ImmunoTools *special* Award 2018

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Characterization of extracellular vesicles obtained from human and mouse cell cultures

My name is Martha Blank and I am working as a PhD student at the Ludwig Boltzmann Institute of Osteology at the Hanusch Hospital in Vienna, Austria. Since the beginning of my Bachelor internship I have been working and exploring the mineralization of bone tissue cells and the enrolment of extracellular vesicles (EVs) during this process.

Extracellular vesicles play an important part in transduction of cellular signals. EVs can contain proteins, DNA, RNA but also ions and are considered to be signalling molecules and shippers. The formation of extracellular particles is a highly regulated process and divide those spherical bodies into two groups: extracellular vesicles that are shed by the cell, also called ectosomes and vesicles that are pre-formed within the cell in so-called multivesicular bodies that are subsequently released from the cell. Those two types differ predominantly in their size. Whereas ectosomes have a size from 100-1000 nm, exosomes appear as much smaller spherical bodies with a range from 30-150 nm (Kalra et al., 2016).
There is evidence that matrix vesicles, a special type of ectosomes, are involved in the initiation of bone tissue mineralization, but maybe also in extra-osseous calcification of blood vessels in the progress of cardiovascular diseases. Immuno-blotting of surface marker proteins, for instance CD9, CD53, CD63, CD81, and Annexin V are used to confirm the isolation of MVs.
The basic composition of matrix vesicles has already been analyzed. As they derive from buds of the cellular membrane, the content is surrounded by a lipid bilayer made of phosphocholine and phosphoethanolamine. Inside of the matrix vesicles PHOSHPO1 was found and embedded in the lipids, tissue non specific alkaline phosphatase, the Pi transporters Pit-1 and Pit-2, ENPP1 as well as annexins II,V,VI which are suggested to transport and bind calcium. PHOSHPO1 should increase internal concentrations of the mineral components leading to nuclei for crystallization (Kirsch, 2012; Millan, 2013; Zhou et al., 2012). By the help of proteins the mineral move into the space of collagens (Nudelman et al., 2013).

Aim of our study is to investigate the impact of matrix vesicles on the initiation of mineral deposition. For that purpose, we will culture bone tissue cells at mineralizing and non-mineralizing conditions. To compare different systems we use mesenchymal murine D1 stem cells that differentiate into osteoblastic cells within two weeks and human osteosarcoma MG-63 (mineralizing) and U2-OS (non-mineralizing) cells. Preliminary results already validated the reliability of these cell systems to study and

modulate the mineralization process. ImmunoTools antibodies will help us to characterize specific differences in protein content of MVs and determine differences in vesicles shed by mineralizing and non-mineralizing cells.

Shape, size, and distribution of isolated MV will be analyzed using the ZetaView®. ZetaView® is used for nanopartical tracking analysis in combination with fluorescence. ImmunoTools FITC-conjugated antibodies against vesicle surface marker proteins, previously confirmed by immuno-blotting, will be used to label isolated MVs and determine their size and distribution. Differences in amount, size or composition of the vesicles might indicate differences in mineralization.

For a better understanding how bone tissue cells shed matrix vesicles cells and how they are transported in the extracellular space the cells will be investigated using confocal laser scanning microscopy. Therefore, the cells will be grown on cover slips, fixed at different stages and treated with ImmunoTools dye-conjugated antibodies against surface marker proteins (CD9, CD53, CD63, CD81, Annexin V, VI). This will allow us to visualize the transport of vesicles out of the cell and understand the communication. Differences in amount and presence of these marker proteins will provide new insights about the function of specific vesicles.

References

Kalra H., Drummen GP., Mathivanan S. (2016). Focus on Extracellular Vesicles: Introducing the Next Small Big Thing.Int J Mol Sci Feb 6;17(2):170.
Kirsch, T. (2012). Biomineralization-an active or passive process? Connective tissue research 53, 438-445.
Millan J. L. (2013). The Role of Phosphatases in the Initiation of Skeletal Mineralization. Calcif Tissue Int 93, 299-306.
Nudelman F., Lausch, A. J., Sommerdijk, N. A. and Sone, E. D. (2013). In vitro models of collagen biomineralization. J Struct Biol 183, 258-269.
Zhou X., Cui, Y. and Han, J. (2012). Phosphate/pyrophosphate and MV-related proteins in mineralisation: discoveries from mouse models. Int J Biol Sci 8, 778-790.

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APC – conjugated anti-human CD9, CD53, CD63, Annexin-V

FITC - conjugated anti-human CD9, CD53, CD63, Annexin-V

PE - conjugated anti-human CD9, CD63, Annexin-V

APC – conjugated anti-mouse Annexin-V

FITC - conjugated anti-mouse CD9, CD81, Annexin-V

PE - conjugated anti-mouse CD81, Annexin-V

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