Introduction:
Tendon healing contains several processes including cell infiltration/migration, angiogenesis, cell proliferation, collagen/extra-cellular matrix (ECM) syntheses and tissue remodeling [1]. The urokinase-type plasminogen activator (uPA) system is composed of one enzyme (uPA), one substrate (plasminogen), two receptors (uPA receptor/uPAR and plasminogen receptor) and three PA inhibitors (PAI-1, PAI-2 and protease nexin 1), which has been implicated in cell migration, angiogenesis, ECM synthesis and tissue remodeling [2]. The uPA system has been briefly studied in skin wound and arterial injury [3, 4]. However, little is known about the role of the system in tendon healing. The aim of this study was to investigate the expression pattern of uPA and its receptor (uPAR) in tendon healing.

Methods:
1. Animal experiments:
   All procedures and protocols were approved by the Animal Care and Ethics Committee of the University of New South Wales, Sydney, Australia. Surgical division of the right Achilles tendon was performed as previously outlined [1]. Healing Achilles tendons were harvested at 4, 7, 14 and 21 days following the surgery. The uninjured left Achilles tendons were used as controls.

2. Northern blot:
   Total RNA was extracted from rat Achilles tendons for the indicated time periods post-surgery. 20ug of RNA was used for Northern blot analysis and the same blot hybridized sequentially with 32P-labelled uPAR cDNA, uPA cDNA and 18S rDNA probes. Each experiment was repeated at least two times.

3. Western blot:
   Protein was isolated from tendon tissues (n = 6, for each time point). 40 ug of protein was separated on 10% SDS-PAGE gel, then transferred to a nitrocellulose membrane. After blocking, the membrane was probed with uPAR/uPA mAb. The immunoreactive bands were quantitated by densitometry.

4. Immunohistochemistry:
   Paraffin-embedded tendon tissue sections were cut to 5 um thickness. After deparaffinizing and hydrating, the sections were incubated with uPAR/uPA mAb, and then incubated with biotinylated immunoglobulins, followed with streptavidin peroxidase. Antigenic sites were visualized using diaminobenzidene as the chromagen.

5. Statistical analysis:
   All data are presented as mean ± SE. Differences among experimental groups were assessed using unpaired two-tailed Student’s t-tests and analysis of variance (ANOVA). The level of statistical significance was accepted at P < 0.05.

Results:
1. uPAR/uPA mRNA accumulation during tendon healing:
   Following tendon division, both of uPAR and uPA mRNA were increased on day 4, reached their maximum level (9-fold increase for uPAR and 11-fold increase for uPA) on day 4 and 7, and then decreased quickly to non-injured control values by 21 days post-injury (Fig 1).

2. Effects of tendon injury on uPAR/uPA protein levels:
   Both uPAR and uPA proteins increased to maximal expression level at day 14 (12.6- and 1.3-fold respectively), then decreased on day 21 post-injury. The quantitative densitometry data are presented in Fig 2.

Discussion and Conclusion:
1. Our results show for first time that uPA, and its receptor uPAR are up-regulated during tendon healing. The protein expression was later than their mRNA expression possibly because of a prolonged post-translational modification.

2. The time courses of day 4, 7 and 14 represent the early and middle phases of tendon healing. The uPAR/uPA were induced in these early and middle phases of tendon healing.

3. The increased expression level of the uPAR protein was much higher (12.6-fold) than uPA (1.3-fold) suggesting that uPAR may be more important than uPA itself in controlling uPA activity.

References: