TRANSGENIC EXPRESSION OF HUMAN BCL-2 IN OSTEOBLASTS IN MICE PREVENTS GLUCOCORTICOID-INDUCED APOPTOSIS.

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Introduction

High dose glucocorticoids lead to bone loss primarily by decreasing bone formation and increasing osteoblast apoptosis. In bone, glucocorticoids have also been shown to induce apoptosis by down-regulating bcl-2, a cell survival regulator. To elucidate the role of apoptosis and to determine if apoptosis affects bone mass, a transgenic mouse (Col2.3bcl-2) was developed that over-expresses bcl-2 in mature osteoblasts.

Methods

Three founder lines for Col2.3bcl-2 mice (CD-1 background) were established with a Type I collagen promoter fragment (2.3 kb Col1a1) driving the 1.7 kb region of human bcl-2 (hbc1-2). Western blot analysis was performed on mouse tissues from brain, spleen, kidney, liver, heart, tendon, skin, vertebrae, calvaria and femurs to confirm tissue specific transgene expression. Varying concentrations of dexamethasone (0.1, 0.3 and 1 mg/kg body weight) was administered ip. to 2 month old mice daily for 72 h. Femurs and calvaria were obtained, fixed and embedded in paraffin. Paraffin sections were dried for 1 h at 56°C, deparaffinized, and rehydrated, followed by a 10 min antigen retrieval in 4N HCl. Nonspecific reactivity was blocked with Power Block (BioGenex, San Ramon, CA) for 30 min. Sections were incubated with 4µg/ml of mouse anti-human Bcl-2 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) in 50 mM Tris HCl, 1% BSA, and then with Peroxidase Labeled Polymer followed by substrate-chromagen solution, from the Envision+ kit. (DAKO, Carpinteria, CA). For the ex vivo studies, osteoblasts from Col2.3bcl-2 (Tg/+ ) and nontransgenic (+/+ ) littermates were isolated by sequential collagenase digestion of calvaria. Cells were plated at a density of 10,000 cells/cm² and were treated with varying concentrations of corticosterone. Immunocytochemical detection and quantification of apoptosis was determined by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) using an In Situ Cell Death Detection Kit (Roche Diagnostics distributed by Boehringer Mannheim, Indianapolis, IN).

Results

Three transgenic lines of Col2.3bcl-2 mice appeared healthy and bred normally, however body weight, body and femur length were smaller than +/- littermates by the age of 2 months. Western blot analysis of tissues revealed hbc1-2 expression in tendon, skin, vertebrae, calvaria and femurs. Immunohistochemistry of hbc1-2 expression in femurs of 2-month Tg/+ revealed transgene protein expression in osteoblasts at the growth plate, the endosteal surface and areas of active bone formation on the periosteal surface of the cortical bone. Hbc1-2 expression was also found in the calvarial osteoblasts. Static histomorphometric analysis of the Tg/+ compared to +/- demonstrated that calvaria width was reduced (103.4±9.2 µm vs. 130.7±5.6 µm, p<0.02). Osteoclast number per bone surface and % osteoclast surface was also reduced (1.6±0.5% vs. 5.5±1.2%, p<0.01, and 4.7±1.7% vs. 15.5±4.3%, p<0.04). The decreased calvaria width in the transgenic mice suggests decreased bone remodeling. A dexamethasone dose-dependent increase in apoptotic calvarial osteoblasts of +/- mice was visualized by TUNEL. To further explore the in vivo induction of apoptosis, 2-month old Tg/+ and +/- mice were treated with 1 mg/kg of dexamethasone or vehicle each day for 7 days and then sacrificed. Paraffin-embedded calvaria were evaluated for apoptotic osteoblasts (Ob) and osteocytes (Os). No significant differences were found in the baseline apoptosis in either (Tg/+ vs. +/- vehicle group. However, glucocorticoids significantly increased Ob and Os apoptosis in +/- (p<0.001) animals, but this effect was significantly blunted in the Tg/+ mice (Table 1). This data demonstrates that in vivo expression of bcl-2 prevents dexamethasone-induced apoptosis in bone cells. Ex vivo analysis of apoptosis in +/- osteoblasts treated for 72 h with 1, 10, 100 and 1000 nM corticosterone, demonstrated a dose-dependent increase in TUNEL staining cells (Figure 1). However, in Tg/+ cells, glucocorticoids had no effect on apoptosis.

Discussion

Bcl-2 was shown to be an important regulator of apoptosis in osteoblasts. Mature osteoblast overexpression of bcl-2 in transgenic mice demonstrated significant inhibition of glucocorticoid-induced apoptosis. Additionally, ex vivo cultures of osteoblasts derived from transgenic and littermate control mice demonstrated suppression of glucocorticoid-induced apoptosis. We have shown that the overexpression of bcl-2 can prevent glucocorticoid-induced apoptosis. The effect of glucocorticoid-induced apoptosis on bone mass is presently under investigation.

Table 1. Bcl-2 Expressing Transgenic Mice (Tg/+ ) Are Significantly Less Responsive To Dexamethasone-Induced Osteoblast Apoptosis Than Wild Type (+/- ) Littermates

<table>
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<th>% Positive TUNEL of Dexamethasone-Treated Mice</th>
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<tr>
<td>Dex +/-</td>
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<td>%Ob</td>
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<td>%Os</td>
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Figure 1. Bcl-2 Protects Against Corticosterone-induced Apoptosis in Transgenic Col2.3Bcl-2 Derived Osteoblasts.

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