deregulated genes included Has1, ADAMTS4, Tnf, IL6, IL18, Inhba, Cd68, Ngf, Ccr2, Wnt 16, Tnfaip6 and Il1r.
Conclusions: Deletion of JNK2 retarded the development of experimental murine OA, showing the importance of the kinase for cartilage homeostasis in vivo and implicating an intracellular signalling pathway in OA for the first time.

68
MEMBRANE-TYPE 1 MATRIX METALLOPROTEINASE CONTROLS OSTEO- AND CHONDROGENESIS BY A PROTEOLYSIS-INDEPENDENT MECHANISM MEDIATED BY ITS CYTOPLASMIC TAIL

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Purpose: We aimed to understand the mechanism by which membrane-type 1 matrix metalloproteinase (MT1-MMP, MMP-14) controls bone and cartilage homeostasis. MT1-MMP, a cell-membrane-bound proteinase with an extracellular catalytic site and a 20-amino acid cytoplasmic tail, degrades the extracellular matrix and plays a key role in postnatal bone formation. MT1-MMP is overexpressed in osteoarthritis, and mutation of MT1-MMP in humans causes the multicentric osteolysis and arthritis disease, Winchester syndrome. The genetic deficiency of MT1-MMP (MT1-MMP$^{-/-}$) in the mouse causes dwarfism, osteopenia and severe arthritis. Conditional deletion of MT1-MMP in bone marrow-derived mesenchymal progenitor cells (BM-MSC) fully recapitulates this phenotype, showing that MT1-MMP controls osteogenic differentiation in MSC. It has been proposed that the phenotype of MT1-MMP$^{-/-}$ mice results from the lack of MT1-MMP proteolytic activity. However, mounting evidence shows a variety of proteolysis-independent functions of MT1-MMP. The unique tyrosine (Y573) in the MT1-MMP cytoplasmic tail is fundamental for the control of intracellular signaling. We have shown that Y573 mediates Ras-ERK1/2 signaling, and that Y573 mutation (Y573D) blocks this function without affecting MT1-MMP proteolytic activity.
Methods: We generated a mouse with the Y573D mutation in MT1-MMP (MT1-MMP Y573D) and characterized its skeletal phenotype by histological and microCT analyses. We isolated BM-MSC and induced them to differentiate into osteoblasts, chondrocytes and adipocytes, using qRT-PCR to analyze gene expression. Mouse C3H10T1/2 MSC were transfected with MT1-MMP cDNA and analyzed for Wnt signaling and Runx2 activation by luciferase reporter assays.
Results: Analysis of the long bones of both homozygote and heterozygote MT1-MMP Y573D mice showed increased trabecular bone relative to wt littermates (BV/TV %; - wt: 10 4±8; homo: 23.2±2.0; Tb.Th (μm) - wt: 20.5±3.8; homo: 40.1±7.0; Tb.N./mm - wt: 10.5±4.2; homo: 19.5 4.2; Tb.Sp (μm): 325.2±52.1; 190.6 49.1; p≤0.01), a phenotype opposite to that of MT1-MMP$^{-/-}$ mice. MT1-MMP Y573D mice also showed marked thinning of articular cartilage, with disorganized tissue architecture, clustering and cloning of chondrocytes, as well as pronounced decrease in bone marrow-associated and total body fat (fat weight/body weight - wt: 0.270±0.0105; homo: 0.0135±0.006; p=0.0029). These findings indicated a defect in MSC differentiation. Therefore, we isolated BM-MSC from wt and homozygous mutant littermates, and induced them to differentiate into osteoblast and chondrocytes, and myeloid precursors were induced to differentiate into osteoclasts. The Y573D mutation dramatically increased MSC expression of osteoblast markers (*ALP, COL1, BSP, OCN, RUNX2*) and strongly downregulated chondrocyte (*ADA4, ADA5, SOX9, MMP13*) and osteoclast markers (*TRACP, CALCR, CTSK, DC-STAMP, MMP9, iNOS*). These findings indicated that Wnt signaling is upregulated in MT1-MMP Y573D-expressing MSC. The canonical Wnt pathway is indeed unique, as it exerts opposite effects on osteoblast and chondrocyte differentiation from MSC. Therefore, we analyzed Wnt signaling and Runx2 activation. We transiently transfected C3H10T1/2 MSC cells in osteoblast medium with the cDNAs for wt MT1-MMP and MT1-MMP Y573D. As controls the cells were transfected with the empty vector (pcDNA) or with MT1-MMP E240A, a mutant devoid of proteolytic activity. MT1-MMP Y573D dramatically upregulated both Wnt signaling (2.8-4.6-fold; p=0.007) and Runx2 activity (8.7-46.5-fold; =0.009) relative to wt MT1-MMP and MT1-MMP E240A.
Conclusions: MT1-MMP controls Wnt signaling by a mechanism independent of extracellular proteolysis and mediated by its cytoplasmic tail. MT1-MMP is a bifunctional protein, with an extracellular proteolytic activity that promotes bone formation through ECM remodeling and a cytoplasmic tail that controls osteogenesis by interacting with a key pro-osteogenic signaling pathway.

69
A DUAL ROLE FOR NOTCH SIGNALING IN JOINT CARTILAGE MAINTENANCE AND OSTEOARTHRITIS

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Purpose: The role of the NOTCH signaling pathway in articular cartilage maintenance and osteoarthritis (OA) is controversial. Using conditional NOTCH loss-of-function murine genetic models, we recently identified the NOTCH signaling pathway as a critical regulator of joint cartilage maintenance, while others have identified an increase in the expression and activation of NOTCH signaling components in post-traumatic murine and human OA. Therefore, we set out to determine whether NOTCH signaling promotes joint cartilage maintenance, contributes to the pathogenesis of OA, or potentially both depending on the strength and/or duration of the signal.
Methods: To solve this question we developed two novel genetic mouse models that allowed for either sustained NOTCH activation that likely resembles pathological NOTCH signaling or transient NOTCH activation that may more closely reflect physiological NOTCH signaling in joint tissues. In vivo changes to joint and cartilage structure and gene expression was monitored using histology, histomorphometry, real-time qPCR, and immunohistochemistry (IHC). Furthermore, we developed an in vitro NOTCH gain-of-function primary chondrocyte culture model, which mimicked our in vivo sustained NOTCH activation mouse model, and performed large scale RNA-sequencing (RNAseq) to identify direct and indirect targets of NOTCH signaling in cartilage. Numerous targets were subsequently validated via in vitro models (primary chondrocytes and ATDC5 cells), as well as, NOTCH-induced and post-traumatic murine OA models using real-time qPCR, Western analysis, and IHC.
Results: Our data demonstrate for the first time that sustained versus transient NOTCH activation in postnatal murine joint cartilages leads to opposing effects on articular cartilage and joint maintenance. This study establishes that sustained NOTCH activation in adult joint cartilage results in a severe, early, and progressive OA-like pathology, while transient NOTCH activation results in increased cartilage extracellular matrix synthesis and joint maintenance under physiological conditions. In vitro and in vivo studies demonstrate the capability of NOTCH signaling to regulate both anabolic (Sox9, Col2a1, Acan, etc⋯) and catabolic gene expression (Mmp13, Adamts4, Adamts5, etc⋯), and RNAseq experiments determined that sustained NOTCH activation suppresses chondrogenic genes but promotes the expression of cartilage-related proteases, fibrotic collagens, and inflammatory factors including Il6. Utilizing both in vivo and in vitro sustained NOTCH activation models we demonstrate that the NOTCH-mediated suppression of chondrogenic regulators, as well as, the activation of fibrotic and catabolic factors may at least be partially mediated via NOTCH/IL6/ERK and NOTCH/IL6/STAT3 signaling mechanisms, respectively. Furthermore, we show that many of the NOTCH-induced signals leading to joint degeneration are similarly affected in a post-traumatic OA mouse model.
Conclusions: Collectively, our data indicate that NOTCH signaling is a critical pathway that regulates joint cartilage homeostasis and in pathological situations of sustained signaling is involved in joint cartilage degradation, fibrosis, and chondrogenic gene suppression. Therefore, an appropriate balance of NOTCH signaling must be achieved throughout life and following joint injury in order to maintain articular cartilage homeostasis and joint integrity.