T. Karonitsch 1,5, J. Holinka 2, K. Dalwigk 3, B. Niederreiter 3, C.W. Steiner 3, M. Bilban 3, R. Windhager 2, G. Steiner 3, J. Smolen 5, H. Kiener 3, G. Superti-Furga 1

1 CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna
2 Department of Orthopedics, Medical University of Vienna
3 Division of Rheumatobiology, Department of Medicine 3, Medical University of Vienna
4 Department of Laboratory Medicine, Medical University of Vienna

Ziele: By integrating various microenvironmetal cues, mTOR has evolved as a critical determinant for the maintenance of tissue homeostasis and tissue function. More recent data, however, also indicate that mTOR directs the cellular response to inflammatory stimuli. It remains elusive, whether or not this also applies to fibroblast-like synoviocytes (FLS), especially in the context of rheumatoid arthritis. Therefore, we aim to define the role of mTOR in regulating the mesenchymal tissue response to inflammation in rheumatoid arthritis (RA).

Methoden: To assess mTOR activity in RA-FLS, immunohistochemistry (IHC) as well as immunoblotting (IB) was performed using phosphospecific antibodies to mTOR, AKT and S6K. 3H-Thymidine incorporation was used to investigate the influence of mTOR inhibition on FLS proliferation. FLS viability was assessed by AnnexinV/7-AAD staining and flow cytometry. To further evaluate the significance of mTOR activity for the mesenchymal inflammatory tissue response, a simplified 3-D model of the synovial tissue was used. The GeneChip PrimeView array was used for gene expression profiling. Expression levels of selected candidates were validated by Q-PCR and ELISA.

Ergebnisse: IHC revealed that the mTOR signalling cascade is activated in rheumatoid synovitis. In vitro, TNF stimulation of RA-FLS with TNF resulted in the activation of mTOR, and the two well-known mTOR substrates AKT and S6K, indicating that TNF is one of the factors that is responsible for the activation of the mTOR pathway in vitro. RA-FLS were exposed to TNF in the presence or the absence of Torin, which is a well-known, specific inhibitor of mTOR activity. Strikingly, mTOR inhibition not only prevented the TNF induced activation of AKT and S6K, but also inhibited RA-FLS proliferation in response to TNF stimulation. In line with these data, Torin also prevented the TNF induced lining layer hyperplasia in a 3-D in vitro organ culture system. Importantly, Torin alone in the absence of Torin, which is a well-known, specific inhibitor of mTOR activity.

Zusammenfassung/Schlussfolgerung: These studies provide insight into the regulatory circuits that determine the synovial mesenchymal tissue response to inflammation and suggest a multifaceted regulatory role for the mTOR signalling circuit in RA-FLS.

Figure 1. A) The serine/threonine kinase mTOR is one of the central molecules, that help to maintain cellular and tissue homeostasis by coupling energy, nutrient and oxygen abundance to the execution of cytoskeletal organization, cell growth and metabolism. B) Moreover, mTOR senses cues from the immune microenvironment. Thus, stimulation of RA-FLS with TNF (10 ng/ml) immediately results in the activation of mTOR itself and the well known mTOR substrates P70S6K and AKT. C) Immunohistochemistry reveals the presence of activated forms of mTOR, and the two well known mTOR substrates 4E-BP and S6 in RA synovitis, especially in the synovial lining layer. A few, positive cells were also found in the synovial sublining.

Figure 2. A) For functional in vitro studies, the specific mTOR inhibitor Torin-1 was applied. Western blot analysis revealed that Torin-1 decreased the TNF induced activation of P70S6K and AKT in a dose dependent manner in RA-FLS, indicating that the phosphorylation of these proteins by TNF depends on mTOR. B) TNF is well known to increase FLS proliferation. The addition of Torin-1 (250 nM), however, completely overruled TNF-induced FLS proliferation in a thymidine incorporation assay. C) Importantly, Torin-1 (250 nM) alone or in combination with TNF (10 ng/ml), had no effect on cell viability. Cell viability was assessed by AnnexinV/7-AAD stainings and flow cytometry. D) Torin also prevented the TNF induced lining layer hyperplasia in a 3-D in vitro synovial organ culture system.

Figure 3. A) To further determine the effect of mTOR activation for the TNF driven mesenchymal inflammatory response, RA-FLS (n=5) were exposed to TNF in the presence or the absence of Torin. TNF-stimulation resulted in the upregualted expression of 587 genes (FDR<0,01). Surprisingly, 141 of those transcripts were found in the synovial sublining.

Conclusions
- mTOR plays an ambivalent role in the rheumatoid mesenchymal tissue response to inflammation
  - mTOR promotes FLS proliferation/hyperplasia
  - mTOR inhibits NFkappaB activation