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Establishment and characterization of fetal and maternal mesenchymal stem/stromal cell lines from the human term placenta

Sharon Q. Qin [a, b, 1], Gina D. Kusuma [a, b, 1], Batla Al-Sowayan [a, b], Rishika A. Pace [a, b], Sandra Isenmann [c], Mark D. Pertile [d, e], Stan Gronthos [c], Mohamed H. Abumaree [f, g], Shaun P. Brennecke [a, b], Bill Kalionis [a, b, *]

[a] University of Melbourne, Department of Obstetrics and Gynaecology, Royal Women's Hospital, Parkville, Victoria, 3052, Australia
[b] Pregnancy Research Centre, Department of Maternal-Fetal Medicine, Royal Women's Hospital, Parkville, Victoria, 3052, Australia
[c] Mesenchymal Stem Cell Group, Division of Haematology, Institute of Medical and Veterinary Science/Hanson Institute/CSCR, University of Adelaide, Adelaide, SA, 5000, Australia
[d] Victorian Clinical Genetics Services (VCGS), Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, Victoria, 3052, Australia
[e] University of Melbourne, Department of Paediatrics, Royal Children's Hospital, Parkville, Victoria, 3052, Australia
[f] King Abdullah International Medical Research Center, College of Science and Health Professions, King Abdulaziz Medical City, National Guard Health Affairs, Mail Code 3124, P.O. Box 3660, Riyadh, 11481, Saudi Arabia
[g] King Saud Bin Abdulaziz University for Health Sciences, College of Science and Health Professions, King Abdulaziz Medical City, National Guard Health Affairs, Mail Code 3124, P.O. Box 3660, Riyadh, 11481, Saudi Arabia

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ABSTRACT

Introduction: Human placental mesenchymal stem/stromal cells (MSC) are an attractive source of MSC with great therapeutic potential. However, primary MSC are difficult to study *in vitro* due to their limited lifespan and patient-to-patient variation.
Methods: Fetal and maternal MSC were prepared from cells of the chorionic and basal plates of the placenta, respectively. Fetal and maternal MSC were transduced with the human telomerase reverse transcriptase (hTERT). Conventional stem cell assays assessed the MSC characteristics of the cell lines. Functional assays for cell proliferation, cell migration and ability to form colonies in soft agar were used to assess the whether transduced cells retained properties of primary MSC.
Results: Fetal chorionic and maternal MSC were successfully transduced with hTERT to create the cell lines CMSC29 and DMSC23 respectively. The lifespans of CMSC29 and DMSC23 were extended in cell culture. Both cell lines retained important MSC characteristics including cell surface marker expression and multipotent differentiation potential. Neither of the cell lines was tumourigenic *in vitro*. Gene expression differences were observed between CMSC29 and DMSC23 cells and their corresponding parent, primary MSC. Both cell lines show similar migration potential to their corresponding primary, parent MSC.
Discussion: The data show that transduced MSC retained important functional properties of the primary MSC. There were gene expression and functional differences between cell lines CMSC29 and DMSC23 that reflect their different tissue microenvironments of the parent, primary MSC. CMSC29 and DMSC23 cell lines could be useful tools for optimisation and functional studies of MSC.

1. Introduction

Human mesenchymal stem/stromal cells (MSC, also known as multipotent stromal cells), are intensively studied for their therapeutic potential and immunomodulatory ability [1–4]. While most of the studies in humans focus on bone marrow MSC, the shortage of tissue supply, relatively low numbers of MSC in the marrow and the invasive procedure for acquisition are disadvantages for

Abbreviations: hTERT, human telomerase reverse transcriptase; MSC, mesenchymal stem/stromal cells.

* Corresponding author. Pregnancy Research Centre, Department of Maternal-Fetal Medicine, Royal Women's Hospital, Parkville, Victoria, 3052, Australia.
E-mail address: bill.kalionis@thewomens.org.au (B. Kalionis).
[1] These authors contributed equally to this work.

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therapeutic applications. The placenta is an attractive source of MSC as invasive procedures are not required, there are high numbers of MSC present in placental tissues and placentas are readily available [5–8].

Placenta-derived MSC differentiate *in vitro* under specific induction conditions into cells with characteristics of the three mesenchymal lineages, and into cells with features of the endodermal and ectodermal lineages [9,10]. This plasticity gives them significant therapeutic potential but in addition, there is evidence that placenta-derived MSC have greater immunomodulatory ability than other sources of MSC [11]. Placental MSC immunomodulation is the subject of current investigations and the mechanisms involved are only partially understood [12,13].

Studying primary adult MSC *in vitro* has limitations because of their limited lifespan, variation in characteristics resulting from differing isolation preparation methods, and patient-to-patient variation between preparations [14–16]. With prolonged culture, MSC show changes in gene expression, slower proliferation and they reach senescence around 20–30 population doublings (i.e. about 5 passages in culture) [17–19]. The experiments involving primary MSC employed cells from passage P2 to passage P5. MSC were not used beyond P5, since several studies show primary MSC undergo apoptosis, senescence or loss of differentiation potential at later passages [20–22].

The creation of cell lines with increased life span, while retaining MSC properties, would be advantageous for developing and testing therapeutic strategies. These cell lines would also serve as *in vitro* models to increase our knowledge of the *in vivo* role of MSC in the placenta.

One way to create such cell lines is to exploit the properties of telomeres. Telomeres shorten as human cells divide and when the telomeres are too short, the cells undergo senescence. In recent years, stable cell lines were produced by transduction of the telomerase gene (hTERT) into the human genome, which resulted in extended life span *in vitro* and well preserved properties and functions [23]. Several MSC cell lines were produced using this method, either by hTERT transduction alone, or by hTERT combined with oncogene transformation to create immortal cell lines, which extended the MSC life span and improved differentiation abilities in some cell lines [24–26]. However, many immortal cell lines undergo significant chromosomal rearrangements, which may alter their original functions. Two fetal chorionic MSC lines, derived from human placenta, were previously published, one of which was by hTERT transduction [17,27].

The fetal side of the placenta is a source of fetal chorionic MSC [7,28–30]. However, the maternal side of the can be used as a source of maternal MSC, presumed to be of decidual origin [31–34]. The source of the maternal decidual MSC is likely to be the *decidua basalis* that remains attached to the maternal side of the placenta following delivery. Recent studies show that fetal and maternal MSC have similar immunophenotypic profiles, and possess common stem cell properties, but they also show gene expression and functional differences [31]. To date, there are no reports of MSC cell lines with extended lifespan, derived from maternal MSC isolated from the maternal side of the placenta.

The aim of this work was to generate fetal and maternal MSC cell lines with extended life span by hTERT transduction and to compare the stem cell and functional properties of these cell lines with their respective primary MSC.

2. Materials and methods

2.1. Tissue collection

The Royal Women's Hospital Human Research Ethics Committee approved this project. Informed patient consent was obtained. Placentae from uncomplicated pregnancies were collected after elective caesarean section or vaginal delivery at term. All placentae were from deliveries 37–41 weeks of gestation at birth. Gestation age was calculated from first trimester fetal ultrasounds of last menstrual period where no scan available. Fetal exclusion criteria included fetal abnormalities, abnormal ultrasounds and fetal growth restriction. Maternal exclusions included any pregnancy related conditions, including but not limited to preeclampsia, HELLP syndrome, thrombocytopaenia and gestational diabetes. Placentae with abnormal gross morphology were also excluded.

2.2. Isolation of fetal and maternal MSC

An incision was made through the fetal membranes adjacent to the site of umbilical cord attachment on the fetal side of the placenta and 50 mg of villous tissue was obtained for fetal chorionic MSC [35]. Samples were carefully dissected under a dissection microscope using a 25 gauge needle to remove any non-villous material. The villous tissue was mechanically diced and digested for 40 min at 37 °C in trypsin (0.25%, Life Technologies, CA, USA). Digested tissue was cultured in Amniomax C100 complete medium (Life Technologies) and cells were passaged by treatment with TrypLE Express (Life Technologies) for 5 min at 37 °C when confluent.

To isolate maternal MSC, 8 g of placental tissue was obtained from a central cotyledon on the maternal side of the placenta and washed in PBS as described elsewhere [32,35–37]. The tissue was mechanically minced and digested overnight in trypsin (0.25%) and DNAse (50 μg/ml, Worthington, NJ, USA) at 4 °C. After removal of the supernatant by centrifugation at 200 g, the tissue was incubated with collagenase (10 mg/ml, Worthington) and DNAse (50 μg/ml) for 30 min at 37 °C. The digested tissue was filtered through a 100 μm stainless steel sieve (Prospectors, Midvale, Australia) and the filtrate was separated by gradient centrifugation for 30 min at 400 g using Histopaque (Sigma Aldrich, St Louis, USA). The mononuclear cell layer was collected and cultured in Mesencult complete medium (Stem Cell Technologies, Vancouver Canada) with penicillin/streptomycin (100 U/ml and 100 mg/ml respectively, Murdoch Children's Research Institute, Melbourne, Australia). All cell cultures were maintained in a humidified 5% CO_2 and 21% O_2 incubator at 37 °C.

Cell purity was assessed against minimal criteria for defining MSC set by International Society of Cell Therapy (ISCT) which include: (i) adherence to plastic, (ii) expression of CD105, CD73, and CD90 and negative expression of CD45, CD34, CD14 or CD11b, CD79α or CD19, HLA-DR, (iii) *in vitro* differentiation into mesenchymal lineages [38].

2.3. Human telomerase reverse transcriptase (hTERT) transduction

Fetal and maternal MSC were isolated from two different placentae from male foetuses. Retroviral expression constructs were generated with the retroviral vector pbabe encoding the full-length hTERT cDNA as previously described [39]. The packaging cell line PT67 was transfected with either the hTERT-containing constructs, or pbabe vector alone, using Fugene-6-reagent (Boehringer Mannheim, Mannheim, Germany), then selected with 800 μg/ml G418 (Sigma Aldrich). Harvested supernatant containing infectious particles from stable PT67 lines was used to transduce cultured fetal chorionic and maternal MSC at passage P2 (1×10^5 cells per well in 6 well plates), in the presence of 5 μg/ml polybrene (Sigma Aldrich). Forty-eight hours after transduction, MSC were selected using puromycin (1 μg/ml) for 10 days and resistant clones were selected. Transduced fetal chorionic and maternal MSC were