Calcium/Calmodulin-Dependent Protein Kinase is Elevated in Human Osteoarthritis and its Inhibition Protects Against Chondrocyte Apoptosis and Catabolism

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Purpose: Posttraumatic osteoarthritis (PTOA) is the number one cause of medical discharge of wounded warriors from active duty, with 28% of affected combat-wounded soldiers experiencing disability. There are no effective treatments to prevent or mitigate PTOA. Intra-articular fracture causes chondrocyte apoptosis, matrix degradation, and suppression of collagen synthesis. We recently reported using an animal model that CAMKK2, a serine-threonine protein kinase, plays a key role in PTOA. We now hypothesize that CAMKK2 is elevated in human OA, and that its blockade will reduce chondrocyte apoptosis and cartilage catabolism.

Methods: This study involving human specimens was approved by the IRB. Osteochondral plugs were collected from patients undergoing total hip arthroplasty for primary osteoarthritis (n = 12/group). Plugs were extracted from damaged and healthier portions of each femoral head. Half of the plugs were placed in liquid nitrogen and underwent RNA isolation for quantitative real-time polymerase chain reaction analysis CAMKK2, MMP13, COL2A1, and ACAN expression. Remaining plugs were formalin fixed and paraffin-embedded for histology and immunohistochemistry (IHC). Safranin O–stained sections were graded for OA using the OARSI (Osteoarthritis Research Society International) scoring system. Samples were also immunostained for CAMKK2, COL2 and matrix metalloproteinase (MMP) 13. Healthy and osteoarthritic chondrocytes from the same femoral head were isolated, cultured, and transfected with scrambled or CAMKK2 siRNA for 3 days, and total protein isolated for immunoblotting to assess CAMKK2, MMP13, BAX and BCL2 levels (n = 3/group). Chondrocytes were also analyzed using flow cytometry to measure cellular apoptosis after 3 days of growth in complete media in the presence or absence of 2 μM STO-609, a CAMKK2 inhibitor.

Results: OA chondrocytes had increased expression of CAMKK2 (2.78 ± 1.86 vs 1.2 ± 0.70, P = 0.02), increased MMP13 (median 20.68 vs 7.46, P = 0.02), decreased ACAN (0.34 ± 0.29 vs 0.55 ± 0.35, P<0.05), and decreased COL2A1 (0.48 ± 0.06 vs 1.09 ± 0.30, P<0.05) mRNA relative to glyceraldehyde 3-phosphate dehydrogenase (GADPH). Osteoarthritic cartilage had higher OARSI scores (3.92 ± 1.00 vs 1.71 ± 1.29, P<0.001), MMP-13 positivity (70.92% ± 6.06% vs 38.22% ± 14.10%, P<0.01) and CAMKK2 positivity (49.45% ± 16.41% vs 14.82% ± 6.17%, P<0.01), whereas COL2 positivity was decreased (48.2% ± 16.7% vs 72.0% ± 8.3%, P<0.01). Human OA chondrocytes had elevated CAMKK2, BAX, and MMP-13. CAMKK2 knockdown (0.35 ± 0.01 vs 1.08 ± 0.09, P<0.001) resulted in lower BAX (0.50 ± 0.04 vs 0.99 ± 0.09, P<0.01) and MMP-13 (0.58 ± 0.05 vs 1.07 ± 0.11, P<0.01) and unchanged BCL2 (0.94 ± 0.24 vs 1.10 ± 0.22, P = 0.53) levels. Treatment with STO-609 attenuated apoptosis in healthy and osteoarthritic chondrocytes (20.8% and 13.8 % vs 9.35% and 7.54%).

Conclusion: OA cartilage possessed enhanced CAMKK2, and its knockdown or inhibition mitigated chondrocyte apoptosis and catabolic marker expression. Our results suggest that CAMKK2 may be a potential therapeutic target for osteoarthritis.