and suppressing mTOR activity, and the direct and independent-PI3K/Akt/mTOR manipulation of HIF-1α and HIF-2α expressions.
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Session: Disease & Treatment — Osteoarthritis

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INTRODUCING Wnt16 ATTENUATES THE SEVERITY OF OSTEOARTHRITIS

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Introduction: Osteoarthritis (OA) is the most common form of arthritis characterized by the degeneration of articular cartilage, intra-articular inflammation, and osteophytes formation. Notably, OA chondrocytes undergo cellular changes that recall hypertrophy and ossification process of the growth plate chondrocytes during endochondral ossification. This "replay" at incorrect time and incorrect location leads to subchondral bone pathological process and ossification (osteophytes formation). Therefore, logically, many genes, including HIF-2α, Runx2 and Hedgehog pathway, regulate the developmental endochondral ossification, may also affect the osteophytes formation in OA pathology. Wnt signaling pathway, one of the crucial pathways, regulates chondrocyte differentiation during embryonic development and after birth. Our preliminary data suggests that Wnt16, the last member of Wnt family currently, regulates cartilage differentiation during embryonic mouse endochondral ossification. We hypothesize that Wnt16 attenuates OA progression by inhibiting chondrocytes hypertrophy and osteophytes formation. Our study aims to investigate the function of Wnt16 in OA pathology and its potential for OA therapy.
Methods: The function of Wnt16 during embryonic skeleton development was examined by whole-mount alizarin red and alcian blue staining in the newborn of the ColIIa-creWnt16$^{flox/flox}$ conditional knockout (cKO) and wild type (WT) mice respectively. Male mice from these two groups at 2 months of age were undergo OA model surgery, and Ad-Wnt16 or Ad-Wnt16-shRNA was intra-articular injected after OA model surgery. Samples were collected at designed time points (Week 2, 4, 6 and 8) for μCT and histological evaluation of the OA progression. The mechanism exploration was mainly performed in vitro by Q-PCR and western blotting to detect the gene expression under Ad-Wnt16 or Ad-Wnt16-shRNA stimulation, and the results were confirmed by histological staining. Results were expressed as mean ± standard deviation. Statistical analysis was carried out using two-way ANOVA with Sidaks multiple comparisons test. P values of less than 0.05 were considered statistically significant.
Results: Wnt16 knockout did not affect the embryonic skeleton development. However, μCT and histological analysis showed the cKO mice developed much severer OA compared to the WT mice. Moreover, intra-articular injection of Ad-Wnt16 significantly inhibited the OA process. For the mechanism exploration, we discovered that many cartilage markers, including ColIIa, aggrecan, SOX9 and Has2, were significantly decreased by IL-1β while promoted by Wnt16. However, parathyroid hormone (PTH) pathway, which can inhibit chondrocytes hypertrophy, was significantly decreased by IL-1β when Wnt16 absence, and significantly promoted by Ad-Wnt16 both showed by Q-PCR and western blotting, and confirmed by histological detection.
Discussion: In our current study, we showed a cartilage protective function of Wnt16 and a potential target for OA therapy. Most studies in this field have focused on blocking molecules that cause cartilage destruction. However, we now demonstrate new ways by applying the tools that nature itself uses to protect cartilage from injury. The OA protective role of Wnt16 suggest it as a potential target for OA therapy and future development of strategies to deliver Wnt16 to joints may prevent severe and permanent disability in patients suffering from cartilage loss.
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SDF-1α/CXCR4 PROMOTES THE HYPERTROPHY OF MSCs AND ARTICULAR CHONDROCYTES BY ACTIVATING THE Wnt/β-CATENIN SIGNALING PATHWAY

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Background: SDF-1α is an extracellular chemokine, also known as CXCL12α, which binds to its cell surface receptor CXCR4. High expressions of CXCR4 and SDF-1α are associated with Rheumatoid arthritis and Osteoarthritis. However the role of SDF-1α in chondrogenic differentiation still unclear. We aimed to investigate the effects of the SDF-1α/CXCR4 axis in activation of the Wnt/β-catenin pathway in the chondrogenic differentiation of MSCs and articular chondrocytes.
Subjects and Methods: MSCs and articular chondrocytes were cultured from mouse. MSC cells were identified by three lines of differentiation and flow cytometry. Passaged MSCs and chondrocytes were pretreated with SDF-1α for 7 days and then analyzed for expression of Sox9, ACAN, Col2 proteins and Alcian blue staining. The cultures were maintained for 14 days and then analyzed for expression of Runx2, MMP-13, Col10, ALP proteins and ALP staining. Western blotting were used to assess the expression of β-catenin, p-β-catenin, GSK-3β, p-GSK-3β, Runx2, MMP-13, Col10 and ALP when chondrocytes were treated with Dickkopf-1, and/or SDF-1α for 24 h.
Results: Alcian blue staining of MSCs and articular chondrocytes revealed that SDF-1α had a limited effect on early chondrogenesis, and the expression of Sox9, ACAN, Col2 showed little change. SDF-1α increased the expression of Runx2, MMP-13, Col10 and ALP in MSCs and primary chondrocytes, implying a role of SDF-1α in chondrocyte hypertrophy. SDF-1α induced the phosphorylation of β-catenin and inhibited the phosphorylation of GSK-3β. The increased expression of hypertrophy markers was significantly attenuated by inhibitors of WNT signaling.
Discussion and Conclusion: SDF-1α promoted the hypertrophy of MSCs and primary chondrocytes, by inhibiting the Wnt/β-catenin signaling. These results suggest that SDF-1α may have a negative effect on articular cartilage and clarify its role in OA progression.
Keywords: MSCs; Articula cartilages; SDF-1α/CXCR4; Hypertrophy; Wnt/β-catenin; Osteoarthritis
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HDAC6 INHIBITION SUPPRESSES CHONDROSARCOMA MALIGNANT PROPERTIES AND RESTORES PRIMARY CILIA EXPRESSION

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Background: Chondrosarcoma is the second common primary malignant bone tumor and characterized by secreting cartilage-like matrix to extracellular environment. Primary cilia is a microtubule-based organelle and functions as "antenna" to detect various stimulus in extracellular environment. Recently, primary cilia is found to be absent in chondrosarcoma but the mechanical of deciliation remains unclear. Histone deacetylases 6 (HDAC6), as a special enzyme to regulate proteins acetylated modification, can deacetylate α-tubulin which is the main structure of primary cilia. In this manuscript, we try to evaluate the role of HDAC6 in regulating chondrosarcoma cell malignant properties and primary cilia expression.
Subjects and Methods: Immunohistochemical assay was used to detect the expression of HDAC6 and primary cilia related Hedgehog pathway proteins in chondrosarcoma tissues. CCK8 cell viability test, transwell and vasculogenic mimic assays were conducted to detect the influences of inhibiting HDAC6 by using inhibitor Tubastatin A and small interfering RNA(siRNA). Immunofluorescence test was used to detect primary cilia expression in different conditions. Western blot and real-time PCR (RT-PCR) assays were used to test related proteins and gene expressions.
Results: Abnormal expressions of HDAC6 and Hedgehog pathway related proteins were found in chondrosarcoma. Inhibition of HDAC6 resulted in down-regulating chondrosarcoma cells viability and proliferation capacities in concentration and time dependent manners and could suppress chondrosarcoma cells invasion and vasculogenic mimicry capacities. Primary cilia restoration was detected after inhibiting HDAC6 by Tubastatin A and siRNA. With the increasing concentration of Tubastatin A, HDAC6 and proliferation relevant protein Cyclin D1 expression were decreased as well as primary cilia related IFT88 and acetylated α-tubulin increased. Small interfering RNA could down-regulate endogenous HDAC6 but IFT88 and acetylated α-tubulin expressed highly in HDAC6 interfering group. The results of RT-PCR suggested that down-regulating HDAC6 could lead a low expression of primary cilia related Hedgehog pathway target genes GLI1 and PTCH1.
Discussion and Conclusion: HDAC6 is a special member of histone deacetylases and mainly deacetylate α-tubulin structure which is crucial to restore cilia assembly. Meanwhile, microtubule, as vital component of cytoskeleton, is indispensable to regulate mitotic spindle and primary cilia transformation, to maintain cell morphology and intercellular junction. HDAC6 can directly deacetylate microtubule structure so that affecting chondrosarcoma malignant properties. In this study, these results have confirmed that inhibition of HDAC6 can suppress chondrosarcoma cells malignant properties such as proliferation, invasion and vasculogenic mimicry capacities, and can restore primary cilia assembly accompanied with affecting related gene and protein expression. In conclusion, promoting primary cilia restoration by targeting HDAC6 may be a potential therapeutic method for chondrosarcoma.
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