

Methodology to improve the efficiency in the migration and detection of mesenchymal stem cells in murine models

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Osteoporosis is a generalised disease of the skeletal system characterised by an imbalance between the bone formation and resorption that leads to bone mass loss and to the deterioration of the microarchitecture of the bone tissue, compromising bone resistance and therefore resulting in a higher bone fragility and an increased susceptibility to fractures¹.

Two stem cells coexist in the bone cavity (bone marrow): the hematopoietic stem cell, which generates all the blood and immune system cells, and the mesenchymal stem cell, responsible for the formation of the skeleton. Osteoblasts or bone-forming cells originate from the differentiation of mesenchymal stem cells. These pluripotent cells can create a wide variety of cell types such as osteoblasts, adipocytes, or chondrocytes²⁻⁴. This characteristic makes them highly interesting candidates for regenerative medicine given their ability to migrate to injured areas to promote the *de novo* generation of bone⁵.

The interest in the use of mesenchymal stem cells in the field of bone metabolism has grown in the early 2000s. Studies have focused primarily on the intravenous treatment of mesenchymal stem cells in children with osteogenesis imperfecta, an inherited enzyme deficiency in collagen synthesis by mesenchymal cells in the bones. This hypothesis is based on observing that bone marrow transplantation can provide stromal cells capable of synthesizing intact type I collagen, replacing the poor cellular function of the patient and improving the symptoms of the disease. The efficacy of the treatment was reported in a study carried out

on six newborn children, showing better growth rates and initial intact bone synthesis⁶. In a second study, these same authors showed that autologous mesenchymal stem cells had normal collagen production in bone cavities, and that transplanted children had growth curves similar to those of transplanted children with allogeneic bone marrow⁷. This pioneering work has served as the basis for the successful application of intravenous mesenchymal stem cells in other clinical entities.

Once introduced into the body, mesenchymal stem cells initiate a process known as homing or nesting in which they are retained in the blood vessels of damaged tissue and are guided to the tissue from these blood vessels by biological mediators such as chemokines, cytokines and adhesion molecules.

To monitor transplanted human cells in animal models, cells previously tagged with a fluorophore are used to detect the signal *in vivo* via magnetic resonance imaging or positron emission tomography⁸. An alternative to these imaging techniques is the detection by real-time quantitative PCR of the presence of transferred human DNA in the organ of interest using Alu elements⁹, a name derived from the presence of a recognition site for the restriction enzyme Alu I. These Alu elements are short sequences of about 300 base pairs, which are repeated throughout the genome, representing roughly 10% of the total. These characteristics and the fact that the appearance of these Alu sequences dates back approximately 65 million years, coinciding with the origin and expansion of



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primates, makes them ideal for detecting human cells¹⁰. However, the limits of detection of the current techniques for studying human genomic DNA do not allow it to be distinguished from other non-human DNA.

In this issue of the Journal of Osteoporosis and Mineral Metabolism, Del Real et al.¹¹ develop a methodology based on the work of Funakoshi et al., using a highly sensitive and specific quantitative real-time PCR method based on Alu sequences to discriminate human cells from rodent cells¹². The aim of this work was to study, by means of PCR analysis of human Alu sequences, the performance to detect human DNA after the infusion of human bone marrow stem cells in immunodeficient mice. These human bone marrow stem cells were obtained from the femoral head of patients undergoing hip replacement surgery.

These authors were able to locate human DNA in the lungs of mice on the first day and 7 days after cell infusions, but this human DNA was inconsistently detected in the liver and the bones, presenting a discrete decrease in human DNA among the days 1 and 7 in the lung, but with clear differences in human DNA levels on day 1 compared to samples that did not contain human DNA.

The authors comment on the need to study the distribution of these cells after their infusion into the blo-

odstream, for which a very sensitive and specific method of detecting small populations of human cells among the cells of the recipient organism is needed. Based on the methodology developed by Funakoshi et al.¹², Del Real et al. were able to detect very low concentrations of human DNA among a high concentration of mouse DNA¹¹. After intravenous infusion of human bone marrow stem cells into mice and between the first 24 hours and the seventh day, these authors were able to verify that human cells were only detectable in the lung, not consistently appearing in either the liver or the bones. As a consequence of this practical limitation, several strategies are being tested to increase the tropism of human bone marrow stem cells to bone tissue, using for this purpose the glycosylation of membrane proteins that allow greater attraction to bone¹³.

Therefore, as previously mentioned, although the use of intravenously infused human mesenchymal cells for regenerative bone treatment is a very promising strategy, there are important methodological limitations as they can become trapped in the lungs and quickly lost. The search for procedures that selectively target these cells to the bone and the ability to improve their monitoring will, in the near future, open up a new therapeutic pathway for the treatment of osteoporosis.



Conflict of interest: The author declares that he has no conflicts of interest.

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