INTRODUCTION:

Paracrine communication among cells, growth factors, and extracellular matrices has an essential role in ligament repair and regeneration. Several growth factors have been identified to stimulate the healing of medial collateral ligament (MCL) injury. We have previously demonstrated that basic fibroblast growth factor (bFGF) acts as the major stimulator in the cellular proliferation and migration of MCL-derived fibroblasts. The migration of MCL cells is also activated by growth and differentiation factor (GDF)-5. In addition, bFGF and GDF-5 individually increase the expression of α1(I) collagen gene (Col1a1), a major ECM component of ligament, in vitro. However, the effect of isolated and combined use of these growth factors in vitro and in vivo remains unclear.

The purpose of this study is to compare the availability of isolated and combined treatments of bFGF and GDF-5 in MCL injury.

MATERIALS AND METHODS:

Cells: Ligament fibroblasts were isolated from MCL of 10-week-old Japanese white rabbits.

Cell proliferation: MCL cells (n = 5) were treated with the indicated concentration of bFGF and/or GDF-5 (0, 1, 10, 100 ng/ml) for 48 hours prior to addition of WST-1.

Cell migration: Using modified Boyden chamber, the membrane was placed over the bottom chamber filled with bFGF and/or GDF-5 at the indicated concentration (0, 1, 10, 100 ng/ml). MCL cells were added to the upper chamber of each well. After 8-hour-incubation, the number of migrated cells through the membrane pore was counted.

MCL injury model and growth factor treatment: Thirty Japanese white rabbits with a body weight of 2.0-2.5 kg were used. A full thickness defect size was 80% wide of MCL injury. No treatment was applied in a group of partial excision control (n = 5). Treatments were performed using hydrogels contained 10 µg of each growth factor (bFGF, GDF-5, and bFGF/GDF-5; n = 3).

Histological and immunohistochemical analyses: Animals were sacrificed at 2 and 4 weeks after surgery. Coronal sections of partially excised MCL lesions were assessed by hematoxylin–eosin (HE) and Masson trichrome (MT) staining. The histology was evaluated using fiber alignment score and morphology of fibroblasts [2]. Quantitative real-time PCR analysis: MCL samples at 2 and 4 weeks after surgery were also harvested for RT-PCR analyses. The primer sets for rabbit Col1a1, Col3a1, glyceraldehyde-3-phosphate dehydrogenase (G3pdh) were used. Amplification of G3pdh was used for normalization. The final expression value was calculated in dividing each expression level of partial excision control.

RESULTS:

Cellular proliferation of MCL fibroblasts were increased with bFGF: GDF-5 single treatment did not influence MCL cell proliferation. Combined use of bFGF and GDF-5 induced similar increase of cell proliferation by bFGF (Fig. 1).

bFGF and GDF-5 stimulated the migration of MCL cells: Combined treatment of bFGF and GDF-5 additively enhanced the migration of MCL cells (Fig. 2).

DISCUSSION:

Growth factors and scaffolds that activate the cellular proliferation and migration of ligament fibroblasts could potentially induce sufficient ECM synthesis and early ligament healing. The present study demonstrated that the combined use of bFGF and GDF-5 stimulated the healing of MCL injury by increasing the proliferation and migration of ligament fibroblasts, and by regulating the collagen synthesis and connective fiber alignment. Combination therapy using bFGF and GDF-5 might have a potential to induce the optimum healing after ligament injury.

REFERENCES: