FIBROBLAST GROWTH FACTOR 2 PROMOTES REGENERATION OF CARTILAGE BY ATTRACTING MESENCHYMAL STEM CELLS TO THE SITE OF CARTILAGE INJURY.

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Introduction

An important response of cartilage to injury is release of fibroblast growth factor 2 (FGF2) from the pericellular matrix. Deletion of FGF2 in mice, leads to worsening cartilage degradation both spontaneously and after surgical induction of osteoarthritis (OA); thereby indicating a chondroprotective role for FGF2. One mechanism by which FGF2 could protect the joint is by promoting intrinsic repair after injury.

Healing of focal cartilage defects in mice has been shown to be dependent upon background strain and age, with young DBA1 mice having superior regenerative capacity. It is presumed that repair is dependent upon mesenchymal stem cell (MSC) migration to the injured site, followed by the process of chondrogenesis. Here we test whether FGF2 is involved in these processes.

Methods

Constitutive $FGF2^{-/-}$ C57bl/6 mice were backcrossed onto the DBA1 strain (10 generations). Full thickness defects were created in the patella groove of 10-week-old male DBA1 wild type (WT) or $FGF2^{-/-}$ mice. Mice were culled 8 weeks post-surgery, histological sections taken and the repair tissue scored blind by two independent assessors using a modified Pineda score, where a score of 1 equates to poor repair and 14 equates to complete repair.

MSCs were either purchased from Lonza (human) or isolated from the bone marrow or the epididymal fat pad of 6 week old WT, $FGF2^{-/-}$ or green fluorescent protein (GFP)-labelled mice by differential adhesion to plastic. MSC markers were confirmed by FACS analysis. A "scratch assay" was used as a surrogate for cell migration using monolayer WT or $FGF2^{-/-}$ MSCs, or WT cells in the presence of the FGFR inhibitor (50nM SB402451). To investigate if FGF2 promoted adhesion of MSCs to damaged tissue, GFP-labelled MSCs were cultured with porcine cartilage explants in serum free medium in the presence/absence of the FGFR inhibitor for 24hrs. Adhesion to the 'cut' (under surface) or 'undamaged' (articular surface) was compared by measuring the number of adherent fluorescent cells under fluorescence microscopy.

To analyse the role of FGF2 in chondrogenesis, human MSCs were cultured in a scaffold-free trans-well chondrogenic disc assay with standard chondrogenic medium (DMEM supplemented with TGFβ3 10ng/µL, ITS 1X, sodium pyruvate 100µg/ml, dexamethasone 100nM, ascorbate-2-phosphate 25µg/ml, L-proline 40µg/ml, L-glutamine 1X) in the presence of FGF2 or the FGFR inhibitor. The discs were analysed histologically and by RT-PCR to assess their chondrogenic nature.

Results

$FGF2^{-/-}$ mice had significantly lower repair scores (mean of 8) compared with the WT controls (mean of 12). Histologically, the $FGF2^{-/-}$ repair tissue was less cellular and poorly stained for proteoglycan, when compared with the WT tissue.

Migration in the scratch assay was strongly FGF2-dependent, seen both with $FGF2^{-/-}$ cells and when the assay was performed with WT MSCs treated with the FGFR inhibitor. Adhesion of WT MSCs to damaged cartilage was also inhibited in the presence of the FGFR inhibitor, whereas the articular

surface showed little adhesion regardless of condition. FGF2 inhibited *in vitro* chondrogenesis; discs expressed reduced type II collagen and aggrecan mRNA and showed lower Safranin-O staining. FGF2 treated discs also showed higher Sirius red staining, which is suggestive of fibrosis.

Conclusion.

These results suggest that FGF2 release after injury is an important signal for promoting cartilage repair. FGF2 has specific roles in MSC migration and adhesion to the damaged tissue but inhibits chondrogenesis, suggesting that optimal repair requires early, but transient release of the growth factor. A second signal, possibly mediated by released TGFβ, is then required for *in vivo* chondrogenesis; the two in concert delivering successful cartilage regeneration.