# How does the viability of bone-restricted osteocytes regulate bone atrophy?

Prof. Dr Aline Bozec and Dr Darja Andreev
Universitätsklinikum Erlangen and Friedrich-Alexander University Erlangen-Nürnberg

Although the skeleton appears stiff and solid, it is, in fact, a dynamic and sensitive network, demanding a fine balance between the bone-resorbing osteoclasts and the bone-forming osteoblasts. Disruption of this homeostasis frequently leads to exaggerated bone degradation by osteoclasts, manifesting in pathological bone loss. Bone metabolism is largely controlled by osteocytes that are terminally differentiated osteoblasts, becoming trapped within the bone matrix (Figure 1).

Osteocytes represent the major cell type within the bone compartment. Nonetheless, for a long time, they were neglected from the field of bone research because they appear biologically inert. Today, their broad contribution to bone remodelling is widely recognised. In particular, osteocytes act as mechanosensors of bone, participate in the recycling of bone minerals and regulate osteoblasts and especially osteoclasts (Bonewald, 2011).

The rather inactive feature of osteocytes allows them to live an average half-life of 25 years. Despite their longevity, excessive osteocyte death is observed upon ageing, notably in bone diseases linked to osteoclast-mediated bone loss, such as osteoporosis, arthritis, fracture, and osteonecrosis (Figure 2A). Up to now, it is not yet known whether increased osteocyte death appears just as a concomitant of bone disease or may even contribute to the disease pathology.
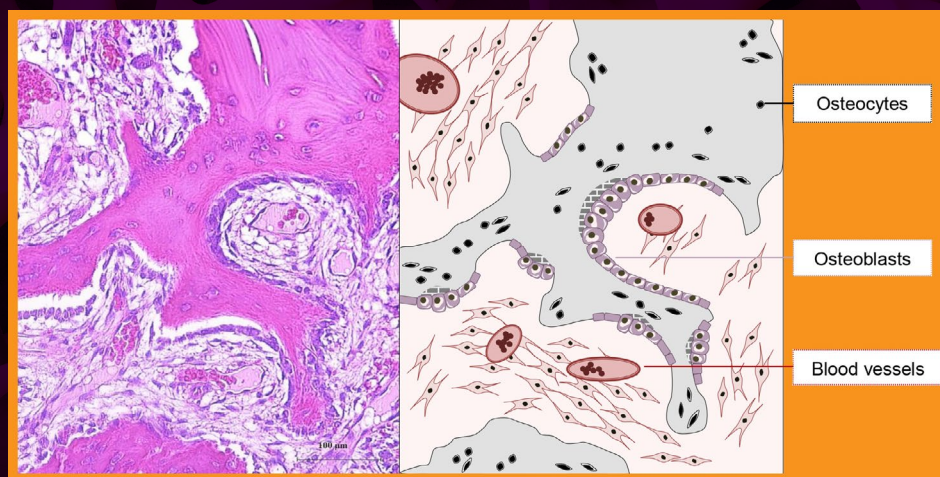
Figure 1: Image of human femoral trabeculae with a corresponding illustration. Section of human femoral bone stained with hematoxylin and eosin. Bone-forming osteoblasts are located around the bone surface. Osteocytes are localised within the bone matrix. The marrow space is infiltrated by blood vessels. Material was provided by the Department of Orthopaedics, University Medical Center Hamburg-Eppendorf, Germany.
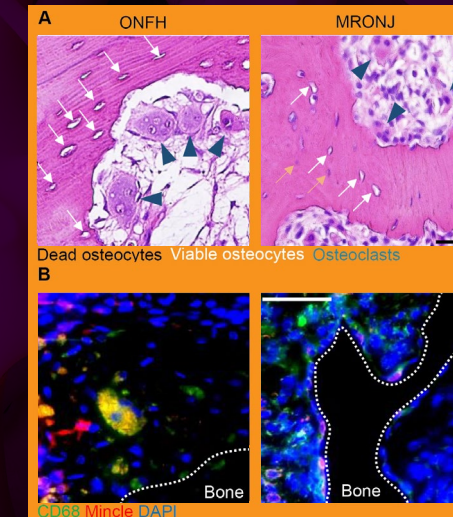
The ODE project

For this purpose, we initiated the ERC Starting Grant project "Unknown functions of Osteocyte DEath (ODE)" to delineate how osteocyte death is induced, in which manner osteocytes die within the bone matrix and which consequences their death has for bone health.

So far, the death of osteocytes is still a mystery. It is believed that factors like oxygen level and nutrients distributed by blood vessels within the bone strongly influence their viability. When shortages occur, osteocytes undergo autophagy to protect themselves from damage. However, when the damage is too severe, the death of the cells is initiated (Komori, 2016). One of the primary goals of the ODE project is to delineate in detail the death process of osteocytes in dependence of different bone conditions, such as arthritis, fracture and osteonecrosis. The use of newly developed imaging techniques and omics platforms will allow us to analyse osteocytes within their remote localisation in the bone matrix. Within this study, we want to answer questions like: Which stress factors (hypoxia, malnutrition, inflammation) cause osteocyte death? Which death pathway (apoptosis, necroptosis, ferroptosis, necrosis) is specifically triggered in osteocytes? Is osteocyte viability important for healthy bone composition, and how is it affected by bone disease?

Generally, the body gets rid of dead cells very quickly by specialised phagocytic cells to prevent sterile inflammation. However, bone is exceptional since phagocytes cannot reach the isolated osteocytes within the mineralised tissue. Thus, at some point, osteocytes are supposed to burst, releasing damage-associated molecular patterns (DAMPs) into the surrounding, thereby priming an inflammatory environment. Last year we published an article describing for the first time that osteocytic DAMPs are able to stimulate osteoclast formation (Andreev et al., 2020). This response is mediated by the C-type lectin receptor, macrophage-inducible C-type lectin (short Mincle), expressed on osteoclasts (Figure 2B). As a pattern recognition receptor (PRR), Mincle is able to recognise microbial compounds as well as DAMPs. These results opened many new questions that will be addressed within the ODE project as: Which DAMPs are released by osteocytes upon death? Are other DAMP sensing PRRs besides Mincle expressed on osteoclasts and have an impact on osteoclast biology?

Conclusively, the ODE project will provide a new understanding of the sensitive balance of bone homeostasis and how stress factors in bone such as inflammation and reduced vascularisation influence the viability of osteocytes. We will decipher in detail which impact the death of osteocytes has on the bone environment and bone strength and how we can avoid this negative influence. On a broader scale, we can use the knowledge gained to better understand the pathogenesis of bone loss and discover potential new pathways that might be targeted in the future to prevent bone loss from happening.

Figure 2: Increased osteocyte death in osteonecrotic lesion is associated with upregulation of Mincle-positive osteoclasts. A) Hematoxylin and eosin staining of human bone slides from patients with osteonecrosis of the femoral head (ONFH) and medication-related osteonecrosis of the jaw (MRONJ). Black arrows indicate dead osteocytes; Yellow arrows show viable osteocytes; Blue triangles depict osteoclasts. B) Immunofluorescence staining (IF) for DAPI (blue), monocytic cells (CD68/ green) and Mincle (red) of human bone slides from ONFH and MRONJ patients. Adapted from Andreev et al., 2020.

References

Andreev, D., Liu, M., Weidner, D., Kachler, K., Faas, M., Gruneboom, A., *et al.* (2020) 'Osteocyte Necrosis Triggers Osteoclast-mediated Bone Loss Through Macrophage-Inducible C-type Lectin', *The Journal of Clinical Investigation.* 130(9), pp.4811–30.

Bonewald, L.F. (2011) 'The Amazing Osteocyte', *Journal of Bone and Mineral Research : The Official Journal of the American Society for Bone and Mineral Research.* 26(2), pp.229–38.

Komori, T. (2016) 'Cell Death in Chondrocytes, Osteoblasts, and Osteocytes', *International Journal of Molecular Sciences,* 17(12). doi: 10.3390/ijms17122045.