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Naves, Díaz M
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Methodology to improve the efficiency in the migration and detection of mesenchymal stem cells in murine models

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Naves Díaz M
Clinical Management Unit for Bone Metabolism. Asturias Central University Hospital (HUCA). Renal Research Network of the Carlos III Health Institute (REDinREN-ISCIII). Institute for Health Research of the Principality of Asturias (ISPA). Oviedo (Spain)

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 fractures[1].

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 chondrocytes[2-4]. 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 bone[5].

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 synthesis[6]. 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 marrow[7]. 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 tomography. 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 elements, a name derived from the presence of a recognition site for the restriction enzyme Alu I.

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 tomography[8]. 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 elements[9], 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

Correspondence: Manuel Naves Díaz (mnaves.huca@gmail.com)

primates, makes them ideal for detecting human cells[10]. 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 cells[12]. 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 bloodstream, 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 DNA[11]. 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 bone[13].

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.

Bibliography

1. Díaz Curiel M. Osteoporosis: concepto. Fisiopatología. Clínica. Epidemiología. Rev Osteoporos Metab Miner. 2018;10 (1 Suplemento):2-4.
2. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science. 1999;284:143-7.
3. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. J Cell Biochem. 2006;98:1076-84.
4. Caplan AI. Why are MSCs therapeutic? New data: new insight. J Pathol. 2009;217:318-24.
5. Han Y, Li X, Zhang Y, Han Y, Chang F, Ding J. Mesenchymal stem cells for regenerative medicine. Cells. 2019;8(8): 886.
6. Horwitz EM, Gordon PL, Koo WK, Marx JC, Neel MD, McNall RY, et al. Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: implications for cell therapy of bone. Proc Natl Acad Sci U S A. 2002;99:8932-7.
7. Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WW, Gordon PL, Neel M, et al. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nat Med. 1999;5:309-13.
8. Chikate TR, Tang L. Tracking and imaging of transplanted stem cells in animals. Methods Mol Biol. 2019; online ahead of print.
9. Schubert R, Sann J, Frueh JT, Ullrich E, Geiger H, Baer PC. Tracking of adipose derived mesenchymal stromal/stem cells in a model of cisplatin-induced acute kidney injury: Comparison of bioluminescence imaging versus qRTPCR. Int J Mol Sci. 2018;19(9): E2564.
10. Batzer MA, Deininger PL. Alu repeats and human genomic diversity. Nat Rev Genet. 2002;3(5):370-9.
11. Del Real A, López-Delgado L, Sañudo C, Pérez-Núñez MI, Laguna E, Menéndez G, et al. Método sensible para monitorizar la migración de las células madre mesenquimales de la médula ósea en modelos murinos. Rev Osteoporos Metab Miner. 2020;12(2):40-44.
12. Funakoshi K, Bagheri M, Zhou M, Suzuki R, Abe H, Akashi H. Highly sensitive and specific Alu-based quantification of human cells among rodent cells. Sci Rep. 2017;7(1): 13202.
13. Sackstein R, Merzaban JS, Cain DW, Dagia NM, Spencer JA, Lin CP, et al. Ex vivo glycan engineering of CD44 programs human multipotent mesenchymal stromal cell trafficking to bone. Nat Med. 2008;14(2):181-7.