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COMMENTARY

Autophagy and differentiation of bone-forming cells

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Commentary on: Onal M, Piemontese M, Xiong J, Wang Y, Han L, Ye S, Komatsu M, Selig M, Weinstein RS, Zhao H, Jilka RL, Almeida M, Manolagas SC, O'Brien CA. Suppression of autophagy in osteocytes mimics skeletal aging. *J Biol Chem* 2013;**288**:17432–17440 and Liu F, Fang F, Yuan H, Yang D, Chen Y, Williams L, Goldstein SA, Krebsbach PH, Guan JL. Suppression of autophagy by FIP200 deletion leads to osteopenia in mice through the inhibition of osteoblast terminal differentiation. *J Bone Miner Res* 2013;**28**:2414–2430.

Autophagy is a physiological process being involved in stressful physiological and mechanical situations; its role in osteoblast/osteocyte function has been first suggested in the murine model using truncation of Nbr1, a selective autophagic receptor for degradation of ubiquitinated proteins. Two recent papers,[1,2] establish the importance of autophagy in osteoblast terminal differentiation and osteocyte functions. The cell specific deletion of autophagy-related proteins in bone-forming cells demonstrates the central role of autophagy in bone development and aging.

Autophagy is the primary intracellular degradation system. During this physiological process cytoplasmic material from various cellular origins (vesicles, intracytoplasmic, endoplasmic reticulum (ER), mitochondria) are packed in autophagosomes. These structures will fuse with lysosomes to form autolysosomes where degradation and recycling takes place.[3] Autophagy is a physiological event, controlled by genes of Atg family and is activated at several steps of cell life. Autophagy is involved during differentiation where unused/altered cellular components are digested/renewed. It is classically activated during starvation, when a cell needs to degrade some of its content to support protein synthesis; this TOR-Autophagic Spatial Coupling Compartment (TASCC)[4] is used to ensure protein secretion. On the other hand, autophagy is also involved during sustained and abundant protein production when ER-protein quality control fails to eliminate abundant unfolded proteins. Autophagy being involved in stressful situations is emerging as a central hub for the understanding of many diseases.[5]

During their differentiation from osteoprogenitors to osteocytes, cells face many physiological and physical challenges. For example, synthesis of abundant extracellular matrix is an important challenge for ER of immature cells while during osteoblast/cyte transition, the drastic reduction of cytoplasmic content (especially mitochondrion) is required while important synthesis of local factors (RANKL, Sost) is effective. We conjuncture that during these particular phases, cells use autophagy making this multifaceted process a new player in biology of bone-forming cells.[6]

The role of autophagy in osteoblast/osteocyte function has been first suggested in the murine model using truncation of Nbr1, a selective autophagic receptor for degradation of ubiquitinated proteins. Nrb1 truncation increases osteoblast differentiation and activity *in vivo*.[7] In this model, truncation of Nrb1 was not osteoblast-specific, which makes it unclear about roles of other bone cells such as osteocytes and/or osteoclasts.

The recent paper of Liu *et al.*[2] demonstrates elegantly and convincingly that deletion of a focal adhesion interacting protein in osteoblasts impairs their terminal differentiation. This paper provides clear evidence for a positive role of autophagy in osteoblast differentiation by selectively deleting FIP200 (focal adhesion kinase family interacting protein of 200 kD) in Osterix expressing cells.

FIP200 is a known inhibitor of focal adhesion kinase and its related kinase Pyk2 and as such could be an important modulator of integrin mediated signaling.[8] Interestingly, FIP200 is a regulatory partner of the ULKs–Atg13–FIP200 complex, which is essential for the induction of autophagy in mammalian cells.[9] The authors establish that FIP200 deletion effects overlap those observed in Atg5- and Atg7-deleted mice models.

Liu and colleagues showed first *in vitro* that primary osteoblastic cells presented altered autophagic capabilities as soon as they reach confluency. Confluency triggers fibronectin fibrillogenesis and collagen type I deposition, both conveying differentiation signals. Such abundant protein secretion may require elevated autophagic capabilities to be sustained (ER-protein quality control and TASCC).

The authors investigate thoroughly the *in vivo* function of osteoblast autophagy by comparing Osx-, Col2.3- and Col3.6-Cre;FIP200fl/fl transgenic models. A similar osteopenia phenotype is observed in all these lines, highlighting the important

role of FIP200 in bone development and osteoblast function. Using various inhibitors of autophagy at various times during osteoblast differentiation *in vitro*, these authors reveal that FIP200 and autophagy are necessary for the switch between proliferative and nodule formation phases of osteoblastogenesis.

Sandilands *et al.*[10] discovered a c-Cbl-mediated autophagic targeting of active Src in cancer cells to maintain cell survival capabilities when the integrin/Src/FAK pathway is disrupted. Focal adhesion comprises many associated proteins among which are FIP200, FAK and Pyk2. Pyk2 is a negative regulator of osteoblastogenesis[11] and in absence of FIP200, Pyk2 may be free to fully exert its negative effects by shuttling to focal adhesions.[12] Thus it is conceivable that Pyk2 itself may be degraded by an autophagic-dependent pathway.

Osteoblasts are short living cells as compared to osteocytes, the latter being able to survive many years; their autophagic capabilities might be particularly elevated or inductible. Of note, excessive autophagy could be responsible for osteocytes apoptosis as previously described under prolonged or high-doses glucocorticoid treatments (see Yao *et al.*[13] for review). Aging is often associated with a decrease in autophagy. Onal *et al.*[1] presented recently evidence that genetic suppression of autophagy (Atg7 deletion) in murine osteocytes causes accelerated skeletal aging in young adult mice. These authors compared Atg7fl/fl and DMP1-Cre;Atg7fl/fl mice models. They showed that bone mass and strength, active forming and resorbing surfaces, as well as bone formation rate decreased while cortical porosity and oxidative stress increased; hallmarks of bone aging. However, in this altered genetic mice model, the expected osteocyte apoptosis was not found. Interestingly, the ageing phenotype is associated with increased reactive oxygen species (ROS) production in Atg7-deleted osteocytes. This increase in ROS may be the result of defect in mitophagy (selective elimination of mitochondria). Indeed, accumulation of ROS producing mitochondria may impair the osteoblast/osteocyte transition switch (cytoplasmic reduction, aged mitochondria removal). In addition excessive ROS production may be also directly responsible for reduction in osteoblast and osteoclast activities seen in DMP1-Cre;Atg7fl/fl mice model.

In conclusion, terminal differentiation of osteoblasts/cytes is controlled by autophagy. However, from loss of stemness of skeletal multipotent cells, proliferation of osteoprogenitors, abundant matrix synthesis in osteoblasts and survival of osteocytes, many different players may be positively and/or negatively regulated by autophagic-dependent processes. Because the capacity of active osteoblasts to survive may change dramatically the amount of bone formed and due to the fact that an active secretion of RANKL and Sclerostin by osteocytes may control bone turnover and strength, manipulating autophagy (in particular, using peptides able to increase it[14]) in bone-forming cells may reveal innovative targets to control bone physiology.

Conflict of Interest

The author declares no conflict of interest.

References

1. Onal M, Piemontese M, Xiong J, Wang Y, Han L, Ye S *et al.* Suppression of autophagy in osteocytes mimics skeletal aging. *J Biol Chem* 2013;**288**:17432–17440.
2. Liu F, Fang F, Yuan H, Yang D, Chen Y, Williams L *et al.* Suppression of autophagy by FIP200 deletion leads to osteopenia in mice through the inhibition of osteoblast terminal differentiation. *J Bone Miner Res* 2013;**28**:2414–2430.
3. Mizushima N, Komatsu M. Autophagy: renovation of cells and tissues. *Cell* 2011;**147**:728–741.
4. Narita M, Young AR, Arakawa S, Samarajiwa SA, Nakashima T, Yoshida S *et al.* Spatial coupling of mTOR and autophagy augments secretory phenotypes. *Science* 2011;**332**:966–970.
5. Rubinsztein DC, Codogno P, Levine B. Autophagy modulation as a potential therapeutic target for diverse diseases. *Nat Rev Drug Discov* 2012;**11**:709–730.
6. Hocking LJ, Whitehouse C, Helfrich MH. Autophagy: a new player in skeletal maintenance? *J Bone Miner Res* 2012;**27**:1439–1447.
7. Whitehouse CA, Waters S, Marchbank K, Horner A, McGowan NW, Jovanovic JV *et al.* Neighbor of Brca1 gene (Nbr1) functions as a negative regulator of postnatal osteoblastic bone formation and p38 MAPK activity. *Proc Natl Acad Sci USA* 2010;**107**:12913–12918.
8. Ueda H, Abbi S, Zheng C, Guan JL. Suppression of Pyk2 kinase and cellular activities by FIP200. *J Cell Biol* 2000;**149**:423–430.
9. Hosokawa N, Hara T, Kaizuka T, Kishi C, Takamura A, Miura Y *et al.* Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. *Mol Biol Cell* 2009;**20**:1981–1991.
10. Sandilands E, Serrels B, McEwan DG, Morton JP, Macagno JP, McLeod K *et al.* Autophagic targeting of Src promotes cancer cell survival following reduced FAK signalling. *Nat Cell Biol* 2012;**14**:51–60.
11. Buckbinder L, Crawford DT, Qi H, Ke HZ, Olson LM, Long KR *et al.* Proline-rich tyrosine kinase 2 regulates osteoprogenitor cells and bone formation, and offers an anabolic treatment approach for osteoporosis. *Proc Natl Acad Sci USA* 2007;**104**(25):10619–10624.
12. Guignandon A, Boutahar N, Rattner A, Vico L, Lafage-Proust MH. Cyclic strain promotes shuttling of PYK2/Hic-5 complex from focal contacts in osteoblast-like cells. *Biochem Biophys Res Commum* 2006;**343**:407–414.
13. Yao W, Dai W, Jiang JX, Lane NE. Glucocorticoids and osteocyte autophagy. *Bone* 2013;**54**:279–284.
14. Shoji-Kawata S, Sumpter R, Leveno M, Campbell GR, Zou Z, Kinch L *et al.* Identification of a candidate therapeutic autophagy-inducing peptide. *Nature* 2013;**494**:201–206.