group; Group 3: OA Group. In group 2–3, osteoarthritids was induced in the right knee joint with ACL+MkMs in rats. Group 2 received continuous infusion of the gin-
senside Rb1 via an osmotic mini-pump, implanted subcutaneously. At four weeks
after treatment, the rat was sacrificed. Interleukin-1 beta (IL-1β) level was eval-
uated by ELISA; cartilage damage was assessed via histology (Safranin-O/fast green
stain) and immunohistochemistry (MMP13, Col X). In the cell study, C5.18 (Rat
chondrocyte cell line) was used. The effect on Rb1 of IL-1β- induced MMP13 or
Col X expression level in C5.18 cells was investigated.

Results: In the in vivo study, characteristics of OA were present in the OA group, con-
trary to the Rb1 treatment group where, in general, less severe damage occurred:  
initially, IL-1β level was significantly decreased; then, cartilage degeneration was
attenuated, by lower histologic damage scores and the percentage of MMP13 or
Col X expression also decreased. In the cell study, the results showed that Rb1 treatment
would relieve the MMP13 or Col X expression level in C5.18 cells in IL-1β induction.

Conclusion: In the present study, we demonstrated that Rb1 can attenuate the  
progression or severity of arthritis via reducing the inflammation level.

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ASSOCIATION BETWEEN ABO BLOOD GROUP AND PRIMARY KNEE
OSTEOARTHRITIS: A CASE-CONTROL STUDY
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Background: Recent studies suggest association between ABO blood group and
inflammation, which was crucial in the pathological process of primary knee oste-
arthritis. This study aimed to investigate whether ABO blood group was asso-
ciated with primary knee osteoarthritis. 

Methods: We performed a retrospective review of patients of primary knee oste-
arthritis as the case group, and a random sampling of healthy blood donors as the
case control group. The severity of knee osteoarthritis at the first outpatient visit
was evaluated by the Kellgren/Lawrence scoring system. Further study was performed
to investigate the expression of blood group antigens in synovial tissue of the
knee in both cases and controls.

Results: 1126 cases and 3029 controls were involved. Logistic regression revealed
that AB blood group was a risk factor of primary knee osteoarthritis (P = 0.025 and
0.048 for univariate and multivariate analysis, respectively), independent of age
(P > 0.973) and sex (P = 0.520). Patients of blood group AB had a higher K/L score
(P = 0.017). Immunohistochemical study indicated association between Leα antigen
and primary knee osteoarthritis (P = 0.029).

Discussions and Conclusions: This study suggested that blood group AB was asso-
ciated with primary knee osteoarthritis, as well as its inflammatory severity. Further
study indicated that Leα antigen, which was related to blood group, was associated
with primary knee osteoarthritis.

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mTORC1 PREVENTS PREOSTEOBLAST DIFFERENTIATION THROUGH THE NOTCH
SIGNALLING PATHWAY
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Introduction: Disruption of the balance between bone formation and resorption
results in loss of bone mass and causes bone diseases such as osteoporosis. Current
therapies for osteoporosis are limited to antiresorptive agents, while bone diseases
due to reduced osteoblast activity, such as senile osteoporosis, urgently require
mechanistic understanding of the signaling pathways and clarifying the underlying
mechanisms may uncover useful therapeutic targets.

Subjects and Methods: We treated osteoblasts and mice with rapamycin (the
mTORC1-specific inhibitor) and established conditional Tsc1 knockout cell and mouse
models (with activated mTORC1). We detected the proliferation rate and
differentiation potential of osteoblasts with impaired or activated mTORC1 and
explored the regulatory mechanisms responsible.

Results: mTORC1 is activated during preosteoblast proliferation but is suppressed
during their differentiation. Inactivation of mTORC1 prevents preosteoblast prolif-
eration but enhances their differentiation in vitro and in vivo. mTORC1 activation in
preosteoblasts produces immature woven bone in mice due to excess proliferata-
tion and reduced expression of Runx2 expression by activating Notch signalling in
preosteoblasts. Preosteoblasts with hyperactive mTORC1 re-ac-
quired the capacity to fully differentiate and mature when subjected to inhibition of
the Notch pathway.

Discussion and Conclusions: mTORC1 signalling has emerged as a critical regulator
of bone formation; however, results from in vitro and in vivo studies on the func-
tion of mTORC1 in osteoblast lineage are inconsistent. Using mTORC1 specific in-
hibitor and conditional knockout cell and mouse models, we elucidated the role of
mTORC1 in osteoblast formation. Here, we report that activation of mTORC1 is
required for preosteoblast proliferation; however, inactivation of mTORC1 is
essential for their differentiation and maturation. Mechanistically, mTORC1 pre-
vented osteoblast maturation through activation of the STAT3/p63/Jagged/Notch
pathway and down-regulation of Runx2. In conclusion, this study clarified the po-
tential role of mTORC1 signalling in the regulation of preosteoblast proliferation
and differentiation and identified Notch signalling and Runx2 as critical down-
stream mediators. Pharmaceutical coordination of the pathways and agents in pre-
ioseoblasts may be beneficial in bone formation.

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ANTI-INFLAMMATORY EFFECT OF JUANBI TANG ON TNF-Tg MICE THROUGH
PROMOTING LYMPHATIC DRAINAGE FUNCTION
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Introduction: Previously, we reported that sufficient lymphatic drainage was
favourable for the treatment of Rheumatoid arthritis (RA) and treatment targeting
drug delivery to RA joints may benefit the patients with insufficient lymphatic
injection of joints. JuanBi Tang (JBT), a Chinese patent medicine, has been
widely used for the treatment of RA in China, which can relieve symptoms of
articular and activate joint function of patients. The aim of this study is to inves-
tigate whether JBT could attenuate inflammation and promote lymphatic drainage
function.

Subjects and Methods: Three-month old TNF-Tg mice and WT littersmates were
used. (1) The effect of JBT on lymphatic drainage function was detected with Indoc-
yanine green near-infrared (ICG-NIR) lymphatic imaging system. (2) The effect of
JBT on ankle joints inflammation were assessed by haematoxylin and eosin (H&E)
staining, Alcian blue/orange G (ABHO) staining, and tartrate resistant acid phospha-
tase (TRAP) staining. (3) The effect of JBT on lymphangiogenesis at inflammatory
ankle joints was detected by using anti-LYVE-1 and anti-podoplanin antibodies for
double immunofluorescence staining. (4) The effect of JBT on lymphangiogenesis
was determined by a zebrafish screening system. (5) The effect of JBT on iNOS
expression and NO production of lymphatic endothelial cells (LEC) were also tested.

Results: Decreased clearance and pulse from ICG-NIR detection indicated TNF-Tg
mice lymph function is impaired, but it was rescued by JBT (p < 0.05). According
to HE staining of ankle sections, JBT (1.2kg/L) could increase TNF-tg mice astra-
gus bone area (1.54x 0.78mm2) in contrast to saline mice (0.75x 0.44mm2, p<0.05),
almost equal to WT mice (1.58x 0.75mm2, p>0.05). The inflammation area around
the astragalus bone of TNF-Tg mice (1.07+0.44 mm2) increased compared with the WT
littermates (0, p<0.05). JBT could reduce the inflamma-
tion area (1.77+0.44mm2, p<0.05). As to Alcian blue/orange G (ABHO) stain, carti-
lage area of the astragalus bone (1.77+0.44mm2) increased more than 20-
fold in the JBT group compared with the saline group. JBT promotes lymphangi-
genesis in the ankle joint of TNF-Tg mice. JBT could also promote the formation of
the lymphatic thoracic duct of zebrafish (48h and 72h, p<0.05). In addition, JBT
blocked iNOS expression and NO production of LEC.

Conclusion: JBT decreases synovial inflammation, bone erosion and cartilage
erosion, and promotes lymphatic drainage function and lymphangiogenesis in
TNF-Tg mice. JBT is a promising agent for treating Rheumatoid arthritis and pro-
moting the lymphatic drainage function might be one of its mechanisms.

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TANSHING-LOADED BONE-TARGETING LIPOSOME ACCELERATES DELAYED
FRACTURE HEALING IN MICE
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Objective: Bone fracture non-union is a major clinical challenge in orthopaedic
practice. In addition to surgical intervention and autologous bone grafts, bone

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morphogenic proteins (BMPs) have also been tested as an adjunct therapy to accelerate the healing of fractures. BMP is an expensive therapy and has not been approved by the FDA for fracture treatment, only for spinal fusion. Clearly, there is an unmet clinical need of a less expensive adjunct therapy for better clinical management of fracture non-union. As a water-soluble mono-
mer isolated from Salivae miltiorrhizae, tanshinol has been proved to be an effective bone anabolic agent with a high therapeutic index. However, its high water solubility and lack of chemical stability, has impeded its clinical application. To address this issue, we have developed a tanshinol-loaded bone-targeting liposome formulation (Tan-BTL). The objective of this study was to examine the potential therapeutic effects of Tan-BTL on fracture repair in mice.

Methods: The bone-targeting liposome (BTL) was labelled by rhodamine B and was used for a hydroxyapatite affinity test in vitro and a bone tissue targeting test in vivo. The BTL was labelled by Iryde 800CW for the observation of its distribution and retention time in the fracture site of mice. Tan-BTL (equivalent tanshinol dose 5 mg/kg, local administration once/week) was used for the treatment of a delayed fracture healing mouse model (induced by daily administration of prednisone at 12 mg/kg for 64 days). Planar X-ray image monitored the healing process of the model for 64 days and the callus was analysed by micro-CT at the 18th day after fracture.

Results: Tan-BTL demonstrated significant hydroxyapatite affinity in vitro. Histological analysis of the distal femur after local administration of the formulation reveals robust targeting and retention at the growth plate, the trabecular bone, the cortical bone, and bone lacuna. A near infrared imaging analysis further confirmed BTL could concentrate on the fracture site of mice and be retained for up to 40 days after local injection. When tested in a delayed frac-
ture healing mouse model (induced by daily administration of prednisone at 12 mg/kg for 64 days), micro-CT and planar X-ray imaging analysis of the callus tissue suggests that Tan-BTL increased callus BV/TV by 54% in a femur fracture of glucocorticoid-treated mice at the 18th day and shortened the fracture healing time from >64 days to 42 days when compared to glucocorticoid-treated mice without treatment.

Conclusion: These results support BTL as a promising targeted drug delivery sys-
tem for local delivery of low molecular weight bone anabolic agents. Specifically, the Tan-BTL formulation tested could be a simple, safe, and effective non-invasive strategy for the treatment of bone fracture non-union.

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231 ANGIOPOIETIN-LIKE PROTEIN 2 INDUCES INTERLEUKIN-6 EXPRESSION IN THE MECHANISM UNDERLYING LIGAMENTUM FLAVUM HYPERTROPHY IN LUMBAR SPINAL CANAL STENOSIS PATIENTS

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Introduction: Chronic inflammation is thought to cause ligamentum flavum (LF) degeneration and hypertrophy in lumbar spinal canal stenosis (LSCS). Angiopoie-
tin-like protein 2 (Angptl2) is highly expressed in hypertrophied LF. Angptl2 regu-
lates interleukin-6 (IL-6) expression in various tissues, thus we investigated whether IL-6 is expressed in hypertrophied LF and, if so, whether Angptl2 induces IL-6 expression in LF in vitro.

Patients and Methods: LF tissue was obtained from LSCS patients and non-LSCS patients. Polymerase chain reaction (PCR) for Angptl2 and IL-6 genes, and immu-
nohistochemistry for IL-6 protein were performed in LF tissue. Fibroblasts from LF tissue were used for in vitro experiments. After Angptl2 recombinant protein treatment, NF-κB activation and IL-6 expression in LF fibroblasts were investigated by immunocytochemistry, PCR, and enzyme-linked immunosorbent assay.

Results: IL-6 mRNA expression was increased in hypertrophied LF tissue from LSCS patients and positively correlated with LF thickness and Angptl2 mRNA expression. IL-6 protein was highly expressed in LF fibroblasts in hypertrophied LF tissue. In vitro experiments demonstrated Angptl2 stimulation promoted NF-κB nuclear translocation and induced IL-6 expression and secretion in LF fibroblasts.

Discussion and Conclusion: This study provides evidence that Angptl2 could be a key molecule causing and promoting inflammation in LF tissue by activating IL-6 expression. IL-6 mRNA expression and IL-6-expressed fibroblasts were increased in hypertrophied LF compared with non-hypertrophied LF. Also, the expression was positively correlated with LF thickness and Angptl2 expression. Our in vitro ex-
periments show that Angptl2 was able to elevate IL-6 expression via integrin α5β1/ NF-κB signalling in LF fibroblasts. Angptl2 could promote inflammation in LF tissue by increasing IL-6 expression and secretion, resulting in LF degeneration and hy-
pertrophy in LSCS patients. Anti-Angptl2 treatment could serve as a target in novel strategies for preventing LSCS and treating it.

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246 MESENCHYMAL STEM CELLS PROMOTE VASCULOGENIC MINICRY IN PROSTATE CANCER THROUGH SDF-1/CXCR4 AXIS AND PI3K/Akt PATHWAY

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Background: Prostate cancer frequently metastasizes to the bone and the interac-
tion between cancer cells and the bone microenvironment has proven to be crucial in the establishment of new metastases. Mesenchymal stromal cells (MSCs) in bone marrow may enhance tumour metastasis through secreting various cytokines that can regulate the behaviour of neighbouring cells. The term vasculoangiogenic mimicry (VM) refers to the unique capability of aggressive tumour cells to mimic the pattern of embryonic vasculoangiogenic networks. VM has been described in prostate cancer and some other highly aggressive tumours and is associated with tumour cell migration and invasion. However, the relationship between MSCs in their native bone marrow microenvironment and VM formation is not clear. Here we investigated the possible role of MSCs in VM by prostate cancer cell lines, focusing primarily on the SDF-1/CXCR4 Axis and PI3K/Akt pathway.

Subjects and Methods: We studied the underlying mechanisms of VM in prostate cancer via the 3D culture system in vitro of PC-3 cells, expression of SDF-1, CXCR4, Akt, p-Akt and VE-cadherin proteins/mRNAs determined by ELISA, immunofluorescence, western blotting, and qRT-PCR, respectively.

Results: In this study, we show the effects of hMSCs on cancer cells are mediated through the SDF-1/CXCR4/Akt signalling pathway. MSCs, when tested in in vitro culture with hMSCs, PC-3 cells were found to have increased the VM formation and CXCR4 expression in vitro. In addition, knocking down of CXCR4 using RNA interference or inhibition of CXCR4 function by an antagonist AMD3100 blocked hMSC-induced VM formation of PC-3 cells. Furthermore, hMSCs increased phosphoryla-
tion of Akt. Additionally, blocking PI3K/Akt Pathway using a PI3K inhibitor LY294002 decreased hMSCs induced VM formations of PC-3 cells. Under in vivo condi-
tions, tumour growth and VM formation was promoted by MSCs in nude mice.

Discussion and Conclusion: To our knowledge, this is the first report discussing the relationship between MSCs and VM of cancer cells. This study establishes that MSCs in the bone microenvironment might promote the VM formations of Prostate can-
cer and points to SDF-1/CXCR4 axis and PI3K/Akt pathway as a potential target for therapeutic intervention. Better understanding of the mechanisms involved in this tumour stoma cell interaction may provide novel targets for the development of treatment strategies for prostate to bone metastasis.

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248 ASSOCIATION OF 3q13.32 VARIANTS WITH HIP TROCHANTER AND INTERTROCHANTER BONE MINERAL DENSITY IDENTIFIED BY A GENOME-WIDE ASSOCIATION STUDY

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Object: Hip trochanter (TRD) and intertrochanter (INT) sub-regions have impor-
tant clinical relevance to subtrochanteric and intertrochanteric fractures, but have rarely been studied by genome-wide association studies (GWAS).

Subjects and Methods: Aiming to identify genomic loci associated with BMD vari-
ation at TRD and INT regions, we performed a GWAS utilising the Framingham heart study (FHS), N = 6,912 as a discovery sample, and utilised the Women’s health initiative (WHI) African-American sub-sample (N = 845), WHI Hispanic sub-sample (N = 446), and Omaha osteoporosis study (N = 963), for replication.

Results: Combining the evidence from both discovery and replication samples, we identified one novel locus, 3q13.32 (rs1949542, discovery p = 6.16x10^-8, replication p = 2.8x10^-4 for INT-BMD; discovery p = 1.3x10^-7, replication p = 4.16x10^-4)