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Article

Rapamycin Maintains the Chondrocytic Phenotype and Interferes with Inflammatory Cytokine Induced Processes

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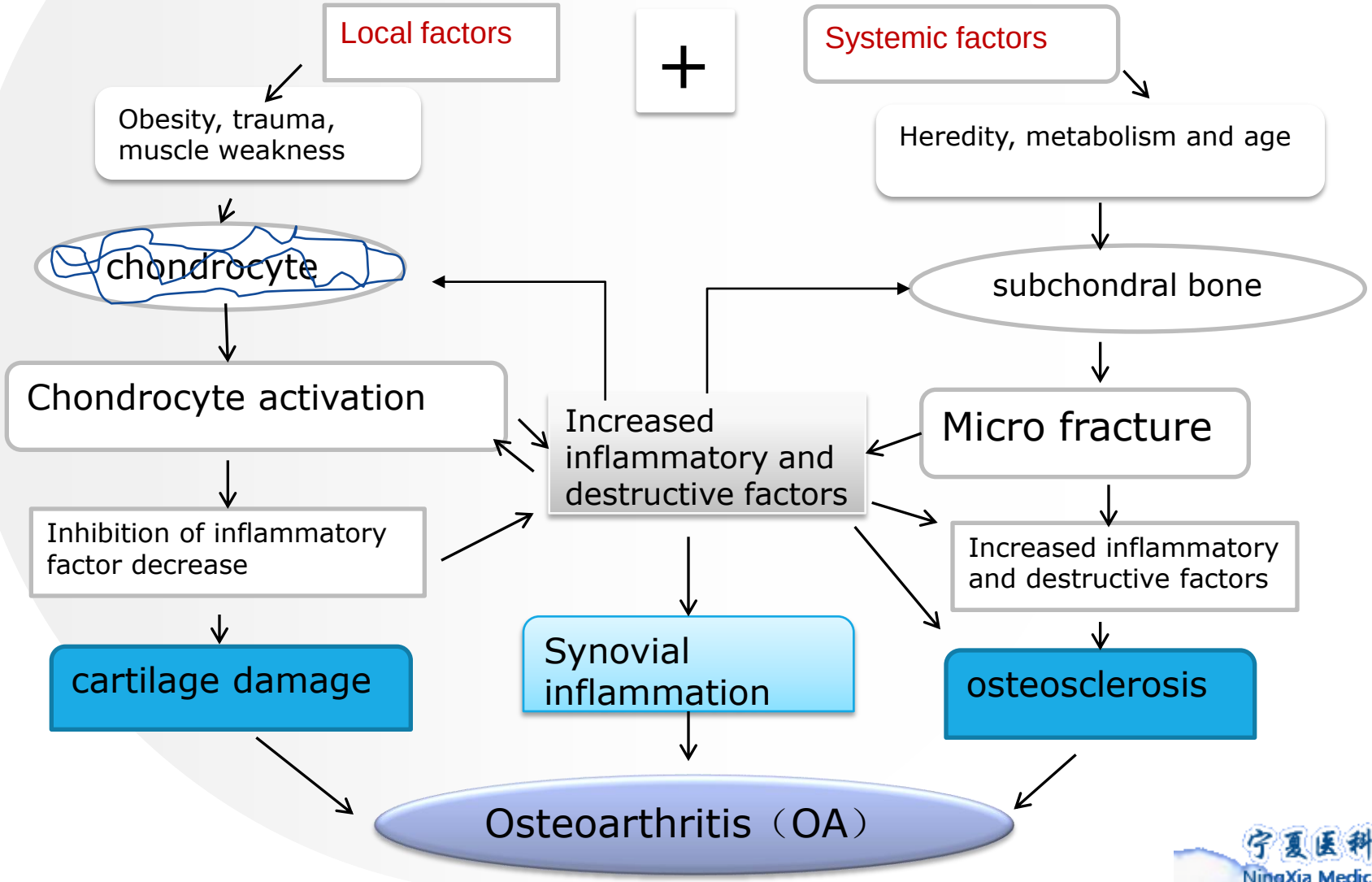
Introduction



mTOR Complex 1 (mTORC1) is composed of mTOR itself, regulatory-associated protein of mTOR，This complex embodies the classic functions of mTOR, namely as a nutrient/energy/redox sensor and controller of protein synthesis，The activity of this complex is regulated by rapamycin, insulin, growth factors, phosphatidic acid, certain amino acids and their derivatives ， mechanical stimuli, and oxidative stress.

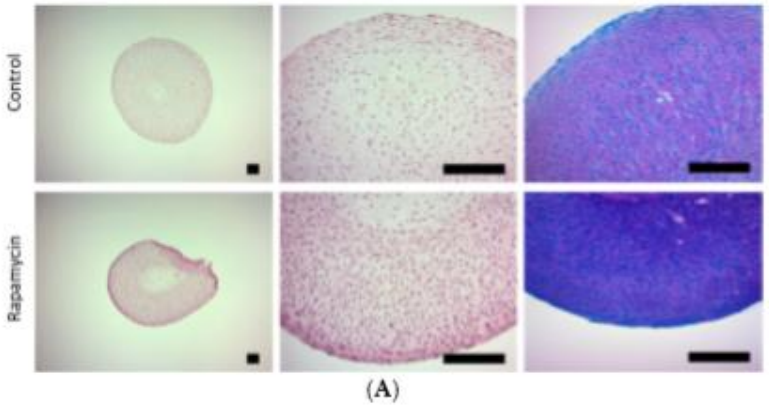
Materials and Methods

- Cultivation of Patient-Derived OA Chondrocytes
- H E Staining
- Immunohistochemical Staining
- Dimethylmethylen Blue (DMMB) Staining
- Sulfated Glycosaminoglycan (sGAG) Assay
- Lactate Dehydrogenase (LDH) Assay
- Caspase 3/7 Assay
- RT-PCR

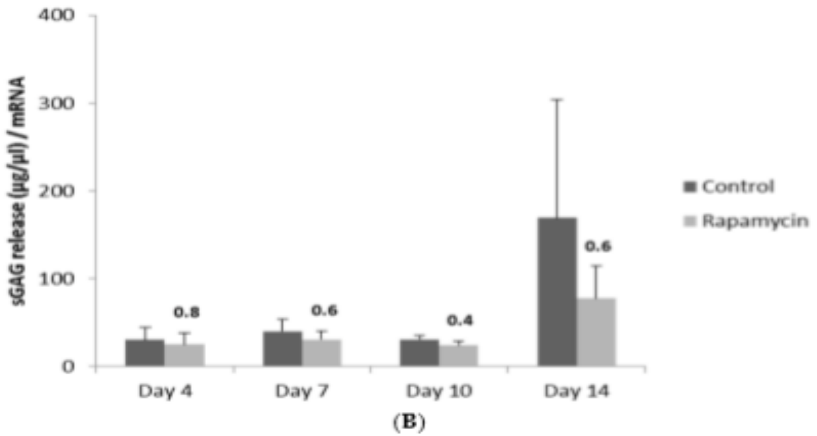
Results：

1.Blocking the Mechanistic Target of Rapamycin Complex (mTORC)1 Prevents Degradation of the Extracellular Matrix

- (A) H E staining was performed to visualize the morphology of the patient-derived osteoarthritic (OA) chondrocyte pellets (DMMB) staining was performed indicating expression of sGAGs
- (B) sGAG release into supernatant of cultured pellets was measured on respective time points indicating degradation of extracellular matrix. Values are normalized to mRNA of pellets isolated on the respective time points.



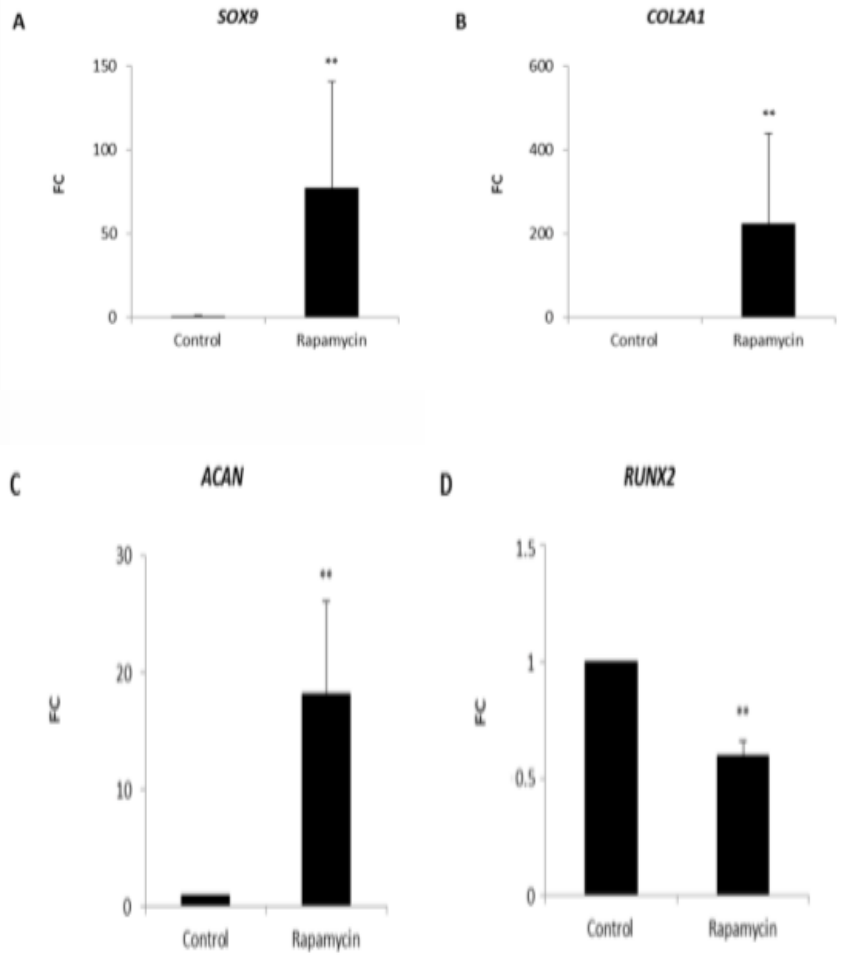
(A)



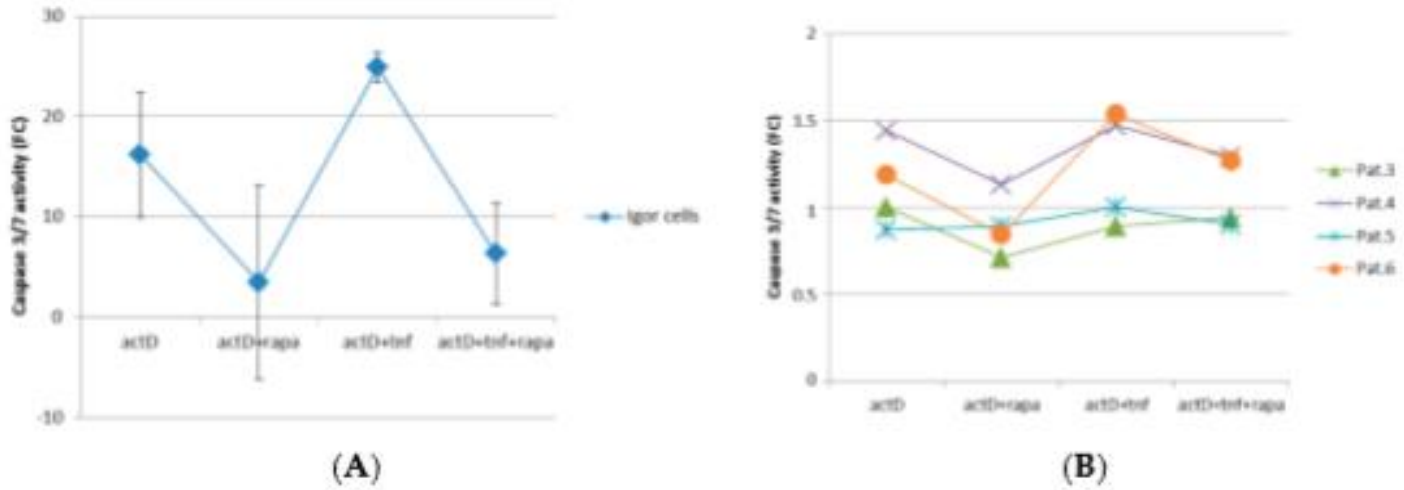
(B)

2.Effects of mTORC1 Inhibition on the Chondrogenic Phenotype of Patient-Derived Osteoarhritic (OA) Chondrocytes

- Collagen type I-chondrocyte constructs were cultured in chondrogenic culture media supplemented without (control) with or rapamycin for 14 days. mRNA was isolated and quantitative real time polymerase chain reaction (RT-PCR) was performed: for the main chondrogenic markers SRY-box (SOX)9, collagen type II α 1 chain (COL2A1) and aggrecan (ACAN) (A–C); and for the hypertrophic marker RUNX2.

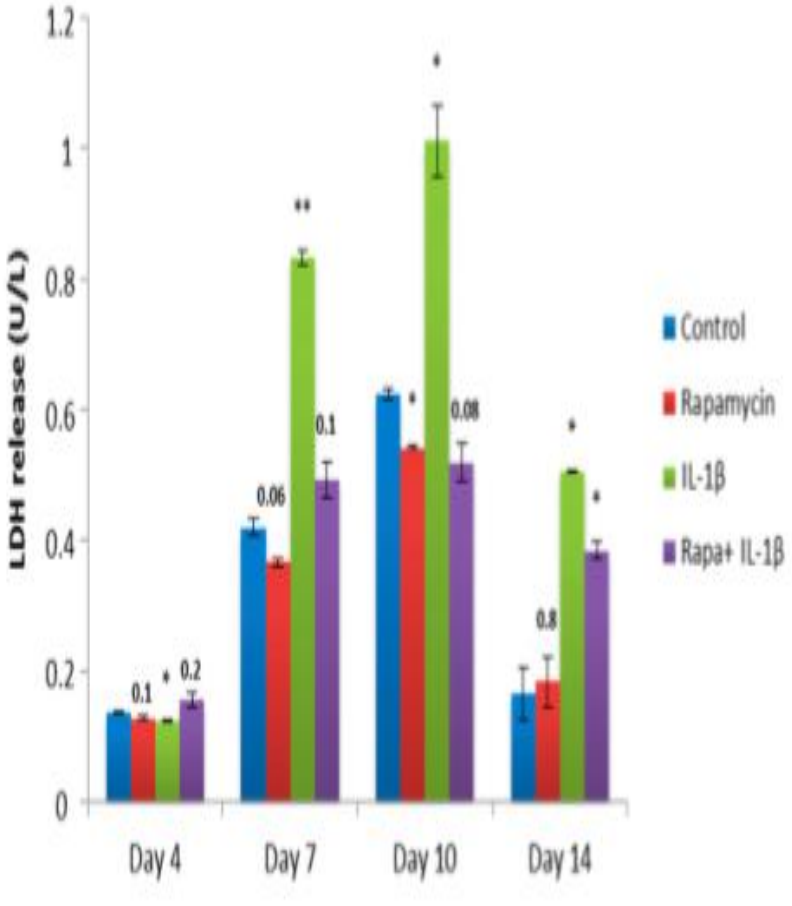


3.mTORC1 Inhibition Prevents Chondrocytes from Undergoing Apoptosis



Caspase 3/7 assay of chondrocytes cultured in a monolayer under inflammatory conditions. To induce apoptosis, chondrocytes obtained from: healthy cartilage (A); and patient-derived OA chondrocytes (B) were cultured with the transcription inhibitor Actinomycin D. To mimic the inflammatory environment present in OA, chondrocytes were treated with tumor necrosis factor α (TNF-α). To assess protective effect of mTORC1 inhibition on OA chondrocytes, cells were also treated with rapamycin

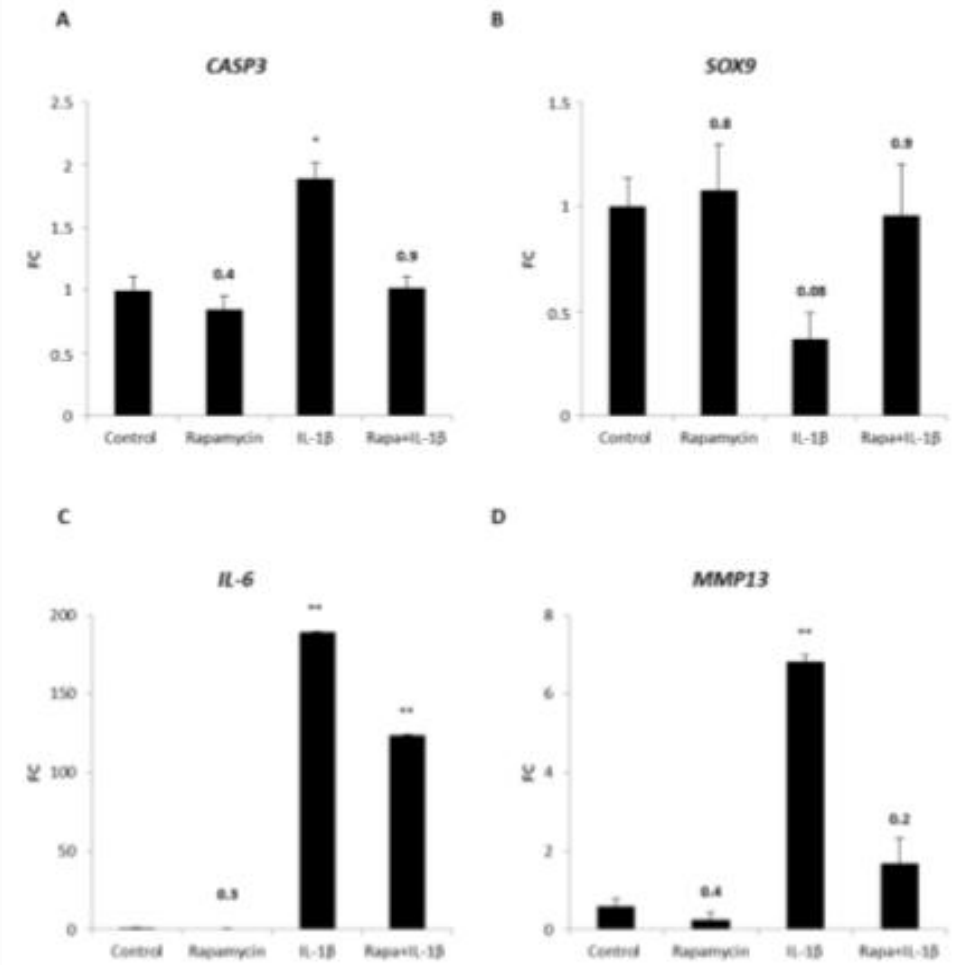
4.Block of mTORC1 Prohibits Cellular Cytotoxicity and Cytolysis of Patient-Derived OA Chondrocytes



- Lactate dehydrogenase (LDH) release of chondrocyte pellets cultured in an OA-model. Patient-derived OA chondrocyte pellets were cultured in media supplemented with or without rapamycin, the inflammatory cytokine interleukin (IL)-1β or rapamycin in combination with IL-1β for 14 days. Chondrocyte pellets cultured with culture media alone are indicated as control. Supernatants were collected on indicated time points and released LDH was measured.

5.Blocking mTORC1 Promotes Chondrogenesis and Suppresses Cartilage Degrading and Inflammatory Processes in an OA-Model

Figure 5. Chondroprotective and anti-inflammatory effect of mTORC1 inhibition on patient-derived OA chondrocyte pellets in an OA-model. Chondrocyte pellets were cultured with media containing rapamycin,IL-1β or rapamycin in combinationwithIL-1β for 14 days.Chondrocyte pellets culturedin chondrogenic culture media alone are indicated as control. CASP3, SOX9, IL-6 and MMP13 mRNAs were analyzed byquantitative real time polymerase chain reaction (RT-PCR)



Discussion

- This study show that rapamycin was able to reduce sGAG release levels compared to control group. The mTORC1 pathway is a major regulator of crucial cellular processes including proliferation, survival and cell growth. In human OA cartilage, mTORC1 is over expressed and in vitro studies in which OA was surgically induced showed that blocking mTORC1 by rapamycin protects cartilage from being degraded.
- We demonstrated that rapamycin had chondrocytes protective effects within the OA-model indicated by reduced LDH and Caspase3/7 levels as well as by a reduced expression of the matrix degrading enzyme MMP13 and the inflammatory cytokine IL-6.

Conclusions

- The present results show that rapamycin is a potent agent to stimulate chondrogenesis and protect chondrocytes during the inflammatory events of OA.
- Its anti-inflammatory properties and its chondroprotective effects make rapamycin a new ray of hope in treating OA and therefore give back quality of life to those who are affected by this progressive disease.

Thank you for your attention!

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