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Evans and Wan reply:

This concerns the correspondence by Zou *et al.*[1] on the physiologic, pharmacologic and pathologic roles of peroxisome proliferator-activated receptor-γ (PPAR-γ) in osteoclasts. Multiple studies, including our own[2] and that of Zou *et al.*[1], demonstrate a direct role for PPAR-γ in enhancing osteoclastogenesis, which would in part explain the increase in bone fractures seen in patients treated with PPAR-γ drugs, such as Actos or Avandia, as compared to untreated controls. Thus, the suggestion by Zou *et al.*[1] that PPAR-γ does not regulate normal osteoclast differentiation is unexpected and inconsistent with our findings that PPAR-γ deficiency leads to osteoclast dysfunction and osteopetrosis[2].

The burden of proof to support the 'serendipitous' expression of PPAR-γ in osteoclasts is high, given its agreed-upon potent pro-osteoclastogenic activity. In our hands, the genetic loss of PPAR-γ in the osteoclast lineage leads to splenomegaly and extramedullary hematopoiesis (EMH), a phenotype consistent with a major role in osteoclastogenesis. Indeed, splenomegaly and EMH are hallmarks of osteoclast defects in multiple osteopetrotic mouse models[3–8]. Furthermore, osteopetrosis, EMH and splenomegaly are pathologically linked in humans[9], and spleen enlargement is a key clinical symptom used for the diagnosis of osteopetrosis in patients[10]. In our original study[2], this phenotype was both conferred and rescued by bone marrow transplants, an observation that is consistent with a defect in the marrow. Of note, Zou *et al.*[1] confirmed our findings[2] and reported the same splenomegaly in their PPAR-γ knockout (KO) mice (~50% increase in spleen/body weight ratio; see Supplementary Figure 2a in Zou *et al.*[1]) when they used the same Tie2-Cre as in our study[2]. Unfortunately, in Zou *et al.*[1], the importance of this phenotype was not discussed, and the spleen sizes in the Vav1- and LysM-PPAR-γ KO mice were not provided. Similarly, Zou *et al.*[1] confirmed our findings and reported reduced expression of Ctsk, an osteoclast differentiation marker and a protease responsible for bone degradation, in LysM-Cre PPAR-γ KO mice as compared to wild-type controls; however, they did not address the significance or relevance of this result. Moreover, other osteoclast differentiation markers (Acp5, Calcr, Car2, MMP9) that exhibited greater reductions (>80%) in basal expression in the Tie2-Cre PPAR-γ-KO in our original report[2] were not interrogated by Zou *et al.*[1]. Importantly, Zou *et al.*[1] did not report serum levels of bone-resorption markers—*in vivo* measurements of osteoclast activity.

In the penultimate sentence of their correspondence[1], the authors allude to a conflict with their own recently published results in Izawa *et al.*[11]. This intriguing study on the PPAR-γ co-factor ASXL2 leads them to conclude that "ASXL2 regulates the osteoclast via two distinct signaling pathways. It induces osteoclast formation in a PPARγ/c-Fos-dependent manner and is required for RANK ligand- and thiazolidinedione-induced bone resorption independent of PGC-1β", which is in agreement with our original study[2]. Izawa *et al.*[11] reported osteopetrosis in ASXL2-KO mice with splenomegaly and high bone mass, which is similar to the phenotype of our Tie2-Cre PPAR-γ-KO mice[2]. By contrast, the opposite phenotype for ASXL2-KO (bone loss rather than bone gain) was reported by this group in an earlier study in Farber *et al.*[12]. These apparent discrepancies highlight the challenges and potential pitfalls in loss- and gain-of-function studies. For example, although fibroblast growth factor 1 (FGF1) is widely used in growth media, the lack of any developmental, reproductive, neurologic or cardiovascular defects in the FGF1-knockout mouse might be considered as proof of no physiologic function of the protein. However, 15 years after the KO mouse was developed, an essential role for FGF1 in the survival of nutrient stress was eventually described by Jonker *et al.*[13].

It is possible that subtle changes in culture conditions or the genetic background of the mice could explain some of the variations in PPAR-γ gain- and loss-of-function studies. On this note, our mice could not be fully inbred, which might lead to some intrinsic genetic variation during the 9 years between the two studies. In addition, Tie2-Cre, Vav1-Cre and LysM-Cre each knock out PPAR-γ at different developmental times and in different sets of cells. Thus, timing and cell cross-talk could produce variable phenotypes both *in vitro* and *in vivo*.

Despite some hard-to-reconcile differences, the suggestion that PPAR-γ is irrelevant to osteoclast function, although possible, is not logical. On balance, the data supports a physiologic role for PPAR-γ in osteoclastogenesis; however, clearly more work needs to be done. Given the established translational impact of pharmacologically activated PPAR-γ on bone loss, additional and careful examination of the differences will no doubt be enlightening.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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