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Biodistribution and retention of locally administered human mesenchymal stromal cells: Quantitative polymerase chain reaction–based detection of human DNA in murine organs

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Abstract

Background. Determining the distributive fate and retention of a cell therapy product after administration is an essential part of characterizing it's biosafety profile. Therefore, regulatory guidelines stipulate that biodistribution assays are a requirement prior to advancing a cell therapy to the clinic. Here the development of a highly sensitive quantitative polymerase chain reaction (qPCR)-based method of tracking the biodistribution and retention of human mesenchymal stromal cells (hMSCs) in mice, rats or rabbits is described. *Methods.* A primer-probe–based qPCR assay was developed to detect and quantify human Alu sequences in a heterogeneous sample of human DNA (hDNA) and murine DNA from whole organ genomic DNA extracts. The assay measures the amount of genomic hDNA by amplifying a 31–base pair sequence of the human Alu (hAlu) repeat sequence, thus enabling the detection of 0.1 human cells in 1.5×10^6 heterogeneous cells. *Results.* Using this assay we investigated the biodistribution of 3×10^5 intramuscularly injected hMSCs in Balb/c nude mice. Genomic DNA was extracted from murine organs and hAlu sequences were quantified using qPCR analysis. After 3 months, hDNA ranging from 0.07%–0.58% was detected only at the injection sites and not in the distal tissues of the mice. *Discussion.* This assay represents a reproducible, sensitive a method of detecting hDNA in rodent and lapine models. This manuscript describes the method employed to generate preclinical biodistribution data that was accepted by regulatory bodies in support of a clinical trial application.

Key Words: *biodistribution, cell therapy, genomic DNA, human Alu sequence, mesenchymal stromal cell, polymerase chain reaction, translational stem cell research*

Introduction

Determining the distributive fate and retention of cell therapy (CT) products after administration are an essential part of characterizing the product's mechanism of action and biosafety profile. The therapeutic cell's phenotype, efficacy and migratory potential are influenced by the formulation of the CT product as well as by the route of administration and the microenvironment in which the cells reside in the host. Concerns surrounding the *in vivo* acquisition of cellular autonomy resulting in ectopic tissue formation prompt regulatory authorities to require stringent preclinical investigations into the biodistribution of the administered CT [1].

For CT products it is vital that reproducible, sensitive and quantitative assays are developed and applied to evaluate the persistence and distribution of cells after administration. Regulatory guidelines stipulate that CT product safety is determined using risk-based approaches such that the assays developed to determine biosafety for the intended host consider and directly address any risks posed to the intended host [1–4]. Unlike small molecule pharmaceuticals, the biological complexity of living cells does not make them suitable for routine absorption, distribution metabolism and excretion and pharmacokinetic testing [1]. As a result, biodistribution assays are a regulatory requirement for advancing a CT to the clinic. Biodistribution studies can provide data on CT product localization or migration over time as well as *in vivo* survival and differentiation in the case of progenitor cell-based CT [4].

The biodistributive profile of a CT product has safety and efficacy implications, addressing questions such as: Are the cells reaching the reparative site of interest in the host? Are they engrafting in numbers sufficient to elicit the desired response? How long do

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they persist in the host? Laboratories worldwide have used a wide range of techniques in attempts to determine the distribution of transplanted cells [5,6]. Microscopic visualization of histological samples has been widely used to detect the presence of the transplanted cells in pre-clinical models using a variety of cell labeling techniques, such as membrane dyes (PKH26, DII [1,1′Dioctadecyl-3,3,3′3′-tetramethylindocarbocyanine perchlorate]) or nuclear dyes (Hoechst 33342, bromodeoxyuridine, DAPI [4′6-diamidino-2-phenylindole]) [6]. However, cellular labeling methodologies are susceptible to dilution with cellular division reducing the label below the limits of detection [6]. Furthermore, the labor-intensive histological techniques required to locate the CT *in vivo* can be subject to sampling error, leading to variability and thus reduced sensitivity and reliability of the results [6].

Genetic modification of the intended CT can allow for the identification of the administered cells without concern about dilution of the label. However, the required use of cellular labeling molecules can have consequences on a CT product functions and may potentially alter the biodistributive fate of the cells [4,7]. Modification of the pre-clinical CT product with genes encoding proteins that can be visualized via microscopy (green fluorescent protein, enhanced green fluorescent protein, red fluorescent protein, yellow fluorescent protein, β-galactosidase and mCherry) can provide quantitative information about cellular location and survival, however, gene silencing in long-term studies may result in a decrease in signal with time [6]. Furthermore, autofluorescence within the tissues of interest and uptake of the fluorescent protein by adjacent cells, such as macrophages, can result in false positives thus compromising the accuracy of the results [6]. Newer technologies are emerging in which non-invasive imaging can provide real-time *in vivo* tracking of the transplanted CT [5,7–9]. Such imaging modalities are exciting because they enable the investigators to obtain dynamic measurements of cellular viability and location after administration. However, their application relies upon suboptimal extensive cell labeling. This strategy may not be ideal because the regulatory authority requires that the pre-clinical studies to support the first in human application must be completed using the final cell product intended for human use [1,2].

Real-time quantitative PCR (qPCR) is a relatively inexpensive technique that bypasses the disadvantages associated with other cellular detection methods [6]. qPCR enables the accurate and sensitive detection of transplanted cells via their cell-specific DNA sequences within the whole host organ, minimalizing sampling errors [6,10]. The Alu sequence remains the marker of choice when assessing the biodistribution of transplanted cells in xenogenic models, due to genomic repetition and species specificity. The human Alu (hAlu) sequence can be amplified and quantified by qPCR from genomic DNA (gDNA) with a high degree of accuracy [6,10].

Here we describe the development of an accurate, reproducible, quantitative and inexpensive qPCR-based method of tracking the biodistributive fate of human cells in xenogenic models. The assay is a primer-probe–based PCR assay using custom-made primers to detect and quantify the hAlu sequences in a heterogeneous sample of human DNA (hDNA) and murine DNA (mDNA) from whole organ gDNA extracts. The assay enables the quantification of human gDNA by amplifying the human-specific hAlu repeat sequence [12] with a sensitivity to detect the DNA equivalent of 0.1 human cell in 1.5×10^6 heterogeneous cells.

Materials and methods

Human bone marrow mesenchymal stromal cell isolation and culture

Human mesenchymal stromal cells (hMSCS) were isolated from adult bone marrow and culture expanded in accordance with local ethical approval and regulatory body–approved good manufacturing practice (GMP) protocols. Upon receipt, the bone marrow aspirate was washed with Dulbecco's phosphate-buffered saline (DPBS) and centrifuged at 900*g*. A 4% acetic acid wash was performed on a sample of the marrow to lyse the red blood cells and enable an accurate mononuclear cell (MNC) count. MNCs, plated at 40–50 million per 175 cm^2, were culture expanded in monolayer with complete medium (α-minimal essential media supplemented with 10% selected fetal bovine serum [FBS]) in 5% CO_2 at 37°C. On day 3, fresh medium was added to the culture. On day 5, the cultures were washed with DPBS to remove non-adherent cells and fresh complete medium was added to each flask. When the monolayer reached 80%–90% confluence, the adherent cells were washed with DPBS and detached from the culture plastic with 0.25% tryspin/ethylenediaminetetraacetic acid (EDTA). The dissociated cells were centrifuged at 400*g* for 5 min. The resultant pellet was resuspended in complete fresh medium and the cellular yield determined. hMSCs were further sub-cultured by seeding 3×10^6 cells in a triple flask through two passages. hMSCs were cryopreserved at a dose of 2×10^6 per mL in FBS combined with 10% dimethyl sulfoxide.

Animal husbandry

Animal care and administration of the hMSCs were conducted in Charles River, a good laboratory practices