

Imaging using osteocalcin-luciferase

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Non-invasive monitoring of gene expression *in vivo* could serve as an efficient tool in developmental and regeneration studies of the skeleton. To date, there are no adequate molecular imaging techniques, which quantitatively monitor gene expression *in vivo* in skeletal development and repair. Our aim is to monitor gene expression in the skeleton, utilizing a real-time molecular imaging system, which quantitatively, and non-invasively, detects bioluminescence *in vivo*. In order to pursue this aim, we use an experimental model that consists of transgenic mice harboring luciferase marker gene under the regulation of human osteocalcin (hOC) promoter and a new light detection cooled charge coupled device (CCCD) camera, applied to monitor the luciferase expression. Since only cells expressing the osteocalcin gene, indicating differentiation to bone, will express the luciferase gene, this novel combination enables the real time *in vitro* or *in vivo* tracking of cells differentiating to bone (Figure 1).

During development, transgenic mice, ranging from 5 days to 5 months, exhibited transgene expression in a wide spectrum of skeletal organs, including calvaria, vertebra, tail and limbs, reaching a peak at 1-month-old mice in most of the skeletal organs. Mesenchymal stem cells (MSCs) isolated from bone marrow of transgenic mice exhibited hOC promoter regulation detected by luciferase expression that correlated with their osteogenic differentiation. Additionally, we have been able to show luciferase expression indicating osteogenic differentiation in two skeletal repair models, bone fracture and marrow ablation¹. Non-invasive CCCD system has revealed in both models a peak of luciferase expression at 6 days post-surgery.

In order to establish the reliability of this system, we compared the results to conservative molecular methods. All quantitative, non-invasive, real-time CCCD measurements correlated with luciferase biochemical assay and luciferase immunohistochemistry, which demonstrated luciferase expression in

hypertrophic chondrocytes and trabecular osteoblasts.

We further utilized this model for the non-invasive detection of fracture repair mediated by genetically engineered MSCs. A non-union calvarial defect was performed in osteocalcin-luciferase mice. The defect was filled with human MSCs that were further infected *in situ* with an AAV-2 vector encoding for the BMP-2 gene under tetracycline regulation (tet-on). A parallel monitoring was performed using μ CT and CCCD. Our results demonstrated a correlation between the intense signal seen in CCCD and bone formation detected by μ CT when the BMP-2 gene was up regulated². Low signal and no bone formation were observed in the control group where BMP-2 was downregulated².

Our studies demonstrate: a) that the CCCD detection system is a reliable quantitative gene detection tool for the skeleton *in vivo*; b) expression of luciferase regulated by hOC promoter is significantly decreased with age in most skeletal sites, and c) that the dynamics of hOC regulation during mice skeletal development and repair in real-time, can be quantitatively and non-invasively monitored.

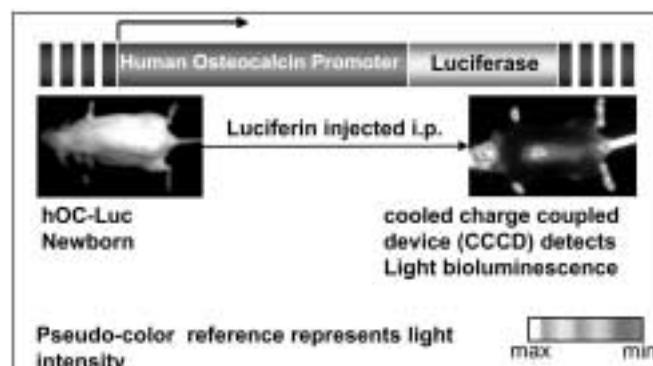


Figure 1. Molecular imaging system monitoring osteogenic differentiation in transgenic mice.

The author has no conflict of interest.

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