Bone Reports 9 (2018) 27–36

Contents lists available at ScienceDirect

Bone Reports

journal homepage: www.elsevier.com/locate/bonr

Bones in human CYP26B1 deficiency and rats with hypervitaminosis A phenocopy *Vegfa* overexpression

Thomas Lind[a,*], Roberta Lugano[b], Ann-Marie Gustafson[a], Maria Norgård[c], Arie van Haeringen[d], Anna Dimberg[b], Håkan Melhus[a], Stephen P. Robertson[e], Göran Andersson[c]

[a] Department of Medical Sciences, Section of Clinical Pharmacogenomics and Osteoporosis, Uppsala University, University Hospital, SE-75185 Uppsala, Sweden
[b] Department of Immunology, Genetics and Pathology, Science for Life Laboratory, The Rudbeck Laboratory, Uppsala University, SE-75185 Uppsala, Sweden
[c] Division of Pathology, Department of Laboratory Medicine, Karolinska Institutet, Karolinska University Hospital, SE-14152 Huddinge, Sweden
[d] Department of Human and Clinical Genetics, Leiden University Medical Center, 2333 ZA Leiden, the Netherlands
[e] Department of Women's and Children's Health, Dunedin School of Medicine University of Otago, 9054 Dunedin, New Zealand

ARTICLE INFO

Keywords:
CYP26B1
Vitamin A
VEGFA
Microhemorrhage
Human
Rat
Bone

ABSTRACT

Angulated femurs are present prenatally both in CYP26B1 deficient humans with a reduced capacity to degrade retinoic acid (RA, the active metabolite of vitamin A), and mice overexpressing vascular endothelial growth factor a (Vegfa). Since excessive ingestion of vitamin A is known to induce spontaneous fractures and as the Vegfa-induced femur angulation in mice appears to be caused by intrauterine fractures, we analyzed bones from a *CYP26B1* deficient human and rats with hypervitaminosis A to further explore Vegfa as a mechanistic link for the effect of vitamin A on bone. We show that bone from a human with *CYP26B1* mutations displayed periosteal osteoclasts in piles within deep resorption pits, a pathognomonic sign of hypervitaminosis A. Analysis of the human angulated fetal femur revealed excessive bone formation in the marrow cavity and abundant blood vessels. Normal human endothelial cells showed disturbed cell-cell junctions and increased *CYP26B1* and *VEGFA* expression upon RA exposure. Studies in rats showed increased plasma and tissue Vegfa concentrations and signs of bone marrow microhemorrhage on the first day of excess dietary vitamin A intake. Subsequently hypervitaminosis A rats displayed excess bone formation, fibrosis and an increased number of megakaryocytes in the bone marrow, which are known characteristics of *Vegfa* overexpression. This study supports the notion that the skeletal phenotype in CYP26B1 deficient human bone is caused by excess RA. Our findings suggest that an initial part of the vitamin A mechanism causing bone alterations is mediated by excess Vegfa and disturbed bone marrow microvessel integrity.

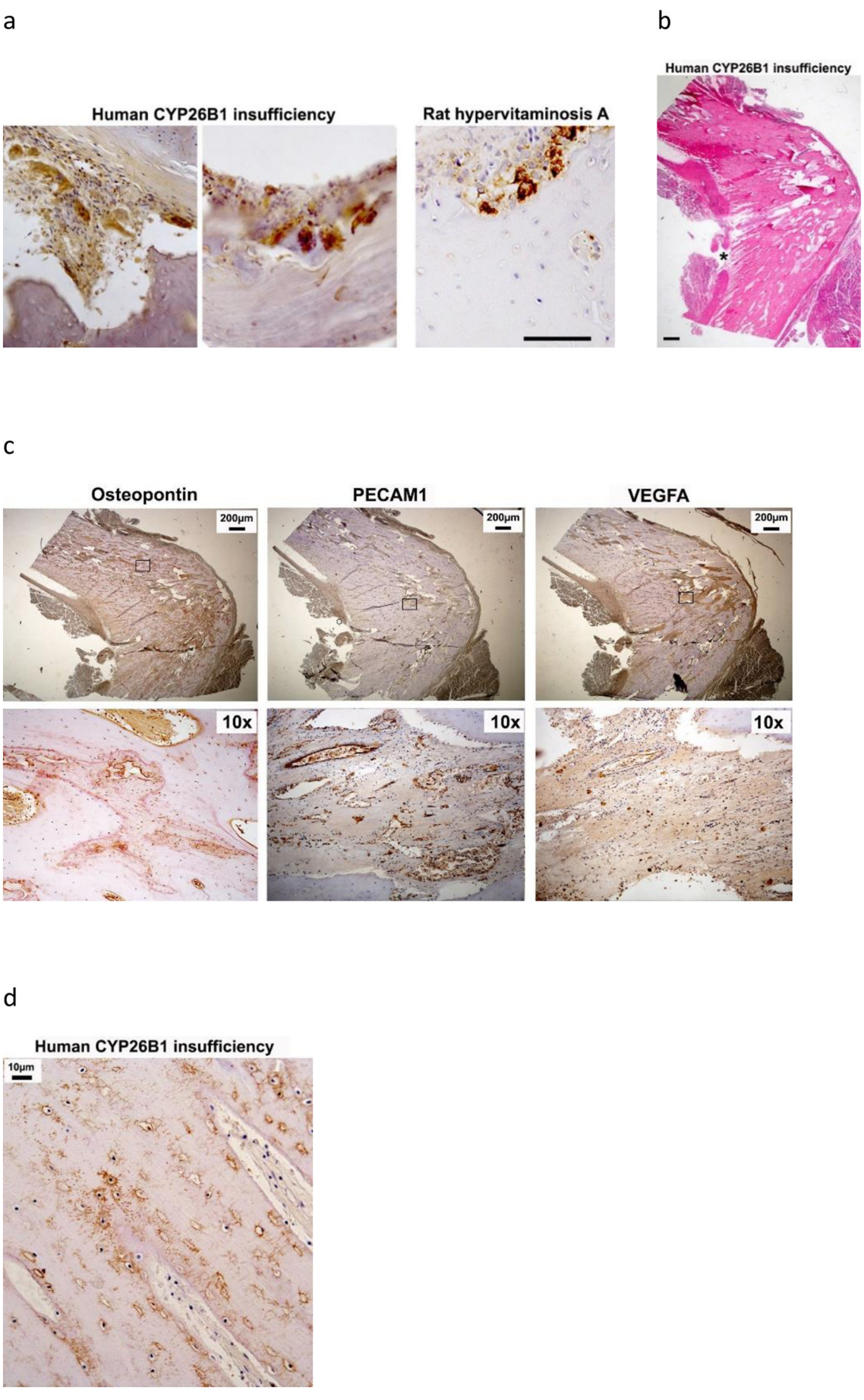
1. Introduction

In humans, biallelic mutations in *CYP26B1*, the gene encoding the major enzyme responsible for degradation of excess intracellular retinoic acid (RA), that lead to a substantial reduction in enzyme activity result in severe skeletal anomalies demonstrating the importance of strict regulation of intracellular RA levels for human bone health (Laue et al., 2011) The major skeletal defects were angulated femora, joint synostosis, advanced bone age and calvarial bone hypoplasia. Unrestricted chondrogenesis and aberrant osteoblast-osteocyte transitioning were proposed as the mechanisms behind the joint synostosis and craniosynostosis (a phenotypic feature associated with a hypomorphic *CYP26B1* mutations), respectively (Laue et al., 2011). However, the mechanism behind the angulated femurs in the *CYP26B1* deficient human remained unexplored.

Vitamin A (retinol) is particularly toxic to bone tissue as it is known to cause spontaneous skeletal fractures in experimental animals when administered in excess (Binkley and Krueger, 2000). No animal species has the capability for *de novo* synthesis of vitamin A and it is thus an essential micronutrient. Ingested vitamin A is fat soluble and quickly absorbed but slowly cleared from the body, thus toxicity can occur either from high-dose exposure during a short time or lower intake over more prolonged periods (Melhus, 2011). In line with this, high intake and elevated serum concentrations of vitamin A in humans have been associated with an increased risk of hip fracture (Melhus et al., 1998; Michaëlsson et al., 2003). We and others have shown that the

* Corresponding author at: Department of Medical Sciences, Section of Clinical Pharmacogenomics and Osteoporosis, Rudbecklaboratoriet, C11, plan 4, SE-75185 Uppsala, Sweden.
E-mail addresses: thomas.lind@medsci.uu.se (T. Lind), roberta.lugano@igp.uu.se (R. Lugano), ann-marie.gustafson@imbim.uu.se (A.-M. Gustafson), maria.norgard@ki.se (M. Norgård), A.van_Haeringen@lumc.nl (A. van Haeringen), Anna.Dimberg@igp.uu.se (A. Dimberg), hakan.melhus@medsci.uu.se (H. Melhus), stephen.robertson@otago.ac.nz (S.P. Robertson), goran.andersson@ki.se (G. Andersson).

https://doi.org/10.1016/j.bonr.2018.06.006
Received 12 March 2018; Received in revised form 5 June 2018; Accepted 16 June 2018
Available online 21 June 2018



(caption on next page)