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Role of tartrate-resistant acid phosphatase (TRAP) in long bone development

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ABSTRACT

Tartrate resistant acid phosphatase (TRAP) was shown to be critical for skeleton development, and TRAP deficiency leads to a reduced resorptive activity during endochondral ossification resulting in an osteopetrotic phenotype and shortened long bones in adult mice. A proper longitudinal growth depends on a timely, well-coordinated vascularization and formation of the secondary ossification center (SOC) of the long bones epiphysis. Our results demonstrate that TRAP is not essential for the formation of the epiphyseal vascular network. Therefore, in wild type (Wt) controls as well as TRAP deficient ($TRAP^{-/-}$) mutants vascularised cartilage canals are present from postnatal day (P) five. However, in the epiphysis of the $TRAP^{-/-}$ mice cartilage mineralization, formation of the marrow cavity and the SOC occur prematurely compared with the controls. In the mutant mice the entire growth plate is widened due to an expansion of the hypertrophic zone. This is not seen in younger animals but first detected at week (W) three and during further development. Moreover, an enhanced number of thickened trabeculae, indicative of the osteopetrotic phenotype, are observed in the metaphysis beginning with W three. Epiphyseal excavation was proposed as an important function of TRAP, and we examined whether TRAP deficiency affects this process. We therefore evaluated the marrow cavity volume (MCV) and the epiphyseal volume (EV) and computed the MCV to EV ratio (MCV/EV). We investigated developmental stages until W 12. Our results indicate that both epiphyseal excavation and establishment of the SOC are hardly impaired in the knockouts. Furthermore, no differences in the morphology of the epiphyseal bone trabeculae and remodeling of the articular cartilage layers are noted between Wt and $TRAP^{-/-}$ mice. We conclude that in long bones, TRAP is critical for the development of the growth plate and the metaphysis but apparently not for the epiphyseal vascularization, excavation, and establishment of the SOC.

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1. Introduction

In mammals, long bones are formed via a cartilage model by a process referred to as endochondral bone formation. In the course of which the primary ossification center (POC) initially develops within the diaphysis, followed by the establishment of the secondary ossification center (SOC) within the epiphysis. Basically, the development of the two ossification centers is quite similar, requiring cartilage matrix mineralization, its resorption, angiogenesis and eventually deposition of the bone matrix. The overall bone growth is, on the one hand, governed by the growth plate located between the two sites of ossification, and responsible for the length growth of the diaphysis. On the other hand, the longitudinal, radial and lateral growth of the epiphysis is controlled by the articular cartilage layers that act as a surface growth plate during the early postnatal period (Alvarez et al., 2005a; Holmbeck and Szabova, 2006; Hunziker et al., 2007; Stempel et al., 2011).

A timely well-coordinated epiphyseal vascularization is essential for a proper histology and function of the growth plate in the long bones (Brashear, 1963; Holmbeck et al., 1999; Maes et al., 2004). Accordingly, in murine species cartilage canals are generated at a certain point of time, shortly after birth (Alvarez et al., 2005b; Blumer et al., 2008a,b; Holmbeck and Szabova, 2006; Kugler et al., 1979). The canals erode the non-mineralized cartilage matrix and thus give blood vessels and bone-forming cells access to the epiphysis for the subsequent development of the SOC. Canal formation is governed by several matrix metalloproteinases (MMPs) and, most notably, membrane-bound type-1 matrix metalloproteinase (MT1-MMP = MMP 14) is critical for this process. As a result, MT1-MMP$^{-/-}$ mice reveal a vascular defect accompanied by delayed ossification (Davoli et al., 2001; Holmbeck et al., 1999; Lee et al., 2009; Zhou et al., 2000). In mice lacking both MMP 9 and MMP 13 epiphyseal development is affected likewise (Ortega et al., 2004; Stickens et al., 2004). Apart from the importance of the MMPs, the establishment of the epiphyseal vascular network is triggered by the vascular endothelial growth factor (VEGF), and VEGF deficiency causes a delayed vascularization and formation of the SOC (Allerstorfer et al., 2010; Maes et al., 2004; Petersen et al., 2002). Taken together, failures in early epiphyseal development result in impaired bone growth leading to dwarfism (Blumer et al., 2008b; Holmbeck et al., 1999; Maes et al., 2004; Stickens et al., 2004; Zhou et al., 2000).

Tartrate-resistant acid phosphatase activity type 5 (TRAP or Acp5) is an iron-containing enzyme that is found in humans and murine species. It occurs in diverse tissues including bone and cartilage (Hayman et al., 2000; Hayman and Cox, 2003). TRAP is, at first, synthesized as a latent proenzyme with low activity, and proteolytic processing generates two subunits of about 16 and 20–23 kDa with enhanced enzymatic activity. The cysteine proteinase cathepsin K has been suggested to be responsible for the proteolytic activation of TRAP (Hollberg et al., 2002, 2005; Yamaza et al., 1998). TRAP is highly expressed in chondroclasts as well as osteoclasts and, therefore, used as a specific histochemical marker for these cells (Burstone, 1959; Minkin, 1982). Both are polynucleated, having the same ultrastructural features. However, chondroclasts attack the mineralized cartilage matrix whereas osteoclasts participate in the resorption of the mineralized bone matrix (Hayman et al., 2000). TRAP prompts the dephosphorylation of bone matrix phosphoproteins like osteopontin and bone sialoprotein and was originally shown to be important for a normal endochondral bone formation (Ek-Rylander et al., 1994; Hayman et al., 1996; Hollberg et al., 2002; Suter et al., 2001). Mice lacking TRAP develop normally, but adults reveal a malformation of the skeleton. Specifically, they have a mild osteopetrotic phenotype with increased bone tissue and mineral density. The bones are brittle and shortened due to a malfunction of the growth plate (Hayman and Cox, 2003; Hayman et al., 1996; Hollberg et al., 2002; Roberts et al., 2007; Suter et al., 2001).

The importance of TRAP during early epiphyseal development of the long bones has not been elucidated. However, the occurrence of the enzyme in the cartilage canals exactly at the onset of their formation (Allerstorfer et al., 2010; Alvarez et al., 2005b; Blumer et al., 2008a) suggests a possible role in the establishment of the vascular network. Loss of TRAP may impair this process, thus altering the epiphyseal development and growth plate structure. To delineate the long-range impact of TRAP during bone development, we investigated the femur in TRAP deficient mice from postnatal day (P) 5 until week (W) 12 and compared the results with wild type (Wt) mice.

2. Materials and methods

2.1. Experimental animals

Mice (129SvEv) with a targeted disruption of the single Acp5 gene that maps to murine chromosome 9 were generated as previously described by Hayman et al. (1996). In brief, homologous recombination was used to disrupt exon 2 of the murine TRAP gene. These mutant mice and their Wt littermates aged 5, 6, 7, 8, 9, 10, and 13 days were obtained from the School of Clinical Veterinary Science, University of Bristol, Langford BS40 5DU, UK. Wt mice of the same inbred strain 129SvEv aged 3, 4, 6, 8, and 12 weeks were obtained from the Central Laboratory Animal Facilities of the Innsbruck Medical University. TRAP$^{-/-}$ mice of corresponding age were obtained from the School of Clinical Veterinary Science, University of Bristol, Langford BS40 5DU, UK. Three animals per age group were used; the sex of the mice was not recorded. The animals were anesthetized with CO_2, and killed by cervical displacement. Subsequently the legs were amputated, the soft tissue was carefully removed, and the distal part of the right and left femur examined. All mice were killed between 9 and 11 a.m. for better comparability at early stages.

2.2. Tissue preparation for histology, histochemistry and immunohistochemistry

The bones were fixed with 4% PFA in phosphate buffer saline (PBS, 0.1 M) for 4 h at room temperature and rinsed in PBS. Until P 13 the femurs were decalcified in 3% ascorbic acid in sodium chloride (0.15 M) for 1–2 days at room temperature, and from each age group only one femur/animal was decalcified. Non-decalcified bones were used for von Kossa staining. The femurs of mice aged 3, 6, 8, and 12 weeks were