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Fibromatosis stem cells rather than bone-marrow mesenchymal stem cells recapitulate a murine model of fibromatosis

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ABSTRACT

Palmar fibromatosis is a benign fibroproliferative tumor of unknown etiology, with a high rate of recurrence after excision. The offending cells of palmar fibromatosis are myofibroblasts and the cellular origin of other myofibroblasts has previously been reported to be the bone marrow. However, further clarification of the relationship between bone marrow precursors and palmar fibromatosis is required. Stem cells (SCs) are known to exist in various tissues, but whether SCs can be isolated from fibromatosis tissue is still unclear. The purpose of this study was to isolate and identify stem cells from human palmar fibromatosis, and to evaluate the differences in the differentiation and fibrogenic capacities of bone marrow stem cells (BMSCs) and fibromatosis-derived stem cells (FSCs). We found that FSCs had better fibrogenic differentiation potential than BMSCs, whereas BMSCs had better adipogenic and chondrogenic differentiation capacities. Treatment with transforming growth factor-β1 increased the expression of α-smooth muscle actin, and types III and I collagen significantly more in FSCs than in BMSCs. An in vivo study further confirmed the results of fibrogenesis and suggested that FSCs can recapitulate the fibromatosis nodule. In summary, their myofibroblastic differentiation both in vivo and in vitro makes FSCs a potential cell source for future applications in murine models of fibromatosis or fibrogenesis.

1. Introduction

Among soft-tissue tumors, fibromatosis is one of the most common benign lesions [1]. When it occurs in important organs or structures, fibromatosis usually causes severe complications and induces morbidity in patients [1]. For example, palmar fibromatosis (also known as Dupuytren's disease) is a nodular fibroproliferative disorder of the palmar aponeurosis, which undergoes irreversible contracture [2,3]. Surgical excision is still the principal treatment option for palmar fibromatosis, but the disease tends to recur. The hallmark of palmar fibromatosis is its clinical course, which can be divided into several stages [2,3]. The initial stage is the proliferative stage, which is characterized by nodule formation, with hypercellular areas full of proliferating myofibroblasts and newly formed capillaries. α-Smooth muscle actin (α-SMA) and type III collagen (Col III) are specific markers of myofibroblasts. The last stage is the residual stage, when nodules are replaced by dense avascular, acellular, type I collagen (Col I)-rich cord containing a smaller population of myofibroblasts. Despite some understanding of the biochemical and cellular processes of palmar fibromatosis, the precursor cells of the myofibroblasts and the pathogenesis of palmar fibromatosis remain unclear [2,3].

Most of adult stem cells (ASCs) are the cell sources for tissue engineering and regeneration. For example, bone-marrow-derived mesenchymal stem cells (BMSCs) have the ability to self-renew and differentiate into multiple lineages of cells, including bone, fat, cartilage [4], and nonmesenchymal tissues, like neurons [5]. ASCs, such as mesenchymal stem cells (MSCs), can be isolated from various tissues, including bone marrow, umbilical cord, and articular synovium [6–8]. However, it is yet to be determined whether they can be isolated from benign tumors, such as palmar

Abbreviations: α-SMA, α-smooth muscle actin; ASC, adult stem cell; MSC, mesenchymal stem cell; BMSC, bone-marrow stem cell; Coll I, type I collagen; Coll III, type III collagen; FSC, fibromatosis-derived stem cell; PBS, phosphate-buffered saline.

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fibromatosis. Myofibroblasts have been identified in palmar fibromatosis and the cellular origin of other myofibroblasts, such as renal myofibroblasts, corneal myofibroblasts, and gastric cancer myofibroblasts, are reported to be from bone marrow [9–11]. Therefore, we hypothesized that ASCs exist in palmar fibromatosis. In this study, we demonstrate the isolation of SCs from palmar fibromatosis and compare them with BMSCs in the expression of their surface markers, their differentiation capacity, and most importantly, their potential for recapitulation of the fibromatosis nodule.

2. Materials and methods

2.1. Cellular isolation and expansion method

In this study, we adhered to the tenets of the Declaration of Helsinki. The isolation of bone marrow MSCs was performed according to a protocol described previously [12]. Fibromatosis tissues were obtained from three patients undergoing excision for palmar fibromatosis after the patients had given their written informed consent. The study protocol and written informed consent forms were approved by the Institutional Ethics Committee/Institutional Review Board of Taipei Veterans General Hospital, Taiwan. The tissues were repeatedly washed in phosphate-buffered saline (PBS; Gibco BRL, Grand Island, NY), and the surrounding tissues were carefully scraped off, leaving only the palmar fibromatosis tissues to be used in our experiment. The tissues were digested with 3 mg/mL collagenase for 3 h. The harvested nucleated cells were then plated at the optimal density and cultured in α-MEM (Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum (FBS), 100 U/mL penicillin (Invitrogen), 100 μg/mL streptomycin (Invitrogen), and 250 ng/mL amphotericin B (Invitrogen). The growth medium was changed every 48 h and the cells were propagated every four days with a 1:5 split ratio until 80% confluence was reached.

2.2. Flow-cytometric analysis

To analyze the cell-surface expression of typical marker proteins, the FSCs and BMSCs were harvested in PBS containing 5 mM EDTA. The cells were reactivated with the following anti-human antibodies: CD34–phycoerythrin (PE), CD73–PE (also referred to as SH3 and SH4), CD90–fluorescein isothiocyanate (FITC; Becton Dickinson, Franklin Lakes, NJ), CD29–FITC, CD44–FITC, CD45–FITC (Beckman Coulter, Krefeld, Germany), CD133–PE (Miltenyi Biotech, Bergisch Gladbach, Germany), CD105–FITC (SH2), or CD166–PE (Immunokontakt, Wiesbaden, Germany). Mouse isotype antibodies were used as the controls (Becton Dickinson). Ten thousand labeled cells were acquired and analyzed with a FACScan flow cytometer (Becton Dickinson) and CellQuest software (Becton Dickinson).

2.3. Differentiation protocols

The FSCs and BMSCs were induced under the following culture conditions: (I) adipogenic medium consisting of α-MEM supplemented with 10% FBS, 50 μg/mL ascorbate-2-phosphate (Nacalai, Kyoto, Japan), 10^{-7} M dexamethasone (Sigma, St. Louis, MO), 50 μg/mL indomethacin (Sigma), 0.45 mM 3-isobutyl-1-methylxanthine (Sigma), and 10 μg/mL insulin (Sigma); (II) osteogenic medium consisting of α-MEM supplemented with 10% FBS, 50 μg/mL ascorbate-2-phosphate, 10^{-8} M dexamethasone, and 10 mM β-glycerol phosphate (Sigma); (III) chondrogenic medium consisting of serum-free high-glucose Dulbecco's modified Eagle's medium supplemented with ITS$^+$ Premix (BD Biosciences, Bedford, MA: 6.25 μg/mL insulin, 6.25 μg/mL transferrin, 6.25 μg/mL selenious acid, 1.25 mg/mL bovine serum albumin [BSA], 5.35 mg/mL linoleic acid), 10^{-7} M dexamethasone, 50 μg/mL ascorbate-2-phosphate (Nacalai, Kyoto, Japan), and 10 ng/mL transforming growth factor-β1 (TGF-β1). On the indicated days after induction, the cells were harvested for histochemical staining study.

Table 1
Primers used for reverse transcription-polymerase chain reaction (RT-PCR) analysis.

Gene	Sense primer	Anti-sense primer
PPARγ2	CCTATTGACCCAGAAAGCGATTC	GCATTATGAGACATCCCCACTGC
LPL	GGTCGAAGCATTGGAATCCAG	TAGGGCATCTGAGAACGAGTC
OP	CTAGGCATCACCTGTGCCATACC	CAGTGACCAGTTCATCAGATTCATC
RUNX2	GTTTGTTCTCTGACCGCCTC	CCAGTTCTGAAGCACCTGA
COL2A1	CCAGGACCAAAGGGACAGAAAG	TTCACCAGGTTCACCAGGATTG
β-actin	GCACTCTTCCAGCCTTCCTTCC	TCACCTTCACCGTTCCAGTTTTT

PPARγ, peroxisome proliferator-activated receptor-γ; LPL, lipoprotein lipase; OP, osteopontin; RUNX2, runt-related transcription factor 2; COL2A1, α-1 type II collagen.

2.4. Reverse transcription (RT) and real-time polymerase chain reaction (PCR) analysis

Total RNA was extracted with TRIzol reagent (Invitrogen, Melbourne, Australia). To initiate first-strand cDNA synthesis, random-sequence primers were used to prime the reverse transcription reactions, and synthesis was catalyzed with SuperScript III reverse transcriptase (Invitrogen). The cDNA was used as the template in a 30 μL PCR reaction mixture containing specific primer pairs (Table 1). In total, 35 cycles of PCR were performed using recombinant *Taq* DNA polymerase (Invitrogen). β-Actin expression was used as the control. The amplification products were resolved by electrophoresis in a 1.5% agarose gel and visualized with ethidium bromide. Real-time amplification of the genes was performed in triplicate using the Assays-on-Demand primers (Applied Biosystems, Foster City, CA) and SYBR Green PCR Master Mix (Applied Biosystems) on a real-time PCR machine (7500; Applied Biosystems), according to the manufacturer's instructions. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression was used as the internal control (Table 2). The results were analyzed with the software supplied by the manufacturer, using the ΔCT method.

2.5. In vivo murine model of fibromatosis nodules

All animal care and experimental protocols were in accordance with the Institutional Animal Welfare Guidelines of Taipei Veter-

Table 2
Primers used for real-time reverse transcription-polymerase chain reaction analysis.

Gene	Primer length	T_m	Sense primer	Anti-sense primer
α-SMA	20	53.7	CATCATGCGTCTGGATCTGG	GGACAATCTCACGCTCAGCA
Col3A1	20	50.2	GGAGAATGTTGTGCAGTTTG	AGGACCAGTAGGGCATGA
Col1A2	20	51.78	GACATGCTCAGCTTTGTGGA	CTTTCTCCACGTGGTCCTCT
GAPDH	21	52.57	ATATTGTTGCCATCAATGACC	GATGGCATGGACTGTGGTCATG

α-SMA, α-smooth muscle actin; Col3A1, α1 type III collagen; Col1A2, α2 type I collagen; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase.