chondrocytes. Rebamipide down-regulated the mRNA expression of IL-1β, TNF, and NF-κB, thus Rebamipide had an anti-inflammatory effect in chondrocytes. Rebamipide down-regulated catabolic factors (MMP3, MMP13, and ADAMTS5) in chondrocytes. Injection of Rebamipide could prevent articular cartilage degeneration for six weeks. These findings indicate that intra-articular injection of Rebamipide prevents matrix breakdown and the development of OA. In conclusion, intra-articular injection of Rebamipide prevented articular cartilage degeneration for six weeks in murine models of osteoarthritis, Rebamipide down-regulated catabolic factors, and up-regulated anabolic factors in human chondrocytes. Rebamipide could be an important candidate for prevention of articular cartilage degeneration.
http://dx.doi.org/10.1016/j.jot.2016.06.064

### 64
### STRUCTURAL SIMULATION OF ADENOSINE PHOSPHATE VIA PLUMBAGIN AND ZOLEDRONIC ACID COMPETITIVELY TARGETS JNK/Erk TO SYNERGISTICALLY ATTENUATE OSTEOCLASTOGENESIS IN A BREAST CANCER MODEL

Han Qiao, Tingting Tang
*Shanghai Ninth Hospital, China*

**Objective**: The treatment of breast cancer and subsequent osteolysis remains a challenge in clinical settings. The aim of this study was to explore the effect and mechanism of combined treatment with zoledronic acid (ZA) and plumbagin (PL), a widely investigated component derived from Plumbago Zeylanica, against breast cancer and cancer-induced osteoclastogenesis.
**Methods**: Quantitative synergism was evaluated using Compusyn software with determined combination index (CI) and drug reduction index (DRI) values. The attenuated malignancies after dual-drug treatment against breast cancer cells were evaluated with a CCK-8 assay, flow cytometry, and a transwell assay. The cancer-associated osteoclast formation *in vitro* after stimulation of receptor activator for nuclear factor-κB ligand (RANKL) or conditioned medium from MDA-MB-231 human breast cancer cells upon murine bone marrow-derived monocytes (BMMs) and human peripheral blood mononucleated cells (hPBMCs), respectively, were assessed with tartrate-resistant acid phosphatase (TRAcP) staining. Western blot, real-time PCR, homogeneous time-resolved fluorescence (HTRF) assay, and molecular docking were used to unravel the underlying synergistic anti-tumorigenesis and anti-osteoclastogenesis mechanisms. Non-invasive imaging system (IVIS), μCT scanning, and immunohistochemistrical analysis were deployed to investigate the anti-tumorigenesis and anti-osteoclastogenesis effects *in vivo* after dual-drug treatment in an intra-tibiae breast cancer model.
**Results**: We found that the combination of PL and ZA triggered cytotoxicity (CI=0.26), induced apoptosis, and inhibited migration of breast cancer cells synergistically. When the breast cancer cells were transfected with specific siRNA against Notch-1, the combination of ZA and PL markedly increased the expression of Bcl-2. Combined treatment also suppressed cell viability of precursor osteoclasts and synergistically inhibited MDA-MB-231 induced osteoclast formation (CI=0.28) with the abrogation of RANKL-induced activation of NF-κB/MAPK pathways. Molecular docking suggested a putative binding area within JNK/Erk protease active sites through structural mimicking of adenosine phosphate (ANP) by the spatial combination of PL with ZA. A HTRF assay further illustrated the direct competitiveness of the dual drugs against ANP docking to phosphorylated JNK/Erk, contributing to the inhibited downstream expression of c-Jun/c-Fos/NFATc-1. Then, *in vivo* testing demonstrated that the combined administration of PL and ZA attenuated breast cancer growth in the bone microenvironment. Additionally, these molecules prevented the destruction of the proximal tibia, with significant reduction of TRAcP positive osteoclast cells and potentiation of apoptotic cancer cells, to a greater extent when combined than when the drugs were applied independently.
**Conclusion**: The combination treatment with PL and ZA could significantly and synergistically inhibit tumorigenesis and suppress osteoclastogenesis both *in vitro* and *in vivo* by modulating Notch-1-Bcl-2 signalling and simulating the spatial structure of adenosine phosphate to competitively inhibit phosphorylation of JNK/Erk.
http://dx.doi.org/10.1016/j.jot.2016.06.065

### 67
### TENASCIN-C PROMOTES THE REPAIR OF FULL-THICKNESS OSTEOCHONDRAL DEFECTS IN MICE

Hironori Unno [a], Masahiro Hasegawa [a], Yuriyo Matsui [a], Naoya Ito [a], Yoshiaki Suzuki [a], Kyoko Imanaka-Yoshida [b], Toshimichi Yoshida [b], Akihiro Sudo [a]
[a]*Department of Orthopaedic Surgery, Mie University Graduate School of Medicine, Japan*
[b]*Departments of Pathology & Matrix Biology, Mie University Graduate School of Medicine, Japan*

**Introduction**: Tenascin-C (TNC) is an extracellular matrix glycoprotein. While the expression is repressed in normal adult tissues, it reappears under pathological conditions such as wound healing, regeneration, and tumorigenesis. In articular cartilage, TNC expression is associated with development and is almost absent in adult cartilage. In diseased joints, including those with osteoarthritis (OA), TNC was highly recurring in cartilage. Our previous studies have demonstrated that full-length TNC promoted chondrocyte proliferation and increased proteoglycan content *in vitro*, and promoted the repair of full-thickness osteochondral defects in rabbits. In this study, we examined the effects of full-length TNC on joint cartilage repair and on synovial inflammation in full-thickness osteochondral defects model mice. In addition, we performed an *in vitro* study to reveal the mechanism of repairing cartilage using full-length TNC.
**Methods**: Full-length TNC was purified from culture supernatant of human glioma cells U-251 MG. Male 8-week-old BALB/c strain mice were used. *In vivo* study: Full-thickness osteochondral defects were created in the centre of the femoral trochlea with a hand micro-drill equipped with a 0.3-mm diameter drill-bit. The defect was filled with TNC (group A: 100μg/ml, group B: 10μg/ml, group C: empty) by direct administration. Mice were sacrificed at 1, 2, 3, and 6 weeks postoperatively (n=10 knees in each groups at every time point), and whole knee joints were removed by dissection. Histological examinations were made using haematoxylin & eosin (H-E) and Safranin-O (Saf-O) staining. Cartilage repair was evaluated using the modified WAKITANI score. Synovitis was evaluated using synovitis score according to Krenn. *In vitro* study: Human cartilage specimens were obtained from patients who underwent total knee arthroplasty for the treatment of OA. Chondrocytes were isolated and cultured and they were treated with 0μg/ml, 1μg/ml, or 10μg/ml of TNC. We compared the expression of numerous kinds of mRNA for every dose by real-time PCR. We evaluated the expression of TNC, inflammatory cytokines [TNF-α, IL-1β], anabolic factors [TGFβ, TIMP3, bFGF], and catabolic factors [ADAMTS4, 5, and MMP3, 13].
**Results**: In vivo study: In Saf-O-staining, the defects in Group A were covered with hyaline-like cartilage at 3 and 6 weeks. Average modified WAKITANI scores were significantly better in group A than group B and C at 3 and 6 weeks [3weeks: group A: 6.2, group B: 7.8, group C: 9.8 ($p<0.05$); 6weeks: group A: 6.4, group B: 7.6, group C: 8.6 ($p<0.05$)]. At 1 and 2 weeks, there was no significant difference in the average scores in all groups. Low-grade synovitis occurred in both groups at each week. There were no significant differences in average synovitis scores between the two groups at each day. *In vitro* study: Both 1μg/ml and 10μg/ml of TNC up-regulated the expression of TNFα and IL-1β. 10μg/ml TNC up-regulated the expression of endogenous TNC, TGFβ, TIMP3 and ADAMTS4, MMP3, MMP13, but 10μg/ml of TNC down-regulated the expression of ADAMTS5.
**Conclusion**: This study demonstrated that full-thickness osteochondral defects in mice with 100μg/ml of TNC were successfully repaired with hyaline-like cartilage without exacerbating synovitis. The *in vitro* study demonstrated that TNC up-regulates itself and anabolic factors. TNC up-regulated the expression of ADAMTS4, but down-regulated the expression of ADAMTS5, which contributed to cartilage degradation. We consider that TNC could repair the cartilage using these mechanisms.
http://dx.doi.org/10.1016/j.jot.2016.06.066

### 75
### ADVANCED MUSCULOSKELETAL IMAGING SYSTEMS ADOPTED FOR STUDYING A NOVEL BONE-TARGETING DELIVERY SYSTEM CARRYING OSTEOPROMOTIVE PHYTOMOLECULE(S) DEVELOPED FOR THE PREVENTION OF OESTROGEN DEPLETION-INDUCED OSTEOPOROSIS

Le Huang
*The Chinese University of Hong Kong, Hong Kong SAR, China*

**Introduction:** Epimedium is a usual herb used in herbal Fufang (formula) in TCM to strengthen bone and Icaritin is the most potential metabolite of Epimedium. This study was designed to develop an Icaritin-containing bone targeting delivery system for evaluation of its prevention effects on estrogen-depletion induced osteoporosis in experimental model.
**Subjects and Methods:** To evaluate the efficacy of Icaritin with targeting liposome delivery system on prevention of osteoporosis in vivo by analyzing the bone quality and microarchitecture by micro-computed tomography (micro-CT) and the organ distribution of the delivery system by using Xenogen IVIS spectrum system. (DSS6)-liposome-Icaritin was firstly synthesized by thin film evaporation method with extruding through polycarbonate filter membranes to obtain unilamellar vesicles. Then the bone targeting molecules DSS6 were attached to the surface of the vesicles. Eighty 4-month-old C57/BL6 female mice were divided into 9 groups (n=10), including Baseline (BL), Sham surgery (SH), Ovariectomized only (OVX), Estradiol for oral administration (O-E2), Icaritin for oral administration (O-ICT), low dose (8 mg/kg, 1/week) targeting liposome delivery system with Icaritin injected via caudal vein (IV-LIP+ICT+DSS6-L), high dose (8 mg/kg, 2/week) targeting liposome delivery system with Icaritin injected via caudal vein (IV-LIP+ICT+DSS6-H), liposome delivery system with Icaritin injected via caudal vein (IV-LIP+ICT, 8 mg/kg, 2/week). Except in the BL and SH groups, ovariectomy surgery (OVX) was performed on other mice. Administration of gavage and IV injection were applied respectively from the day right after the surgery and lasted for 6 weeks. All mice were sacrificed 6 weeks after surgery. Lumbar spine and the lower limbs were harvest for bone quality analysis. Proximal tibia was scanned by micro-CT (Scanco micro-CT 40). Trabecular bone was identified and the parameters including bone mineral density (BMD),