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Primary cilia disassembly down regulates mechanosensitive hedgehog signalling: a feedback mechanism controlling ADAMTS-5 expression in chondrocytes

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SUMMARY

Objective: Hedgehog signalling is mediated by the primary cilium and promotes cartilage degeneration in osteoarthritis. Primary cilia are influenced by pathological stimuli and cilia length and prevalence are increased in osteoarthritic cartilage. This study aims to investigate the relationship between mechanical loading, hedgehog signalling and cilia disassembly in articular chondrocytes.

Methods: Primary bovine articular chondrocytes were subjected to cyclic tensile strain (CTS; 0.33 Hz, 10% or 20% strain). Hedgehog pathway activation (Ptcp1, Gli1) and A Disintegrin And Metalloproteinase with Thrombospondin Motifs 5 (ADAMTS-5) expression were assessed by real-time PCR. A chondrocyte cell line generated from the Tg737OPPK mouse was used to investigate the role of the cilium in this response. Cilia length and prevalence were quantified by immunocytochemistry and confocal microscopy.

Results: Mechanical strain upregulates Indian hedgehog expression and activates hedgehog signalling. Ptcp1, Gli1 and ADAMTS-5 expression were increased following 10% CTS, but not 20% CTS. Pathway activation requires a functioning primary cilium and is not observed in Tg737OPPK cells lacking cilia. Mechanical loading significantly reduced cilium length such that cilia became progressively shorter with increasing strain magnitude. Inhibition of histone deacetylase 6 (HDAC6), a tubulin deacetylase, prevented cilia disassembly and restored mechanosensitive hedgehog signalling and ADAMTS-5 expression at 20% CTS.

Conclusions: This study demonstrates for the first time that mechanical loading activates primary cilium-mediated hedgehog signalling and ADAMTS-5 expression in adult articular chondrocytes, but that this response is lost at high strains due to HDAC6-mediated cilia disassembly. The study provides new mechanistic insight into the role of primary cilia and mechanical loading in articular cartilage.

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Introduction

The primary cilium is a singular, immotile organelle elaborated by the majority of cells during interphase. Structurally the cilium comprises a centriole-derived basal body from which projects an axoneme composed of acetylated microtubules. The axoneme is sheathed in a specialised ciliary membrane and forms a compartment which functions as a hub for numerous signalling pathways such as Wnt, PDGF- and hedgehog signalling. The majority of chondrocytes exhibit a primary cilium and the size of this signalling compartment is influenced by pathological stimuli, including mechanical and inflammatory cytokines. This may be responsible for the increase in primary cilia length and prevalence observed in osteoarthritic cartilage. Previous studies have established that the chondrocyte primary cilium is required for mechanotransduction and the response to osmotic loading, with genetic inhibition of primary cilium assembly leading to the development of osteoarthritic-like cartilage.

The primary cilium is assembled and maintained by intr-flagellar transport (IFT), a microtubule based motility present in the axoneme. IFT conveys axonemal precursors and signalling proteins along the length of the cilium towards the distal tip and returns them to the basal body. Genetic mutation of IFT proteins and/or primary cilium assembly leading to the development of osteoarthritic-like cartilage.

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the molecular motors that drive this process disrupt cilia structure
and function and are responsible for a group of related disorders
termed ciliopathies13. Given their shared requirement for IFT, a
correlation is often observed between the length of the cilium and
its function15–17. Indeed, Tran et al. reported that a 27% reduction in
retrograde transport is sufficient to disrupt both cilia morphology
and hedgehog signalling18. Alterations in cilium length have been
reported in several pathological conditions including osteoarthritis
(OA)18,19.

Hedgehog signalling regulates the activity of a family of bi-
functional transcription factors called Gli proteins20,21. A func-
tioning primary cilium is essential for this pathway. In the absence
of hedgehog ligands, Gli proteins are modified within the ciliary
compartment promoting formation of transcriptional repressors22.
Binding of the hedgehog ligand to its receptor, Patched (Ptc1),
releases the repression of a second transmembrane protein
Smoothened (Smo)23. Upon activation, Smo traffics into the cilium
where it promotes the formation of Gli activators and the expres-
sion of hedgehog target genes24,25.

Hedgehog signalling is aberrantly activated in osteoarthritic
cartilage where it promotes chondrocyte hypertrophy and the
expression of catabolic enzymes such as matrix metalloproteinase
13 (MMP-13) and A Disintegrin And Metalloproteinase with
Thrombospondin Motifs 5 (ADAMTS-5) leading to cartilage
degeneration26,27. The level of pathway activation correlates with
the severity of the OA phenotype. Inhibiting hedgehog signalling
attenuates disease severity in OA models highlighting the potential
of this pathway as a therapeutic target21,22.

Indian hedgehog (Ihh) is the major hedgehog ligand expressed
in cartilage and regulates chondrocyte proliferation and differen-
tiation during development28. Ihh expression is increased in early
cartilage lesions24 and in osteoarthritic cartilage in association with
chondrocyte hypertrophy24,29. However the mechanisms responsible
for this are unknown. While several reviews in vitro and in vivo studies have shown that Ihh expression is regulated by mechanical stimuli
during skeletal development25,26; the mechanosensitivity of this
pathway has not been examined in healthy adult chondrocytes.

This study tests the hypothesis that hedgehog signalling is
mechanosensitive in adult articular chondrocytes and examines the
role of the primary cilium in this response. We show that me-
chanical strain promotes Ihh expression and hedgehog pathway
activation. However, while mechanosensitive Ihh expression was
found to be independent of cilial function, pathway activation did
not occur at high magnitude strain as a result of primary cilia
disassembly. We identify a role for the tubulin deacetylase histone
deacetylase 6 (HDAC6) in mechanosensitive cilia disassembly and
show that inhibition of this enzyme prevents disassembly and re-
stores mechanosensitive hedgehog signalling and ADAMTS-5
expression. Thus we reveal the chondrocyte primary cilium struc-
ture–function relationship and how this is modulated by me-
chanical loading. We propose that cilia disassembly is
chondroprotective, reducing enzymatic cartilage degradation in
response to high magnitude strain.

Methods
Cell isolation and culture

Forefeet from freshly slaughtered adult bovine steers (aged 18–24 months) were obtained from a local abattoir and primary
articular chondrocytes isolated by enzymatic digestion as previ-
ously described10. Chondrocytes were cultured in Dulbecco's
Modified Eagles Medium (DMEM) supplemented with 10% (v/v)
fetal calf serum (FCS), 1.9 mM t-glutamine, 96 U/ml penicillin,
96 μg/ml streptomycin, 19 mM 2-[4-(2-hydroxyethyl)piperazin-1-
yl]ethanesulfonic acid (HEPES) buffer, and 0.74 mM L-ascorbic
acid (Sigma Aldrich). Cells were seeded onto collagen 1 coated
Bioflex® membranes and cultured at 37°C, 5% CO2 for 6 days
without passage.

Conditionally immortalised wild-type (WT) and Tg737060K
(ORPK) mouse chondrocyte cell lines were generated as previously
reported10. Chondrocytes were maintained in DMEM supple-
mented with 10% (v/v) FCS, 88 U/ml penicillin, 90 μg/ml strepto-
mycin and 2.5 mM L-glutamine. Immortalised cells were
maintained under permissive conditions at 33°C, 5% CO2 in
the presence of 10 mM IFN-γ. For experiments, cells were seeded onto
collagen I coated Bioflex® membranes and cultured for 24 h
then washed and cultured under non-permissive conditions at 37°C
without IFN-γ for 4 days. WT chondrocytes express Acan and Col2a
and have been shown to exhibit mechanosensitive gene expression
and proteoglycan production in response to dynamic compression21.

Application of mechanical strain

Uniform, equibiaxial cyclic tensile strain (CTS) was applied to
chondrocytes using the Flexcell FX4000-T system with circular
loading posts with a diameter of 25 mm. To ensure only chon-
drocytes that experience a uniform strain field were studied; the
cells at the periphery of each well were removed by aspiration 3
days prior to loading. Cells were subjected to 10% or 20% CTS for 1 h
at 0.33 Hz under serum-free conditions. For unstrained controls,
chondrocytes were cultured in an identical manner but without the
application of strain.

Immunofluorescence and confocal microscopy

Chondrocytes were fixed with 4% paraformaldehyde (PFA) for
10 min followed by detergent permeabilisation with 0.25% Triton
X-100 and blocking for 1 h with 5% goat serum. The Bioflex™
membrane was excised and cells were incubated with primary
antibodies at 4°C overnight in a humidified chamber. Mouse
monoclonal anti-acetylated tubulin, clone 611B-1 (1:2000; Sigma
Aldrich) and rabbit polyclonal anti-Arl13b (1:2000; ProteinTech)
were used for the detection of the ciliary axoneme. Rabbit pol-
yclonal anti-HDAC6 (1:250, Abcam) was used for the detection of
HDAC6 in the cilia. Following repeated washing in PBS, cells were
incubated with appropriate Alexa488 and Alexa594 conjugated
secondary antibodies (Molecular Probes) for 1 h at room tempera-
ture and nuclei were detected with 1 μg/ml 4',6-diamidino-2-
phenylindole (DAPI). Following repeated washing, membranes
were mounted with Prolong gold reagent (Molecular Probes).

Samples were imaged using a Leica TCS SP2 confocal microscope
with a 63×, 1.3-NA lens (Leica Microsystems, Wetzlar, Germany).
Primary cilia length and prevalence were measured as described
previously10. In summary, confocal Z-stacks were obtained
throughout the entire cellular profile with a Z-step size of 0.5 μm
and an image format of 1024 × 1024 pixels. This produced an xy
pixel size of 0.232 μm × 0.232 μm. Z-stacks were reconstructed
and an xy maximum intensity projection generated for measure-
ment of cilia prevalence and length using Image J software.

RNA extraction, cDNA synthesis and real-time PCR

Total RNA was isolated from individual wells immediately after
loading using an RNeasy Kit (Qiagen) according to the manufac-
turer’s protocol. RNA integrity was confirmed by gel electro-
phoresis. For each sample 1 μg total RNA was converted to cDNA using
the Quantitect reverse transcription kit (Qiagen) according to
the manufacturer’s instructions. This kit incorporates a DNase step for
removal of genomic DNA prior to cDNA synthesis. Both RNA and
cDNA were quantified using the Nanodrop ND–1000 spectropho-
tometer (LabTech, East Sussex, UK).

For real-time PCR, reactions were performed in 10 μl volumes
containing 1 μl cDNA (diluted 1:2), 5 μl KAPA SYBR® FAST Universal
2 × qPCR Master Mix (KAPA Biosystems) containing SYBER-green
dye, 0.2 μl ROX reference dye and 1 μl optimised primer pairs
(listed in Table 1). An annealing temperature of 60 °C was used for
PCR reactions and fluorescence data was collected using the
MX3000P qPCR instrument (Stratagene). Samples were run in
triplicate to minimise pipetting errors. Data was analysed using the
relative standard curve method and target genes were normalised
to GAPDH28,29, the expression of which was not signi-
cantly altered by the experimental conditions used in this study (see Fig. S1).

Data analyses

All statistical analyses were conducted using Graph Pad Prism
6.01 (GraphPad, La Jolla, CA, USA). Data is presented as mean values
with 95% confidence intervals (CI). For primary articular chon-
drocytes an experiment is defined as follows: cells were isolated
from a pool of cartilage taken from a minimum of three donors to ensure
a heterogeneous population of cells for each experiment. For
work using cell lines an experiment is defined as a single passage of
the cells. Experiments were performed in duplicate unless other-
wise stated. For real-time PCR, each analysis comprised 6–10 re-
peats per condition (defined as n through the manuscript) where
one replicate was a single well of a tissue culture plate. For primary
cilia length, prevalence and Ki-67 data, a replicate or
one replicate was a single well of a tissue culture plate. For primary
chondrocytes in a strain dependent manner

Results

Mechanical strain upregulates Ihh and activates hedgehog signalling
in primary chondrocytes in a strain dependent manner

In primary bovine articular chondrocytes, the expression of Ihh
was increased in response to both 10% and 20% CTS relative to the
unstrained (noCTS) control [10%: P = 0.026; 20%: P = 0.043;
Fig. 1(A)]. Hedgehog pathway activation was assessed by moni-
toring changes in the expression of Gli1 and Ptch1. The expression
of these genes was increased following 10% CTS [Gli1: P = 0.007;
Ptch1: P = 0.046; Fig. 1(B) and (C)] indicative of pathway activation.
Despite the induction of Ihh, pathway activation was not observed in response to 20% CTS [Gli1: P > 0.999; Ptch1: P > 0.999; Fig. 1(B)
and (C)].

The primary cilium is required for mechanosensitive hedgehog
signalling in chondrocytes

An immortalised chondrocyte cell line generated from the
Tg737ORPK mouse was used to investigate the role of the primary
cilium in the mechanosignalulation of hedgehog signalling. In ORPK
chondrocytes primary cilia are disrupted due to a hypomorphic
mutation in the Ift88 gene, the protein product of which is essential for
IFT and thus cilia assembly and maintenance91,10. In WT cultures
80 ± 13% of cells elaborated a primary cilium with a mean length of
3.25 ± 0.3 μm [Fig. 2(A)–(C)]. By contrast, only 13 ± % of cells
exhibited a cilium in ORPK chondrocyte cultures [Fig. 2(B)]. Further-
more, the cilia elaborated by ORPK cells were shorter than those in WT cells with a mean length of 0.18 ± 0.3 μm [P < 0.001; Fig. 2(C)].

Chondrocytes were treated for 24 h with 1 μg/ml recombinant
Indian hedgehog ligand (r-Ihh) to establish the role of the cilium in
ligand-mediated hedgehog signalling [Fig. 2(D) and (E)]. In WT
chondrocytes, the expression of Gli1 and Ptch1 was increased in
response to r-Ihh treatment confirming pathway activation [Gli1: P < 0.001; Ptch1: P = 0.020; Fig. 2(D) and (E)]. In ORPK chondrocytes
the expression of these genes was not altered by r-Ihh treatment
[Fig. 2(C)] indicating the cilium/IFT is required for this response. To determine the role of the cilium in mechanosensitive hedgehog signalling, WT and ORPK chondrocytes were subjected to 10% CTS for 1 h
[Fig. 2(F)–(H)]. The expression of Ihh was increased by CTS in both
WT and ORPK chondrocytes relative to the noCTS control [WT:
P = 0.028; ORPK: P = 0.015; Fig. 2(F)]. In WT chondrocytes, Gli1
eexpression was increased following 10% CTS (P = 0.036) suggesting hedgehog signalling is activated [Fig. 2(G)]. Ptch1 expression was
also increased although the difference was not significant, this
suggests the pathway is activated to a lesser extent in response to
mechanical strain compared to r-Ihh treatment [Fig. 2(H)]. In ORPK
chondrocytes, Gli1 and Ptch1 expression were not altered by strain
[Fig. 1(B)] indicating that the primary cilium is not required for mechanosensitive Ihh expression but is necessary for signal transduction and pathway activation.

Primary cilia disassemble in a strain dependent manner

Previous studies have shown that mechanical stimuli trigger
cilia disassembly in numerous cell types including chon-
drocytes15,30,31. Given the importance of the cilium for chon-
drocyte hedgehog signalling, changes in cilia length and prevalence
were monitored in response to strain by confocal microscopy. Primary
bovine articular chondrocytes were subjected to strain then
immediately fixed and processed for immunofluorescent labelling.
Primary cilia were labelled for the axoneme marker acetylated α-
tubulin and for Arl13b, which labels the ciliary membrane. Within
the cilia Arl13b and acetylated α-tubulin fully overlapped
[Fig. 3(A)]. Primary cilium prevalence was not altered in response to mechanical strain [Fig. 3(B)]. However, primary cilia length was
significantly reduced in cells subjected to 20% CTS relative to the
noCTS control [P < 0.001; Fig. 3(A) and (C)]. Mean cilia length was
reduced from 2.48 μm ± 0.1 to 2.11 μm ± 0.1, a disassembly of approximately 15.1% which was highly reproducible.

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Yjocar.2013.12.016
Fig. 1. Mechanical strain upregulates Ihh expression and activates hedgehog signalling in articular chondrocytes. Changes in (A) Ihh, (B) Gli1 and (C) Ptch1 gene expression for primary bovine articular chondrocytes subjected to CTS for 1 h at 10% or 20% strain. Data is expressed as a fold change relative to the mean of the unstrained (noCTS) control group. Data represents mean ± CI (n = 8). Data was analysed by two-way ANOVA with post hoc Bonferroni corrected t-test.

Fig. 2. The primary cilium is required for chondrocyte hedgehog signalling. (A) Representative confocal maximum intensity Z projections showing primary cilia immunofluorescently labelled in WT and ORPK chondrocytes cultured in the absence of strain. The cilium was labelled with antibodies directed to acetylated α-tubulin (red) while nuclei were counterstained with DAPI (blue). Scale bar represents 20 μm. (B) Primary cilia prevalence (n = 10) and (C) primary cilia length for WT and ORPK chondrocytes (n = 10). Statistical significance was assessed by Student’s t test. Changes in (D) Gli1 and (E) Ptch1 gene expression for WT and ORPK chondrocytes treated with 1 μg/ml r-Ihh for 24 h. Changes in (F) Ihh, (G) Gli1 and (H) Ptch1 gene expression for WT and ORPK chondrocytes subjected to 10% CTS for 1 h. Gene expression data is expressed as a fold change relative to the mean of the untreated or unstrained control group. Data is presented as mean ± CI (n = 6–10). Statistical significance was assessed by two-way ANOVA with post hoc Bonferroni corrected t-test.
Primary cilia assembly and disassembly are intrinsically linked with the cell cycle and cilia resorption occurs upon cell cycle re-entry. Cell-cycle status was examined using the nuclear marker Ki-67. Nuclear Ki-67 is expressed by cells during all active phases of the cell cycle (G1, S, G2, and mitosis), but is absent from resting cells (G0). Bovine articular chondrocytes were subjected to CTS for 1 h then cultured for a further 24 h prior to fixation. There was no difference in the proportion of Ki-67 positive cells between strained and unstrained groups [Fig. 3(D)] indicating ciliary resorption does not occur as a result of cell cycle re-entry. Moreover, there was no significant difference in % viability between strained and unstrained cells which remained above 98% throughout the course of the study.

Mechanosensitive cilia disassembly requires HDAC6

Previous studies have implicated the tubulin deacetylase HDAC6 in cilia resorption which is proposed to modulate cilia length through the deacetylation and subsequent destabilisation of ciliary microtubules\(^\text{18,32,33}\). To explore the role of HDAC6 in mechanosensitive cilia disassembly, HDAC6 function was inhibited using trichostatin A (TSA) and tubacin. TSA is a broad spectrum HDAC inhibitor while tubacin specifically targets the tubulin deacetylase domain of HDAC6\(^\text{31}\). Following protocols used in previous studies, primary bovine articular chondrocytes were pre-incubated with 7 nM TSA, 500 nM tubacin or the carrier DMSO (control) for 3 h prior to and during the application of 20% CTS\(^\text{34}\). Both TSA and tubacin prevented strain-induced cilia disassembly indicating HDAC6 is required for this response [Fig. 4(A)]. Consistent with this finding, examination of HDAC6 distribution in articular chondrocytes revealed that while the majority of HDAC6 staining is cytoplasmic, HDAC6 is also found within the ciliary axoneme [Fig. 4(B)]. HDAC6 staining within the cilium is punctate and occurs along the entire length of the axoneme [Fig. 4(B), insert].

The data presented so far indicates that HDAC6 inhibition rescues the cilia disassembled in response to 20% CTS. If the absence of hedgehog pathway activation at this strain magnitude is due to HDAC6-dependent axoneme destabilisation and cilia disassembly then inhibiting tubulin deacetylation would be expected to rescue mechanosensitive hedgehog signalling. Consistent with this hypothesis, tubacin treatment restored mechanosensitive hedgehog signalling such that the expression of Ptch1 was increased following 1 h 20% CTS relative to the noCTS control [Fig. 4(C)]. By contrast, the expression of Gli1 was not increased by mechanical strain \(P = 0.956;\) Fig. 4(D)].

Cilia disassembly inhibits mechanosensitive ADAMTS-5 expression

Despite reports that Ihh functions as a mechanotransduction mediator to promote chondrocyte proliferation during embryonic development\(^\text{16,35}\); cell-cycle status was not altered by strain in articular chondrocytes suggesting proliferation is not increased in these cells [Fig. 3(D)]. Previous studies have reported that hedgehog signalling promotes ADAMTS-5 expression in human articular...
Bonferroni corrected Data is expressed as a fold change relative to the mean of the untreated or unstrained control group. Statistical significance was assessed by two-way ANOVA with post hoc Bonferroni corrected t-tests. (B) Representative confocal maximum intensity Z projection showing HDAC6 (green) localised to the primary cilium immunofluorescently labelled for acetylated α-tubulin (red). Nucleus counterstained with DAPI (blue). Scale bar represents 20 μm, lower panel shows magnified view of the cilium. Ptc1 (C) and Gli1 gene expression was not upregulated in response to 20% CTS in the presence of tubacin (500 nM) or the carrier DMSO (control). Data is expressed as a fold change relative to the mean of the untreated or unstrained control group. Statistical significance was assessed by two-way ANOVA with post hoc Bonferroni corrected t-tests.

Discussion

In this study, primary articular chondrocytes were subjected to variable levels of mechanical strain in 2D culture. For the first time we demonstrate that hedgehog signalling is mechanosensitive in adult chondrocytes and that pathway activation requires the primary cilium. Moreover we show that the chondrocyte primary cilium disassembles in response to CTS in a strain dependent manner. Cilium disassembly in chondrocytes requires HDAC6-dependent tubulin deacetylation and functions to inhibit mechanosensitive hedgehog signalling and ADAMTS-5 expression.

Chondrocytes experience a variety of mechanical or physico-chemical stimuli during physiological joint loading such as compressive, tensile and shear strain, fluid flow, electrical streaming potentials and changes in pH and osmolarity (for review see36). While tensile strain may only be a small component of this physiological load the effects of tensile strain on chondrocyte function have been widely studied in 2D culture.37 In the context of the current study, this model is advantageous as it provides a sensitive and highly reproducible system in which to reliably measure small changes in cilium length.

Mechanosensitive hedgehog signalling has previously been reported in immature chondrocytes26,35 but this study is the first to demonstrate the response in adult chondrocytes. This has important consequences for understanding the aberrant activation of hedgehog signalling in OA which promotes cartilage degeneration through the upregulation of ADAMTS-521. In agreement with our findings, Shao et al. recently used chemical disruption of the ciliary structure to demonstrate a requirement for the cilium in mechanosensitive hedgehog signalling in growth plate chondrocytes33. In contrast with the current study, Ihh expression was shown to be down-regulated by cilia disruption perhaps highlighting a key difference between mature and immature cells. Indeed, during skeletal development hedgehog signalling promotes chondrocyte hypertrophy and thus further Ihh expression33,40 however the function of hedgehog signalling for healthy articular cartilage is unclear.

The present study demonstrates that mechanical loading reduces primary cilium length in a strain dependent manner. In articular cartilage, the mean length of chondrocyte primary cilia in the superficial zone is 1.1 μm. This is approximately 27% shorter than reported for deep zone chondrocytes for which mean cilium length is 1.5 μm.41 Our data suggests that zonal variations in a chondrocytes mechanosensitive environment might be directly responsible for these differences as several studies have reported that the cells of the superficial zone are subjected to the greatest levels of both compressive and tensile strain41. The extent of cilium disassembly observed in the current study is less dramatic than the 30% reduction in length observed following prolonged dynamic compression of chondrocytes in agarose.39 This suggests that different types of stimuli, or differences in the intensity and number of cilia.
duration of the loading regime might regulate cilia structure to
different extents. Indeed, a similarly low level of cilia disassembly
was observed for chondrocytes in situ during short-term osmic
loading where cilia length was reduced by just 10%42.

The influence mechanosensitive disassembly exerts over
the function of the cilium as a flow sensor is well studied15,43,44.
However, while cilia disassembly is hypothesised to modulate
other signalling pathways this has not been demonstrated in
chondrocytes prior to this study. For the first time we show that
loading-induced chondrocyte cilia disassembly influences mecha-

nosedative hedgehog signalling and ADAMTS-5 expression at high
strains. Remarkably, the 15.1% reduction in cilia length observed at
20% strain was sufficient to completely inhibit mechanosensitive
hedgehog pathway activation and ADAMTS-5 expression. These
data indicate that hedgehog signalling is highly sensitive to changes
in primary cilia length. In osteoarthritis cartilage there is a 50%
increase in the number of ciliated cells and an increase of approx-
imately 20% in mean primary cilia length9. Our data suggests that
this modest increase will be more than sufficient to influence cilia-
mediated hedgehog signalling and may therefore explain the
aberrant upregulation of this pathway reported in OA11.

In the present study the inhibition of HDAC6 prevented strain-
induced cilia disassembly and at least partially restored mecha-
nosedative hedgehog signalling (Fig. 4) and ADAMTS-5 expression
at 20% strain [Fig. 5(B)]. It is unclear how tubulin deacetylation is
influencing this pathway. However the findings agree with pre-
vious studies which show that HDAC6 is required for cilia disas-
sembly in response to heat shock and that this inhibits hedgehog
signalling13. While destabilisation of the axoneme might be
influencing IFT and the traffic of Gli proteins through the cilium,
previous studies have also linked ciliary dysfunction to changes in
whole cell HDAC6 activity and tubulin acetylation levels45. While,
gross changes in the level of whole cell tubulin acetylation were
not observed in this study (not shown), subtle changes in the
acetylation status of cytoplasmic microtubules could potentially
affect cellular function by modulating kinesin motor activity and
thus microtubule transport throughout the cell46. Alternatively,
the actin regulatory protein cortactin is reportedly a substrate of
HDAC6 and the increased activity of this enzyme could affect
ciliary structure through changes in actin dynamics47. Gradilone
et al. have recently reported that increased HDAC6 expression in
cholangiocarcinoma disrupts hedgehog signalling and promotes
tumour growth through the modulation of cilia structure18.

HDAC6 inhibition was able to restore cilia function and rescue the
signalling defect such that a reduction in tumour growth was
observed. This and the current study highlight HDAC6 as a
potentially targetable element which could be used to adjust cilia
structure—function in vivo.

In summary, we have shown that mechanically induced cilia
disassembly occurs in an HDAC6 dependent manner in healthy
articular chondrocytes and attenuates mechanosensitive hedgehog
signalling and ADAMTS-5 expression at higher strains. We propose
that low levels of mechanical loading may activate hedgehog-
mediated matrix degradation, but that cilia disassembly at higher
strains provides a chondroprotective mechanism such that hedgehog
signalling is prevented. The study highlights the sensitive
relationship between primary cilia length and hedgehog sig-
nalling. Thus the aberrant upregulation of hedgehog signalling
reported in OA41 may be directly related to an increase in cilia
length3. Consequently this study provides further evidence of the
importance of primary cilia structure in cartilage development,
health and disease.

Author contributions

All authors were involved in the drafting of this article or
revising it critically for important intellectual content, and all
authors approved the final version to be published.

Study conception and design: Thompson, Knight, Chapple.

Acquisition of data: Thompson.

Analysis and interpretation of data: Thompson, Knight, Chapple.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary data

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