

Bone & Joint Research



Supplementary Material

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Patient/donor inclusion and exclusion criteria

Following written consent of all individuals/their relatives, the following criteria were applied:

- Cadaver: samples from patients deceased less than 24 hours were included. Patients who had died during a hospital stay and were directly transferred to the pathology department. Only patients with no prior shoulder surgery, no documented shoulder problems, and no macroscopic signs of osteoarthritis during tissue sampling were included.
- Proximal humerus fracture: samples from patients who qualified for osteosynthesis were included. Patients had no signs of osteoarthritis or prior shoulder problems.
- SLAP: samples from patients with symptomatic biceps tendinopathy and without signs of rotator cuff involvement were included. Patients were scheduled for elective biceps tenodesis after failure of conservative treatment.
- RCT: samples from patients with symptomatic rotator cuff tears involving supraspinatus tendon with or without subscapularis tendon were included. Patients were scheduled for elective arthroscopic rotator cuff repair, and tissue samples were collected during surgery. Patients with revision surgery after prior rotator cuff repair were excluded.
- OA: patients with documented history of progressive shoulder problems due to osteoarthritis which underwent elective shoulder arthroplasty surgery were included. Patients with a detached long biceps tendon due to degeneration before the surgery were excluded.

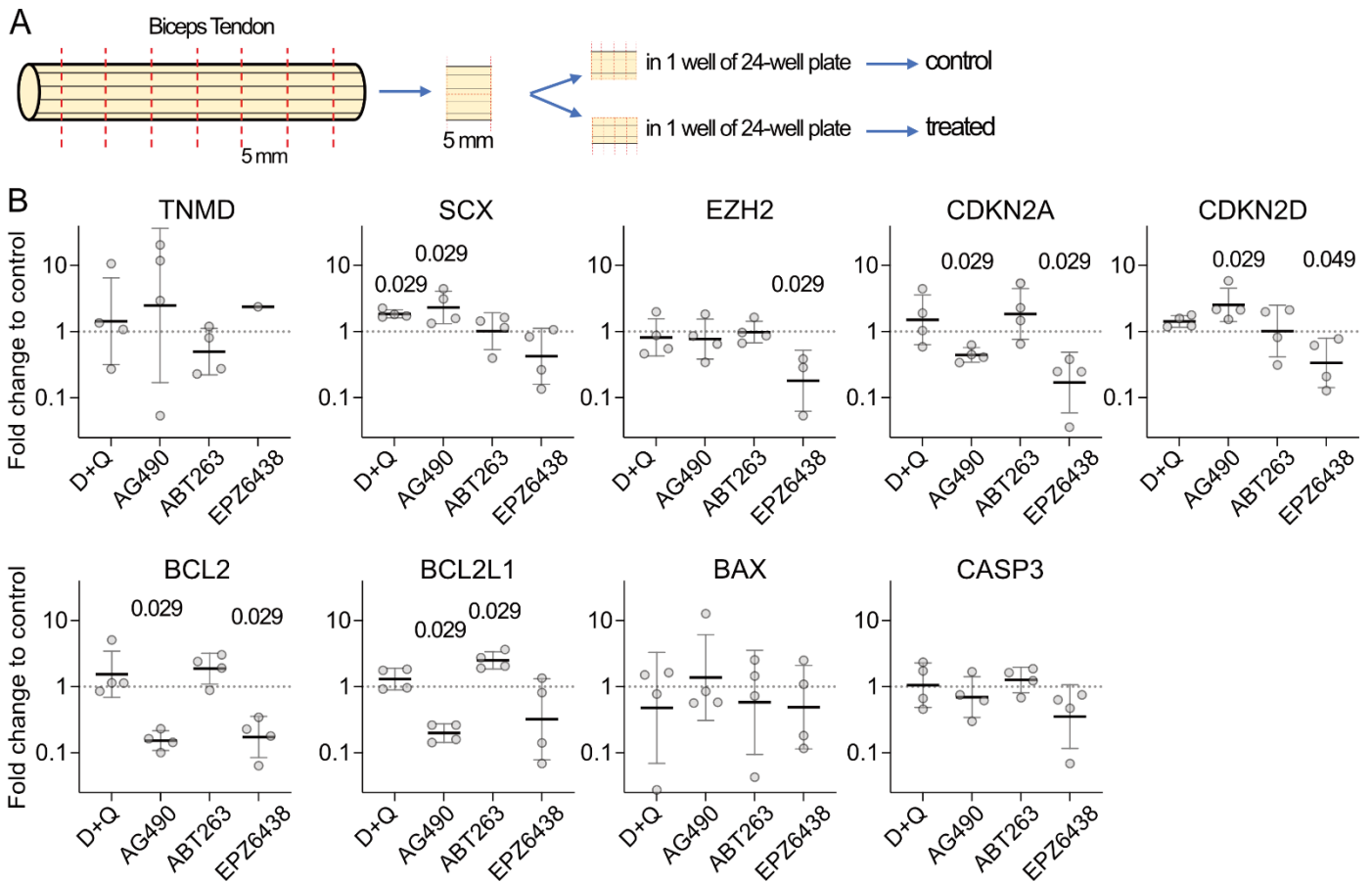


Fig. a. a) Schematic of experimental setup. b) Fold-changes of gene expression in tendon tissues after six days of incubation with therapeutic compounds compared to expression levels in adjacent untreated control tissue segments (n = 4 donors). Dashed line visualizes the relative expression level in controls. Dots are the means of three technical replicates. Solid lines show the geometrical means (geometrical SDs). p-values of Mann-Whitney U tests are indicated in the graphs if p ≤ 0.05. D + Q, Dasatinib + Quercetin. BAX, BCL2-associated X protein; BCL2, B cell lymphoma 2; CASP3, Caspase 3; CDKN2A, cyclin dependent kinase inhibitor 2A; EZH2, Enhancer of Zeste 2; SCX, scleraxis; TNMD, tenomodulin.

Table i. Patient demographic data.

Variable	Age, yrs	Sex	Symptom duration	Surgery
Healthy (from Pathology)				
Subject 1 <i>Fig1,2,3,4,6</i>	84	Male	N/A	N/A
Subject 2 <i>Fig1,2,3,4,6</i>	72	Female	N/A	N/A
Subject 3 <i>Fig1,2,3,4</i>	57	Male	N/A	N/A
Subject 4 <i>Fig1,2,3,4</i>	87	Male	N/A	N/A
Proximal humerus fracture				
Patient 1 <i>Fig1,2,3,4,6</i>	53	Male	1 week	ORIF
Patient 2 <i>Fig1,2,3,4</i>	53	Male	1 month	ORIF
Patient 3 <i>Fig1,2,3,4</i>	57	Female	1 day	ORIF
Patient 4 <i>Fig1,2,3,4</i>	60	Female	2 days	ORIF
Patient 5 <i>Fig1,2,3,4,6</i>	78	Female	1 day	ORIF
Patient 6 <i>Fig1,2,3,4</i>	52	Male	1 day	ORIF
SLAP lesion				
Patient 1 <i>Fig1,2,3,4</i>	26	Female	4 months	Biceps tenodesis
Patient 2 <i>Fig1,2,3,4</i>	38	Female	2 months	Biceps tenodesis
Patient 3 <i>Fig1,2,3,4,6</i>	46	Male	11 months	Biceps tenodesis

Patient 4 ^{Fig1,2,3,4,6}	50	Female	3 months	Biceps tenodesis
Patient 5 ^{Fig1,2,3,4}	44	Male	2 months	Biceps tenodesis
Patient 6 ^{Fig1,2,3,4}	43	Female	7 months	Biceps tenodesis
Patient 7 ^{Fig1,2,3,4}	57	Female	3 months	Biceps tenodesis
Patient 8 ^{Fig1,3,7}	60	Female	6 months	Biceps tenodesis
Rotator cuff tear				
Patient 1 ^{Fig1,2,3,4,6}	57	Male	5 months	ARCR
Patient 2 ^{Fig1,2,3,4,6}	64	Male	2 years	ARCR
Patient 3 ^{Fig1,2,3,4,5}	55	Male	2 years	ARCR
Patient 4 ^{Fig1,2,3,4}	62	Female	1 year	ARCR
Patient 5 ^{Fig1,2,3,4}	59	Female	2 years	ARCR
Patient 6 ^{Fig1,2,3,4}	74	Male	6 months	ARCR
Patient 7 ^{Fig1,2,3,4,7}	46	Male	2 months	ARCR
Patient 8 ^{Fig7}	69	Male	> 2 years	ARCR
Patient 9 ^{Fig7}	47	Male	4 months	ARCR
Patient 10 ^{Fig7}	56	Female	3 months	ARCR
Osteoarthritis				
Patient 1 ^{Fig1,2,3,4,6}	67	Male	> 9 years	Arthroplasty
Patient 2 ^{Fig1,2,3,4}	61	Male	> 10 years	Arthroplasty
Patient 3 ^{Fig1,2,3,4}	65	Male	> 1 year	Arthroplasty
Patient 4 ^{Fig1,2,3,4,6}	75	Male	> 10 years	Arthroplasty

ARCR, arthroscopic rotator cuff repair; N/A, not applicable; ORIF, open reduction and internal fixation; SLAP, superior labrum anterior to posterior tear.

^{Fig} Superscript indicates for which Figure(s) patients' material was used.

Table ii. Modified Bonar Score (adapted from Fearon et al³⁴ and Streit et al³⁵).

Variable	Grade 0	Grade 1	Grade 2	Grade 3
Cell morphology (4 FOVs, 20x, brightfield)	Inconspicuous elongated, spindle-shaped nuclei with no obvious cytoplasm on light microscopy	Increased roundness: nucleus becomes more ovoid-to-round in shape without conspicuous cytoplasm	Increased roundness and size: nucleus is round, slightly enlarged, and a small amount of cytoplasm is visible	Nucleus is round, large with abundant cytoplasm and lacuna formation (chondroid change)
Collagen arrangement (1 FOV, 10x, polarized)	Collagen arranged in tightly cohesive, well demarcated bundles with a smooth, dense, bright homogeneous polarization pattern with normal crimping	Diminished fibre polarization: separation of individual fibre bundles but with maintenance of overall bundle architecture; non-homogeneous polarization	Bundle changes: separation and loss of demarcation of fibre bundles, giving rise to expansion of the tissue overall and clear loss of normal polarization pattern	Marked separation of fibre bundles with complete loss of architecture
Cellularity (1 FOV, 10x, brightfield)	Mainly discrete cells	Hypercellular, in runs and/or increased cell numbers	Areas of hypo- as well as hyper-cellularity	Area of assessment is mostly acellular

Vascularity (10 FOVs, 40x, brightfield)	Inconspicuous blood vessels coursing between bundles	Occasional cluster of capillaries, < 1 per ten high power fields	1 to 2 clusters of capillaries per ten high power fields	Areas with greater than 2 clusters per ten high power fields
Ground substance* (1 FOV, 10x, brightfield)	No myxoid material	Myxoid material present in small quantities between discrete bundles	Myxoid material present in moderate-to-large amounts with loss of demarcation of bundles	Abundant myxoid material with loss of bundles

*Ground substance relied on the quantity of bluish amorphous 'myxoid' material seen in the tendon on haematoxylin and eosin staining.
FOV, field of view.

Table iii. TaqMan assays (ThermoFisher Scientific, USA) used for gene expression analyses.

Gene symbol	Gene name	Assay ID
ACTB	Beta actin (tested as reference gene)	Hs99999903_m1
TBP	TATA-box binding protein (tested as reference gene)	Hs00427620_m1
BAX	BCL2-associated X protein	Hs00180269_m1
BCL-2	B cell lymphoma 2	Hs00608023_m1
BCL2L1	BCL2-like protein1 encoding for BCL-xL	Hs00236329_m1
CASP3	Caspase 3	Hs00234387_m1
CDKN2A/p16	Cyclin dependent kinase inhibitor 2A	Hs00923894_m1
CDKN2D/p19	Cyclin dependent kinase inhibitor 2D	Hs00176481_m1
COL1A1	Collagen type 1	Hs00164004_m1
COL3A1	Collagen type 3	Hs00264051_m1
EZH2	Enhancer of zeste 2 polycomb repressive complex 2 subunit	Hs01016789_m1
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase (reference gene)	Hs2758991_g1
MMP1	Matrix metalloproteinase 1	Hs00233958_m1
MMP3	Matrix metalloproteinase 3	Hs00968305_m1
SCX	Scleraxis	Hs03054634_g1
TNMD	Tenomodulin	Hs00223332_m1

Table iv. Association of age and tissue degeneration (Modified Bonar Score) with either "Fracture" (above double line) or "Healthy" (below double line) as reference. p-values were defined by Student's *t*-test on the estimated coefficient divided by its standard error as part of the mixed model statistical analysis.

Variable	Option	Coefficient (95% CI)	p-value
Age		0.02 (-0.03 to 0.06)	0.509
Aetiology	Fracture	Reference	
	Healthy	-0.82 (-2.44 to 0.80)	0.320
	SLAP	4.97 (3.51 to 6.43)	< 0.001
	RCT	5.47 (4.23 to 6.72)	< 0.001
	OA	6.03 (4.36 to 7.70)	< 0.001
Age		0.02 (-0.03 to 0.06)	0.509
Aetiology	Fracture	0.82 (-0.80 to 2.44)	0.320
	Healthy	Reference	
	SLAP	5.79 (3.77 to 7.81)	< 0.001
	RCT	6.30 (4.76 to 7.84)	< 0.001
	OA	6.85 (5.11 to 8.60)	< 0.001

OA, osteoarthritis; RCT, rotator cuff tear; SLAP, superior labrum anterior to posterior tear.

Table v. Association of location and tissue degeneration (Modified Bonar Score). P-values were defined by Student's *t*-test on the estimated coefficient divided by its standard error as part of the mixed model statistical analysis.

Variable	Option	Coefficient (95% CI)	p-value
Age		0.02 (-0.01 to 0.06)	0.212
Degenerative		5.79 (4.78 to 6.79)	< 0.001
Location	Intra-articular	Reference	
	Bicipital groove	-0.05 (-1.01 to 0.90)	0.911
	Extra-articular	-0.98 (-1.98 to 0.03)	0.058

Table vi. Quantification of p19 and/or EZH2 expressing cells. Proportion of cells expressing p19 and EZH2 alone or in combination quantified by QuPath for all patient groups (n = 2 donors per group with n = 4 to 7 quantified regions). Values are means and SDs.

Variable	Healthy	Fracture	SLAP	RCT	OA	p-value
p19 +	7.5% (6.3%)	13.7% (2.3%)	38.9% (31.3%)*§	85.7% (21.6%)*†††	11.4% (10.6%)§	< 0.001
EZH2 +	11.5% (10.3%)	24.6% (10.4%)	29.5% (16.4%)§	82.2% (17.8%)*†††	4.3% (5%)§	< 0.001
p19 - / EZH2 -	86.6% (13.5%)	73.3% (8.5%)	56.2% (21.1%)*§	5.5% (8%)*†††	88.8% (10.6%)§‡	< 0.001
p19 + / EZH2 -	0%	2.1% (2.1%)	13.7% (13.7%)	12.6% (15.4%)	7.6% (6.3%)	0.146
p19 - / EZH2 +	4.1% (5%)	13.0% (8.6%)	4.2% (8.2%)	9.1% (14.1%)	0.5% (0.6%)	0.261
p19 + / EZH2 +	7.5% (6.3%)	11.6% (2.7%)	25.3% (12.5%)§	73.1% (25.5%)*†††	3.8% (4.8%)§	< 0.001

Significant differences defined by analysis of variance with Tukey's post-hoc testing corrected for multiple comparison.

*p < 0.05; **p < 0.01; ***p < 0.001 vs healthy group

†p < 0.001 vs fracture group

‡p < 0.05 vs SLAP group

§p < 0.001 vs RCT group

OA, osteoarthritis; RCT, rotator cuff tear; SLAP, superior labrum anterior to posterior tear.

Table vii. Senescence-associated secretory phenotype (SASP) quantification. Secretion of SASP (all in pg/ml by Luminex; except IL8* in ng/ml by ELISA) by tendon tissue biopsies (n = 5 donors) cultured for six days in medium alone (Untreated Control) or in the presence of IL1 β (Control + IL1 β) to activate SASP and upon treatment with AG490 and EPZ-6438. Values are means (SD).

Variable	Untreated control	Control + IL1 β	AG490 + IL1 β	EPZ-6438+ IL1 β
ADAMTS13	10 (5.3)	71 (19.2)	20 (1.8)	51 (23.1)
MMP3	1,1190 (2,093.0)	12,825 (404.7)	8,417 (1,848)	9,902 (1,298)
MMP13	38 (26.5)	236 (239.7)	47 (8.1)	49 (9.7)
IL6	758 (984.1)	19,114 (3,773)	39 (15.6)	8,322 (6,981)
IL8*	22 (1.7)	1,330 (342.5)	19 (29.4)	862 (551.1)
TNF α	12 (3.18)	36 (6.4)	20 (2.0)	27 (6.8)
MCP1/CCL2	4,795 (6,003.2)	8,438 (4,497)	21 (7.3)	457 (888.2)
MCP2/CCL8	11 (6.5)	207 (175.7)	22 (0.9)	44 (28.8)
MCP3/CCL7	10 (3.9)	76 (28.1)	12 (1.2)	16 (3.8)
GRO1/CXCL1	58 (76.4)	10,234 (2,753)	24 (1.0)	299 (375.4)
GRO2/CXCL2	78 (96.2)	6,742 (4,452)	28 (11.8)	4,442 (3,890)
MIP1 α /CCL3	16 (3.5)	262 (347.7)	23 (3.4)	93 (127.2)