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Morphology and size of stem cells from mouse and whale: observational study

 OPEN ACCESS

Martin J Hoogduijn *scientist*¹, Johanna C van den Beukel *PhD student*¹, Lidewij C M Wiersma *PhD student*², Joeske Ijzer *assistant professor*³

¹Department of Internal Medicine, Erasmus MC, Rotterdam, Netherlands; ²Department of Virology, Erasmus MC, Rotterdam, Netherlands; ³Department of Pathobiology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands

Abstract

Objective To compare the morphology and size of stem cells from two mammals of noticeably different body size.

Design Observational study.

Setting The Netherlands.

Participants A humpback whale (*Megaptera novaeangliae*) and a laboratory mouse (*Mus musculus*).

Main outcome measures Morphology and size of mesenchymal stem cells from adipose tissue.

Results Morphologically, mesenchymal stem cells of the mouse and whale are indistinguishable. The average diameter of 50 mesenchymal stem cells from the mouse was 28 (SD 0.86) μm and 50 from the whale was 29 (SD 0.71) μm . The difference in cell size between the species was not statistically significant. Although the difference in bodyweight between the species is close to two million-fold, the mesenchymal stem cells of each were of similar size.

Conclusions The mesenchymal stem cells of whales and mice are alike, in both morphology and size.

Introduction

The class Mammalia contains species of extensively different phenotype and body size. The family Cetacea contains the largest mammals, whales. Cetaceans vary in body size, with the rare porpoise species vaquita (*Phocoena sinus*) measuring 1.5 m in length and with a bodyweight of 55 kg and the blue whale (*Balaenoptera musculus*)—the largest animal ever known to have lived—measuring up to 30 m in length and with a bodyweight of 180 000 kg. Mice (genus *Mus*) represent the other end of the mammalian size spectrum, with the common house mouse measuring about 8 cm in length, with a tail of approximately the same length, and a bodyweight on average of 20 g. The common laboratory mouse (C57BL/6, or the “black

6” mouse) is of the same size and weight. Although organs are sized in proportion to the size of the animal, suborgan structures do not reflect this difference. For instance, capillaries in all mammals range from 5 to 10 μm , the optimal size for oxygen exchange.

In recent decades interest in stem cells has been growing, from both a basic biological and a therapeutic perspective. In response to this, we compared the morphology and size of mesenchymal stem cells from two mammalian species of noticeably different body size.

Methods

The whale

The humpback whale (*Megaptera novaeangliae*) is a baleen whale (contains special structures in its mouth that enable it to filter food from the water) of the subfamily Balaenopteridae, and ranges in length from 12 to 16 m with a bodyweight of approximately 36 000 kg.

On 12 December 2012 a humpback whale became stranded on a sandbank near Texel on the Dutch coast. After several failed attempts to get the whale back in the sea, it died on 16 December and necropsy was performed two days later.

Sample collection

Samples of blubber, subcutaneous adipose tissue, and visceral adipose tissue were removed from the whale, stored in phosphate buffered saline, transferred to the laboratory at the Erasmus MC, and kept overnight at 4°C. The next day we sectioned and stained the tissues with haematoxylin and eosin (fig 1A-C¹). Multiple small muscle-like structures were seen in the blubber sections.¹ These were not observed in the subcutaneous adipose

tissue. Owing to severe autolysis of the carcass, the visceral adipose tissue was of poor quality. Under continuous shaking we minced up and digested the three tissues with sterile 0.5% collagenase type IV (Sigma-Aldrich, St Louis, MO) solution for 30 minutes at 37°C. After washing the digested material, we seeded the cell suspensions in culture flasks in α minimum essential medium (Lonza, Verviers, Belgium) supplemented with combined penicillin and streptomycin and 15% fetal bovine serum (Lonza). The cell suspensions were kept at 37°C in 5% carbon dioxide and 20% oxygen, and at 95% humidity. We changed the medium every three or four days. After two days we stopped culturing the visceral adipose tissue because of a bacterial infection. After three weeks we stopped culturing blubber because of the absence of living cells in the cultures. However, in the culture derived from subcutaneous adipose tissue we observed a few living cells that resembled mesenchymal stem cells (fig 2↓). We subcultured these cells when confluency was 90%. For further experiments we used cells that had been subcultured four times.

The mouse

The house mouse (*Mus musculus*) is one of the most common mammalian species in the world. In 1921, an American researcher, Clarence Cook Little, inbred the C57BL/6 mouse strain from the house mouse. This strain provides consistent data in experiments.

On 2 January 2013 we collected, sectioned, and stained interscapular brown adipose tissue, subcutaneous fat, and abdominal fat from a laboratory mouse (fig 1D-F). We isolated mesenchymal stem cells from the abdominal fat using the same procedures as for the humpback whale. In the culture we observed cells that resembled the morphology of both human mesenchymal stem cells and cells cultured from the humpback whale adipose tissue (fig 3↓).

Mesenchymal stem cells

Mesenchymal stem cells are the precursor cells of mesenchymal tissues. They can be expanded in culture, and differentiated into osteogenic, adipogenic, chondrogenic, and myogenic lineages in culture.² Mesenchymal stem cells have been identified in nearly all tissues,³ with adipose tissue being the most accessible. Mesenchymal stem cells are usually routinely cultured from human or mouse adipose tissue, but there are reports on cells derived from ovine and pig adipose tissue. A study from 2011 showed that mesenchymal stem cells can be isolated from adipose tissue of the brown bear.⁴ Although fierce, the brown bear is only a mediocre species to use for comparison of the size of mesenchymal stem cells from small and large mammals. Comparing cells from a mouse with those from a whale covers a far larger body size spectrum and is therefore more informative.

In addition to their capacity for differentiation, mesenchymal stem cells have trophic properties and support the survival, proliferation, and differentiation of other cell types. Furthermore, they have potent immunomodulatory properties and inhibit the proliferation of activated T cells, modulate the differentiation of B cells, and promote the differentiation of macrophages into a regulatory phenotype.⁵⁻⁷ These beneficial properties make mesenchymal stem cells candidates for application in regenerative medicine and immune therapy. Early clinical trials are exploring this in conditions such as graft versus host disease⁸ and Crohn's disease,⁹ and in organ transplantation.¹⁰ As mesenchymal stem cells need to be administered in relatively large numbers, obtaining sufficient cells of good quality is

challenging. A universal source of mesenchymal stem cells is a potential solution to this problem.

The size of mesenchymal stem cells from mouse and whale is of general biological interest. We therefore trypsinised the fourth subculture of mesenchymal stem cells from both species and placed a sample of the cell suspension in a counting window. The cells were then photographed and their size measured using Axiovert software (Zeiss, Oberkochen, Germany).

Results

The average diameter of 50 mesenchymal stem cells from the mouse and 50 from the whale was 28 (SD 0.86) μ m and 29 (0.71) μ m, respectively (fig 4↓). The difference in size of the cells between the species was not statistically significant. Despite these mammals differing in bodyweight close to two million-fold, their mesenchymal stem cells were of similar morphology and size.

Discussion

Mice and whales have noticeably different body sizes, but their mesenchymal stem cells are of equal morphology and size in culture.

Species that differ vastly in size and are far apart on the phylogenetic tree are known to have molecular machinery of similar proportions. Bacteria use adenine, guanine, cytosine, and thymine to replicate their genetic code, as do whales. At a cellular level we now know that the size of mesenchymal stem cells between small and large mammals does not differ. Size starts to matter only beyond the level of the single mesenchymal stem cell. The whale's bodyweight is approximately two million times that of the mouse. Does the whale also have two million times more mesenchymal stem cells? If this was the case, and if mouse or whale adipose tissue were being considered for study or perhaps for veterinary therapeutic applications, the option would be to breed a large number of mice, or wait for a whale to become stranded on a beach.

Every study can be improved and even the present study does have limitations. The study could be further improved by obtaining mesenchymal stem cells from a larger whale species, preferably the blue whale, and from a smaller mouse species. The authors therefore call on investigators with access to beached blue whales or with mesenchymal stem cells from the African pygmy mouse or bumblebee bat, to come forward and collaborate with us.

We thank Naturalis Biodiversity Center, Leiden, Netherlands, for its support of this study and all members of the necropsy teams for their extensive technical assistance.

Contributors: MJH isolated and cultured the mesenchymal stem cells, carried out *in vitro* experiments, and wrote the manuscript. He is the guarantor. JCB performed immunohistochemistry and approved the manuscript. LW and JJJ carried out the autopsy on the whale, collected adipose tissue, and approved the manuscript.

Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Ethical approval: This study was approved by the Erasmus MC Animal Experiments Committee. Laboratory mice at the Erasmus MC are kept according to institutional regulations.

What is already known on this topic

Class Mammalia contains species of extensively different phenotype and body size

What this study adds

Mesenchymal stem cells are of similar size in very large and very small mammals

Data sharing: No additional data available.

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Figures

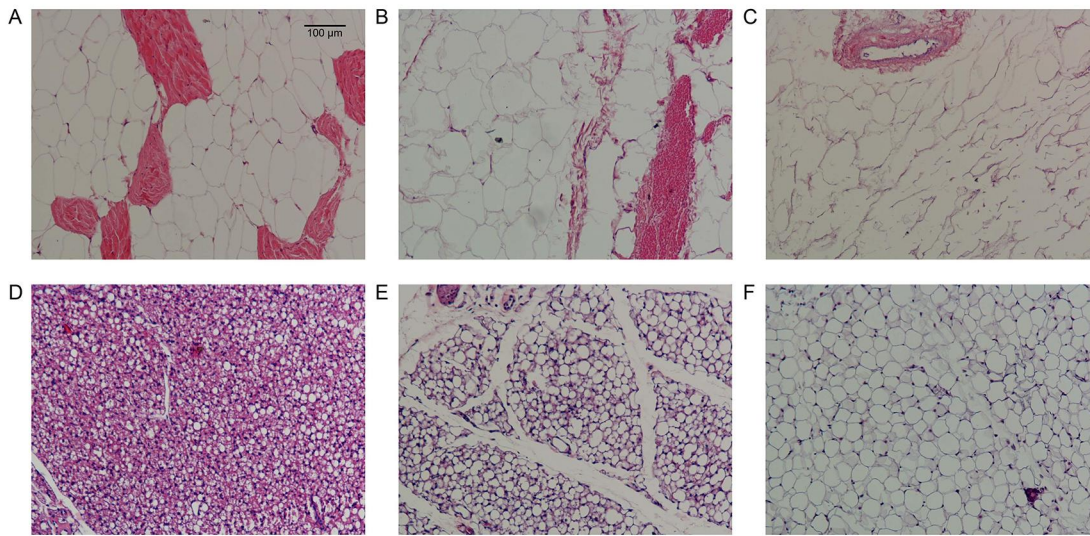


Fig 1 Sections of haematoxylin and eosin stained whale and mouse adipose tissue. Whale (A) blubber, (B) subcutaneous adipose tissue, and (C) visceral adipose tissue, and mouse (D) interscapular brown adipose tissue, (E) subcutaneous adipose tissue, and (F) abdominal adipose tissue

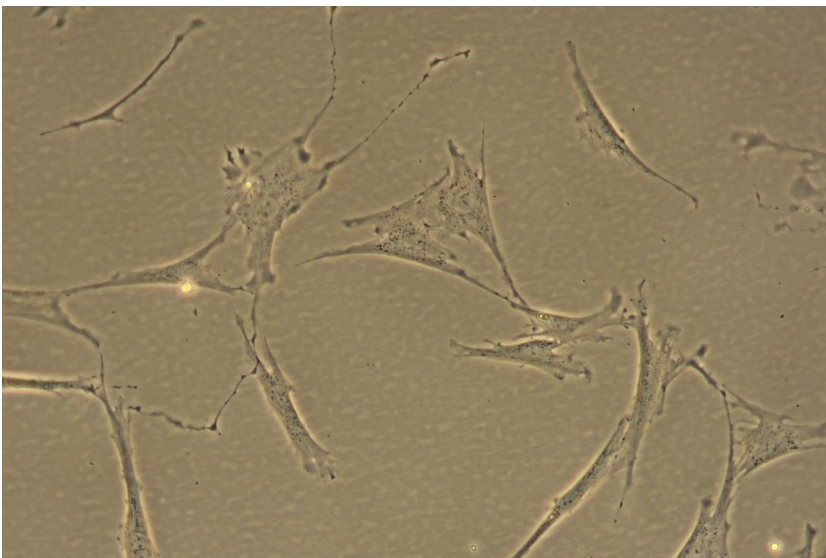


Fig 2 Morphology of cultured whale mesenchymal stem cells after four subcultures

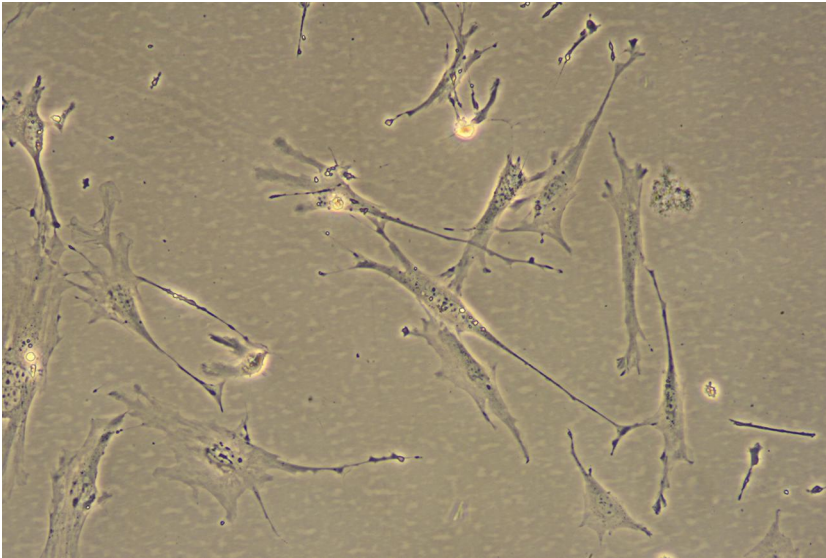


Fig 3 Morphology of cultured mouse mesenchymal stem cells after four subcultures

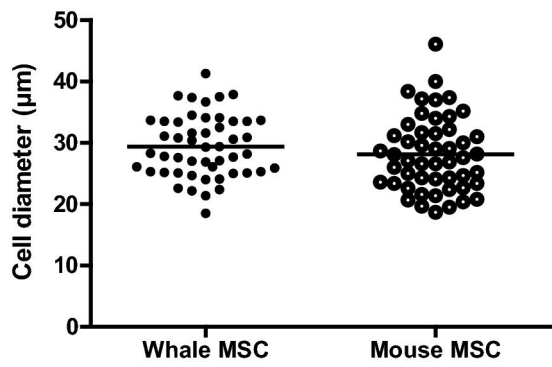


Fig 4 Diameter of trypsinised whale and mouse mesenchymal stem cells after four subcultures