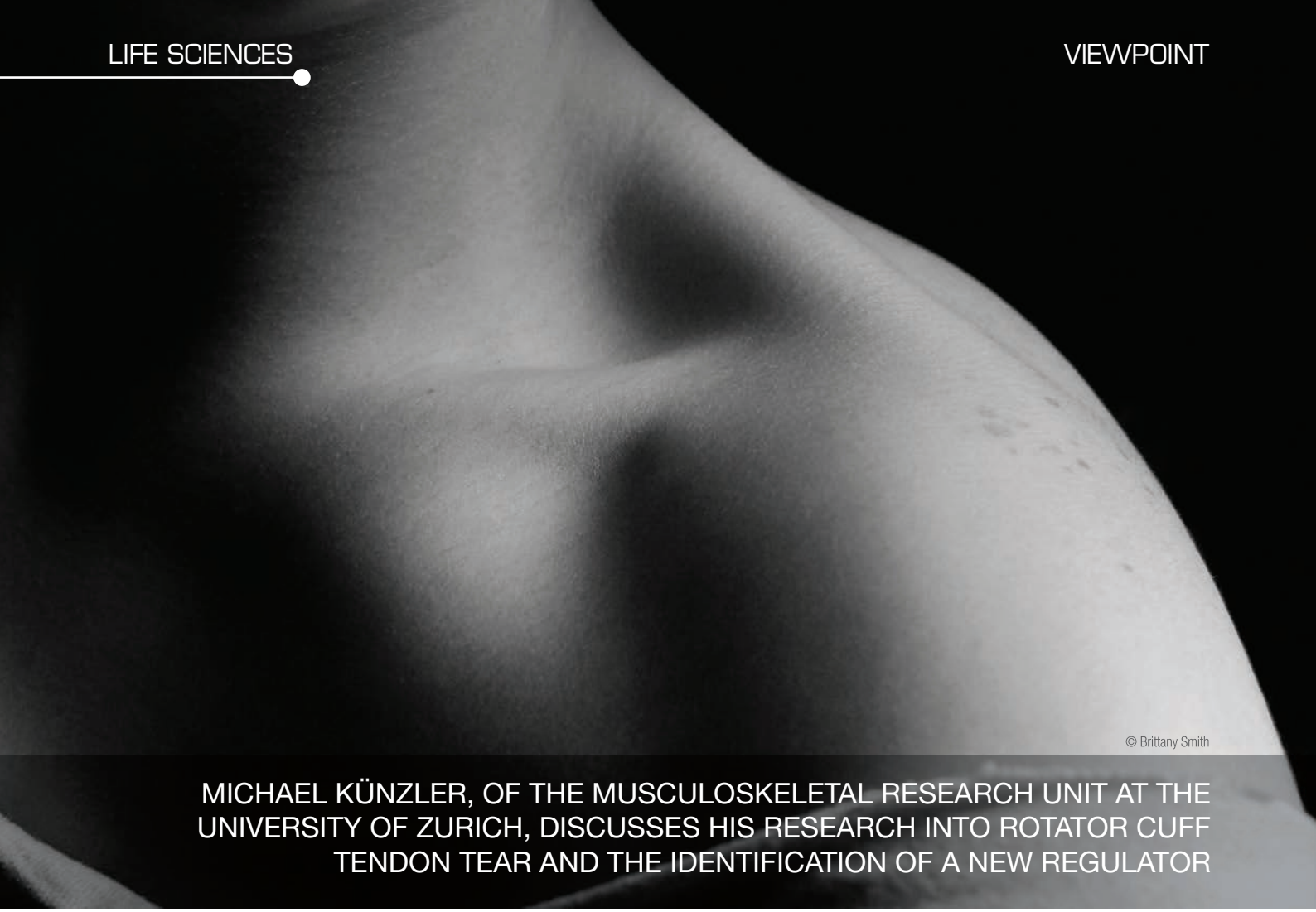

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MICHAEL KÜNZLER, OF THE MUSCULOSKELETAL RESEARCH UNIT AT THE UNIVERSITY OF ZURICH, DISCUSSES HIS RESEARCH INTO ROTATOR CUFF TENDON TEAR AND THE IDENTIFICATION OF A NEW REGULATOR

# Repairing rotator cuff tendons

The shoulder is the joint with the largest range of motion in the human body due to its unique anatomy, with the large humeral head moving on the rather small glenoid (the dimensions are similar to a golf ball on the tee). This high mobility comes at the expense of bone stability, in that the different soft tissue structures including the labrum, ligaments, and the rotator cuff muscles provide dynamic and static stability. Furthermore, the rotator cuff muscles not only provide dynamic stability but also move the humeral head in all directions, thereby allowing overhead movements of the arm. Moving the arm during daily activities, combined with a prominent bony shoulder roof, cause repetitive micro-injuries to the tendon. These micro-injuries drive the tendon to undergo degeneration, thereby rendering the tendon vulnerable to traumatic ruptures.

Due to the long lasting pathogenesis of tendon degeneration with repetitive micro-injuries over a long period of time, minor impacts or even normal movements of the arm can be sufficient to finally tear the tendon. This is why rotator cuff tears are mainly observed in patients over the age of 60 years. However, especially in those whose job entails a lot of overhead movement and athletes who throw objects, where the soft tissue is subjected to repetitive high loads, the degeneration is accelerated, thus leading to earlier tearing.

## Rotator cuff tear causes muscle degeneration

With complete rotator cuff tear, mechanical force transmission of the muscle is completely interrupted. This impairs the patient from moving the arm in the specific direction of the muscle pull. Subsequent to the loss of mechanical load, the muscle undergoes distinct degenerative changes. During the initial inflammatory phase macrophages and neutrophils invade the muscle and clear the debris from muscle fibres that underwent apoptosis. The macrophages then switch to become regulatory macrophages in order to orchestrate the attempt of the tendon and muscle to regenerate. This attempt is doomed to failure because of the gap that emerged between the tendon stump and the insertion on the humeral head (it can be several centimetres).

With the continuing loss of load, the muscle fibres shorten and the muscle volume decreases, leading to atrophy of the muscle,

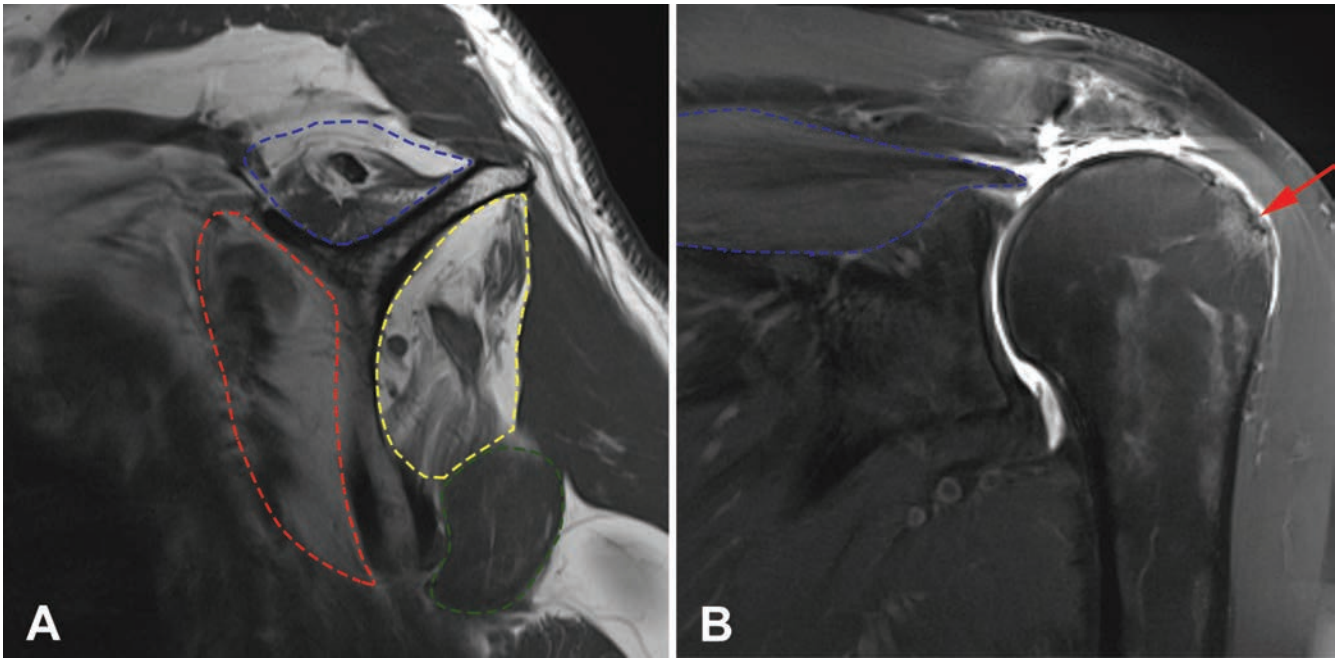



**Fig. 1 MRI arthrography of a human shoulder joint with rotator cuff tear. A: Sagittal plane of the joint showing the scapula with the four rotator cuff muscles in a T1 weighted MRI scan. The supraspinatus (dashed blue line), infraspinatus (dashed yellow line) and subscapularis muscle (dashed red line) show signs of severe atrophy and fatty infiltration with most of the muscle tissue (dark) replaced by fat (bright). The lines show the hypothetical border of the muscles. Only the teres minor muscle (dashed green line) appears normal. B: Coronal plane of the shoulder joint in a T2 weighted MRI scan. The image shows a tear of the supraspinatus muscle (dashed blue line) with the tendon stump (darker signal at the tip of the muscle) severely retracted from the normal insertion site at the humeral head (red arrow) to the upper border of the glenoid. Fat content cannot be assessed in this sequence**

thereby causing the tendon to retract even further. With this retraction, the pennation angle of the muscle increases, thus enlarging the extracellular space between the muscle fibres and allowing for fat cells to grow into this space.

The process of muscle atrophy and tendon retraction takes weeks to months and results in muscle fibres being replaced by fat cells, while adipocytes infiltrate the increased intermyofibrillar space from the vasculature. This fatty infiltration is the irreversible end point of muscular degeneration and thereby determines the success of surgical repair.

As shown in Fig. 1, in clinical routine fatty infiltration is staged in MRI scans of the shoulder. When the volume of intramuscular fat mass exceeds the remaining muscle mass, surgical repair is no longer accomplishable because the tendon has retracted too far and the surgeon is unable to mobilise the tendon enough to suture it back to its insertion on the humeral head. In addition, at these stages too many muscle fibres have been replaced by fat, and this has impaired the muscle's ability to produce sufficient force to move the arm, even if mobilisation and suturing of the tendon during surgery has been successful.

## Prone to failure

Surgical repair is the choice of therapy for patients presenting with clinical signs of rotator cuff tear that have been confirmed by MRI scans, with fatty infiltration being less than 50% and low retraction. During the procedure, the tendon is sutured back to its insertion on the humeral head arthroscopically. Various suture techniques, anchors, and implants have been developed to optimise the repair.

Nonetheless, despite the advancements in surgical technique, the repair is prone to failure with re-tear rates ranging from 13% up to 93%. This insufficient healing rate is mainly due to the fact that the tendon is unable to form a functionally stable neo-enthesis. Instead, the tendon tries to heal by forming mechanically weak scar tissue that is unable to bear high loads. Knowledge on how the tendon can be directed from building scar tissue into forming a mechanical strong neo-enthesis is still lacking.

## Animal models

In past decades, investigations of the cellular and molecular mechanisms leading to degeneration after rotator cuff tear and the improvement of the healing after repair underwent a huge shift from clinical patient-centred studies (which mainly aimed to improve surgical technique) to preclinical experimental animal models.

The shift happened because in these models it is possible to standardise the injury pattern and control the influencing factors such as the exposure to pathogens (which may trigger unwanted inflammatory response, dietary uptake) and, to some extent, daily activity. With these advantages, animal studies are superior to *in vitro* studies for the translation of the findings into clinical application.

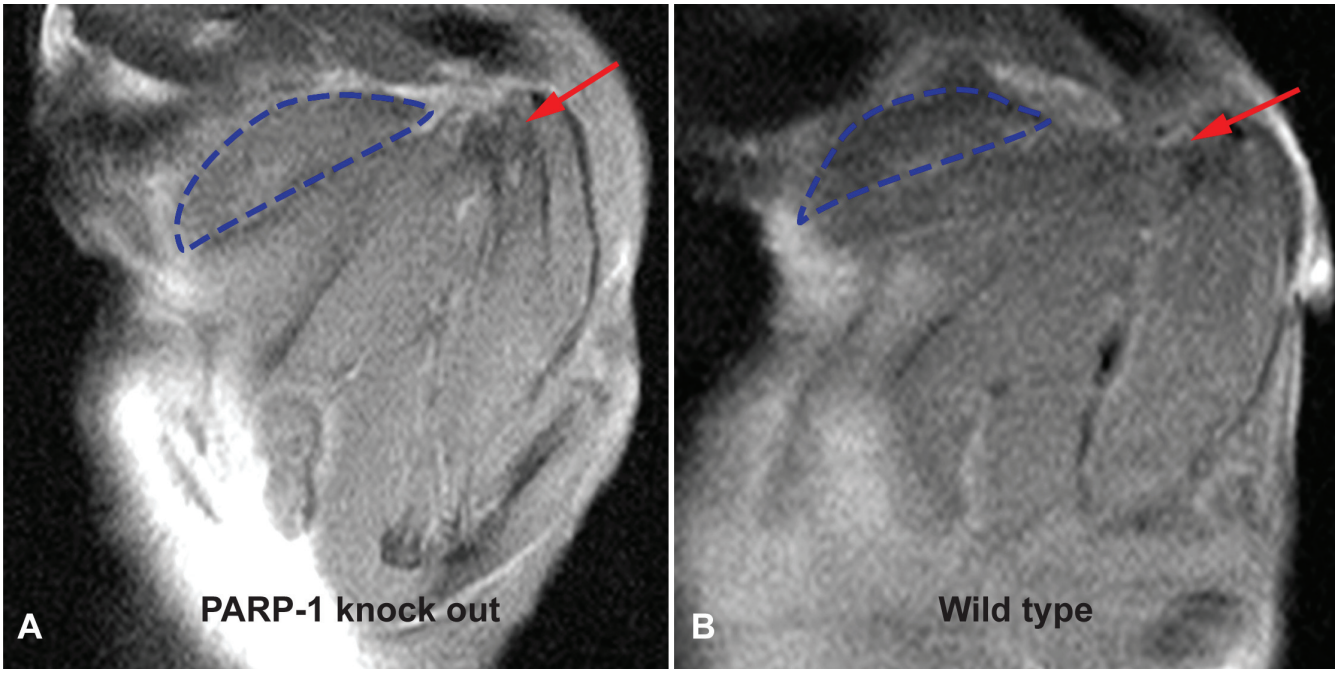



**Fig. 2 MRI scan of the rat shoulder 12 weeks after tenotomy and neurectomy of the supraspinatus muscle acquired with a 4.7 T PharmaScan MRI system (Bruker, USA). The dashed blue lines enfold the borders of the supraspinatus muscle and the red arrow points to the muscle's insertion site on the humeral head in PARP-1 knock out mice (A) and wild type mice (B). Retraction was significantly lower in the PARP-1 knock out group compared to the wild type group**

The animal species used vary from small rodents such as mice, rats or rabbits, to larger animals including dogs and sheep. All these species are established models for the investigation of molecular factors influencing the degeneration after rotator cuff tears because they have a shoulder anatomy that is comparable to that of humans. However, despite recent advancements the exact interplay of the degenerative mechanisms is still not well understood and an upstream regulator that orchestrates them is so far yet to be found.

The Center of Applied Biology and Molecular Medicine (CABMM) of the University of Zurich, together with the Musculoskeletal Research Unit (MSRU) of the Vetsuisse Faculty at the University of Zurich, has been working with sheep and rabbit models for rotator cuff tear for some years and has gained significant knowhow in conducting these studies, many of which have contributed important knowledge to the understanding of the molecular mechanisms that lead to degenerative changes after rotator cuff tear.

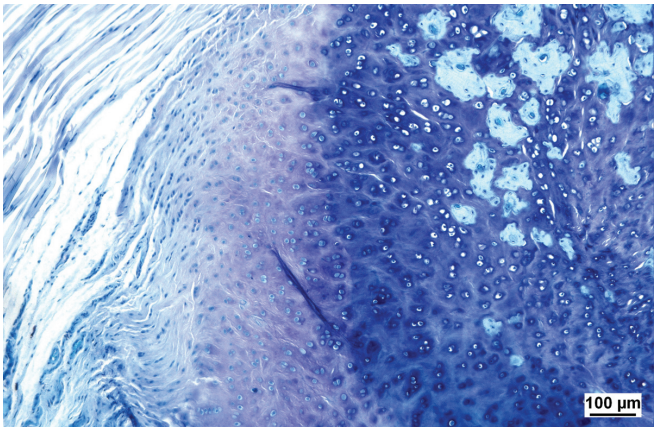
## A regulator of muscle degeneration

Most recently, the MSRU in collaboration with the Shoulder, Elbow and Sports Medicine of the Inselspital University Hospital of Bern have established a model with genetically modified mice for the investigation of the molecular mechanisms after rotator cuff tear. Using this new model, we have found a regulator that is involved in the orchestration of the early inflammatory response, muscle atrophy and retraction, and fatty infiltration – the four most important pathological features after rotator cuff tear.

The regulator is a polymerase that catalyses the post-translational modification of various proteins and thereby regulates vital cell processes. Poly[ADP-ribose] polymerase 1 (PARP-1) belongs to the PARP superfamily that contains 17 proteins and plays a fundamental role in cell cycle modulation, DNA damage repair, apoptosis, and transcription of various genes mainly of the inflammatory cascade.

For the study, PARP-1 knockout mice were used and compared to wild type mice. The animals underwent surgical transection of the rotator cuff tendon from the humeral insertion as well as neurectomy of the nerve that innervates these muscles. In absence of PARP-1, unloading of the muscle after rotator cuff tear dampens the inflammatory reaction during the initial phase and consequently causes fewer muscle cells to undergo apoptosis and degradation. The muscles lacking PARP-1 maintained a greater ability to regenerate and finally regained a larger muscle

**Enthesis of a normal rat shoulder**



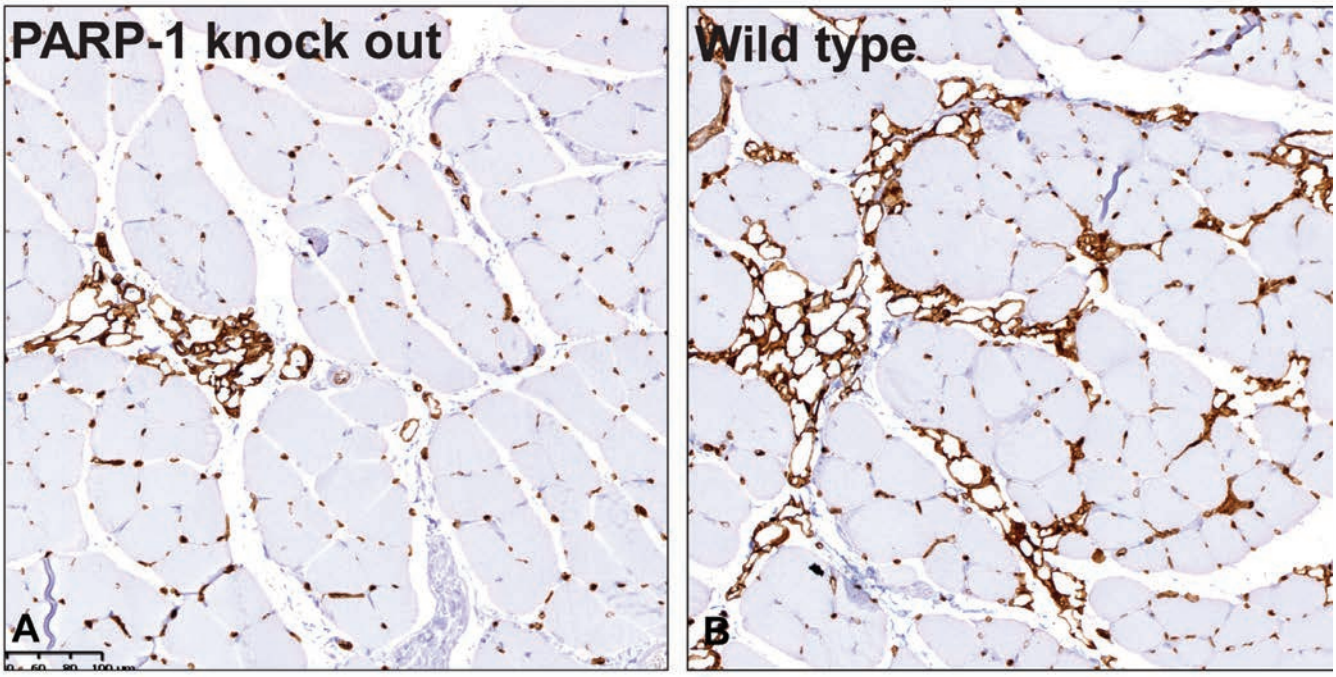



**Fig. 3 Histological cross-sections at the midsection of the supraspinatus muscle of experimental mice with rotator cuff tear at a 20x magnification 12 weeks after surgery. The samples were stained with an antibody against fatty acid binding protein 4 (FABP4), a protein mainly expressed on the cell surface of adipocytes. A shows a representative sample from the PARP-1 knock out group with only little intermyofibrillar fat. B shows a sample from the wild type group with extended inter and intramyofibrillar fatty infiltration. In the semi-quantitative analysis the PARP-1 knock out group had significantly less fatty infiltration compared to the wild type group**

volume as well as having less retraction after three months (see Fig. 2A and B).

With the lower pennation angle, fewer adipocytes infiltrated from the vasculature into the narrower intermyofibrillar space and less muscle fibres were replaced by fat cells, consequently reducing the overall fatty infiltration compared to the wild type mice (see Fig. 3A and B). With the description of this novel regulator of muscle degeneration, a potential approach to influence the outcome after rotator cuff tear was established.

The finding of this regulator could help to establish a therapeutic approach for the prevention of further damage to the muscle. However, translation into clinical application needs further work in order to generate a better understanding of the influence of PARP-1 on the different pathological changes in the muscle.

## Translation into clinical application

Another potential therapeutic application is the improvement of the healing after repair. PARP-1 has strong interactions with the profibrotic pathways of the transforming growth factor (TGF-ß) family. TGF-ß is known to induce remodelling after rotator cuff tear, leading to the formation of inferior scar tissue (a process that also happens during the healing after repair).

In our mouse model, TGF-ß was significantly downregulated in the mice lacking PARP-1 in the first week after the tear. Based on this finding, we hypothesised that if PARP-1 is absent or inhibited during the healing after repair then the formation of mechanically inferior scar tissue is reduced, thereby directing the tendon tissue towards the formation of a stronger neo-enthesis. To investigate this hypothesis, our group is currently conducting a study in a rat model where the rotator cuff tear is repaired immediately and PARP-1 is inhibited pharmaceutically. Preliminary results from the project, which is being conducted in collaboration with the Orthopaedic Biomechanics Laboratory of the University of California, USA, point towards an improvement of histological healing when PARP-1 is inhibited.

This research raises hopes to find ways to improve the outcome after rotator cuff tears. Yet, many questions remain unanswered and lots of energy and resources will be needed to translate these findings into clinical applications.

**Michael Künzler**
**Musculoskeletal Research Unit**
**The CABMM**
**University of Zurich**

**http://www.cabmm.uzh.ch/ResearchplatformP/MusculoskeletalResearchUnit.html**