Podoplanin expression in osteochondral grafts in a rabbit model

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INTRODUCTION

Podoplanin (PDPN) is a type I transmembrane O-glycoprotein, which is expressed specifically in lymphatic endothelial cells and has been widely used as a marker for lymphatic vessels. PDPN expression has been reported in chondrocytes and osteocytes. However, there are no reports of PDPN expression in osteochondral grafts. In this study, PDPN expression in osteochondral grafts of a rabbit model was analyzed using anti-rabbit PDPN monoclonal antibody.

METHODS

Production of osteochondral graft model: Female Japanese white rabbits (13 weeks old) underwent general anesthesia using an intramuscular injection of ketamine hydrochloride mixed with xylazine. A 4-cm paramedian incision was made. The patella was dislocated toward the lateral side, and the knee joint was exposed. A full-thickness cylindrical osteochondral defect (5 mm in diameter and 2 mm in depth) was created on the patellar groove in the left knee using an Osteochondral Autograft Transfer System harvest cylinder (Arthrex). An osteochondral plug from the contralateral patellar groove (3.5 mm in diameter and 5 mm in length) was harvested using a MOSAICPLASTY harvest cylinder (Smith & Nephew). The plug was grafted into the distal portion of the osteochondral defect (Figure 1). The patella was returned to its normal position, and the wound was closed using an absorbable suture. After 3-week, 6-week and12-week postoperative periods, rabbits were euthanized with a lethal dose of sodium pentobarbital. The distal part of the femur was resected. The femurs were fixed in 4% paraformaldehyde phosphate buffer solution at 4°C for 3 days, decalcified with 20% EDTA (pH 7.4) at 4°C for 3 to 4 weeks, and then embedded in paraffin.[1)]

Immunohistochemical analyses: Four-μm-thick histologic sections were deparaffinized in xylene and rehydrated. Then, antigen retrieval was performed using proteinase K for 6 min. Sections were incubated with 5 μg/ml of PMab-32[2)] for 1 h at room temperature followed by treatment with Envision+ kit for 30 min. The color was developed using 3, 3-diaminobenzidine tetrahydrochloride (DAB) for 1 min, and then the sections were counterstained with hematoxylin.

RESULT

At 3 weeks postoperatively, PDPN was expressed in the osteochondral graft but not in the non-grafted defect. Non-chondral tissue was observed in the non-chondral graft area. Similar results were obtained at 6 and 12 weeks postoperatively: PDPN expression was strongest at 3 weeks postoperatively, and PDPN expression was weaker and comparable at 6 and 12 weeks postoperatively.

DISCUSSION

We established a novel anti-rabbit PDPN monoclonal antibody, PMab-32, which is useful for immunohistochemical analysis, flow cytometry, and Western blot analysis in the previous study[2)]. In this study, we revealed that PDPN was expressed in osteochondral grafts, but not expressed in the non-grafted defect areas. Non-grafted defect was covered with non-chondral tissue, where there were no chondrocytes stained with PMab-32. Significance of PDPN expression is unknown but might be useful in evaluation osteochondral graft viability. PDPN expression was strongest at 3 weeks postoperatively, which might indicate inflammation.

SIGNIFICANCE

PDPN was expressed in osteochondral grafts of a rabbit model. PMab-32, the first monoclonal antibody useful for rabbit immunohistochemistry, was able to evaluate podoplanin expression in osteochondral grafts. It may also have a potential for assessing the maturity of osteochondral grafts.

REFERENCE

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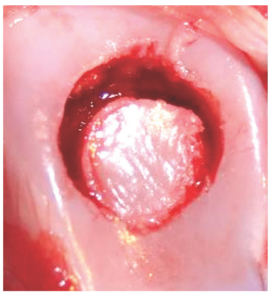

Fig. 1. The plug was grafted into the distal portion of the osteochondral defect.

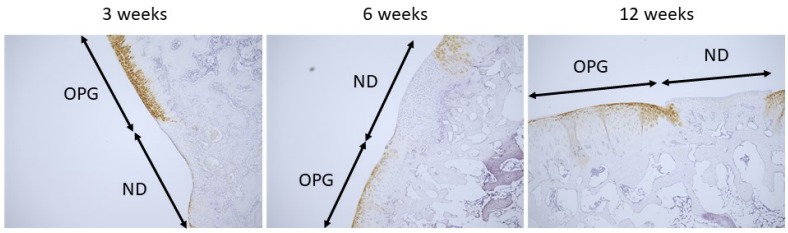

Fig. 2. PMab-32 staining.
OPG, osteochondral plug graft; ND, nongrafted defect.