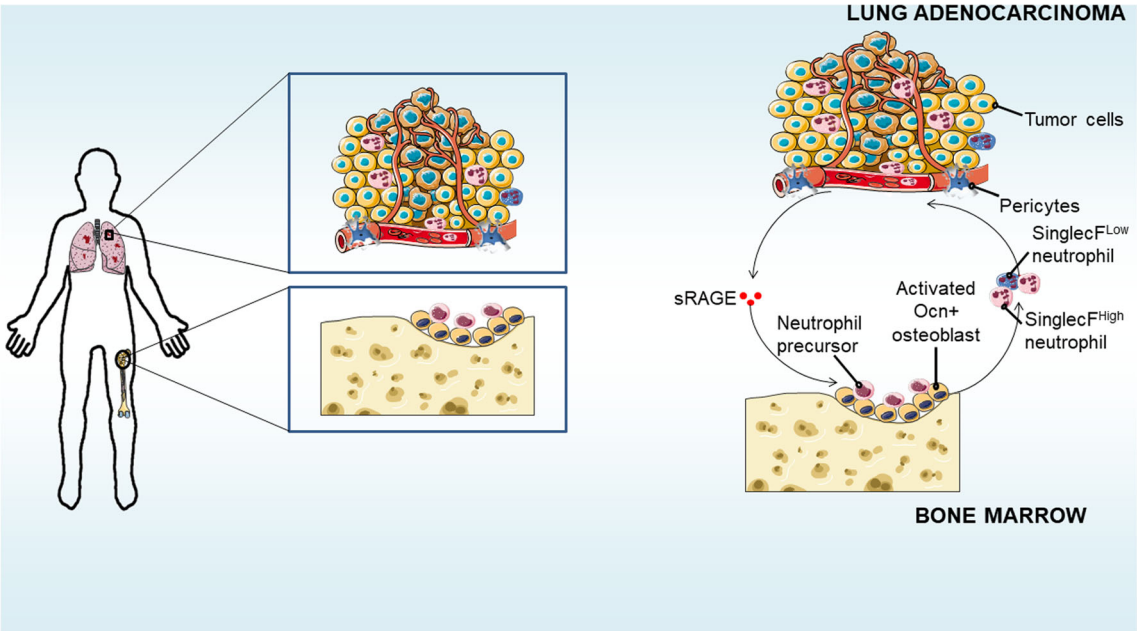

Fig. 1 Osteoblasts prime neutrophils that promote tumor growth in the lung. The relationship between the lung tumor and its surroundings plays a pivotal role in determining whether and how malignant cancer cells grow. The study of Engblom and colleagues now reveals a novel and very important function of systemic cross-talk between lung tumor and the bone that regulates cancer progression [42]. Lung tumors increase the number of osteocalcin (Ocn)-expressing osteoblasts through tumor-secreted factors. These osteoblasts prime SiglecF^{high}–expressing neutrophils, which in turn expand lung tumor growth *in vivo*. With the appearance of state-of-the-art techniques, future studies will reveal in detail the cellular and molecular components that regulate the lung tumor microenvironment

occupy as much as half of its mass in some tumors [44]. Thus, cancer malignant growth does not rely exclusively on its intrinsic genetic and epigenetic alterations. Besides its capacity to spread to secondary tissues as metastasis, the primary tumor can also interact with distant organs by tumor-induced systemic factors [45]. Such factors, e.g., CXCL12, osteopontin, VEGF, TGFβ, and G-CSF, may affect hematopoiesis in the bone marrow microenvironment, altering the formation of other immune cells, in addition to neutrophils, including monocytes, macrophages, and lymphocytes [46, 47]. The recruitment of these cells may comprise the tumor microenvironment, influencing tumor angiogenesis, invasion, and immune suppression [47–49]. It will be important to explore how these other immune cells influence neutrophil mobilization to the lung tumor microenvironment, and whether tumor-driven inflammatory signaling to other immune cell populations is involved in this process. Interestingly, neutrophils, together with other immune cells, also participate in the preparation of the pre-metastatic niche for the initial seeding of cancer cells in the lung [48, 50–56]. These immune cells are recruited to the pre-metastatic lung, enhancing pulmonary metastasis by production of tumor growth-promoting factors. The role of other cells from outside the lung in the establishment of the pulmonary tumor niche is still not completely determined. Also, the details about the cross-talk between distinct immune cell subsets involved in the arrival of tumor cells to the lung remain to be examined. Further studies are required to evaluate the importance of neutrophils' interactions with other immune cells in the pulmonary tumor growth. Importantly, future studies should also explore whether tumor location is important for its immune-modulatory roles.

The lung neoplasic cells actively interact with the immune system. Lung adenocarcinoma microenvironment presents infiltration from several immune cells, including lymphocytes, macrophages, and dendritic cells [57–60]. These interactions may be essential for the shaping of lung cancer growth and development. For instance, the presence of macrophages is associated with poor survival [58], while dendritic cells are linked to prolonged survival [59]. Despite this knowledge, how exactly different immune subsets interact with SiglecF^{high}–expressing neutrophils and affect lung adenocarcinoma progression remains to be elucidated (Fig. 2). Understanding the degree to which immune cells are modulated in the lung adenocarcinoma microenvironment during cancer progression will provide insights into how the lung tumor and immune cells shape each other as they evolve.

2.2 Tumor-activated osteoblasts

It is known from previous studies that metastatic tumor cells promote increased bone formation *via* osteoblasts activation [61]. Increased bone growth originates mineralized tissue where malignant cancer cells reside, causing more osteoblastic lesions, stimulating cancer growth [62]. A recent study showed that the newly formed osteoblasts derive from bone marrow endothelial cells when those are stimulated by metastatic cancer cells [63, 64]. Engblom and colleagues introduce a new concept: osteoblasts can be remotely activated by tumor cells remotely even when those are not present in the bone [42]. Future studies will reveal what is the origin of newly formed osteoblasts activated by lung tumor, and whether they are also derived from endothelial cells. Also, it will be

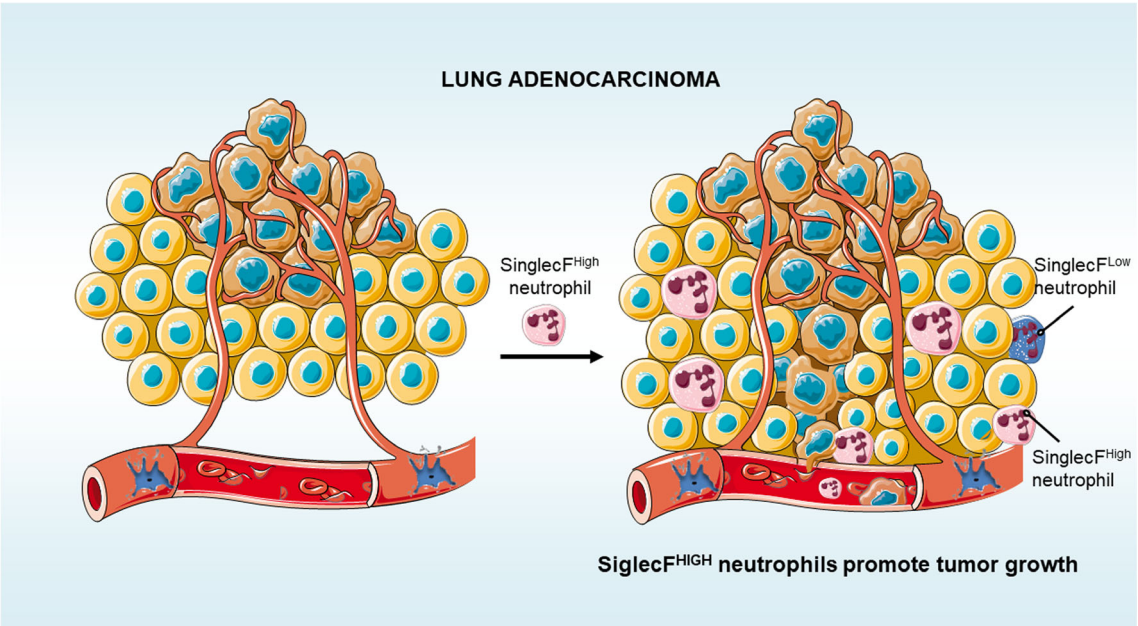

Fig. 2 SiglecF^{high}–expressing neutrophils expand tumor growth *in vivo*. SiglecF^{high}–expressing neutrophils promote lung adenocarcinoma

important to explore whether, in osteoblastic lesions caused by metastatic malignant cells, Siglec^{high}–expressing tumor-promoting neutrophils are also being generated.

Osteoblasts have been shown to be heterogeneous. Nonetheless, Engblom and colleagues consider osteoblasts as a homogeneous cell population in their study [42]. Osteoblasts from distinct bones differ in their embryonic origins. For instance, craniofacial bones osteoblasts derive from the neural crest, while long bones osteoblasts derive from the mesoderm. Osteoblasts also differ in the way they form the bone. In the clavicle and in the craniofacial skeleton, it happens *via* intramembranous ossification, while in the remaining skeleton, through endochondral ossification [65–67]. Osteoblasts from varying bones utilize distinct molecules for their activities. For instance, 1,25-dihydroxyvitamin D(3) and parathyroid hormone affect differently osteoblasts from distinct sites [68]. Long bone metaphysis is highly sensitive to parathyroid hormone, while calvaria is not [69]. Also, hypoxia-inducible factor α and Indian hedgehog are essential for osteoblastic activity during endochondral ossification but not during intramembranous ossification [70, 71]. Inorganic and organic matrix composition produced by calvaria and long bones osteoblasts also differ [72, 73]. Due to the crucial role played by osteoblasts discovered by Engblom et al. (2017), the question arises as to whether the osteoblast subpopulations from different bones differ in their capacity to promote lung tumor growth.

Moreover, the main findings from Engbom et al. (2017) study are based on the data obtained from Ocn-Cre mice [42]. It is known that osteocalcin gene is expressed by mature osteoblasts [74]. Note however that expression of osteocalcin is not restricted to mature osteoblasts. Other osteoblast lineage cells, including hypertrophic chondrocytes, pre-osteoblasts, and osteocytes may also express osteocalcin [75–78]. In addition, osteocalcin is expressed in megakaryocytes [79], platelets [79], and brain [80]. Furthermore, a recent study shows, by using high-resolution microscopy of bone sections and flow cytometry, that, additionally to mature osteoblasts, Ocn-Cre mice exhibit Cre recombinase activity in the majority of CXCL12-abundant reticular (CAR) cells and arteriolar pericytes [81]. Thus, it is possible that the effect on Siglec^{high}–expressing tumor-promoting neutrophils could be due to a different cell type, other than osteoblasts. To perform osteoblasts-specific targeting, a more specific mouse model should be used in future studies, i.e., mouse 2.3 kb Col1α1-Cre mice [82].

Engblom and colleagues reveal in their work that osteoblasts prime SiglecF^{high}–expressing neutrophils to promote lung tumor growth [42]. However, it remains unclear whether this priming needs direct contact between osteoblasts and neutrophils, or whether it depends on molecules secreted by osteoblasts to the bone marrow milieu or even to the circulation that will induce the formation of SiglecF^{high}–expressing neutrophils. Interestingly, a recent study showed that the lung microenvironment serves as a niche to a subtype of hematopoietic stem cells that produce all hematopoietic lineages, including neutrophils [83, 84]. It remains unknown whether the neutrophils produced in the lung are also primed by the bones through osteoblasts-derived molecules present in the circulation, and what percentage of tumor-promoting SiglecF^{high}–expressing neutrophils originate from the lung. Also, it will be important to analyze whether some cells in the lung microenvironment are targeted in Ocn-Cre mice as discussed above [81].

2.3 Bone marrow niche for tumor-activated neutrophils

The hematopoietic stem cells, which give rise to all blood and immune cells throughout life, reside in a specific complex niche in the bone marrow, that supports the homeostasis of these cells, composed by osteoblasts, osteocytes, osteoclasts, endothelial cells, adipocytes, smooth muscle cells, pericytes, fibroblasts, macrophages, megakaryocytes, lymphocytes, hematopoietic progenitors, neutrophils, peripheral innervations, and Schwann cells [9, 10, 18, 19, 64, 85–93]. Recent studies have revealed distinct contributions of several cellular components of the bone marrow to different functions of hematopoietic stem cells regulation. Nothing is known about the needed niche for the production of tumor-promoting SiglecF^{high}–expressing neutrophils in the bone marrow. It remains to be revealed whether there is a specific niche in the bone marrow where those SiglecF^{high}–expressing neutrophils are being formed.

Bone marrow neutrophils cooperate with macrophages in several important pathophysiologic functions, including immunomodulatory, inflammatory, and phagocytic activities [94, 95]. Although it is known that bone marrow macrophages enhance lung metastasis [96], the details of these mechanisms remain poorly understood. Future studies should explore whether the macrophage is an important niche cell for neutrophil priming by osteoblasts (Fig. 3). Engblom and colleagues revealed that genetic ablation of osteoblasts, by using Ocn-Cre-iDTR mice, is sufficient to interrupt lung tumor progression. Their findings also indicate that SiglecF^{high} neutrophils support cancer progression by promoting the differentiation of tumor-associated macrophages [42]. Surprisingly, their own results also show that genetic ablation of macrophages, by using CD169-DTR mice, did not suppress lung tumor growth. As previous data demonstrates that macrophages drive lung tumor growth [97], future studies will clarify this issue, which possibly could be methodological.

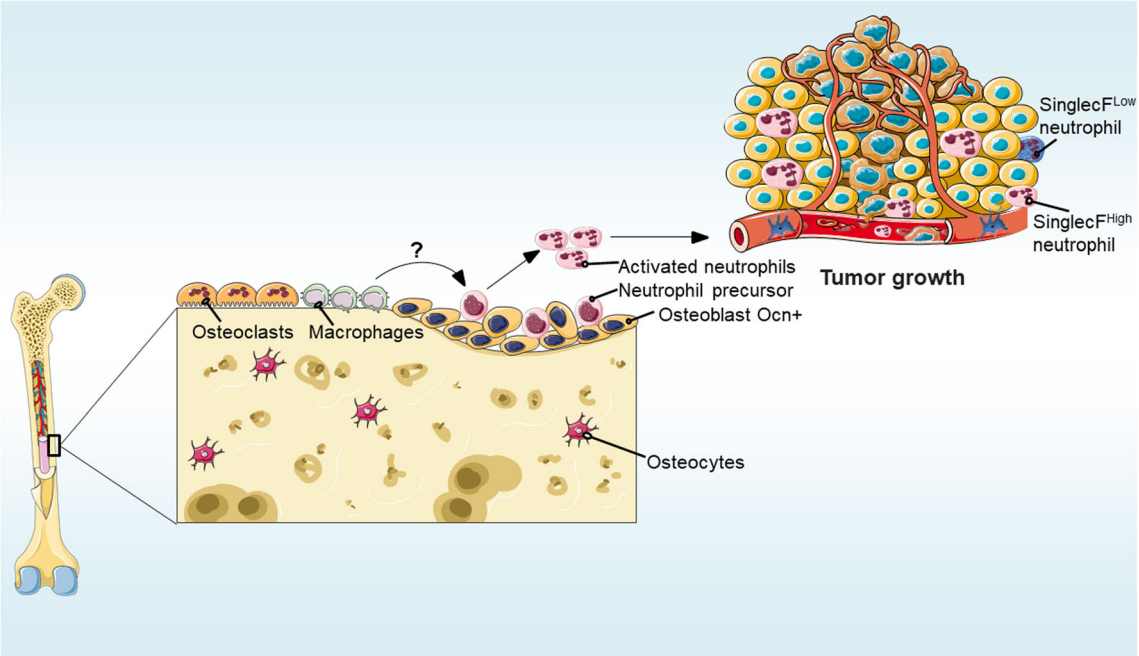

Fig. 3 Do bone marrow macrophages modulate neutrophil activation? The details of the mechanisms by which bone marrow macrophages enhance lung metastasis remain poorly understood. Future studies should explore whether macrophages are important niche cells for neutrophil priming by osteoblasts

2.4 Role of SiglecF expression in neutorphils during tumor progression

Engblom and colleagues show that neutrophils expressing SiglecF induce tumor growth [42]. Siglecs (sialic acid binding immunoglobulin-like lectins) are well-characterized (immunoglobulin-type) lectins identified by an amino-terminal V-set immunoglobulin domain that mediates sialic acid residues binding on glycoproteins and glycolipids, followed by varying numbers of C2-set immunoglobulin domains [98–100]. These proteins were first identified as receptors important for tolerance induction, pathogen recognition and uptake, and regulation of cell activation [100]. Siglecs are expressed in a variety of immune and non-immune cells in the central nervous system, prostate, kidney, placenta, amniotic epithelium, and others [100–105].

SiglecF is a component of Siglecs family and was first described in eosinophils [100]. Binding of SiglecF to a specific glycan present in the asthmatic airway potentiates eosinophil apoptosis in this condition [106]. Although this suggests a suppressive role for SiglecF on eosinophils, the function of SiglecF in other cells, in which its expression has also been detected, such as activated T cells [107], macrophages [108], and neutrophils [42, 107, 109], remains unclear. As multiple cell types in the tumor microenvironment express SiglecF, conditional deletion of this antigen specifically from tumoral neutrophils, by the creation of SiglecF floxed mice crossed with neutrophil-specific inducible CreER drivers, will reveal the exact role of SiglecF in neutrophils during tumor development.

The knowledge on Siglecs biology is based mainly on experiments with mouse models due to the limitations associated with mechanistic studies in humans. Human Siglec8 was identified as the murine functional paralog of SiglecF [110, 111]. The findings in mice imply the possible value of Siglecs as therapeutic targets in humans. Further investigation of these proteins is required to supply proof of concept for targeting Siglecs in cancer.

2.5 Lung tumor microenvironment

The capacity to eliminate single genes in specific cellular populations in adult mice has allowed us to answer specific questions regarding the roles of different cell types in the regulation of several physiopathologic conditions. In the lung tumor microenvironment, the exact contribution of non-malignant cells that may play important roles in stimulating osteoblasts remains uncertain. It also remains unclear whether lung cancer cells signal directly to osteoblasts, or indirectly *via* other cells from the lung microenvironment. Engblom and colleagues suggest, by *in vitro* experiments, that tumor-derived soluble RAGE stimulates osteoblasts to regulate pro-tumoral neutrophil formation [42]. Nevertheless, several cell types in the lung tumor microenvironment can produce soluble RAGE and other tumor-associated molecules [112]. RAGE has not been conditionally deleted from tumor cells, so there is no

direct evidence that cancer cells are the only/main functionally important source of this other osteoblasts-stimulating molecules. The generation of RAGE floxed mice to be crossed with cell type-specific inducible CreER drivers will allow us to specifically delete RAGE in several cells from the lung tumor niche. In addition to studies in genetic mouse models, transcriptomic and single cell analysis of various cells in the lung tumor microenvironment represents fundamental tools that will help us understand the roles of different cells from the lung tumor microenvironment in the communication with osteoblasts.

The lungs are a prevalent location to where metastatic cancer cells home, and several pre-metastatic modifications have been described in this organ [113]. The pre-metastatic lung niches include both resident cells and cells recruited from other organs [114, 115]. It will be interesting to examine whether the cells that form the pre-metastatic lung niches communicate with osteoblasts before the tumor cells seeding. Also, it is still unknown whether cancer cells from other organs that metastasize to the lung also stimulate osteoblasts. Investigation and characterization of cells from the lung microenvironment that play important roles in setting the pre-metastatic niche are necessary to develop targeted therapies to revert alterations of local microenvironment, inhibiting metastatic establishment [114]. As recent studies showed that lung pericytes are essential components of the lung pre-metastatic niche [116], exploring whether lung pericytes communicate with osteoblasts in the bones seems promising (Fig. 4). In addition to promoting existent lung tumor growth, it should be explored whether SiglecF^{high}–expressing neutrophils are also able to form the pre-metastatic niche in the lung. Moreover, several malignant cancer cells have a predilection to metastasize also to the bones, which comprise fertile ground for the accommodation and growth of malignant metastatic cells [117]. Future studies should elucidate whether this activation of osteoblasts activity by lung tumor cells also creates a pre-metastatic niche in the bone.

2.6 Clinical relevance

Lung cancer, like other oncological disorders, is heterogeneous. Thus, this disease has various histological types. The two main lung cancer categories have been defined: non-small cell lung cancer and small cell lung cancer. Around four fifth of lung cancers are non-small cell lung cancers, which have distinct molecular profiles and clinical course forms of the disease [118, 119]: lung adenocarcinoma, squamous cell carcinoma, large cell neuroendocrine carcinoma, adenosquamous carcinoma, and large cell carcinoma. These cancer types arise from diverse locations within the lung [120, 121]. Engblom and colleagues analyzed in their studies lung adenocarcinomas [42]. It remains to be explored whether the communication with the bone is a common feature to all types of lung tumors, or it is specific to adenocarcinoma. Furthermore, future studies should explore whether other types of solid

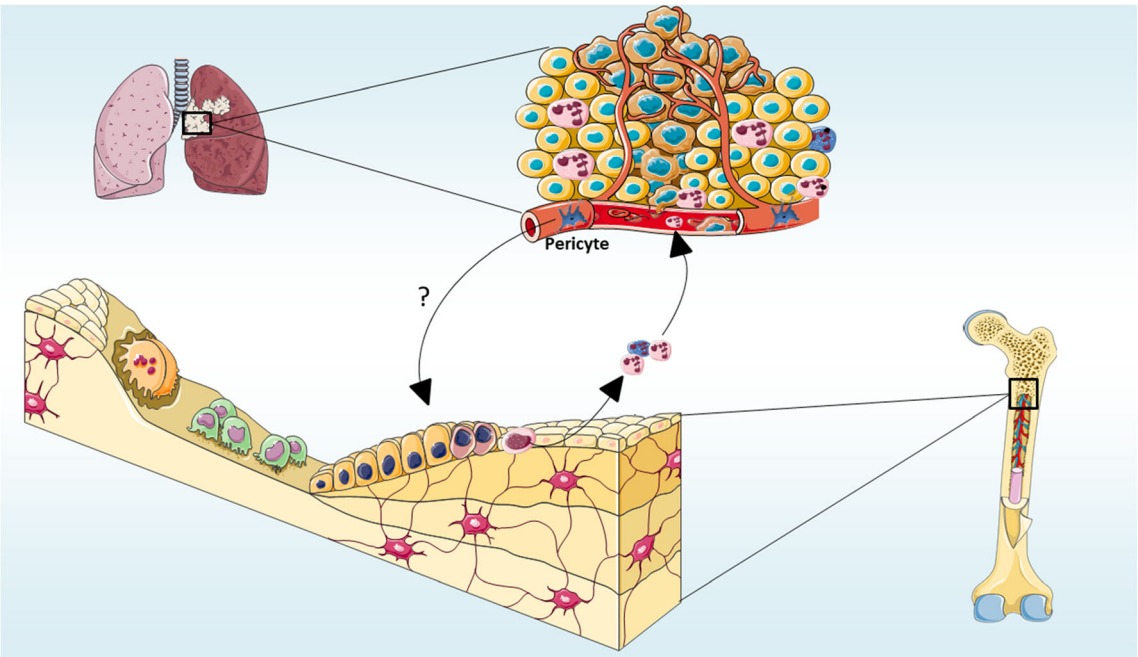

Fig. 4 Do pulmonary pericytes communicate with osteoblasts in the bones to induce tumor development? Lung pericytes are essential components of the lung pre-metastatic niche. Whether adenocarcinoma pericytes communication is important for osteoblasts to activate Siglec^{high}–expressing neutrophils remains unknown

tumors also communicate with osteoblasts, even in the absence of bone metastasis.

Several pulmonary disorders, characterized by deregulated inflammation in the lung microenvironment, have a big risk of developing into lung cancer [122–124]. Common inflammatory mediators, including TGFβ, TNFα, PGE2, HGF, and IL1β, drive chronic obstructive pulmonary disease, such as pulmonary fibrosis, and emphysema. These mediators also play essential roles in the regulation of bone marrow homeostasis, affecting immune cell survival, migration, proliferation, and differentiation [125–130]. Thus, it is possible that these molecules, altered in these pulmonary disorders, participate in bone marrow alterations that will affect lung cancer development. As the neutrophils that promote lung tumor growth are primed by osteoblasts in the bone [42], it will be interesting to explore at what stage of lung cancer development the cross-talk with the bone starts. Also, it remains unknown whether the lungs in these pre-neoplasic conditions are already receiving Siglec^{high}–expressing neutrophils primed in the bone marrow. If yes, targeting these neutrophils should be explored as a potential way to inhibit the evolution of these inflammatory disorders into lung cancer.

Age is one of the main risks factors for lung cancer [131]. During aging of the hematopoietic system, myeloid cells increase in number, and their properties are affected; for instance, aged neutrophils migrate less in response to stimuli [132]. It will be interesting to explore whether this increase in number corresponds to the appearance of tumor-promoting SiglecF^{high}–expressing neutrophils.

To translate animal research on tumor-promoting SiglecF^{high}–expressing neutrophils to humans, specific markers selectively expressed in cells equivalent to these ones must be validated in human tissues. Engblom and colleagues performed RNA-sequencing in SiglecF^{high}–expressing neutrophils isolated from mice [42]. A detailed analysis of this data may provide new specific surface markers to develop therapies that will target these receptors in patients with lung cancer.

2.7 Future directions and conclusions

In conclusion, the study by Engblom and colleagues reveals a novel unexpected role of osteoblasts in lung tumor progression. However, our understanding of lung tumor biology still remains limited, and the complexity and interactions of different cellular components and molecules of the lung microenvironment during tumor progression should be elucidated in future works. A great challenge for the future will be to translate the research from mouse models into patients. Whether cancer cells at an early stage of human lung adenocarcinoma promote, neutrophil priming *via* osteoblasts remains to be determined. Improving the availability of the human lung and bone marrow samples will be essential to reach this goal.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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