Karolinska Institutet

Department of Women's and Children's Health

Effects of Proteasome Inhibitors on Chondrogenesis and Linear Bone Growth

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Skandiasalen, Astrid Lindgrens Barnsjukhus, Plan 1, Karolinska Sjukhuset

Fredagen den 31 Maj, 2013, kl 09.00

av

Emma Eriksson

BSc

Huvudhandledare:
Professor Lars Sävendahl, MD, PhD
Dept of Women's and Children's Health
Karolinska Institutet, Stockholm, Sweden

Bihandledare:
Assoc. Professor Dionisios Chrysis, MD, PhD
Dept of Pediatrics
Pediatric Endocrinology Unit
Medical School, University of Patras, Greece

Assoc. Professor Vladimir Bykov, MD, PhD
Dept of Women's and Children's Health
Oncology-Pathology Unit, Cancer Centrum Karolinska (CCK)
Karolinska Institutet, Stockholm, Sweden

Fakultetsopponent:
Professor Francesco De Luca, MD, PhD
Section of Endocrinology and Diabetes
Drexel University College of Medicine, Philadelphia, USA

Betygsnämnd:
Assoc. Professor Rachel Sugars, PhD
Dept of Dental Medicine
Karolinska Institutet, Stockholm, Sweden

Professor Maria Masucci, PhD
Dept of Cellular and Molecular Biology
Karolinska Institutet, Stockholm, Sweden

Assoc. Professor Ingrid Öra, MD, PhD
Dept of Pediatric Oncology and Hematology
Skåne University Hospital, Lund University, Sweden

Stockholm 2013

ABSTRACT

Linear bone growth occurs at the growth plate, a thin layer of cartilage between the epiphysis and metaphysis of long bones. In the growth plate, resting/stem-like chondrocytes divide and generate the highly proliferative chondrocytes, which further differentiate into the enlarged hypertrophic form before being substituted by bone, a process called endochondral ossification. A precise balance between different factors affecting chondrocyte proliferation, differentiation/hypertrophy, matrix synthesis, and cell death within the growth plate must exist to ensure normal bone growth. Anti-cancer therapy can interfere with any of these processes, thereby affecting chondrogenesis and bone growth negatively. Proteasome inhibitors (PIs, e.g., MG262 and bortezomib) are a new, novel class of anti-cancer drugs. Bortezomib is approved for the treatment of adult hematologic malignancies, and is currently under clinical trials with pediatric cancers. So far, any undesired secondary side effects are yet unknown in treated children.

The aim of this thesis was to address whether PIs affect linear bone growth and bone homeostasis, and if so, what the underlying cellular mechanisms are, and to find potential ways to protect bone growth during anti-cancer treatment.

In the first study (Paper I), the effect of the non-clinically used PIs, MG262 and lactacystin, were investigated both in vitro and in vivo. Here we report for the first time that systemic administration of MG262 specifically targets the growth plate, and impairs linear bone growth in treated mice. The effect is linked to increased apoptosis of resting/stem-like chondrocytes in a caspase-dependent and independent manner. Inhibition of p53 and apoptosis-inducing-factor (AIF) were able to partly rescue from MG262-induced chondrocyte apoptosis.

Since bortezomib is in pediatric clinical trials, it is even more important to delineate any possible secondary side effects on linear bone growth and bone homeostasis (Paper II). Our results demonstrate that a clinically relevant dose of bortezomib specifically and efficiently impairs the ubiquitin/proteasome system (UPS). Consequently, young mice display severe growth failure during treatment, as well as after a follow-up period of 6 months post-treatment. This effect was mediated through a local action of bortezomib in the growth plate, causing increased resting/stem-like chondrocyte apoptosis and decreased differentiation. We also show that bortezomib mainly acts via the intrinsic apoptotic pathway, in which p53 and Bax appear to be the key regulators triggering apoptosis. In addition, cultured human growth plate cartilage was confirmed to be highly sensitive to bortezomib.

In an attempt to rescue bone growth during bortezomib treatment, we utilized pharmacological inhibition of Bax by the synthetic peptide analog to endogenous humanin, [Gly14]-Humanin (HNG) (Paper III). We made the novel finding that HNG can rescue bone growth during bortezomib treatment by protecting resting/stem-like growth plate chondrocytes. Importantly, HNG did not interfere with the desired anti-cancer effect of bortezomib as tested and verified in tumor xenograft models as well as several human tumor cell lines. HNG also protected cultured human growth plate cartilage from the cytotoxic effects of bortezomib.

In conclusion, our observations confirmed in vivo and in vitro, including human growth plate cartilage, suggest that bone growth could potentially be suppressed in children treated with PIs. We hereby propose that bone growth and bone mineralization should be closely monitored in ongoing pediatric clinical trials. In addition, HNG may have the capacity to prevent PI-induced bone growth impairment without interfering with the desired anti-cancer effect.

ISBN 978-91-7549-130-1