Bone & Joint Research

Supplementary Material

10.1302/2046-3758.1110.BJR-2022-0013.R2

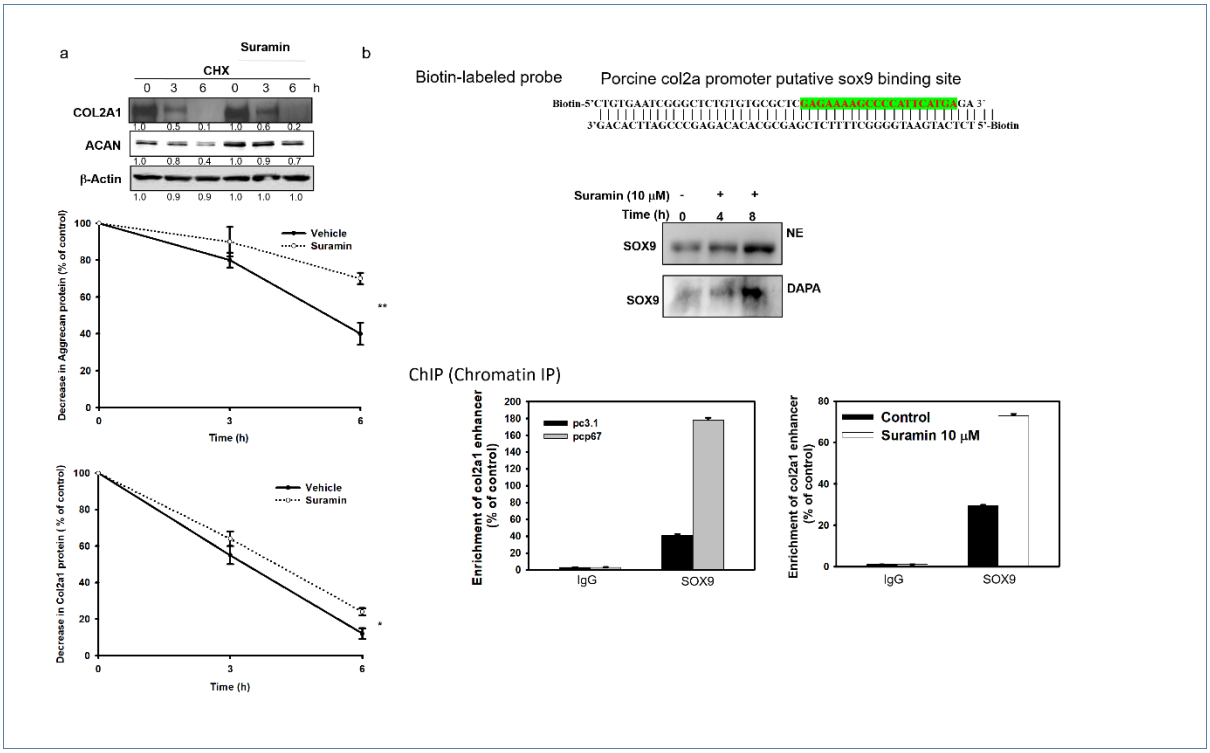

Fig a. a) Disc nucleus pulposus (NP) cells were incubated with or without 10 μM suramin for one hour followed by addition of 30 μg/ml cycloheximide (CHX) for various time periods. Western blot was performed and anti-aggrecan (ACAN) and collagen, type II, alpha 1 (COL2A1) antibody was used for immunoblotting. b) Nuclear extracts from 10 μM suramin-stimulated chondrocytes for different timepoints as indicated were collected, and these were incubated with a biotin-conjugated oligonucleotide of the Col2a1 promoter subjected to DNA affinity precipitation assay (DAPA). The pulled-down proteins were washed and determined by western blot with antibodies against SRY-box transcription factor 9 (SOX9). Chondrocytes were transiently transfected with or without p67 overexpression plasmid or treated with 10 μM suramin for eight hours, and then subjected to chromatin-immunoprecipitation (ChIP) assay. After ChIP assay manipulation, the chromatin

fragments were immunoprecipitated with SOX9 antibody, and the co-immunoprecipitated DNA was amplified by polymerase chain reaction (PCR). Total input chromatin (Input) was included in the PCR as control. Additional control includes a precipitation performed with non-specific immunoglobulin G (IgG).

The ARRIVE Essential 10: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	After harvesting the porcine chondrocytes, the cells separated single animal species: porcine
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	Six porcines will be used and harvested According to the previous study
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori*. If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	The same age and sex matured porcine. these criteria were no exclusion n=6
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	The experiments were not performed random Laboratory animals have independent numbered cages to
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	Allocation: research assistant, members of animal center
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	Molecular detection including Western n>=3
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	statistical methods: t-test N/A
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Lanyu pig ,male, 6-8 mo old (mature) ~20 Livestock Research Institute, Council of
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	Before the surgery , 0.2 mg/kg dormicum and 40 μg/kg After the cartilage animal's medial to investigate the
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	A representative result is presented — N/A