PL-ISSN 0015-5497 (print), ISSN 1734-9168 (online)
Folia Biologica (Kraków), vol. 62 (2014), No 2

doi:10.3409/fb62_2.143

## Short Communication

# Are Chondroclasts and Osteoclasts Identical?

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*Accepted February 19, 2014*

WŁODARSKI K. H., BRODZIKOWSKA A., KUZAKA B. 2014. Are chondroclasts and osteoclasts identical? Folia Biologica (Kraków) **62**: 143-147.

Brief characteristics of cells termed "osteoclasts" and "chondroclasts" are outlined and reasons to consider them as the same cell type, able to resorb calcified matrix, are discussed.

Key words: Osteoclasts, chondroclasts, identification.

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Multinucleated cells which resorb calcified cartilage in the process of endochondral osteogenesis have been termed "chondroclasts", while multinucleated cells removing bone are termed "osteoclasts". Moreover, the resorption of calcified cartilage matrix is executed not exclusively by multinucleated cells, but also by mononuclear ones, which is not the case with bone matrix

For a long time both terms were in use, implying a functional differentiation. However, several workers, despite the separation of hard tissue clastic cells into osteoclasts and chondroclasts (SCHENK *et al*.1967; BROMLEY & WOLLEY 1984; LEVINSON & SILBERMAN 1992; ARANA-CHAVEZ & BRADASCHIA-CORREA 2009; KNOWLES *et al*. 2012), suggested that the same cell is responsible for removal of both bone and calcified cartilage. In recent comprehensive textbooks of histology, the term "chondroclasts" is not mentioned (GARTNER & HIATT 2007; KIRSZENBAUM 2007).

The morphology and origin of cells able to resorb calcified cartilage is briefly reviewed.

## Mononuclear cells resorbing cartilage

An electron microscopic study of replacement of cartilage by bone in endochondral ossification stated that resorption of calcified cartilage matrix is executed by mononuclear cells rich in rough endoplasmatic reticulum, associated with capillary walls, (SASAKI *et al*. 1996).The expression of vascular endothelial growth factor (VEGF) by hypertrophied chondrocytes, required for vascular invasion into cartilage (KANG *et al*. 2010) would support this opinion. These cartilage-resorbing mononuclear cells are of fibroblastic appearance, with numerous phagolysosomes and dense bodies, and some cartilage-ingesting cells extend long cytoplasmic processions toward opened lacunae. These mononuclear cells remove transverse septa of hypertrophic cartilage and phagocytose degenerated, hypertrophic chondrocytes. Transverse septa, in contrast to the longitudinal septa separating chondrocyte columns, are not calcified. Longitudinal septa, the matrix of which is calcified, persist, as they are only superficially resorbed by mononuclear cells, and become covered by osteoblasts secreting osteoid which calcifies, thus forming the primary bone trabeculaes. Thus the primary bone trabecula is composed of a cartilaginous medulla and of a cortical covering of newly-formed bone.

Mononuclear cartilage-ingesting *cells* which remove uncalcified septal cartilage matrix were termed "septoclasts". They secrete the proteolytic

enzyme cathepsin, and lack antigens expressed on osteoclasts and macrophages (LEE *et al*. 1995).

Uncalcified cartilage in the erosion zone is resorbed by perivascular cells. They extend finger-like cytoplasmic processes toward uncalcified cartlage septa, show neither alkaline phosphatase nor tartrate-resistant alkaline phosphatase (TRAP) activity, and fail to express CD44. These cells are specifically stained by Dolichos Biflorus agglutinin (DBA) and are described as a novel type of cell degrading cartilage (NAKAMURA & OZAWA 1996).

Perivascularly located cells include macrophages. These cells accumulate $^{35}S$ sulphate and cause degradation of non-mineralized cartilaginous matrix and also destroy hypertrophic chondrocytes. These tissue-specific macrophages were named chondroclasts (RODIONOVA 1986).

In birds the bulk of uncalcified cartilage is resorbed by mononuclear phagocytes, while multinucleated chondroclasts resorb calcified cartilage (ROACH & SHEARES 1989). The fusion of mononuclear clastic elements into multinucleated chondroclasts depends, according to SMETANA and VILM (1992), on biochemical properties of the cartilage matrix – a high level of chondroitin sulphate has an inhibitory effect on the fusion of these cells.

## Multinucleated cells resorbing cartilage

Multinucleated cells resorbing cartilage in arthritic conditions and in endochondral ossification are morphologically and histochemically similar to osteoclasts. At first they lack a ruffled border: such multinucleated cells were termed "chondroclasts". This term is defined by their close association with mineralized and unmineralized cartilage (SAVOSTIN-ASING 1975; BROMLEY & WOOLLEY 1984; BETLEX-GALLARD *et al*. 1990). They express macrophage/osteoclast markers and exhibit an osteoclast-like phenotype: tartrate-resistent alkaline phosphatase (TRAP+), metalloproteinase MMP9, cathepsine, they do not express CD12-, HLA DR-, but do express CD45+, CD51+ and CD68+, markers of the macrophage lineage (KNOWLES *et al*. 2012).

The concept that multinucleated osteoclasts and multinucleated chondroclasts are identical is supported by their ability to release glycosaminoglycans (GAG) by *in vitro* generated multinuclear, mature osteoclasts and by macrophages cultured on cartilage slices (KNOWLES 2012). However, the concept of identity of osteoclasts and chondroclasts is not universally accepted. NORDHAL *et al*. (1998) have compared ultrastructural and functional features of chondroclasts and osteoclasts in endochondral bone formation.

On the basis of semiquantitative TRAP distribution, which showed a difference in extracellular and intracellular distribution between osteoclasts and chondroclasts, they postulate that despite ultrastructural similarity, these cells differ not only with respect to location, but possibly also by their mode of action. Also SAVOSTIN-ASHING and ASHING (1975) report some peculiar features of multinucleated chondroclasts, namely the spanning of several opened chondrocytic lacunae and fusion with hypertrophic chondrocytes resident in the chondrocytic lacunae. Cells responsible for mineralized tissue resorption are termed collectively as "clastic cells" (ARANA-CHAVEZ & BRADASCHIA-CORREA 2009).

## Bone resorption by osteoclasts

Mature osteoclasts are large multinucleated cells able to resorb organic bone matrix following their demineralization by secreted protons and chloride ions.

Osteoclasts release hydrolytic enzymes which decompose organic bone matrix after dissolution of hydroxyapatite crystals (SUDA *et al*. 1992; for a review see WŁODARSKI and WŁODARSKI 2006). The release of protons and of hydrolytic enzymes takes place in the pole of the cell which is in close contact with resorbing bone. At the site of adhesion to bone the cytoplasm of osteoclasts develop a system of cytoplasmic extensions, forming the so called "ruffled border". The sealing of this border with bone surface is provided by an interaction of osteoclast adhering molecules with bone matrix vitronectin. This seal ensures local activity of exocytosed lysosomal proteolytic enzymes (AMLING & DELLING 1996; SUDA *et al.* 1997; for review see WŁODARSKI & WŁODARSKI 2006). The morphological manifestation of osteoclast activity is an erosion of bone surface, forming resorption pits or Howship's lacunae.

The mechanisms for destruction of minerals and collagen are fundamentally different (KNESE 1972). Osteoclasts function as "mineraloclasts" and as "collagenoclasts". When in "mineraloclast" mode, on contact with mineralized bone the cell develops a "brush border" of microvilli with associated cytoplasmic vesicles. The vesicles contain crystalline "needles" and possibly some are of mitochondrial origin. The cell cytoplasm is well-endowed with lysosomes, mitochondria and rough endoplasmic reticulum.

Non-mineralized collagen fibers are degraded by multinucleated cells, termed "collagenoclasts". Collagen fibrils before or in destruction are oriented vertically to the chondroclast surface. The sparse cytoplasmic processes and decomposed collagen fibers are in contact with the "collageno-

clasts". Their cytoplasm is rich in rough endoplasmic reticulum, the site of collagenolytic enzyme synthesis (KNESE 1972).

An autoradiographic study of bone rudiment chondrocytes exposed to $^{3}$H-thymidine concluded that hypertrophic chondrocytes of calcified cartilage survive dissolution of their matrix and transform into both chondroclasts and osteoblast/osteocytes (CRELIN & KOCH. 1967). A more recent study by LEE *et al*. (1998) of the removal of chondrocytes during endochondral fracture healing conflicts with CRELIN and KOCH's postulate. They reported apoptosis by TUNEL assay (method for detecting DNA fragmentation by labeling the terminal end of nucleic acids) in hypertrophic cartilage and were unable to detect expression of osteocalcin mRNA, which they did in osteoblasts. Thus the transdifferentation of hypertrophic chondrocytes into osteocytes is unlikely.

The morphological characteristics of multinucleated chondroclasts and osteoclasts are outlined in the Table 1.

## Regulation of osteoclastogenesis

Mature osteoclasts are formed by the fusion of mononuclear precursor cells. Proliferation of osteoclast precursor cells is stimulated by numerous cytokines, such as TGF-alpha (transforming growth factor), EGF (epidermal growth factor), GM-CSF (granulocytes macrophages-colony stimulating factor). Bone marrow stromal cells secrete a ligand for the RANK receptor, present on "clastic" precursor cells. Interaction of this ligand with the RANK receptor activates the transcription factor NF kappa beta (NFkB) which activates several genes needed for osteoclast maturation of the precursor cells.

Osteoprotegerin (OPG), another molecule produced by stromal cells, is a decoy receptor with high affinity for RANK. OPG competes with RANK for RANK ligand, and thus antagonizes RANK (for review see WŁODARSKI & WŁODARSKI 2006; SOYSA *et al.* 2012).

## Regulation of chondroclastogenesis

The factors which regulate osteoclastogenesis also regulate chondroclastogenesis. Estrogen impairs chondroclast differentiation and reduces osteoclast number in rats (TURNER *et al*. 1994). An extensive study by OTA *et al*. (2009) points to the role of chondrocytes in chondroclastogenesis. Chondrocytes produce osteoprotegerin (OPG), a decoy receptor of RANK (receptor activator of

Table 1

Brief characteristics of multinucleated chondroclasts and osteoclasts

| Feature | Chondroclast | Osteoclast | References |
|---|---|---|---|
| Actin ring | present | absent | OTA *et al*. 2009 |
| Resorption of GAG | yes | no | OTA *et al.* 2009 |
| Multinuclearity | yes | yes | LEWINSON & SILBERMAN 1992, SMETANA & VILIM 1992, SAVOSTIN-ASHING & ASHING 1975, BETTEX-GALLAND *et al.* 1990, NORDHAL *et al.* 1998 |
| Ruffled border | weak/absent | well developed | LEWINSON & SILBERMAN 1992, NORDHAL *et al.* 1998 |
| Clear zones | in question | present | NORDHAL *et al.* 1998 |
| TRAP activity | present | present | SAVOSTIN-ASHING & ASHING 1975, NORDHAL *et al*. 1998 |
| TRAP accumulation | intracellular | extracellular | NORDHAL *et al*. 1998 |
| TRAP mRNA | strong expression | moderately expressed | NORDHAL *et al.* 1998 |
| CD44+ | present | present | NAKAMURA & OZAWA 1996 |
| Typical localization | epiphys./metaphys. | metaphyseal/diaphyseal | NORDHAL *et al*. 1998 |
| Mitochondria | abundant | abundant | LEWINSON & SILBERMAN 1992, SAVOSTIN-ASHING & ASHING 1975 |
| Lysosomes | abundant | abundant | SAVOSTIN-ASHING & ASHING 1975 |
| RER | sparse | sparse | SAVOSTIN-ASHING & ASHING 1975 |
| Infoldings at foci of contact with calcified matrix | present | present | SAVOSTIN-ASHING & ASHING 1975 |
| Amebiod processes extending into lacunae | present | absent | SAVOSTIN-ASHING & ASHING 1975 |
| Phagocytosis of cartilage matrix and of hypertrophic chondrocytes debries | yes | no | LEWINSON & SILBERMAN 1992 |

nuclear factor kappa beta) ligand – the main regulator of osteoclast differentiation (SOYSA *et al.* 2012; for a review see WŁODARSKI & WŁODARSKI 2006).

Chondrocytes with knock-down gene for osteoprotegerin (OPG-/-) support generation of multinucleated osteoclasts from spleen cells. These osteoclasts also degraded glycosaminoglycans produced by chondrocytes. Factors regulating RANK-ligand (RANK-L) expression (1,25-dihydroxyvitamin D, PGE2, PTHrP, TNF alfa, IL-1) in chondrocytes increased RANKL and decreased OPG leading to activation of chondroclastogenesis. These factors regulate generation from mononuclear cells of multinucleated cells, resorbing both calcified cartilage and bone.

Chondrocytes with OPG gene deletion grafted into kidney recruit TRAP-positive multinucleated chondroclasts, able to resorb glycosaminoglycans and support the *in vitro* formation of multinucleated chondroclasts from splenocytes, (OTA *et al*. 2009).

Another study (MASUYAMA *et al*. 2006) reported chondrocyte-dependent *in vitro* osteoclastogenesis.

As chondoclasts and osteoclasts are TRAP-positive and have similar ultrastructural features it is difficult to classify them as separate cell types. Support for this view is given by KIM *et al*. (2009) and STRASSLE *et al*. (2010) who demonstrated that bisphosphonate-induced inhibition of osteoclasts inhibited the resorption of calcified cartilage.

Cloned macrophages from bone marrow, obtained by co-culturing with chondrocytes, express osteoclast marker enzymes and have degraded cartilage matrix and hydroxyapatite, but failed to express calcitonin receptors, a mature osteoclast marker (MASUDA *et al.* 2001). Thus the cells are similar, but not identical, to the osteoclasts. Thus chondroclasts and osteoclasts, both able to resorb calcified matrix, are formed by the fusion of mononuclear cells of haemetopoietic origin and common mechanisms govern their differentiation (MASUDA *et al.* 2001).

Conclusion: Multinucleated cells present in the zone of calcified cartilage in the model of endochondral osteogenesis, termed on the basis of their location as chondroclasts, are now regarded as slightly modified osteoclasts. Histological, ultrastructural, and biochemical features as well as regulatory mechanisms of differentiation and function of chondroclasts and osteoclasts are very similar and thus justify regarding them as a single cell type with the ability to exhibit both clastic modalities.

## Acknowledgements

We thank Dr. Wyn PARRY from Liverpool University for critical reading of the manuscript and stylistic amendments to the text.

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