Concise Report

A case of chondromatosis indicates a synovial stem cell aetiology

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Objective. To evaluate cell cultures derived from intrasynovial nodules from a patient with primary synovial chondromatosis (PSC) for aberrant numbers/differentiation of osteochondroprogenitor cells.

Methods. Cell cultures were established from PSC synovial nodules, or normal bovine or human osteoarthritis (OA) synovia (for comparison). Multi-lineage potential was determined using well-characterized in vitro culture systems to assess osteogenic, chondrogenic and adipogenic capability.

Results. Primary PSC cell cultures were typically fibroblastic but contained islands of dense cell clusters/nodules, some of which were isolated and cultured separately [putative osteochondroprogenitis (pOCP) cultures]. OA synovial cultures had barely detectable levels of alkaline phosphatase (AP) that increased (0.006±0.008 to 0.141±0.000 nmol p-nitrophenol/min/cm²) with dexamethasone treatment. AP activity was higher in primary PSC cell cultures and further enhanced by dexamethasone (from 0.076±0.022 to 5.735±0.000 nmol p-nitrophenol/min/cm²). Histochemically, AP was localized as discreet areas within PSC cultures. No AP activity was detected histochemically in OA or normal bovine synovial cultures. The pOCP cultures had high basal AP (5.036±0.439 nmol p-nitrophenol/min/cm²) and spontaneously formed mineralized nodules, which increased in number under standard osteogenic conditions. Under chondrogenic conditions, micromass or pellet-cultured pOCP cells spontaneously synthesized a matrix containing glycosaminoglycans and collagen II. In adipogenic medium, the number of lipid-containing cells was increased.

Conclusions. Compared with cultures established from OA or normal synovia, cell cultures established from PSC synovial nodules were enriched in osteochondroprogenitors, which, unlike normal mesenchymal cells, differentiated along chondrogenic and osteogenic lineages in the absence of dexamethasone.

Key words: Primary synovial chondromatosis, Disease aetiology, Stem cells, Osteochondroprogenitors, Mesenchymal progenitors, Chondrogenesis, Osteogenesis.

Introduction

Primary synovial chondromatosis (PSC) is a rare disorder. Although the aetiology is unknown, it is accepted that the condition has a proliferative component [1] suggestive of a benign metaplasia of the synovium. However, the phenotype and origin of the cells causing the metaplasia and production of multiple intrasynovial cartilaginous nodules remain to be unknown. One possibility is a mesenchymal progenitor cell origin. Mesenchymal progenitor cells (MPCs) have a high proliferation potential and can differentiate into several mesenchymal lineages including cartilage, bone, tendon, ligament and adipocytes [2]. MPCs are found in low numbers in osteoarthritic synovial fluid [3] and normal human synovium, and are induced in vitro to differentiate along chondrogenic, osteogenic and adipogenic lineages [4]. Recently, fibroblast growth factor 3-receptor (FGFR3), a marker of cartilage progenitors and mesenchymal stem cells, was observed on a limited number of cells within PSC synovium and superficial layers of cartilage nodules, and its ligand, FGFR9, was present in PSC but not in normal synovial fluid [5]. Our objective was to determine whether intrasynovial cartilaginous nodules from a patient with primary synovial chondromatosis (PSC) may have had a stem cell origin. We investigated this by assessing whether cell cultures isolated from synovial nodules had enhanced multi-lineage capability compared with cultures established from normal bovine or human OA synovia.

Patient and methods

A male nurse, now 42 years old, first presented in 1992 with pain and swelling of the knee. A loose body was identified radiologically and removed arthroscopically. At arthroscopy, no OA was evident. Synovial inflammation was noted but a biopsy showed ‘non-specific inflammatory changes’. In 1994, a definite effusion was noted, and CT and X-ray examination showed evidence of synovial chondromatosis in the region of the infra-patellar fat pad. This was excised and histology confirmed typical changes of synovial chondromatosis with proliferation of the synovium and calcification of multiple intra-synovial cartilaginous nodules.

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Further pain and swelling of the knee occurred and X-ray examination showed typical changes of synovial chondromatosis in the posterior aspect of the joint. A localized synovectomy was performed and, as before, there was gross thickening of the synovium with calcified, cartilaginous nodules in large numbers in the affected area. PSC was confirmed histologically and samples of the abnormal tissue were taken for cell isolation and histology. Synovial tissue was also collected from two female patients with osteoarthritis, aged 67 and 69 years at operation for knee replacement. All patient tissues were collected with informed consent and ethical approval.

**Cell culture**

PSC nodules were digested (2 h, 37°C) with a mixture of bacterial collagenase (Clostridium histolyticum, Sigma, 2 mg/ml) and neutral protease (Bacillus polymyxa, Sigma, 1 mg/ml). The isolated cells were cultured as monolayers (PSC cultures) in Dulbecco’s Modification of Eagle’s Medium containing non-essential amino acids, 10 mM Hepes buffer, 10% fetal calf serum and 25 μg/ml L-ascorbic acid (basic medium). Samples of bovine or human osteoarthritic synovium were treated in the same way. Confluent cultures were passed using a non-enzymic, cell-dissociating medium (Invitrogen) according to the manufacturer’s instructions. Cell cultures at passage 2–3 were used for experimentation.

Cell nodules that formed in PSC monolayers were isolated using cloning rings and non-enzymic cell dissociating medium. Cells from several nodules were combined and cultured in basic medium to yield cultures referred to as putative osteochondroprogenitors (pOCP). The numbers of pOCP cells were expanded in monolayer culture and used at passage 5–17 for experimentation.

**In vitro osteogenesis assays**

Osteogenesis was performed as described previously [6, 7]. Monolayers were incubated in basic medium containing 10^{-8} M dexamethasone then basic medium containing 10^{-8} M dexamethasone and 10 mM β-glycerophosphate. Alkaline phosphatase activity was quantified using p-nitrophenol phosphate (1 mM, n ≥ 3) and the enzyme was localized in cell cultures with naphthol phosphate AS-BI (50 μg/ml) and fast red (1 mg/ml). Mineralized nodules were stained with alizarin red (0.1%, pH 5.5, 30 min).

**In vitro chondrogenesis assays**

Chondrogenesis was assessed as described previously [8, 9]. Cell pellets (2 × 10^6 cells/pellet) or micromass cultures (4 × 10^5 cells/micromass) of pOCP cells were incubated in serum-free basic medium containing 10 μg/ml insulin, 5.5 μg/ml transferrin, 4.7 μg/ml linoleic acid, 5 ng/ml sodium selenite, 1.75 mg/ml bovine serum albumin, with or without 10^{-7} M dexamethasone, and 10 ng/ml transforming growth factor β1 (TGFβ1). After 21 days, pellet cultures were harvested for quantitative determination of sulphated glycosaminoglycans (GAGs) [10] or embedded in optimal cutting temperature, OCT, cryo-embedding medium (Invitrogen) according to the manufacturer’s instructions. Micromass cultures [4] were incubated for 7 or 14 days. After 7 days, micromasses were fixed with methanol and stained with alcian blue (1% in 1N HCl, pH 1.0) to detect GAGs. After 14 days, micromasses were fixed with 4% paraformaldehyde and stained and collagen I and II were visualized immunohistochemically [11].

**Adipogenesis assay**

Confluent cultures of pOCP cells were treated with adipogenic induction medium [4] and then maintained for 5 days in basic medium containing 10 μg/ml insulin. They were fixed with 2% glutaraldehyde and stained with oil red O solution to detect triglyceride accumulation.

**Results**

**Histology and cell culture**

Excised tissue showed a typical pathology of PSC [12] with dense cartilaginous and calcified nodules embedded within a hyperplastic synovium.

Cells from bovine or OA synovium tissue exhibited a spindle-shaped, fibroblastic morphology typical of synovial fibroblasts. Cell cultures derived from PSC synovial nodules contained predominantly spindle-shaped, fibroblastic cells typical of synovial fibroblasts. However, these cultures (PSC) also contained discreet, raised cell clusters or nodules containing cells of rounded morphology (Fig. 1a). Isolation and culture of the cell clusters yielded monolayer cultures (pOCPs) with a polygonal morphology and rapid proliferation (i.e. pOCPs reached confluency within 3-4 days compared with 2-3 weeks for OA or bovine synoviocytes). At confluence, the pOCP cells exhibited a cobblestone appearance with dense nodular cell clusters.

**In vitro osteogenesis**

Dexamethasone enhanced alkaline phosphatase activity in the PSC synovium cultures (0.076 ± 0.022 to 5.735 ± 0.000 nmol p-nitrophenol/min/cm²). The enzyme was localized as discreet areas within the cultures (Fig. 1b), suggesting a sub-population of cells (pOCP) that had differentiated in response to dexamethasone. However, the steroid did not induce nodule formation or mineralization in the PSC synovium-derived cultures in the presence of β-glycerophosphate. OA synoviocyte cultures had virtually undetectable alkaline phosphatase activity that increased with dexamethasone (0.006 ± 0.008 to 0.141 ± 0.000 nmol p-nitrophenol/min/cm²). No histochemical staining for alkaline phosphatase was detected in normal bovine (results not shown) or OA synoviocyte cultures (Fig. 1b) suggesting a low level of osteochondroprogenitor cells in these cultures. Neither nodule formation nor mineralization was induced by dexamethasone in bovine or OA synoviocyte cultures.

Confluent pOCP cultures expressed high alkaline phosphatase activity and formed nodules, some of which mineralized on addition of β-glycerophosphate (Fig. 1c); indicating spontaneous osteogenic differentiation. In common with its effect on MSC differentiation, dexamethasone increased alkaline phosphatase activity in pOCP cultures (5.036 ± 0.439 to 7.087 ± 0.156 nmol p-nitrophenol/min/cm²) and promoted the number of nodules. Addition of β-glycerophosphate with dexamethasone increased the number of mineralized nodules (Fig. 1a), whereas alkaline phosphatase in these mineralized cultures was reduced (5.432 ± 1.400 nmol p-nitrophenol/min/cm²), indicating mature mineralized nodules [6].

**In vitro chondrogenesis**

pOCP pellet and micromass cultures without dexamethasone spontaneously produced a matrix containing sulphated GAGs, collagen II and barely detectable collagen I (Fig. 2a and b); all features characteristic of a chondrocytic phenotype. Collagen II deposition was also observed in cultures treated with dexamethasone and/or TGFβ1. Addition of TGFβ1 alone, or with dexamethasone, promoted collagen I accumulation in pellet and micromass cultures, but had no effect on the level of matrix GAGs compared with control (results not shown). However, dexamethasone alone reduced matrix GAGs (8.05 ± 2.11 to...
4.71 ± 1.31 µg/pellet). In pellets treated with TGFβ1 plus dexamethasone, the peripheral third of the cells were of elongated appearance (results not shown). Deposition of collagen I, lack of stimulation of proteoglycan accumulation and induction of a fusiform morphology suggested that TGFβ1 and dexamethasone promoted differentiation of the pOCP cells towards a hypertrophic chondrocyte phenotype [13].

**In vitro adipogenesis**

Control pOCP cultures contained few lipid-containing cells, but their numbers increased markedly on exposure to adipogenic induction medium (Fig. 2c).

**Discussion**

Using in vitro culture systems, we investigated the multi-lineage potential of cells derived from intrasynovial cartilaginous nodules of a patient with primary synovial chondromatosis. Primary cultures (PSC) contained dense cell clusters which were isolated and cultured separately (pOCP cultures). The marked rise in alkaline phosphatase of dexamethasone-treated PSC cultures indicated that they contained putative osteoprogenitors which differentiated to an osteogenic phenotype with dexamethasone. Histochemical localization of alkaline phosphatase in the PSC monolayers showed a sub-population of cells which differentiated in response to dexamethasone. Dexamethasone also promoted alkaline phosphatase activity in OA synovial cell cultures, but to a lower extent than the PSC cultures and was not detected histochemically. This suggested an enrichment of osteochondro-progenitors in the PSC cultures compared with normal bovine or OA synovium cultures.

Under appropriate conditions in vivo [14, 15] or in vitro [8, 16], MPCs proliferate and differentiate to hypertrophic chondrocytes, osteoblasts and adipocytes. pOCP cells were highly proliferative, formed nodules and spontaneously differentiated along chondrogenic and osteogenic pathways, and were induced to differentiate along an adipocyte lineage. Hence, the pOCP cells were multipotent and may have caused the cartilaginous and mineralized nodules observed in the PSC patient. In contrast, MPCs from normal synovium required dexamethasone to initiate differentiation [4].

OA synovial tissue was collected from two female patients who were older than the PSC patient. It is possible that age may influence the number of putative MSCs present in normal synovium. However, neither basal nor dexamethasone-induced alkaline phosphatase was detected in bovine synoviocytes from...
skeletally mature, young animals, indicating few MPCs in the bovine cultures. Obtaining normal human synovium was not ethically feasible.

Our findings from one PSC patient could suggest a deregulation of MPC differentiation in joints affected by PSC. The aetiology of highly proliferative osteoprogenitor cells with a predilection to differentiate abnormally in a restricted number of articular joints in this patient remains unknown, but may involve a somatic mutation in a key gene governing MPC differentiation. Recently, it was reported that a deregulation of hedgehog signalling (due to a mutation in the target gene, Gli3) is a predisposition for synovial chondromatosis [17]. Increased cell proliferation may result from increased FGF2 secretion by the PSC nodule cells. Evidence for the presence of FGF2 and its receptor FGFR1 in synovial chondromatosis (and absence in OA) was recently reported [18].

The authors have declared no conflicts of interest.

References


Clinical Vignette

Chronic shoulder pain and diaphragmatic endometriosis

A 25-yr-old Caucasian woman was referred by her GP with a 3 yr history of right shoulder pain. The shoulder pain frequently occurred at the start of menses and was responsive to ibuprofen. Clinical examination, shoulder radiographs and blood tests (CRP, rheumatoid factor) were all normal.

The MRI of the shoulder and right hemi-diaphragm showed small areas of high signal on T1, T2 and STIR sequences at the lateral right hemi-diaphragm, consistent with areas of recent haemorrhage, suggestive of ectopic endometrial tissue (Fig. 1). Positive identification and ablation of the endometrial tissue by laparoscopy was thought to be a low-yield procedure so the patient commenced the continuous progestogen—only pill which caused amenorrhoea and resolution of her symptoms.

Endometriosis affects women predominantly aged 25–35 yrs, most commonly causing pelvic disease (ovaries, peritoneum) whilst extra-pelvic endometrial deposits (diaphragm, umbilicus) are rare. When present, diaphragmatic endometriosis can be associated with chest pain, right upper quadrant pain and chronic shoulder tip pain [1, 2]. The pain is referred from the hormonally influenced endometrial tissue located on the diaphragm, due to the common innervation of C5 and is an important differential in young women with shoulder tip pain but without clinical findings.

The patient provided written consent according to the Declaration of Helsinki.

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Fig. 1. A Coronal T1 SE MR image showing a small area of high signal at the right hemi-diaphragm, consistent with endometriosis (see arrow).