HHS Public Access

Author manuscript

Ann Rheum Dis. Author manuscript; available in PMC 2024 April 10.

Published in final edited form as:

Ann Rheum Dis. 2023 February ; 82(2): 262–271. doi:10.1136/ard-2021-221269.

Targeting FoxO transcription factors with HDAC inhibitors for the treatment of osteoarthritis

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Abstract

Objectives: Osteoarthritis (OA) features aging-related defects in cellular homeostasis mechanisms in articular cartilage. These defects are associated with suppression of Forkhead Box O (FoxO) transcription factors. FoxO1 or FoxO3 deficient mice show early onset OA while FoxO1 protects against oxidative stress in chondrocytes and promotes expression of autophagy genes and the essential joint lubricant proteoglycan 4 (PRG4). The objective of this study was to identify small molecules that can increase FoxO1 expression.

Methods: We constructed a reporter cell line with FoxO1 promoter sequences and performed high-throughput screening (HTS) of the Repurposing, Focused Rescue, and Accelerated Medchem (ReFRAME) library. Hits from the HTS were validated and function was assessed in human chondrocytes, meniscus cells and synoviocytes and following administration to mice. The most promising hit, the histone deacetylase inhibitor (HDACI) Panobinostat was tested in a murine OA model.

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Author Contributions: M.K.L initiated project. M.K.L and M.G. were responsible for study design. K.N. and N.O. developed the reporter cell lines. K.J. supervised the drug screening. E.C. and A.C.C. performed the drug screening. H.O. and H.K. conducted in vitro studies and performed the mouse model studies. H.O and M.O. performed pain testing and histological analyses. M.K.L and H.O. wrote the manuscript. Y.H. performed mechanistic studies. All authors approved the final manuscript.

Patient and public involvement: Patient and/or the public were not involved in the design, or conduct, or reporting or dissemination plans of this research.

Patient consent for publication: Obtained.

Ethics approval: All experimental procedures involving mice were conducted with the approval of the Scripps Animal Care and Use Committee. Human joint tissues were collected following informed written patient consent with approval from Scripps IRB Committee.

Results: Among the top hits were HDACI and testing in human chondrocytes, meniscus cells and synoviocytes showed that Panobinostat was the most promising compound as it increased the expression of autophagy genes and PRG4 while suppressing the basal and IL-1β induced expression of inflammatory mediators and extracellular matrix degrading enzymes.

Intraperitoneal administration of Panobinostat also suppressed the expression of mediators of OA pathogenesis induced by intraarticular injection of IL-1β. In a murine OA model, Panobinostat reduced the severity of histological changes in cartilage, synovium and subchondral bone and improved pain behaviors.

Conclusion: Panobinostat has a clinically relevant activity profile and is a candidate for OA symptom and structure modification.

Keywords

Osteoarthritis; FoxO; Histone deacetylase inhibitor; Panobinostat

INTRODUCTION

Osteoarthritis (OA) is the most prevalent joint disease with aging[1], abnormal mechanical loading[2], metabolic syndrome[3], female sex[4], and potentially alterations of the gut microbiota[5] as its major risk factors. Despite considerable efforts in OA drug discovery and develpment there are no approved disease modifying OA drugs (DMOAD) that would halt or slow disease progression which ultimately leads to the need for joint replacement surgery[6]. Potential reasons for the failures in DMOAD development are persisting challenges in OA clinical trials design[7,8], heterogeneity of OA patient populations and a diverse set of OA pathogenesis pathways and molecular therapeutic targets[9].

Our focus in the discovery of mechanisms and molecules was on aging-related changes in joint cartilage, and our more recent findings point to deficient cellular homeostasis mechanisms, including autophagy[10,11] and proteasome function[12]. In our search for dysregulated transcription factors that might contribute to the abnormal cellular homeostasis mechanisms we investigated Forkhead Box O (FoxO) transcription factors which are known to regulate fundamental mechanisms of cellular aging[13,14]. We found that the expression of FoxO1 and FoxO3 is suppressed in human OA cartilage and in mouse models where it precedes cartilage and meniscus damage[15,16]. In chondrocytes, FoxO increase cellular resistance to oxidative stress[17,18]. Cartilage-specific deletion of FoxO leads to abnormal postnatal cartilage maturation and more severe spontaneous OA and experimental OA induced by destabilization of the medial meniscus (DMM)[19]. The early onset of OA in these mice appears first as a disruption of the superficial zone with suppression of PRG4 and autophagy genes, considered as drivers of the increased disease severity[19]. We also found that viral ectopic FoxO1 expression increased PRG4 and synergized with transforming growth factor-β stimulation. In OA chondrocytes, overexpression of FoxO1 reduced inflammatory mediators and cartilage-degrading enzymes, increased protective genes, and antagonized interleukin-1β (IL-1β) effects[18].

Collectively, these findings of FoxO suppression in OA and its protective functions in chondrocytes and cartilage suggest that it is a promising DMOAD target. The goal of the present study was to discover small molecules that can induce FoxO1 and test hit compounds in vitro and in vivo.

METHODS

Detailed experimental procedures are described in the online Supplementary Materials and Methods and Supplemental Tables.

RESULTS

Screening of ReFrame library

To find compounds that upregulate FoxO1 expression for OA treatment, we prepared a monoclonal human SW1353 chondrosarcoma cell line containing a FoxO1 0.8 kb promoter-luciferase construct (see methods for details) and screened the Repurposing, Focused Rescue, and Accelerated Medchem (ReFRAME) Library. The ReFRAME library is composed of 11,948 small molecules that have reached clinical development or undergone significant preclinical profiling[22]. The primary screen was performed at 10 μM drug concentrations. Hit selection cutoffs for the primary screen were selected at 100% increase in luciferase activity compared to DMSO control. To validate the hits obtained from our primary screen, compounds were re-spotted in duplicate in a 10-point, 3-fold dilution dose response. There was a dose-dependent increase in luciferase activity for 59 compounds (46% hit confirmation rate), with EC_{50} values as low as 0.33 μM. Among the top 59 hits were 24 histone deacetylase inhibitors (HDACI) including Panobinostat with the lowest EC_{50} of 0.42 μM (Table 1; Supplementary Figure 1). The ReFame library contains an additional 24 compounds that are classified as HDACI which did not significantly increase luciferase levels (Supplementary Table 1).

Hit confirmation

We selected the top four HDACI (Panobinostat, Vorinostat/SAHA, Givinostat/ITF2357 and Dacinostat/LAQ824) for further testing. In SW1353 chondrosarcoma cells, Panobinostat induced the largest increases in the expression of FoxO1 and its target gene Sesn3 (Figure 1A) compared to the 3 other HDACI. In human OA chondrocytes, Panobinostat was similar to Dacinostat and Givinostat and superior to Vorinostat in inducing the expression of FoxO1, Sesn3, MAP1LC3 and PRG4, and it was superior to the other HDACI in suppressing IL-6 and MMP-13 (Figure 1B). In normal human chondrocytes, Panobinostat Dacinostat and Givinostat also increased FoxO1, Sesn3, MAP1LC3 and PRG4 (Figure 1C). The drugs did not change IL-6 and MMP-13 as the basal levels of these genes in normal cells were very low.

Panobinostat effects on chondrocytes in vitro

The effects of Panobinostat were profiled in more detail in human OA chondrocytes, and genes related to cellular homeostasis and cartilage ECM were analyzed. Panobinostat did not affect cell viability at 20 nM but caused a small reduction at 40 nM with the higher

doses up to 320 nM not causing additional changes in viability (Supplementary Figure 2). Panobinostat increased expression of FoxO1 and the homeostasis genes Sesn3 and MAP1LC3. Among the cartilage ECM genes, it increased PRG4 at all concentrations tested, while increasing ACAN at 5–10 nM but decreasing it at 40–160 nM (Figure 2A). Col2A1 was not increased at any dose but decreased at 10–160 nM. Dio2, an OA risk gene[31], was increased at lower doses but suppressed at higher doses of Panobinostat (Figure 2A). In SW1353 human chondrosarcoma cells, Panobinostat increased FoxO1, Sesn3, PRG4 and Col2A1, while LC3 and ACAN were not significantly changed (Figure 2B).

Next, we analyzed genes related to inflammation and cartilage ECM degradation. Panobinostat suppressed the basal levels of IL-6, MMP13, PTGES2 and NOS2 in OA chondrocytes. In response to IL-1β treatment, these genes were further increased compared to vehicle control, and Panobinostat dose-dependently suppressed the IL-1β effect on above four genes (Figure 3).

As HDAC inhibitors are known to not only change histone acetylation but also affect other signaling mechanisms, we determined which of the Panobinostat effects were dependent on FoxO1. Treatment of chondrocytes with the FoxO1 inhibitor AS1842856 significantly suppressed the effect of Panobinostat on MAP1LC3 but not on the other genes that were analyzed, including PRG4, SESN3, COL2A1 and ACAN (Supplementary Figure 3).

Panobinostat in synoviocytes and meniscus cells

To extend the analysis of Panobinostat on other joint tissue cells, we included meniscus cells and synoviocytes. In meniscus cells (Figure 4A), Panobinostat dose-dependently increased FoxO1, Sesn3 and MAP1LC3. PRG4 was increased significantly at 10 nM but not at the higher doses. Similar effects were observed in synoviocytes (Figure 4B). The basal levels of IL-6 were not significantly changed by Panobinostat in either cell type. The IL-1β effect on MMP13 in synoviocytes was significantly suppressed at all doses of Panobinostat, and IL-1β-induced IL-6 was suppressed by 160 nM Panobinostat in both cell types (Figure 4).

Effects of Panobinostat on gene expression in normal mouse joints

To explore Panobinostat effects on joint tissues in vivo, we injected the drug intraperitoneally into normal C57BL/6J mice (n=6). Following injection on day 0, 2 and 4, Panobinostat at 100 μ g/kg increased FoxO1, Prg4 and Acan while suppressing Nos2 at 2.5 mg/kg (Figure 5A). To test whether the drug can suppress OA pathogenic genes, normal C57BL/6J mice (n=8) were injected intraperitoneally with Panobinostat at 2.5 mg/kg and 2 hours later with IL-1β (5 ng/joint) in the right knee and with saline in the left knee. Six hours after IL-1β injection, cartilage and synovium were collected for RNA isolation and PCR analysis. Panobinostat significantly suppressed the IL-1β effect on Mmp3, Mmp13 and Nos2 in cartilage (Figure 5B) and synovium (Figure 5C).

Analysis of structural changes and pain behaviors in mice with experimental OA

The DMM model of experimental OA[25,26] was used to test whether intraperitoneal injections of Panobinostat (100 μg/kg or 2.5 mg/kg) can improve joint pathology and pain behaviors. First, we measured knee swelling following the early post-surgical period after

DMM surgery as an indicator of acute anti-inflammatory effects of Panobinostat. Mice developed detectable swelling at the knee with DMM surgery, and this swelling decreased to basal levels by day 9. The Panobinostat 2.5 mg/kg group (n=14) showed significantly less swelling compared to the control group on days 1, 3 and 5 (Supplementary Figure 4).

Eight weeks following DMM surgery, the OARSI scores for cartilage pathology were significantly and dose-dependently reduced by Panobinostat (Figure 6A). In a separate experiment, mice were treated with Panobinostat (1 mg/kg) for 12 weeks and this also significantly improved OARSI scores (Figure 6B). Scoring of subchondral bone showed that both concentrations of Panobinostat significantly reduced the thickness of the subchondral bone plate and the area of cancellous bone (Figure 6C). Synovitis scores were significantly reduced at 1 mg/kg (Figure 6D).

Von Frey testing for evaluating mechanical allodynia[30,32] showed a significant improvement at 4 weeks in response to both dosages of Panobinostat when probing was done with the 1g filament in the 8-week experiment (Figure 6E).

In the separate 12-week DMM experiment, improvements in von Frey testing were seen with the 1g filament at 4 and 12 weeks (Figure 6F). Testing with the 2g filament did not show significant differences in the two experiments (Supplementary Figure 5).

There were no apparent systemic adverse reactions to the Panobinostat injections. All animals completed the study without animal deaths or signs of distress.

DISCUSSION

The motivation to discover inducers of FoxO expression was prior findings that FoxO1 expression is reduced in OA-affected human and mouse cartilage[15,16]. In addition, the FoxO deletion in mice leads to spontaneous OA and more severe surgical OA, and FoxO1 overexpression of FoxO has protective effects in joint cells in vitro and in OA animal models[19]. Here, we performed drug screening with the ReFRAME library[22] using SW1353 human chondrosarcoma clones with a reporter construct containing a region from the human FoxO1 promoter. This collection of known drugs or advanced compounds that have optimized pharmacokinetics, safety, known mechanisms and that have been tested in humans leverages this information for more rapid application in new indications. We found that HDACI represented the largest class of hit compounds. In vitro hit validation was first performed with SW1353 cells and subsequently with normal and OA human chondrocytes. We tested Panobinostat, the most potent HDACI, in human chondrocytes, synoviocytes and meniscus cells and in an OA animal model and performed mechanistic analyses in vitro and in vivo.

The ReFRAME library contains 11,948 compounds and 48 compounds that have HDACI activity[22]. Among the HDACI, 24 had significant activity in the primary and secondary screens, while the other 24 compounds showed no significant effects (Table S1).

HDACs fall into four classes: class I HDACs (HDACs 1, 2, 3, and 8), class II HDACs (HDACs 4, 5, 6, 7, 9, and 10), class IV (HDAC 11) and class III (sirtuin family). Class II

HDACs are further divided into two subgroups: class IIa, which has a large C-terminus, and class IIb, which has two deacetylase domains[33].

HDACI are classified based on the presence of a metal chelating group, and further subdivided by the chemical architecture of the cap and linker region. All HDACI, except for sirtuin inhibitors, have a group that can chelate with Zn^{2+} in HDACs[34]. There are two main HDACI classes, hydroxamates and non-hydroxamates. Non-hydroxamates include short-chain fatty acids, benzamides, cyclic tetrapeptides and sirtuin inhibitors[35,36]. Benzamides demonstrate relative isoform selectivity targeting primarily Class I HDACs, with weaker activity against Class II HDACs[37,38]. The hydroxamic acid group includes the potent non-selective HDAC inhibitors Panobinostat, Givinostat, Trichostatin A (TSA) and Vorinostat/SAHA.

We found that most of the hit HDACI are hydroxamates (Table S2), including the top 4 hits, Panobinostat, Vorinostat/SAHA, Givinostat/ITF2357 and Dacinostat/LAQ824 which were analyzed in more detail in the present study. The main difference between hit and non-hit compounds in the FoxO1 screen is the inhibition of Class IIa HDACs (Table S3) by hydroxamates. Non-hydroxamates exhibit very weak or no activity against the Class IIa HDACs[35,36]. By contrast, Panobinostat and the other top hits are pan HDACI with potent activity against Class IIa HDACs at low nanomolar concentrations[39].

Among the HDACI in the ReFRAME library, Panobinostat had not only the lowest EC_{50} of all HDACI in the primary screen and also a more favorable in vitro profile compared to the other three top inhibitors (Vorinostat/SAHA, Givinostat/ITF2357 and Dacinostat/LAQ824) from the same class when tested on human chondrocytes where it increased the expression of autophagy and cellular homeostasis genes (sestrin3, LC3), PRG4 and Acan which are known FoxO target genes[40], while suppressing genes encoding mediators of inflammation (IL-6, iNOS, PTGES2), and ECM degradation (MMP3, MMP13). The anti-inflammatory activities have been observed for several HDACI, especially for broad spectrum HDACI. HDACI interfere with expression and/or secretion of IL-1β, IL-6, and TNF-β[41]. Importantly, Panobinostat inhibited the effects of IL-1β, a potent inducer of OA mediators in chondrocytes.

The Panobinostat effects in vitro showed linear dose-responses for the induction of Sesn3 and MAPLC3 and linear dose responses for suppression of inflammatory mediators (IL-6, iNOS, PTGES2) and ECM degrading enzymes (MMP3, MMP13). The ECM genes (PRG4, ACAN, COL2A1) were induced at low Panobinostat concentrations (<40 nM) but suppressed dose-dependently at higher doses. In vivo analyses also showed that Panobinostat had more favorable effects in stimulating PRG4 at 100 μg/kg as compared to 1.5 mg/kg. These findings are consistent with the notion that global HDACI have a tendency of a dual response with divergent effects at low versus high concentrations[42]. It is assumed that the role of diverse HDACs associated with single pathophysiology are quite different and the overall inhibition effect of HDACI also varies according to concentration.

The HDAC enzymes appear to be the major target of the HDACI[43]. HDACs have an important role both in transcription regulation and in protein modification. Histone

deacetylation leads to chromatin compaction and repression of mRNA synthesis, which are likely to be involved in at least some of the effects observed in chondrocytes. Besides the role in transcription repression, HDACs also function as regulators in posttranslational modification and deacetylate non-histone proteins including both, transcription factors, such as E2F, p53, c-Myc, NF-κB and FoxO, and signal mediators, such as Stat3, Smad7, mitogen-activated protein kinases (MAPK) and β-catenin homeostasis[33,44]. HDAC4 is known to be a major regulator of chondrocyte hypertrophy and abnormal expression of HDAC4 in OA cartilage suggests its involvement in promoting the catabolc activity of chondrocytes associated with OA pathogenesis[45]. HDAC4 is also an upstream mediator of MAPK and promotes ADAMTS4, ADAMTS5, and COX2 expression in rat articular chondrocytes and stimulates IL-1β[46,47]. HDAC7 evokes cartilage damage and ECM degradation through the over-expression of MMP3 and MMP13, which is consistent with the inhibition of HDAC7 in vitro leading to suppression of inflammatory induced MMP13 gene expression[48,49].

Prior studies tested HDAC and HDACI in the context of OA[50]. HDACI suppressed mediators of ECM degradation in human OA chondrocytes[45,51,52]. The pan HDACI (Trichostatin A) and specific Class I HDACI (Valproic acid and MS275) prevented the expression of MMPs in human OA chondrocytes[53,54]. TSA and MS275 suppressed RUNX2, ADAMTS5 and MMP3 through the inhibition of p38 MAPK, ERK1/2 and JNK activation in human chondrocytes[55]. TSA is the only HDACI that was previously tested in OA animal models. Systemic administration of TSA prevented cartilage destruction in a mouse model of surgically induced OA[54] and in a rabbit experimental OA model[56].

The present results show that Panobinostat induced several FoxO target genes, including PRG4, autophagy genes, and sestrin3 which functions as an antioxidant to reduce oxidative damage in cells[57]. FoxO and Panobinostat also share the ability to reduce the basal or IL-1β induced expression of inflammatory mediators and ECM degrading enzymes. Thus, Panobinostat replicates some but not all protective effects of FoxO1 in joint tissues which is consistent with the Panobinostat effects beyond HDAC inhibition.

The present study has used DMM which is a model of posttraumatic OA. To determine utility of Panobinostat in primary OA, testing in spontaneous aging-related animal models would be required.

In summary, the present in vitro and in vivo results suggest that Panobinostat has desirable activities in protecting against OA. Concerns have been raised about using systemic administration of broad spectrum HDACI to treat OA, which are related to their adverse effects. Panobinostat has been approved for the treatment of patients with multiple myeloma where it induces hematological adverse events[58]. However, the present study used much lower concentrations (2.5 mg/kg and 100 μg/kg) than what is typically used in animal model studies of tumors (10 mg/kg). For application in OA, an alternative and feasible route would be the development of sustained release formulations of Panobinostat for intraarticular administration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding:

This study was supported by NIH grants AG049617 and AG059418.

Competing interests:

No financial support or other benefits have been obtained from any commercial sources for this study and the authors declare that they have no competing financial interests.

Data and materials availability:

All data are included in the manuscript and supplemental material.

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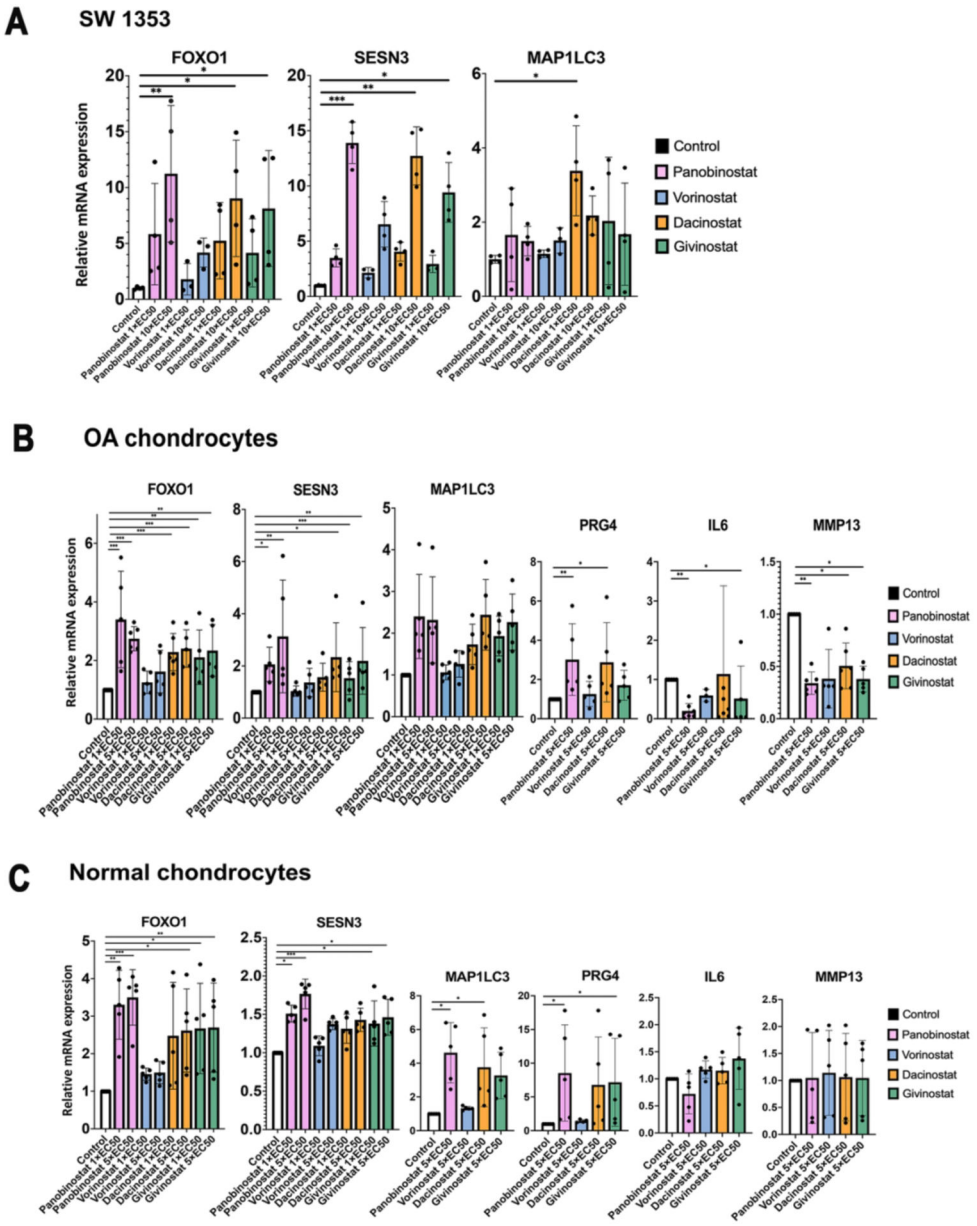

Figure 1. Comparison of top four HDACI.

(A) SW1353 human chondrosarcoma cells were cultured in the presence of the indicated EC_{50} values of Panobinostat, Vorinostat, Givinostat and Dacinostat for 24 h, and RNA was isolated for qRT-PCR analysis. Independent experiments (N=3) were performed where each condition was tested in duplicate.

(B) Human OA chondrocytes (passage 1) from 5 donors were incubated with the indicated doses of Panobinostat, Vorinostat, Givinostat and Dacinostat for 24 hours and RNA was isolated for qRT-PCR analysis.

(B) Normal human chondrocytes (passage 1) from 5 donors were incubated with the indicated doses of Panobinostat, Vorinostat, Givinostat and Dacinostat for 24 hours and RNA was isolated for qRT-PCR analysis.

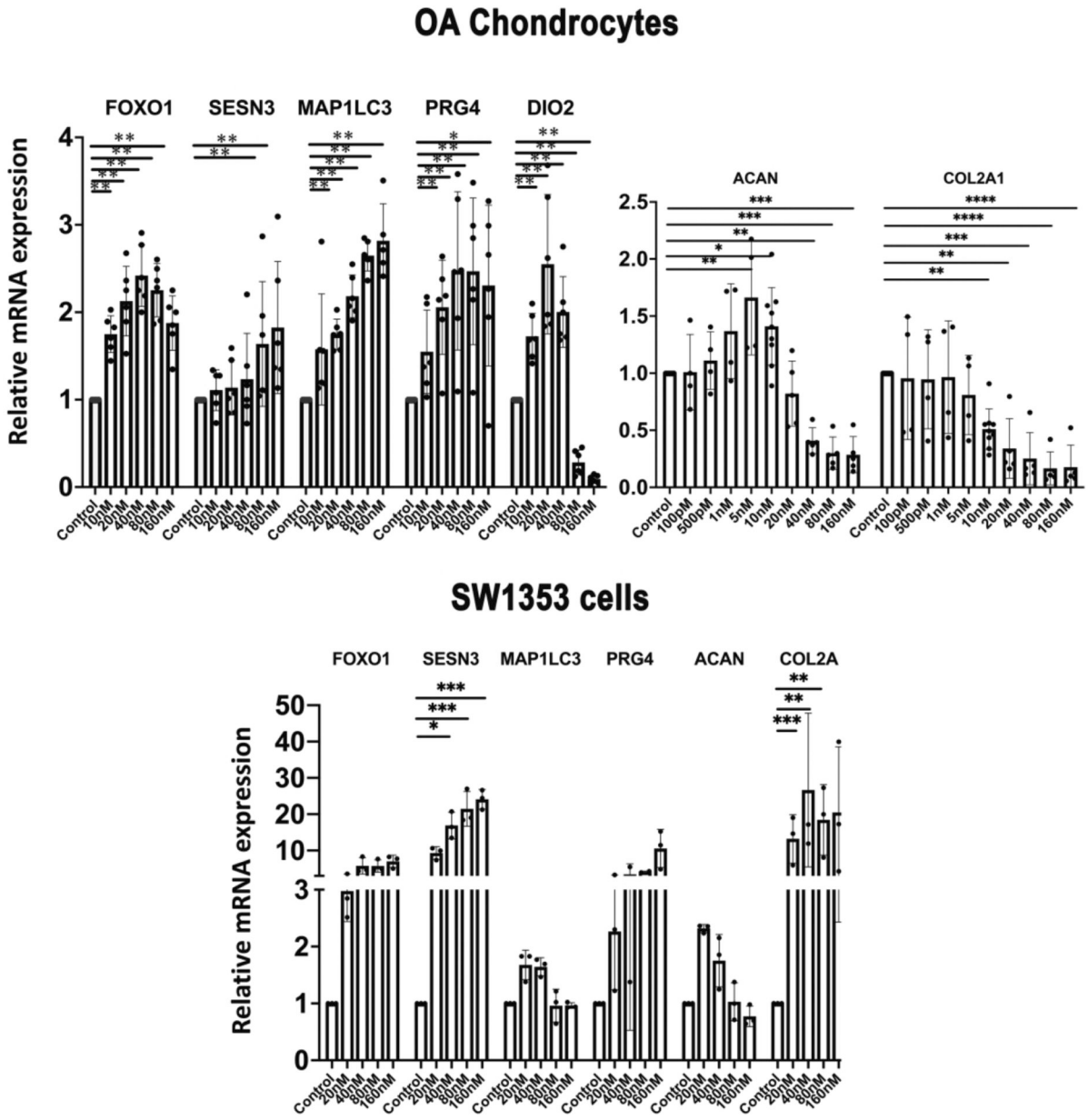

Figure 2. Panobinostat and homeostasis and cartilage ECM genes.

(A). Chondrocytes

Human OA chondrocytes (passage 1) from 5 donors were incubated with the indicated doses of Panobinostat for 24 h and RNA was isolated for qRT-PCR analysis.

(B). SW1353 human chondrosarcoma cells

SW1353 cells were treated with the indicated doses of Panobinostat for 24 h and RNA was isolated for qRT-PCR analysis. Independent experiments (N=3) were performed where each condition was tested in duplicate for each condition.

****= p<0.0001; ***= p<0.001; **= p<0.01; *= p<0.05

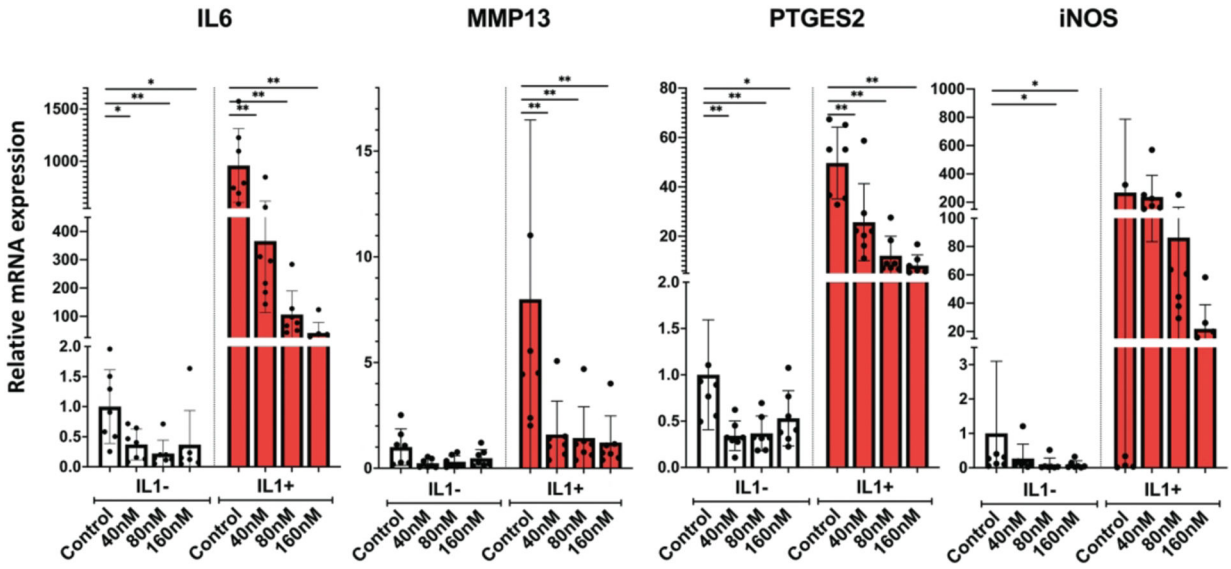

Figure 3. Panobinostat and catabolic genes.

Human OA chondrocytes (passage 1 from 6 donors) were pre-incubated with the indicated doses of Panobinostat for 24 hours. IL-1β (1 ng/ml) was added during the last 6 h and RNA was isolated for qRT-PCR analysis

**= $p<0.01$; *= $p<0.05$

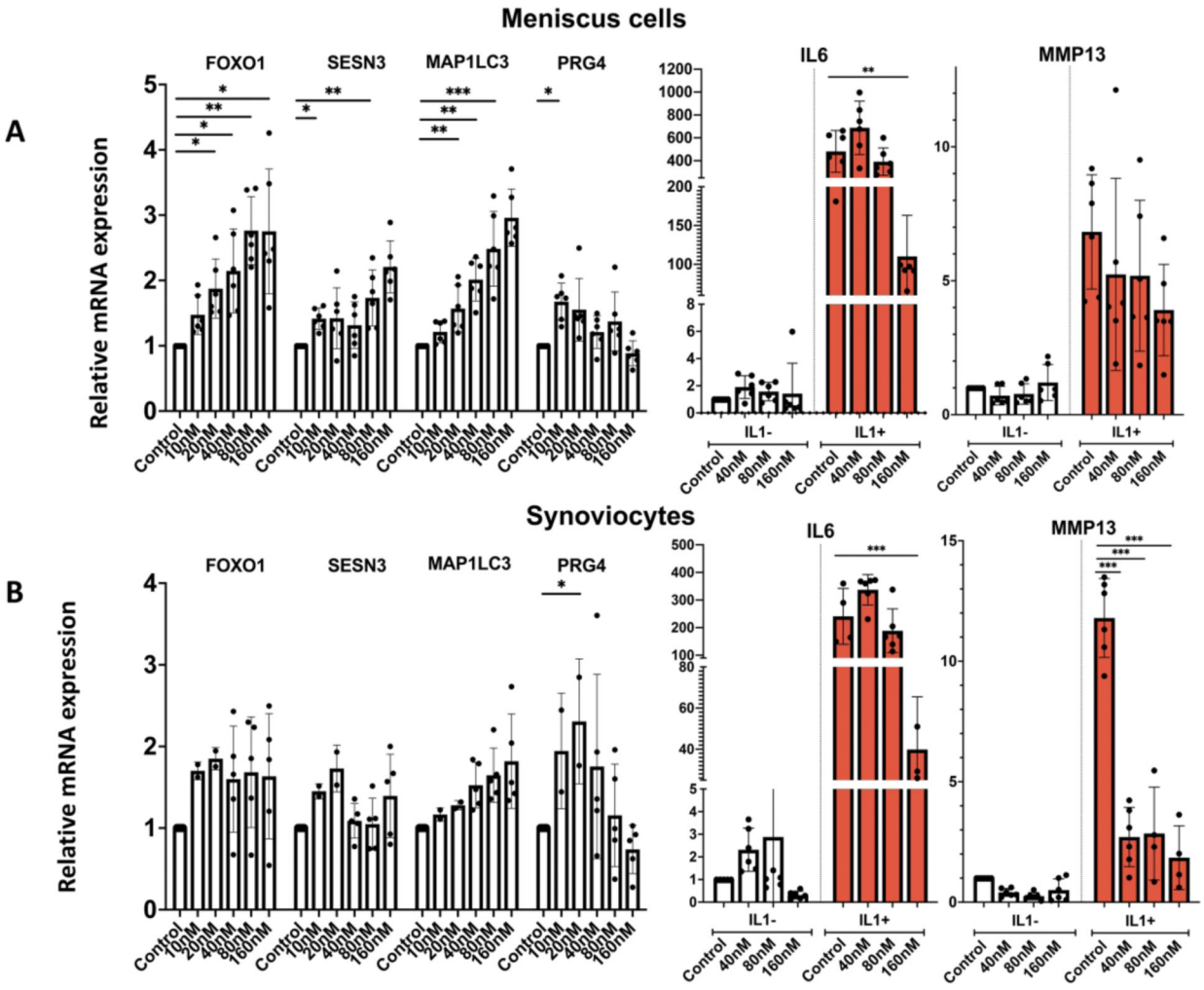

Figure 4. Panobinostat effects on human meniscus cells and synoviocytes.

Human OA meniscus cells at passage 1 from 6 donors (Panel A) and OA synoviocytes at passage 1 from 6 donors (Panel B) were treated with Panobinostat for 24 h when IL-1β (1 ng/ml) was added for additional 6 h and RNA was isolated for RT-qPCR analysis.

***= p<0.001; **= p<0.01; *= p<0.05

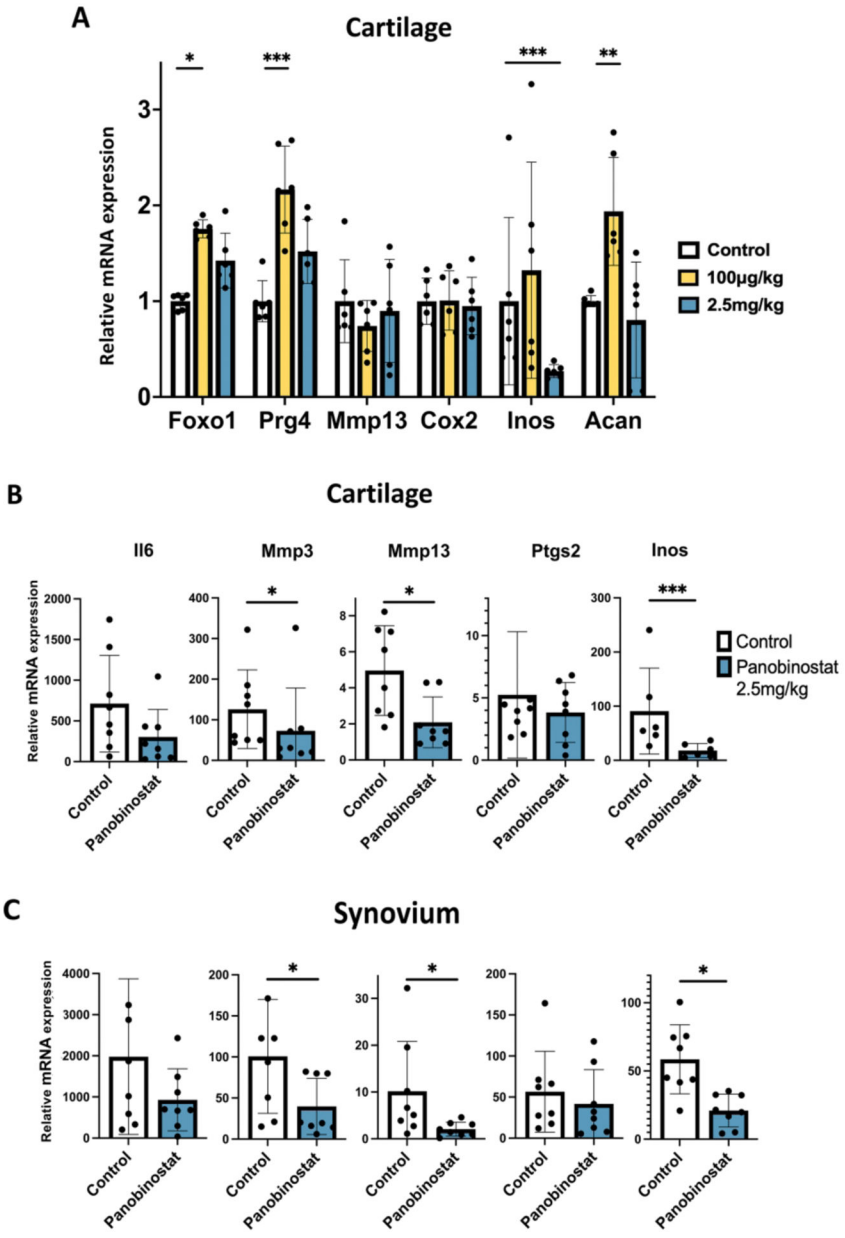

Figure 5. Panobinostat effects on joint tissues in normal mice.

(A). C57BL/6J mice (n=6) received intraperitoneal injections of Panobinostat on Day 0, day 2 and Day 4 and were euthanized 1–3 h after the last injection.

(B). C57BL/6J mice (n=8 each) received one intraperitoneal injection of vehicle and Panobinostat (2.5 mg/kg) and 2 h later IL-1β (50 ng in 10 μl of 5% dextrose) was injected into the right knee and 10 μl of 5% dextrose injected into the left knee. Six h after the IL-1β injection the mice were euthanized. Cartilage and synovium were resected for RNA isolation and RT-qPCR analysis. Data are shown as fold increase in response to IL-1β in Control animals and in Panobinostat treated animals.

***= p<0.001; **= p<0.01; *= p<0.05

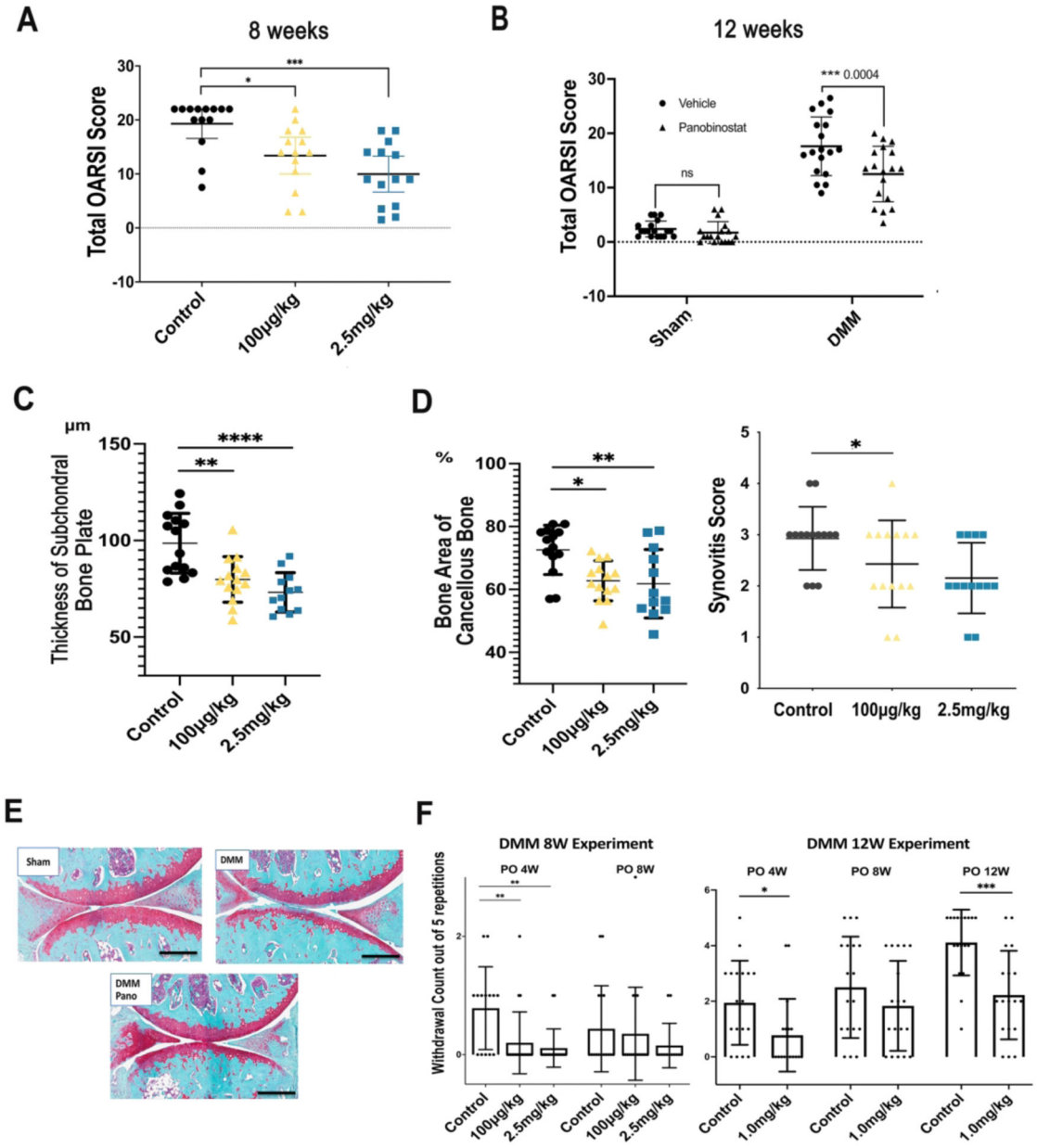

Figure 6. Analysis of structural changes and symptoms in mice with experimental OA.

Male C57BL/6J mice (n=70), 16-weeks old, were subjected to DMM surgery on the right knee and sham surgery on the left knee. Fourteen mice were randomly assigned to one of three groups, Control (IP injection of 10 µl/g body weight) of 5% dextrose with 0.72% DMSO, Panobinostat 100 µg/kg, and Panobinostat 2.5 mg/kg. IP injections were given every other day starting from one-week post-surgery. Two separate experiments were performed with treatment duration for 8 or 12 weeks after DMM.

(A). Knee cartilage changes were scored according to the OARSI system following 8-weeks of Panobinostat treatment (100 µg/kg or 2.5 mg/kg).

(B). Knee cartilage changes were scored according to the OARSI system following 12-weeks of Panobinostat treatment (2.5 mg/kg).

(C). The area of subchondral bone plate and subchondral cancellous bone were measured by Image J at 8 weeks after DMM.
(D). Synovitis score was measured at 8 weeks after DMM.
(E). Representative images of Safranin-O stained knee sections from sham surgery control mice, mice with DMM and mice with DMM and Panobinostat (Pano) treatment (2.5 mg/kg). Scale bars, 200 μm
(F). Von Frey testing for evaluating mechanical allodynia was performed with 1 g filaments at the indicated time points after DMM in the 8-week and 12-week Panobinostat treatment experiments.
****= $p<0.0001$; ***= $p<0.001$; **= $p<0.01$; *= $p<0.05$

Table 1.

HDAC inhibitors with significant activity in the FoxO1 drug screen.

Drug	EC_{50}
Panobinostat (LBH589)	4.15329E-08
Vorinostat (SAHA)	8.62446E-08
Dacinostat (LAQ824)	1.43602E-07
Givinostat	1.52729E-07
QUISINOSTAT	1.55606E-07
AR-42	2.78339E-07
Pracinostat (SB939)	3.15689E-07
Abexinostat (PCI-24781)	3.67361E-07
Ivaltiniostat (CG-200745)	4.13034E-07
JNJ-16241199	4.30184E-07
EVP-0334	4.8753E-07
CUDC-101	5.14518E-07
M344	9.07171E-07
Belinostat (PXD101)	1.41954E-06
CAY10603	1.67958E-06
CHR-3996	1.79217E-06
Resminostat (RAS2410)	2.20604E-06
Scriptaid	2.29618E-06
Rocilinostat (ACY-1215)	2.51576E-06
Pyroxamide	3.47248E-06
CY-190602	5.18846E-06
Oxamflatin	6.39766E-06
CXD101	6.60552E-06
Tacedinaline	5.14518E-07