Title: Osteoarthritis treatment via GLP-1 mediated gut-joint axis by targeting intestinal FXR signaling

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Abstract:
Whether gut-joint axis exists to regulate osteoarthritis is unknown. In two independent cohorts, we identified altered microbial bile acid metabolism with reduced glycoursodeoxycholic acid (GUDCA) in osteoarthritis. Suppressing farnesoid X receptor (FXR), the receptor of GUDCA, alleviated osteoarthritis through intestine-secreted glucagon-like peptide-1 (GLP-1) in mice. GLP-1 receptor blockade attenuated these effects, while GLP-1 receptor activation mitigated osteoarthritis. Osteoarthritis patients exhibited a lower relative abundance of *Clostridium bolteae*, which promoted formation of ursodeoxycholic acid (UDCA), a precursor of GUDCA. Treatment with *Clostridium bolteae* and FDA-approved UDCA alleviated osteoarthritis through the gut FXR-joint GLP-1 axis in mice. UDCA use was associated with lower risk of osteoarthritis-related joint replacement in humans. These findings suggest that orchestrating the gut microbiota-GUDCA-intestinal FXR-GLP-1-joint pathway offers a potential strategy for osteoarthritis treatment.

Main Text:

The gut microbiota has emerged as a critical regulator of host health by producing a diverse range of microbial metabolites to impact physiological and pathophysiological processes (*1-3*). These metabolites can affect innate and adaptive immune systems (*4, 5*), and regulate key metabolic pathways in the hosts (*6, 7*). While previous research demonstrated the functional roles of microbial metabolites in systematic immune and metabolic disorders, few studies explored whether metabolites play a role in conditions with localized effects, such as joint diseases.

Osteoarthritis (OA), a prevalent localized joint disease often referred to as "wear and tear" arthritis, is a common complex disorder with multiple risk factors including mechanical overloading (*8-11*). OA is a leading cause of activity limitations, disability, and chronic pain, affecting over 595 million people worldwide (*12, 13*). Nevertheless, no disease-modifying drugs for OA are currently available (*11, 14*). Recent studies identified gut microbiota dysbiosis in OA (*15, 16*) and linked several gut-derived microbial metabolites to the disease (*17-20*). However, these findings are correlational, and the existence of a functional gut-joint axis has never been established. Identifying such an axis and its mediators would provide insights into OA pathogenesis and facilitate the development of treatment approaches.

Bile acids (BAs), an important and abundant class of microbial metabolites, act as dynamic signaling molecules through the receptors such as farnesoid X receptor (FXR) and Takeda G protein-coupled receptor 5 (TGR5) (*21-23*). These receptors influence multiple organs beyond gut and play critical roles in improving metabolic dysfunction (*23-25*). Given that BA receptors are promising drug targets with U.S. FDA-approved ligands, understanding the importance of BA metabolism and signaling, as well as its potential relevance to OA, may offer better translational opportunities (*26*).

In targeted metabolomics studies encompassing two independent cohorts totaling 1868 individuals, we identified alterations in microbial BA metabolism with reduced glycoursodeoxycholic acid (GUDCA) in OA. Inhibition of intestinal FXR signaling, through either GUDCA supplementation or intestine-specific FXR knockout, ameliorated OA by enhancing intestine L-type cell-secreted glucagon-like peptide-1 (GLP-1). Metagenomics of 981 individuals and functional analyses in mice revealed that *Clostridium bolteae* (*C. bolteae*) participated in the production of ursodeoxycholic acid (UDCA), a precursor of GUDCA, which mitigated OA. UDCA, a U.S. FDA-approved FXR antagonist, attenuated OA through the intestinal FXR-GLP-1-joint axis in mice. Furthermore, UDCA use was associated with a lower risk of the clinically relevant endpoint of OA-related joint replacement in a cohort of 5972 individuals. Our findings reveal a connection between the gut microbiota and OA and suggest that targeting gut microbiota and the downstream BA-FXR-GLP-1 signaling pathway could be promising in OA treatment.

RESULTS

Identification of altered BA metabolism featuring GUDCA decrease associated with OA in two independent cohorts

To investigate the role of BAs in OA, we conducted targeted metabolomics of plasma BAs in a discovery cohort (n = 1714) and verified plasma BAs associated with OA in an independent validation cohort (n = 154) (Fig. 1A). Baseline characteristics of participants in each cohort are shown in tables S1 and S2. In the discovery cohort, OA patients (defined as having at least one knee with Kellgren-Lawrence [KL] grade ≥ 2 and self-reported pain, n = 421) were older (70.2 vs. 60.7 years, $P < 0.001$), had a larger proportion of female (80.8% vs. 50.0%, $P < 0.001$), and had a higher mean BMI (24.2 vs. 23.6 kg/m^2, $P = 0.016$) than controls (defined as participants with both knees free of pain and without KL grade ≥ 2, n = 1293). In the independent validation cohort, OA patients (n = 77), using the same definitions as the discovery cohort, had a higher BMI (24.8 vs. 23.8 kg/m^2, P = 0.015) compared with age- (± 1 year) and sex-matched controls without OA (n = 77).

In the discovery cohort, we compared the sums and ratios of various BA categories, as well as individual BAs, between OA patients and controls. The overall compositions of BAs in OA patients and controls are shown in Fig. 1B. We identified that UDCA species (the sum of UDCA, GUDCA, and tauroursodeoxycholic acid [TUDCA]) displayed a significant inverse association with OA prevalence (P = 0.010, Fig. 1C, table S3). This association was further supported by the positive relationships between the lower ratio of UDCA species to total BAs and UDCA species to chenodeoxycholic acid (CDCA) species (the sum of CDCA, glycochenodeoxycholic acid [GCDCA] and taurochenodeoxycholic acid [TCDCA]), as well as the higher ratio of primary bile acids (PBAs) to secondary bile acids (SBAs) and increased OA prevalence (Fig. 1C, table S3).

GUDCA and TUDCA are the primary forms of UDCA in enterohepatic circulation (*27*). Our analysis revealed that GUDCA (P = 0.004) and TUDCA (P = 0.006) exhibited the most statistically significant inverse associations with OA prevalence (Fig. 1D, table S3). Similar patterns were observed when correlating altered BA indexes with OA severity indicators, including KL grade, joint space narrowing, osteophytes, laterality (number of affected knee joints), and pain (Fig. 1E, table S4). These results indicate a positive relationship between reduced UDCA species (as evidenced by lower levels of GUDCA and TUDCA) and OA. Thereafter, we verified these associations in an independent cohort. The BA compositions of OA patients and controls are shown in fig. S1A. Consistent with findings from the discovery cohort, lower levels of GUDCA were associated with a higher prevalence of OA (fig. S1B, table S5), and more severe OA (fig. S1C, table S6). Together, the findings from these two independent cohorts suggest that decreased levels of UDCA species, specifically GUDCA, might play a pathogenic role in OA.

GUDCA mitigated OA progression by antagonizing intestinal FXR signaling

Next, we investigated the impact of GUDCA using two OA mouse models, including surgical destabilization of the medial meniscus (DMM) and age-related spontaneous models (*28*). In the DMM model, 12-week-old male C57BL/6J mice were treated with oral gavage of GUDCA or vehicle and assessed for OA progression eight weeks post DMM surgery (Fig. 2A). Histological analyses revealed that GUDCA treatment mitigated cartilage degradation (Fig. 2B) and significantly reduced Osteoarthritis Research Society International (OARSI) score ($P < 0.001$, Fig. 2C). Immunohistochemical analyses further showed increased expression of anabolic factor aggrecan (ACAN) and reduced expression

of a disintegrin and metalloproteinases with thrombospondin type 1 motif 5 (ADAMTS5), a catabolic factor (Fig. 2, D and E). Micro-computerized tomography (μCT) revealed that GUDCA treatment inhibited osteophyte formation following DMM surgery (Fig. 2, F and G). Similar results were observed in aged male C57BL/6J mice treated with daily GUDCA gavage, starting at 18 months of age and continuing for six months (fig. S2, A to G).

Given that UDCA species, as SBAs, are produced by gut bacteria and exert biological effects primarily through interactions with BA receptors TGR5 and FXR (*27, 29*), we next investigated whether the therapeutic effects of GUDCA on OA are mediated by these two receptors. After GUDCA administration, the BA profile in the ileum was significantly changed, with GUDCA and TUDCA increased in DMM model ($P = 0.007$ and $P = 0.006$, respectively, Fig. 2H). GUDCA and TUDCA also significantly increased in spontaneous OA model ($P = 0.019$ and $P = 0.002$, respectively, fig. S2H). To determine whether TGR5 contributes to the effects of GUDCA, we treated TGR5-overexpressed cells with GUDCA and measured cellular cyclic adenosine monophosphate (cAMP) activity, an indicator of TGR5 activation, using TGR5 agonist INT-777 as positive control (*30*). Our results suggested that GUDCA did not activate TGR5 (Fig. 2I). To test this notion further, we assessed OA progression in TGR5 knockout ($Tgr5^{-/-}$) mice and wide-type littermates treated with GUDCA or vehicle post DMM surgery (fig. S2I). Western blot analysis confirmed the efficiencies of TGR5 knockout (fig. S2, J and K). The protective effect of GUDCA on OA remained significant in $Tgr5^{-/-}$ mice (fig. S2, L to M). These results suggest that the effects of GUDCA are independent of TGR5.

Next, we assessed whether GUDCA influences FXR activity using the AlphaScreen assay (*31*). In the presence of CDCA, a naturally abundant FXR agonist in the intestine, we found that GUDCA acted as an FXR antagonist with an IC_{50} value of 67.5 μM (Fig. 2J). Since fibroblast growth factor 5 (FGF15, known as FGF19 in humans) is a direct target of FXR (*32*), we evaluated the effect of GUDCA on *Fgf15* transcription using ex vivo organoid cultures (Fig. 2K). GUDCA treatment significantly decreased *Fgf15* mRNA levels ($P < 0.001$, Fig. 2L), consistent with findings in the intestine of GUDCA-treated DMM mice (fig. S3, A to C). Additionally, we found elevated circulating FGF19 levels in OA patients ($n = 40$) compared with controls ($n = 40$), indicating the activation of intestinal FXR signaling in OA (Fig. 2M, table S7). Since FXR is also expressed in the liver (*33*), we examined whether GUDCA exerts any effects on hepatic FXR signaling but observed no significant alterations in the livers of GUDCA-treated mice (fig. S3D). These results suggest that the function of GUDCA on OA is mediated by intestinal FXR.

To further confirm the role of intestinal FXR in OA, we treated DMM-operated mice with Fexaramine, an intestine-restricted FXR agonist (*34*). Our results showed that pharmacological activation of FXR exacerbated OA progression (fig. S3, E to G). We also performed DMM surgery on intestine-specific FXR knockout ($Fxr^{\Delta IE}$) mice and the control floxed ($Fxr^{fl/fl}$) mice, followed by eight-week oral gavage of GUDCA or vehicle (Fig. 2N). Knockout efficiencies were confirmed by western blots (fig. S3, H and I). OA progression in $Fxr^{\Delta IE}$ mice was significantly attenuated compared with $Fxr^{fl/fl}$ mice ($P < 0.001$, Fig. 2, O and P). The anti-OA effects of GUDCA were abolished after intestine-specific FXR knockout (Fig. 2, O to R), demonstrating the role of intestinal FXR as a pathogenic factor

in OA development and underscoring its importance as the main target for the therapeutic effects of GUDCA.

Intestinal FXR knockout alleviated OA via increasing the population of GLP-1-expressing enteroendocrine cells and promoting GLP-1 secretion

Next, we explored how intestinal FXR affects joint pathology and the underlying mechanism linking the two organs (intestine and joint). GLP-1, released from gut L cells in response to the presence of glucose and other nutrients in the gut lumen, is essential for glucose homeostasis (*35*). Recent studies indicated that FXR inhibition in L cells stimulates GLP-1 production and secretion (*36, 37*), suggesting GLP-1 as a potential mediator linking the intestine and the joint. As anticipated, knockout of intestinal FXR significantly elevated serum GLP-1 levels ($P < 0.001$, Fig. 3A) and GUDCA treatment improved glucose tolerance ($P < 0.001$, fig. S3, J to L) in mice. Furthermore, intestinal organoids from both intestinal FXR knockout mice and GUDCA-treated mice revealed an increase of GLP-1-positive cells (GLP-1^+, also known as the L cells) (Fig. 3, B and C), and GUDCA treatment also increased the number of GLP-1^+ cells in the intestine of DMM mouse model (fig. S3, M and N). Notably, GUDCA treatment did not further increase the number of GLP-1^+ cells in intestinal FXR knockout mice (Fig. 3, B and C), indicating that the increase of GLP-1^+ cells by GUDCA is dependent on intestinal FXR.

Since L cells are differentiated from $Lgr5^+$ intestinal stem cells (ISCs) (*38*), their abundance may be influenced by ISC activity. Specifically, FXR agonism with GW4064 reduced the number of buddings in cultured organoids (fig. S4, A and B), suggesting impaired ISC stemness. Consistently, GW4064 reduced mRNA levels of ISC markers *Lgr5* and *Olfm4* (Fig. 3D). Furthermore, GW4064 treatment decreased the number of L cells (Fig. 3, E and F), as well as the expression of enteroendocrine cell (EEC) marker *Chga*, and L cell-specific marker *Gcg* (fig. S4C), supporting the hypothesis that FXR suppresses L cell number by impairing ISC stemness. Additionally, we observed a significant increase in $Olfm4^+Ki67^+$ ISCs (proliferative $Lgr5^+$ ISCs) in the intestines of $Fxr^{\Delta IE}$ (intestinal FXR knockout) mice compared to $Fxr^{fl/fl}$ controls (Fig. 3, G and H), demonstrating enhanced ISC proliferation after intestinal FXR deletion.

To further establish the role of FXR in ISCs, we generated $Fxr^{fl/fl}$; *Lgr5*-eGFP-IRES-creERT2 ($Fxr^{\Delta ISC}$) mice, allowing for specific knockout of FXR in ISCs through tamoxifen induction (a 4-day addition of 4-OH tamoxifen in vitro or a 3-day continuous administration of tamoxifen per day in vivo). ISCs were sorted by flow cytometry and the successful knockout of FXR in ISCs was confirmed (fig. S4, D and E). Supporting the findings from FXR agonist experiments, we observed that FXR deletion increased the number of GFP^{high} cells (representing $Lgr5^+$ cells) compared to *Lgr5*-eGFP-IRES-creERT2 mice (fig. S4F) and upregulated mRNA expression of *Lgr5* and *Olfm4* in the intestines of $Fxr^{\Delta ISC}$ mice compared to $Fxr^{fl/fl}$ controls (Fig. 3I), confirming that ISCs activity was enhanced by FXR deficiency. Notably, FXR knockout in $Lgr5^+$ ISCs promoted budding and was sufficient to increase the number of L cells in cultured organoid, as well as GLP-1 secretion (Fig. 3, J to L and fig. S4, G and H) and improved glucose tolerance (fig. S4, I to K) in vivo. GUDCA administration didn't further improve glucose tolerance (fig. S4, L to N). These results suggest that GUDCA modulates L cell abundance and their secretion

of GLP-1 through ISC proliferation. To determine whether FXR in ISCs regulates the differentiation of EECs, we investigated an established model in which EECs differentiation is induced by inhibition of both Wnt and Notch signaling (*39*). GW4064 did not further impact the differentiation of ISCs to L cells once the proliferation of ISC was completely depleted (fig. S4, O and P). These results suggest that FXR in ISCs regulates the number of L cells likely through ISC proliferation rather than differentiation.

Moreover, we observed lower levels of circulating GLP-1 levels in OA patients (n = 40) compared with controls (n = 40) (Fig. 3M, table S7). We also examined the levels of GLP-1 in both synovial fluids of knee and plasma among OA patients and found that GLP-1 is present in synovial fluid and its levels are highly correlated with the levels of plasma GLP-1 (Pearson correlation r = 0.873, $P < 0.001$) (fig. S5A). Consistent with previous studies that identified the intestine as the primary source of GLP-1 (*40*), in situ hybridization of *Gcg* mRNA (encoding proglucagon, the precursor to GLP-1) in mouse joint tissue confirmed the absence of GLP-1^+ cells in the joint, suggesting the intestine as the origin of GLP-1 (fig. S5B). These observations suggest that circulating GLP-1, which is primarily produced in the intestine, can reach the knee joints. Intestine-derived GLP-1 may exert protective effects in OA by activating the GLP-1 receptor (GLP-1R), which we found to be expressed in cartilage and synovium (fig. S5C). To investigate this further, we administered exendin 9-39 amide, a specific antagonist of GLP-1R, via intra-articular injection twice a week for eight weeks in $Fxr^{\Delta IE}$ mice and $Fxr^{fl/fl}$ mice post DMM surgery, to achieve local inhibition GLP-1-GLP-1R signaling in the joint (fig. S5D). We found that blocking this signaling in the joint cavity mitigated the ameliorating effects of intestinal FXR knockout on cartilage degradation (fig. S5, E to H). Similarly, intra-articular exendin 9-39 amide abolished the therapeutic effects of GUDCA on OA progression (Fig. 3, N to P). Conversely, intra-articular injections of the FDA-approved GLP-1R agonist liraglutide (Fig. 3Q) significantly attenuated cartilage degradation (P = 0.008, Fig. 3, R and S). To confirm the direct protective effects of GLP-1 on OA, we treated primary murine chondrocytes with liraglutide. We observed that liraglutide promotes chondrocyte anabolism and inhibits catabolism (fig. S5, I and J). In addition, using data from the UK IQVIA Medical Research Database (IMRD), we examined the association between GLP-1 receptor agonists (GLP-1RAs) use and the risk of knee replacement in patients with knee OA and diabetes mellitus (n = 3816, table S8). We observed a lower risk of knee replacement in GLP-1RAs new users compared with non-users, with a rate difference of -5.4 (95% confidence interval [CI]: -10.0 to -0.8) per 1000 person-years and a multivariable-adjusted hazard ratio (HR) of 0.73 (95% CI: 0.54 to 0.99) (table S9). Collectively, these findings suggest that intestinal FXR inhibition mitigates OA progression through circulating GLP-1, supporting the existence of an inter-organ signaling pathway, that is GUDCA-intestinal FXR-GLP-1-joint GLP-1R.

C. bolteae is a crucial gut microbe for UDCA species production in gut-joint communication

Since gut microbiota play a pivotal role in converting hepatically synthesized PBAs into secondary forms such as UDCA species, within the intestinal lumen (*41*), we investigated whether gut dysbiosis initiates the above-mentioned gut-joint axis and influences the OA progression. To achieve this, we performed shotgun metagenomic sequencing on

participants who provided qualified stool samples in the previous discovery cohort study, comprising 221 OA patients and 760 controls (Fig. 4A). Our analysis revealed significant differences in α-diversity (Shannon index) and β-diversity (Bray-Curtis distance) between OA patients and controls, at the gene level ($P < 0.001$ and $P = 0.020$, fig. S6, A and B) and species level ($P < 0.001$ and $P = 0.039$, fig. S6, C and D). The gut microbiota in both groups were dominated by *Prevotella spp*, *Bacteroides spp*, *Faecalibacterium prausnitzii*, *Agathobacter rectale*, and *Escherichia coli* (fig. S6E). Notably, OA patients had a lower relative abundance of *C. bolteae* and a higher relative abundance of *Clostridium sp001517625*, *Blautia obeum*, *CAG-279 sp00043795*, *Agathobaculum butyriciproducens*, *Lachnospira rogosae*, *Sutterella wadsworthensis*, and *Gemmiger formicilis* (Fig. 4B). Comprehensive statistics of the microbial species associations with OA are presented in table S10.

Next, we determined the association between altered microbial species and BA metabolism indexes within the same cohort (Fig. 4C and table S11). *C. bolteae* showed the strongest positive correlations with GUDCA, TUDCA, UDCA species, the ratio of UDCA species to CDCA species, and the ratio of UDCA species to total BAs. A lower relative abundance of *C. bolteae* linked to more severe OA (table S12), suggesting that a reduction in *C. bolteae* may contribute to OA progression. Furthermore, after categorizing the discovery cohort into high and low GUDCA groups, we observed a significantly higher relative abundance of *C. bolteae* in the high GUDCA group ($P < 0.001$, Fig. 4D). These data implicate *C. bolteae* in BA metabolism, especially UDCA species, in OA patients. Furthermore, the phylogenetic tree analysis showed that *C. bolteae* shares 98% identity with *Ruminococcus gnavus JCM 6515 AB910745*, a known UDCA producer possessing 7α- and 7β-hydroxysteroid dehydrogenases (HSDHs), indicating functional similarity (fig. S6F).

Next, we examined the effect of *C. bolteae* colonization on BA metabolism and OA progression in vivo. C57BL/6J mice were colonized by *C. bolteae* through oral gavage, and OA progression was assessed eight weeks after DMM surgery (Fig. 4E). *C. bolteae* administration significantly altered BA profile (fig. S6G), characterized by increased absolute UDCA levels ($P = 0.018$, Fig. 4F) and a higher proportion of UDCA in total bile acids ($P = 0.026$, Fig. 4G). Additionally, the proportion of CDCA in total bile acids was significantly decreased ($P = 0.006$, Fig. 4H), while absolute CDCA levels tended to be lower in the *C. bolteae* administration group (Fig. 4I). These results suggested that *C. bolteae* contributes to the observed change in BA metabolism in OA. Furthermore, the *C. bolteae* colonization demonstrated greater intestinal FXR antagonism, evidenced by lower expression of *Fgf15* mRNA, along with increased number of intestine L cell and elevated serum GLP-1 levels (Fig. 4, J to M), and, importantly, ameliorated OA with reduced cartilage degradation (Fig. 4, N to Q). Together, these findings support the notion that the gut symbiont *C. bolteae* is a key microbial source for UDCA species production during OA progression and drives the GLP-1-mediated gut-joint axis via altering BA metabolism.

UDCA, an FDA-approved FXR antagonist, alleviated OA progression through the gut-joint axis

UDCA, a SBA and precursor of GUDCA, is approved by the U.S. FDA for the treatment of primary biliary cholangitis and is known for its ability to antagonize intestinal FXR, either directly or through its conversion into the more potent FXR antagonist GUDCA in vivo (*24, 42*). Its favorable safety profile with long-term use (*43*) makes it a potential candidate for treating OA. To determine the therapeutic efficacy of using UDCA to treat OA, we performed oral gavage of UDCA or vehicle to male C57BL/6J mice of the DMM model (Fig. 5A). UDCA treatment decreased cartilage degradation (Fig. 5, B to E) and osteophyte formation (Fig. 5, F and G), altered BA profiles in the ileum with an increase in UDCA species (Fig. 5H), and also increased L cells (fig. S7, A to C), reduced intestinal FGF15 expression (fig. S7, D and E). Similar improvements were observed in aged male C57BL/6J mice treated with UDCA for six months (fig. S7, F to M). In addition, we treated $Fxr^{\Delta IE}$ and $Fxr^{fl/fl}$ mice with UDCA or vehicle after DMM surgery and assessed OA progression (fig. S7N). UDCA treatment reduced cartilage degradation in $Fxr^{fl/fl}$ but not $Fxr^{\Delta IE}$ mice (fig. S7, O to R). These results establish that UDCA exerts its effects on OA through FXR-mediated gut-joint axis.

Lastly, we conducted an electronic healthcare records-based cohort study using data from the IMRD, incorporating The Health Improvement Network (THIN), a Cegedim database, to interrogate the effect of UDCA use in humans (Fig. 5I). We identified 1338 UDCA new users (mean age 61.6, 88.6% female) who had no prior use of UDCA before entering the study, and 4634 propensity score-matched non-users (mean age 62.2, 89.2% female). Baseline characteristics between the matched cohorts were well-balanced, with all standardized differences < 0.1 (table S13). As shown in Fig. 5J and table S14, UDCA new users had a lower risk of OA-related knee replacement than non-users, with a rate difference of -1.4 (95% CI: -2.2 to -0.5) per 1000 person-years and a HR of 0.46 (95% CI: 0.23 to 0.91). Sensitivity analyses excluding patients who underwent knee replacement within three or six months after the index date confirmed these results (table S14). Collectively, these experimental findings from mouse models and observational data in humans suggest that the use of UDCA, an FDA-approved drug, can ameliorate OA progression through the intestinal FXR-GLP-1-joint axis, and also identify intestinal FXR as a promising therapeutic target in OA treatment.

DISCUSSION

Gut microbiota dysbiosis is implicated in individuals with OA (*44, 45*). Fecal transplantation and microbiota depletion have been shown to impact OA progression (*17, 46*). These prior studies led to the proposal of a gut-joint axis in OA (*47*). However, the biological underpinnings of such a proposed axis, including the microbial taxa, gut cell type, signaling pathway, and its potential as a therapeutic target, were unknown. Metabolites, the functional mediators of gut microbiota, traverse the intestinal barrier to enter the systemic circulation (*48*). Metabolic perturbations are reported in OA (*20, 49*). However, it was unclear whether metabolites mediate gut-joint interaction in OA.

This study yielded several key findings: (i) we elucidated a pathway through which gut microbial metabolites influence OA progression and uncovered the existence of a functional and targetable gut-joint axis; (ii) we identified intestinal FXR as a viable therapeutic target to mitigate OA by increasing intestinal L cells-derived GLP-1; (iii) we

found intestinal FXR plays an important role in maintaining intestinal homeostasis and modulate L cell numbers through ISC proliferation; (iv) we discovered *C. bolteae* as a bacterial modulator of this gut-joint axis through its production of UDCA species; and (v) we demonstrated that UDCA, an FDA-approved drug, alleviates OA through the gut-joint axis in mice, and revealed that UDCA use is associated with a reduced risk of OA-related joint replacement in humans. A schematic illustration depicting the gut microbiota-GUDCA-intestinal FXR-GLP-1-joint axis in OA progression is presented in Fig. 5K. Since FXR is a known druggable target (*24*) and oral medications have high local bioavailability in the intestine (*50*), these findings provide the foundation for developing oral disease-modifying OA drugs.

A recent double-blind, randomized, placebo-controlled trial (RCT) demonstrated that among individuals with obesity and knee OA experiencing moderate-to-severe pain, weekly treatment with the injectable semaglutide, a novel GLP-1RA, resulted in significantly greater reductions in knee pain than placebo (*51*). It should be noted that this study did not evaluate impact of semaglutide on cartilage degeneration (*51*). However, previous preclinical studies have shown that GLP-1RAs have anti-cartilage degrading effects in OA (*52-54*). Furthermore, our cohort study in patients with knee OA and diabetes mellitus revealed that GLP-1RAs treatment was associated with a lower risk of knee replacement. Collectively, these findings suggest that GLP-1RAs could serve as potential therapeutic agents for OA.

Various studies have proven that intestinal FXR-GLP-1 exerts a pivotal role in improving metabolic diseases (*55-58*), most of which attribute the increased production of GLP-1 to FXR-mediated *Gcg* transcription in L cell (*36*). Here, we confirmed the effects of GUDCA as a FXR antagonist on inducing intestinal GLP-1 production and found that these effects are dependent on ISC proliferation. The intestinal epithelium is a highly regenerative tissue that promotes selective nutrient absorption while acting as a barrier to harmful lumen contents (*59*). Notably, $Lgr5^+$ ISCs at the base of the crypt sense changes in the gut microbiota and their metabolites, and subsequently differentiate into various lineages during the renewal process (*60, 61*), including L cells. Our study reveals that FXR activation negatively impacts ISC stemness. Wnt signaling is known to maintain the stemness of ISCs, and consistent with our findings, antagonism of intestinal FXR has been shown to facilitate Wnt signaling and enhance ISC self-renewal, ameliorating gastrointestinal damage caused by aspirin (*62*). Inhibition of Wnt signaling dampens ISCs proliferation and combined with Notch inhibition induces the differentiation of L cells (*39*). Here, we provide evidence that intestinal FXR, especially FXR in ISCs, plays an important role in maintaining ISC and L cell number, and under defective ISC proliferation and active L cell differentiation, activation of FXR cannot result in a reduced number of L cells.

The discovery that bacteria *C. bolteae* can promote UDCA formation offers insights into the role of gut microbiota in BA metabolism, expanding the repertoire of microbial species involved in this crucial process. UDCA is produced from CDCA through a two-step epimerization process involving 7α-HSDH and 7β-HSDH (*63*). Genes encoding 7α-HSDH are found in a range of bacteria, including species from the *Clostridium* and *Bacteroides* genera, whereas genes encoding 7β-HSDH have been identified in *Clostridium absonum*,

Collinsella aerofaciens, *Ruminococcus gnavus*, and *Ruminococcus torques (63, 64)*. The evolutionary relationship between *Ruminococcus gnavus JCM 6515 AB910745* and *C. bolteae* suggests shared genetic and functional characteristics that enable both to perform similar BA conversion. Further investigations into the molecular mechanisms underlying this shared capability could uncover conserved pathways or enzymes involved in BA metabolism.

Repurposing existing drugs for OA treatment is an attractive option due to lower costs and shorter timelines for drug development (*65*). UDCA, which is approved by the U.S. FDA for treating specific liver diseases (*66*), is available as an oral medication. Our study found that UDCA effectively alleviates OA progression in both surgical and spontaneous OA mouse models acting through gut-joint axis we identified. Analysis of a large UK medical database further revealed that individuals initiated on UDCA treatment had a reduced risk of subsequent knee replacement for OA. This nominates UDCA as a potential therapy for OA, a chronic condition, with the potential for long-term use based on its favorable tolerability and safety profile (*67*). Randomized clinical trials are warranted to establish UDCA as a disease-modifying drug to benefit OA patients.

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Supplementary Materials

Materials and Methods

Figs. S1 to S7

Tables S1 to S16
MDAR Reproducibility Checklist

Fig. 1. Profiling of BA metabolism in OA based on a natural population. (**A**) Workflow of the targeted metabolomics screening of plasma BAs. BMI, body mass index. (**B**) BA composition of OA patients and controls. (**C**) Sums and ratios of different BAs categories of OA patients and controls. Error bars depict the 95% CI of odds ratio (OR). UDCAs/Total BAs, the ratio of UDCA species to total BAs. UDCAs/CDCAs, the ratio of UDCA species to CDCA species. (**D**) Associations between levels of individual BAs and OA. The right whisker extends from the hinge to the largest value no further than 1.5 * inter-quartile range from the hinge, and the left whisker extends from the hinge to the smallest value at most 1.5 * inter-quartile range of the hinge (left panel). Error bars depict the 95% CI of ORs (middle panel). (**E**) Associations between altered BAs indexes and OA severity indicators. *P* values were calculated using logistic regression (C, D) and proportional odds logistic regression (E). *, $P < 0.05$; **, $P < 0.01$.

Fig. 2. GUDCA mitigated OA progression by antagonizing intestinal FXR signaling. (**A**) Schematic of the time course. 12-week-old male C57BL/6J mice with gavage of GUDCA were euthanized eight weeks after DMM surgery. (**B** to **G**) Representative images of Safranin O/Fast green stained sections, scale bar = 200 μm (**B**), the severity of cartilage degeneration analyzed using the OARSI score (**C**), representative images of immunohistochemical staining of ACAN and ADAMTS5, scale bar = 100 μm (**D**), quantification of positive staining of ACAN and ADAMTS5 (**E**), representative three-dimensional μCT images showing osteophytes (**F**), and quantification of osteophyte number and size (**G**) under indicated treatments. n = 7 mice/group. (**H**) BA profile in the ileum under indicated treatments. n = 7 mice/group. (**I**) cAMP assay of negative control (DMSO), positive control (TGR5 agonist, 10 μM INT-777), and GUDCA. n = 4 replicates/treatment. (**J**) AlphaScreen assay to assess whether GUDCA is a FXR antagonist in the presence of CDCA. n = 3 replicates/treatment. **(K)** Schematic of the time course. Intestine organoid was cultured with or without GUDCA in Matrigel for 48h. **(L)** Relative mRNA abundance of intestinal *Fxr* and *Fgf15* under indicated treatments. n = 5 mice/group. (**M**) Plasma FGF19 levels in OA patients and controls. n = 40 participants/group. The upper whisker extends from the hinge to the largest value no further than 1.5 * inter-quartile range from the hinge, and the lower whisker extends from the hinge to the smallest value at most 1.5 * inter-quartile range of the hinge. (**N**) Schematic of the time course. DMM surgery was performed in 12-week-old male $Fxr^{fl/fl}$ and $Fxr^{\Delta IE}$ C57BL/6J mice. GUDCA was administered daily after DMM surgery. (**O** to **R**) Representative images of Safranin O/Fast green stained sections, scale bar = 200 μm (**O**), the severity of cartilage degeneration analyzed using the OARSI score (**P**), representative images of immunohistochemical staining of ACAN and ADAMTS5, scale bar = 100 μm (**Q**), and quantification of positive staining of ACAN and ADAMTS5 (**R**) under indicated treatments and genotype. n = 7 mice/group. *P* values were determined by one-way ANOVA with Tukey's correction (C, E, G, H, I, P, R), two-tailed Student's t-test (L), or conditional logistic regression (M). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; ns, not significant. Data are shown as mean ± SEM unless specified.

Fig. 3. Intestinal FXR knockout alleviated OA via increasing the population of GLP-1-expressing enteroendocrine cells and promoting GLP-1 secretion. (**A**) Serum GLP-1 levels of $Fxr^{fl/fl}$ and $Fxr^{\Delta IE}$ mice treated with vehicle or GUDCA for eight weeks after DMM surgery. n = 7 mice/group. (**B** and **C**) Representative images (**B**) and quantification (**C**) of immunofluorescent staining of GLP-1 positive cells in intestinal crypts isolated under indicated treatments and genotypes. Scale bar = 50 μm. n = 4 biological replicates/group. (**D**) Relative mRNA level of intestinal stem cell (ISC) marker *Lgr5* and *Olfm4* of mouse organoid treated with vehicle or GW4064 for 72 hours. n = 4 biological replicates/group. (**E** and **F**) Representative images (**E**) and quantification (**F**) of immunofluorescent staining of GLP-1 positive cells in intestinal crypts isolated from C57BL/6 mice and treated with vehicle or GW4064 in vitro. Scale bar = 50 μm. n = 4 biological replicates/group. (**G** and **H**) Representative images of immunofluorescent staining (**G**) and quantification (**H**) of Olfm4 and Ki67 in intestinal slice from $Fxr^{fl/fl}$ or $Fxr^{\Delta IE}$ mice. Scale bar = 50 μm. n = 5 mice/group. **(I)** Relative mRNA level of ISC marker *Lgr5* and *Olfm4* of intestine from $Fxr^{fl/fl}$ or $Fxr^{\Delta ISC}$ mice after 3-day induction with 100 mg/kg tamoxifen. n = 6 or 7 mice/group. **(J)** Serum GLP-1 level of $Fxr^{fl/fl}$ or $Fxr^{\Delta ISC}$ mice after 3-day induction with 100 mg/kg tamoxifen. n = 9 mice/group. **(K and L)** Representative images (**K**) and quantification (**L**) of immunofluorescent staining of GLP-1 positive cells in intestinal crypts isolated from $Fxr^{fl/fl}$ or $Fxr^{\Delta ISC}$ mice treated with 4-OH tamoxifen for 4 days. Scale bar = 50 μm. n = 4 biological replicates/group. (**M**) Plasma GLP-1 levels in OA patients and controls. n = 40 participants/group. The upper whisker extends from the hinge to the largest value no further than 1.5 * inter-quartile range from the hinge, and the lower whisker extends from the hinge to the smallest value at most 1.5 * inter-quartile range of the hinge. (**N**) Schematic of the time course. DMM surgery was performed in 12-week-old male C57BL/6J mice. GUDCA was administered daily, and intra-articular exendin 9-39 amide injection was performed twice a week, three days after DMM surgery. (**O** and **P**) Representative images of Safranin O/Fast green stained sections (**O**) and the severity of cartilage degeneration analyzed using the OARSI score (**P**) of knee joints under indicated treatments. Scale bar = 200 μm. n = 8 mice/group. (**Q**) Schematic of the time course. DMM surgery was performed in 12-week-old male C57BL/6J mice. Intra-articular liraglutide injection was administered twice a week. (**R** and **S**) Representative images of Safranin O/Fast green stained sections (**R**) and the severity of cartilage degeneration analyzed using the OARSI score (**S**) of knee joints under indicated treatments. Scale bar = 200 μm. n = 8 mice/group. *P* values were determined by one-way ANOVA with Tukey's correction (A, C, P, S), two-tailed Student's t-test (D, F, H, I, J, L) or conditional logistic regression (M). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; ns, not significant. Data are shown as mean ± SEM unless specified.

Fig. 4. *C. bolteae* is a crucial gut microbe for UDCA species production in gut-joint communication (**A**) Workflow of the shotgun metagenomic screening of microbiome. (**B**) Volcano plot of microbial species associated with OA. Significantly different taxa ($Q < 0.2$) are colored according to phylum. (**C**) Correlations between differential microbial species and differential BA indexes. (**D**) Relative abundance of *C. bolteae* in different GUDCA categories. The right whisker extends from the hinge to the largest value no further than 1.5 * inter-quartile range from the hinge, and the left whisker extends from the hinge to the smallest value at most 1.5 * inter-quartile range of the hinge. (**E**) Schematic of the time

course. After three days of treatment with an antibiotic cocktail to clear gut microbiota, 12-week-old male C57BL/6J mice were subjected to DMM surgery and then administered *C. bolteae* or saline by gavage every other day. (**F** to **I**) Absolute UDCA concentration (**F**), UDCA in total bile acids (%) (**G**), CDCA in total bile acids (%) (**H**) and absolute CDCA concentration (**I**) under indicated treatments. n = 7 mice/group. (**J**) Relative mRNA abundance of intestinal *Fxr* and *Fgf15* mRNAs under indicated treatments. (**K** and **L**) Representative images (**K**) and quantification (**L**) of immunofluorescence staining of GLP-1 positive cells in mice intestine under indicated treatments. Scale bar = 50 μm, n = 7 mice/group. (**M**) GLP-1 levels in serum of saline and *C. bolteae.* group after eight weeks of colonization**.** n = 7 mice/group. (**N** to **Q**) Representative images of Safranin O/Fast green stained sections, scale bar = 200 μm (**N**), the severity of cartilage degeneration analyzed using the OARSI score (**O**), representative images of immunohistochemical staining of ACAN and ADAMTS5, scale bar = 100 μm (**P**), and quantification of positive staining for ACAN and ADAMTS5 (**Q**) of knee joints under indicated treatments. n = 7 mice/group. *P* values were determined by the Microbiome Multivariable Associations with Linear Models (B, D), partial Spearman's rank correlation test (C), two-tailed Student's t-test (J, L, M, O, Q), Mann Whitney test (F and G) and Welch's t test (H and I). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; ns, not significant. Data are shown as mean ± SEM.

Fig. 5. UDCA alleviated OA progression through the gut-joint axis in mice and was associated with a reduced risk of OA-associated knee replacement in human. (**A**) Schematic of the time course. 12-week-old male C57BL/6J mice with gavage of UDCA were euthanized eight weeks after DMM surgery. (**B** to **G**) Representative images of and Safranin O/Fast green stained sections of knee joints, scale bar = 200 μm (**B**), the severity of cartilage degeneration analyzed using the OARSI score (**C**) representative images of immunohistochemical staining of ACAN and ADAMTS5, scale bar = 100 μm (**D**), and quantification of positive staining for ACAN and ADAMTS5 (**E**) of knee joints under indicated treatments, representative three-dimensional μCT images showing osteophytes (**F**), and quantification of osteophyte number and size (**G**) under indicated treatments. n = 7 mice/group. (**H**) BA profile in the ileum under indicated treatments. n = 7 mice/group. (**I**) Study design of the electronic healthcare records-based cohort study for the relation of UDCA to the risk of OA-related knee replacement. (**J**) Risk of OA-related knee replacement between UDCA new users and non-users among patients with primary biliary cholangitis. Cox-proportional hazard models were used to estimate the HR and its CI. (**K**) A schematic illustration that depicts the GUDCA/UDCA-intestinal FXR-GLP-1-joint axis in OA progression. HR, hazard ratio; CI, confidence interval. *P* values were determined by one-way ANOVA with Tukey's correction (C, E, G, H), *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; ns, not significant. Data are shown as mean ± SEM.