Introduction: We have previously reported FGF-2 dependent activation of the extracellular signal-receptor kinase (ERK) pathway when articular cartilage was cut or mechanically loaded (1,2), and have since demonstrated that FGF-2 is bound to perlecain in the pericellular matrix. These findings suggest a significant role for FGF-2 in cartilage homeostasis. To study the function of FGF-2 in vivo, progression of spontaneous and surgically-induced osteoarthritis in FGF-2 knockout mice was compared to wild-type littermates.

Materials and Methods: Mice. Fgf2-/- mice were maintained on a 50% 129Sv/50% Black Swiss background. Only male mice were used.

Surgical induction of OA. Surgical induction of OA was performed by micro-surgical release of the anterior horn of the medial meniscus from its tibial attachment (3). Sham surgery comprised medial capsulotomy only.

Histological assessment. OA scoring: coronal knee specimens were sectioned at 80 μm intervals and Saffranin O stained. Severity of cartilage destruction was assessed using a 6 point score (0-normal; 1-surface fibrillations; 2-delamination of superficial cartilage; 3-ulceration of non-calcified cartilage only; 4-clefts extending into subchondral bone; 5-ulceration into calcified cartilage, ≤80% cartilage loss; 6-ulceration into subchondral bone and/or >80% cartilage loss). Scoring was by two blinded observers. All articular surfaces within each section (medial femur, medial tibia, lateral femur, lateral tibia) were graded separately, then added to give a section score (0-24). The histological summed score was calculated from the sum of the 3 highest section scores to indicate both severity and extent of cartilage damage (0-72). Cartilage histomorphometry was performed using MCID Analysis software.

Real-time RT PCR. RNA was purified from snap-frozen whole joints, and reverse transcribed with random hexamer primers. Real time PCR was performed using TaqMan® gene assays.

Pharmacological rescue of Fgf2-/- mice. Recombinant FGF2 was administered subcutaneously at a dose of 1 μg every other day, beginning 10 days before surgical OA induction and continuing until sacrifice. Control animals were injected with an equal volume of PBS (vehicle).

Femoral head explant culture. Femoral head cartilage was microdissected from the hips of 6 week old mice and rested for 48h in serum free medium (DMEM). This was followed by stimulation with human IL1α (1 ng/ml), with or without FGF2 (50 ng/ml, 1h prior to IL1 treatment) for 24h.

Results: Articular cartilage from adult Fgf2-/− and Fgf2+/+ (wild-type) mice had similar histomorphometry, and similar physical characteristics based on nanoindentation. This suggests that loss of FGF-2 does not impair chondrogenesis.

Fgf2-/- mice developed more severe spontaneous OA than Fgf2+/+ littermates. Following DMM surgery, Fgf2-/- mice developed cartilage degradation at an accelerated rate compared to wild-type controls (Fig 1A,B). Administration of FGF-2 to Fgf2-/- mice slowed the progression of surgically-induced OA to levels comparable to that seen in wild-type mice treated with vehicle only (Fig 1C).

Adams5 mRNA increased in joints of both Fgf2+/+ and Fgf2-/- mice 2 weeks after DMM surgery compared to sham operated and non-operated controls. This increase was significantly greater in Fgf2-/- mice. Adams4 and Mmp13 mRNA were also induced in knees following DMM surgery, but there was no further increase in the Fgf2-/- joints.

To verify that FGF2 directly influenced ADAMTS5 levels, we used the pro-inflammatory cytokine interleukin (IL)-1α to drive ADAMTS5-mediated aggrecan degradation in murine hip cartilage explants from either Fgf2-/- or Fgf2+/+ mice. Aggrecanase was significantly higher in Fgf2-/- cartilage following IL1 stimulation as assessed by total PG release and generation of ALGS and AEGE neoepitope fragments (Fig 2), and this was associated with a significant increase in expression of Adams5, but not Adams4 or Mmp13 mRNA.