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Human pseudoarthrosis tissue contains cells with osteogenic potential

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ABSTRACT

Introduction: Nonunion is a challenging problem that may occur after certain bone fractures. The treatment of nonunion is closely related to its type. To develop an effective treatment strategy for each type of nonunion, biological analysis of nonunion tissue is essential. Pseudoarthrosis is a distinct pathologic entity of nonunion. To understand the pathology of pseudoarthrosis, we investigated the cellular properties of pseudoarthrosis tissue-derived cells (PCs) *in vitro*.

Patients and methods: PCs were isolated from four patients with pseudoarthrosis and cultured. Cells were evaluated for cell-surface protein expression by using flow cytometry. Osteogenic differentiation capacity was assessed by using Alizarin Red S staining, alkaline phosphatase (ALP) activity assay, and reverse transcription polymerase chain reaction (RT-PCR) after osteogenic induction. Chondrogenic differentiation capacity was assessed *via* Safranin O staining and RT-PCR after chondrogenic induction.

Results: PCs were consistently positive for the mesenchymal stem cell-related markers CD29, CD44, CD105, and CD166, but were negative for the haematopoietic-lineage markers CD31, CD34, CD45, and CD133. Alizarin Red S staining revealed that PCs formed a mineralised matrix that was rich in calcium deposits after osteogenic induction. ALP activity under osteogenic conditions was significantly higher than that under control conditions. Gene expression of ALP, Runx2, osterix, osteocalcin, and bone sialoprotein was observed in PCs cultured under osteogenic conditions. Induced pellets were negatively stained by Safranin O staining. Gene expression of aggrecan, collagen II, collagen X, SOX5, and SOX9 was not observed.

Conclusion: We have shown for the first time the properties of cells in patients with pseudoarthrosis. Our results indicated that osteogenic cells existed in the pseudoarthrosis tissue. This study might provide insights into understanding the pathology of pseudoarthrosis and improving the treatment for pseudoarthrosis.

Introduction

Approximately 18.3 million fractures are sustained in the United States each year [1], and 5–10% of the fractures fail to heal and result in delayed union or nonunion [2,3]. Delayed union indicates fractures that consolidate in a period longer than normal, whereas nonunion is indicates fractures that do not consolidate within a certain period. According to the U.S. Food and Drug Administration, a nonunion is when a minimum of 9 months has elapsed since injury and the fracture site shows no visibly progressive signs of healing for a minimum of 3 months [4,5]. Some researchers proposed the definition of nonunion as a failure of a fracture to heal in 6 months in a patient in whom progressive repair has not been observed radiographically within 3–6 months after the onset of the fracture [6,7], whereas other defined nonunion as failure to heal after only 3 months [8]. Therefore, the definition of nonunion has remained controversial.

Several classification systems of nonunion have been proposed based on the presence or absence of infection, radiographic features, clinical findings, biologic activity, location, and shape. According to the AO classification, nonunion is classified as hypertrophic nonunion, avascular/avital nonunion with or without bone loss, atrophic nonunion, and pseudoarthrosis [7]. Among these nonunion types, pseudoarthrosis is a distinct pathologic entity [9]. Although the definition of pseudoarthrosis has not been established yet, the typical features of pseudoarthrosis were often reported as follows: gross motion at the fracture site on physical examination, and evidence of the existence of a pseudocapsule and

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fluid collection between the fracture gap [7,9]. Nonunion does not always include a false joint, gross motion, and synovial fluid. Hence, pseudoarthrosis is distinguished from nonunion as "true pseudoarthrosis" or "synovial pseudoarthrosis" [9,10].

There have been limited investigations about the pathogenesis of each type of nonunion considering local cell biology [11–14]. Previously, we demonstrated that the cells derived from hypertrophic nonunion tissue can differentiate into osteogenic, chondrogenic, and adipogenic cells *in vitro*, indicating hypertrophic nonunion tissue contains multilineage mesenchymal progenitor cells [12]. However, it is still unknown whether pseudoarthrosis tissue contains multipotent mesenchymal progenitor cells similar to hypertrophic nonunion tissue, and there have been no reports of detailed cellular analysis in pseudoarthrosis tissue. In this study, we examined whether pseudoarthrosis tissue-derived cells (PCs) had the capacity for osteogenic and chondrogenic differentiation *in vitro*.

Patients and methods

Patient characteristics

Four consecutive patients with pseudoarthrosis were enrolled in this study (Table 1). The patients were two men and two women, with a mean age of 65 years (range, 58–71 years). The fracture sites were as follows: one humeral diaphysis, one humeral metaphysis, one femoral diaphysis, and one clavicle diaphysis. All patients underwent surgery after conservative therapy, and the mean duration from the fracture to the surgery was 14.8 months (range, 4–26 months). In all the cases, rigid internal fixation using a locking plate and resection of pseudoarthrosis tissue were performed. Autologous bone grafting was performed in all the cases except case 2. Bony union was achieved in all the cases. Pseudoarthrosis was defined as the presence of all the following: (1) gross motion at the fracture site on physical examination; (2) bridging bone on 0 of 4 cortices on anteroposterior and lateral radiographs; (3) computed tomography (CT) showing no purposeful cross-sectional area of healing; and (4) evidence showing the existence of pseudocapsule and fluid collection between the fracture gap at the surgery [9,15]. Images of a representative case are shown in Fig. 1. The radiograph showed no bridging bones on the 4 cortices. Three-dimensional computed tomography (CT) showed no cross-sectional area of healing. Magnetic resonance imaging (MRI) showed that the fracture gap had low-signal intensity on the T1-weighted image and high-signal intensity on the short tau inversion recovery (STIR) image, indicating the existence of fluid collection within the gap. The Ethics Committee of Kobe University Hospital approved this study, and informed consent was obtained from all the patients.

Isolation of pseudoarthrosis tissue

A small amount of pseudoarthrosis tissue (pseudocapsule) was obtained during the surgical treatment of their pseudoarthrosis. The pseudoarthrosis site was exposed *via* careful incision, and the pseudocapsule was obtained with a special care for not contaminating the bone, periosteum, and muscle. Some pieces of tissue were used for histological analysis, and the others were used for cell culture.

Table 1
Cells sample date from four patients.

PC No.	Gender	Age	Fracture site	Duration from fracture (months)	Treatment option	Result
1	M	58	Clavicle diaphysis	26	Plate fixation, resection of pseudoarthrosis tissue, autologous bone grafting	Union
2	F	65	Humeral metaphysis	19	Plate fixation, resection of pseudoarthrosis tissue	Union
3	M	67	Humeral diaphysis	10	Plate fixation, resection of pseudoarthrosis tissue, autologous bone grafting	Union
4	F	71	Femoral diaphysis	4	Plate fixation, resection of pseudoarthrosis tissue, autologous bone grafting	Union

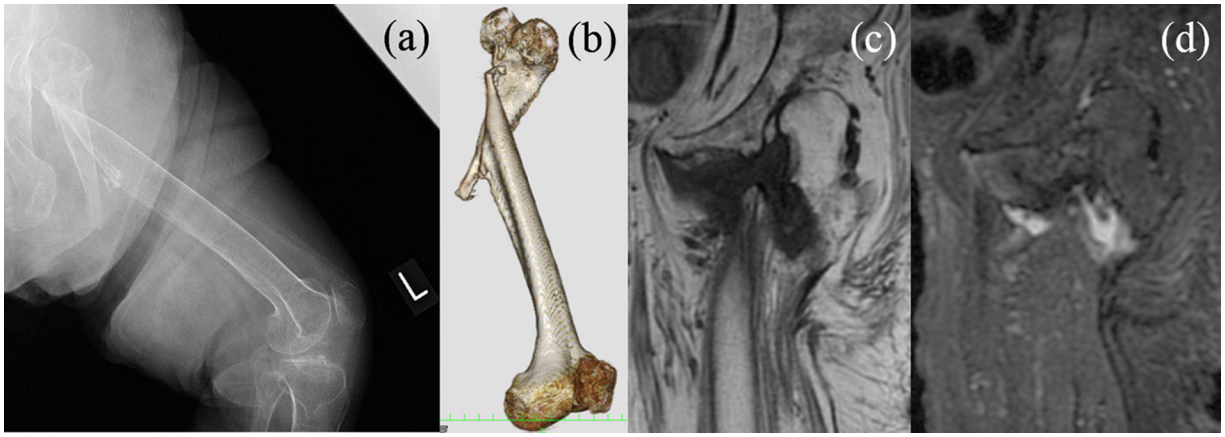

Fig. 1. A 71-year-old woman with pseudoarthrosis at the femoral diaphysis (case 4). (a) Radiograph showing the fracture ununited. (b) A three-dimensional computed tomography image showing no cross-sectional area of bony healing. Magnetic resonance imaging appearance of the pseudoarthrosis on (c) a T1-weighted image and (d) a STIR image, suggesting the existence of fluid collection within the bony gap.