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Analysis of skeletal phenotypes in thyroid hormone receptor mutant mice

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Introduction

Childhood hypothyroidism causes delayed endochondral ossification, impaired bone mineralization and growth retardation. In contrast, thyrotoxicosis induces accelerated growth, advanced bone age and increased mineralization, but leads to premature closure of the epiphyseal growth plates and short stature. In severe cases accelerated intramembranous ossification results in early closure of the skull sutures and craniosynostosis. In adults, thyrotoxicosis causes increased bone turnover, accelerated bone loss and a greater than 3-fold increase in osteoporotic fracture. Thyroid hormone (T3) binds to nuclear T3 receptors (TR α and TR β), which act as hormone-dependent transcription factors that regulate target gene expression in response to T3. Concentrations of circulating thyroid hormones are maintained in a narrow physiological range by the hypothalamic-pituitary-thyroid (HPT) axis, in which TR β controls activity of a negative feedback loop. The TR α and TR β receptors are expressed in temporo-spatial specific patterns during development and in different ratios in individual tissues.

Methods

To investigate the molecular mechanisms underlying abnormalities of skeletal development and adult bone turnover that result from alterations of thyroid status, we characterised the skeletal phenotypes of TR mutant mice harboring dominant negative mutations (TR α 1^{PV/+}, TR α 1^{R384C/+}, TR β ^{PV/PV}) or deletions (TR α ^{0/0}, TR β ^{-/-}) of the genes encoding TR α and TR β . Murine skeletal development was characterized by light microscopy using histological methods that included alizarin red and alcian blue staining of whole skeletal preparations, von Kossa staining of undecalcified histological sections of long bones and alcian blue and van Gieson staining of decalcified sections. Dynamic aspects of endochondral ossification were assessed by analysis of long bone and tail growth, and histomorphometric measurement of epiphyseal growth plate zones and mid-diaphyseal cortical bone. Intramembranous ossification was assessed by measurement of cranial indices and skull suture and fontanelle areas. Skeletal thyroid status was determined by in situ hybridization analysis of T3-target gene expression. In adult mice, 3D analysis of bone micro-architecture was determined in near mid-line longitudinal sections of macerated long bones by BSE SEM using an automated digital system (Zeiss DSM962). In further studies, long bones, tail

vertebrae and incisors were embedded in poly-methyl-methacrylate and optically flat standard longitudinal section planes were obtained by diamond micro-milling. The quantitative distribution of micro-mineralization densities was determined in these specimens by comparison to halogenated dimethacrylate standards using qBSE SEM.

Results

Endochondral ossification, linear growth and skeletal mineralization were retarded in TR α null mice (TR $\alpha^{0/0}$) and more severely delayed in TR α dominant-negative mutants (TR $\alpha^{1^{PV/+}}$, TR $\alpha^{1^{R384C/+}}$). In contrast, these parameters were all advanced in TR β null mice (TR $\beta^{-/-}$), with a more severe phenotype evident in TR β dominant-negative mutants (TR $\beta^{PV/PV}$). TR α mutant mice displayed increased cortical bone width, and up to an 8 fold increase in trabecular bone volume with increased trabecular thickness, plate-like morphology and greater micro-architectural complexity (Figs. 1,2). In contrast, analysis of all these parameters including quantitation of bone micro-mineralization density revealed that TR β mutants were markedly osteoporotic with thinner trabeculae of rod-like morphology. Analysis of downstream signalling pathways involving the T3-target genes FGFR1, FGFR3, GHR and IGF-1R revealed a phenotype of skeletal hypothyroidism in all TR α mutant mice but skeletal thyrotoxicosis in TR β mutants. We further demonstrated that TR α is expressed at 12-fold higher levels in bone than TR β , whereas TR β is known to be predominantly expressed in hypothalamus and pituitary. Accordingly, all TR α mutant mice were systemically euthyroid whereas TR $\beta^{PV/PV}$ and TR $\beta^{-/-}$ displayed pituitary resistance to thyroid hormone with inappropriately elevated circulating thyroid hormone levels.

Conclusions

Our analysis of this large series of TR mutant mice with differing genetic backgrounds unequivocally demonstrates that TR α is the predominant TR isoform expressed in bone, and shows that the skeletal effects of disrupted TR β signalling result from its effects on systemic thyroid status. Thus, TR α plays the major physiological role in skeletal development, linear growth and the maintenance of adult skeletal integrity in vivo.

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Fig. 1. BSE SEM image (field width 584 microns) demonstrating trabecular bone architecture in distal femur of 14 week old wild-type mouse. 22 images at 33 micron focus intervals were processed using AutoMontage software to produce this all-in-focus image (see Boyde Scanning 2004 26 265–269).

Fig. 2. BSE SEM image (field width 584 microns) demonstrating trabecular bone architecture in distal femur of 14 week old TRalphaR384C/+ mutant mouse. 22 images at 33 micron focus intervals were processed using AutoMontage software to produce this all-in-focus image.