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**Editorial**

# Growth plate borderline chondrocytes: a new source of metaphyseal mesenchymal precursors

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The fate of growth plate chondrocytes has been a subject of debate for many years. The short report by Mizuhashi and colleagues published in this issue of JBMR[(1)] describes studies elucidating the fate of a small subset of growth plate cells, the borderline chondrocytes that lie at the periphery of the zones of proliferative and hypertrophic chondrocytes. The authors demonstrate that these cells make a substantial contribution to the population of mesenchymal precursor cells in the metaphysis, thus giving rise to osteoblasts forming metaphyseal bone.

The cartilage template that precedes ossification of most bones is a transient tissue that provides mechanical stability, while allowing for rapid tissue turnover during modelling of the developing bone. Following initiation of ossification, the growth plate persists as the source of longitudinal growth, with the vast majority of its

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cells undergoing an orderly series of morphological changes, progressing from the resting state to proliferation followed by hypertrophy. The most mature hypertrophic chondrocytes reside at the ossification front, and apparently disappear without trace as the growth plate is invaded by blood vessels, osteoclasts, osteoblast precursors and bone marrow cells. Many scientists have grappled with the fate of this disappearing population, and two possibilities were considered. On the one hand, in recent decades many authors assumed that hypertrophic chondrocytes all die, and debated whether this occurred by apoptosis or another form of physiological death[2,3]. The second possibility, that hypertrophic chondrocytes survive and differentiate into metaphyseal osteoblasts, was proposed in the nineteenth century and reappeared sporadically in the literature (discussed by Yang *et al*[4]), however the methods available until recently (static morphological methods, invasive vital staining or culture of cells removed from their normal tissue environment) were unable to provide definitive proof that this occurs.

With the advent of lineage tracing methods based on Cre-Lox technology in mice, there was a sudden revival of interest in the fate of hypertrophic chondrocytes. Since 2014, several groups have published evidence that at least some hypertrophic chondrocytes indeed survive and contribute to the metaphyseal mesenchymal population[4-6], however not all marrow mesenchymal precursors present in adults can be attributed to this source.

In the current study, Mizuhashi *et al* have turned their attention to another population, the borderline chondrocytes, which are the outermost cells of the growth plate, in contact with the perichondrium[7]. *Pthrp-creER* mice were pulsed with tamoxifen on their day of birth (P0) to fluorescently label borderline chondrocytes

(observed at P2), then the distribution of the labelled cells followed over several weeks. By P3, labelled cells were observed in the metaphysis, where their numbers increased up to P14 before tailing off. By crossing the *Pthrp-creER* mice with mice expressing GFP under the control of either the *Col1(2.3kb)* or the *Cxcl12* promoter, the authors demonstrated that the borderline chondrocyte-derived cells in the metaphysis include osteoblasts and Cxcl12-abundant stromal cells, respectively.

What exactly are growth plate borderline chondrocytes? They are smaller than the adjacent proliferative and hypertrophic chondrocytes, and somewhat flattened against the edge of the growth plate, but surrounded by cartilage matrix. Mizuhashi *et al* demonstrate that the *Pthrp-creER*-marked cells express the typical chondrocyte markers *Col2a1* and *Acan,* and a small proportion also express the hypertrophy markers *Col10a1* and Runx2; only a very small proportion divide while in their border location, in contrast to the adjacent perichondrial cells. The perichondrium faced by the borderline chondrocytes forms part of the ossification groove of Ranvier, which gives rise to the periosteal bone collar surrounding the growth plate. Since the description of this groove in 1873, developmental biologists have deliberated as to the origin and fate of the borderline chondrocytes (discussed in Shapiro et al 1977[8]). Some argued that they arise from the perichondrium and contribute to appositional expansion of the growth plate, while Ranvier had proposed that expansion in girth results from interstitial growth, and that the borderline chondrocytes move from the growth plate into the groove, contributing to bone formation there. The results of the current study appear to settle the question of their fate (metaphyseal mesenchymal precursors and their derivatives), but do not address their origin. The authors speculate that the borderline chondrocytes arise from PTHrP-negative chondrocytes within the upper zone of the growth plate. This conclusion is consistent with the

earlier observation that resting zone chondrocytes labelled *in vivo* with a vital stain translocate from the centre of the growth plate to its periphery within 2 days[9].

It was already clear that a high proportion of osteogenic cells in the marrow of endochondral bones arise from cells that had once expressed some chondrocytic features, since *Col2-cre*-targeted cells had been shown to comprise 80% of marrow osteoblasts and 90% of *Cxcl12*-expressing stromal cells at P3[10]. However, not all these cells have arisen from the growth plate, since during embryonic life *Col2-cre*-targeted cells are also present in the perichondrium and include osteoblasts of the periosteal bone collar[10]. Zhou *et al*[5] estimated that ~60% of osteocalcin-positive trabecular and endosteal osteoblasts in one-month old mouse bones are derived from hypertrophic (*Col10a1*-expressing) chondrocytes. Mizuhashi *et al* present no information about the proportion of metaphyseal osteoblasts arising from borderline chondrocytes (i.e. cells marked by *Pthrp-creER* at P0), but it is likely that some or all of them are included in the 60% estimate of Zhou *et al*, since during their residence at the growth plate border, some expressed *Col10a1*. It was also already clear that growth plate precursors of osteogenic cells comprise a diverse population, since some of the authors of the current manuscript recently described identification of a *Pthrp*-expressing population of resting zone chondrocytes, that are first detectable at P3, give rise to columnar chondrocytes that undergo hypertrophy, and ultimately become metaphyseal osteoblasts and stromal cells[6]. Intriguingly, marrow stromal cells derived from this sub-population of growth plate chondrocytes fail to differentiate into adipocytes, which are normally amongst the repertoire of marrow stromal cell progeny. The identification of borderline chondrocytes as another sub-population of mesenchymal precursor cells fits one more piece into the complex puzzle of the origins of the osteogenic cells in the marrow of endochondral bones.

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