suggests that further optimisation of alginate is required before it could be considered a suitable scaffold for the development of cartilage with structural integrity.

Cell Signaling

290

DANCING TRANSCRIPTION FACTORS: WHAT MAKES SOX9 MOVE?

K. Govindaraj, M. Karperien, J.N. Post. *Developmental BioEngineering, MIRA Institute for Biomedical Technology & Technical Medicine; Univ. of Twente, Enschede, Netherlands*

Purpose: Osteoarthritis (OA) is a multifactorial, degenerative joint disease, affecting millions of people worldwide, and for which no cure is available. One of the main obstacles in finding a cure is that the pathophysiology of OA at the molecular level is as yet unknown. Multiple signaling pathways, such as WNT, BMP, TGF-β, FGF, IGF, and HIF are involved and their interplay at various stages of chondrocyte development and disease still needs to be explored. Methods like qPCR, immunofluorescence and western blotting are traditionally used to understand signaling mechanisms. Though these methods are versatile, they are indirect and not suitable for live cell analysis. As such there is a need for a quick and direct read out of the signaling interplay. We hypothesize that the mobility of transcription factors is directly linked to their activity and that this can be measured by the biophysical technique Fluorescence Recovery After Photobleaching (FRAP). In principle, the mobility of the transcription factors is dependent on their binding to their targets. For example: An active transcription factor is transiently bound to DNA, rendering it immobile whereas an unbound transcription factor will be more highly mobile.

Here, we have applied FRAP to study the mobility of SOX9 (the master transcription factor regulating cartilage development and homeostasis) in response to upstream signals, such as those activated by WNT. In addition, we perturbed the WNT signaling pathway using the WNT antagonists DKK1 and FRZB that we have previously identified to be key in controlling the chondrocyte phenotype.

Methods: C20/A4 cells, immortalized juvenile coastal chondrocytes, were grown on glass coverslips and transfected with SOX9-mGFP or mGFP as a control. Recombinant proteins, WNT3A, DKK1, and sFRP-3 (FRZB), were obtained from R&D systems and were added 24 hours post-transfection at concentrations between 1 and 200 ng/ml. C20/A4 were incubated with the recombinant proteins for at least 30 minutes. FRAP was performed on a Nikon A1 confocal microscope. FRAP measurements were performed in at least 15 cells per condition and the values were averaged to get the final curve. Results were analyzed using Matlab™. To correlate protein mobility with DNA binding, Chromatin immunoprecipitation (ChIP) assays are performed. To study the molecular mechanism underlying increased SOX9 mobility we perform protein half-life determination studies.

Results: Treatment of SOX9-mGFP transfected C20/A4 cells with WNT3A significantly decreased the SOX9 immobile fraction from 39% to 22% (Fig.1 and Fig.2.). This is in line with observations that WNT3A treatment reduces the expression of the SOX9 regulated cartilage specific genes ACAN and COL2A1. Interestingly, the WNT antagonists DKK1 and FRZB did not restore the SOX9 mobility to its control level, but increased the mobility of SOX9 even more. This in line with the finding that DKK1 does not restore the gene expression of ACAN and COL2A1 after WNT treatment of chondrocytes for 24h.

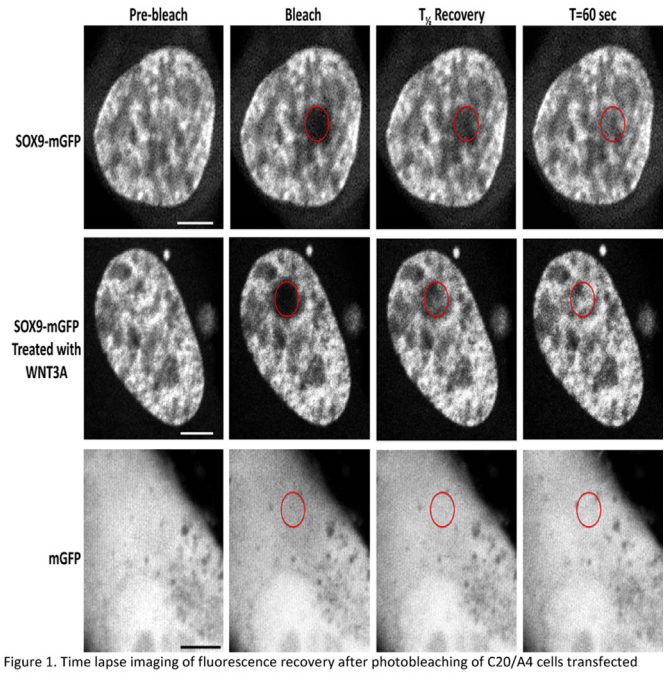

Figure 1. Time lapse imaging of fluorescence recovery after photobleaching of C20/A4 cells transfected with mGFP (control) or SOX9-mGFP with and without WNT3A. Scale bar 5μm.

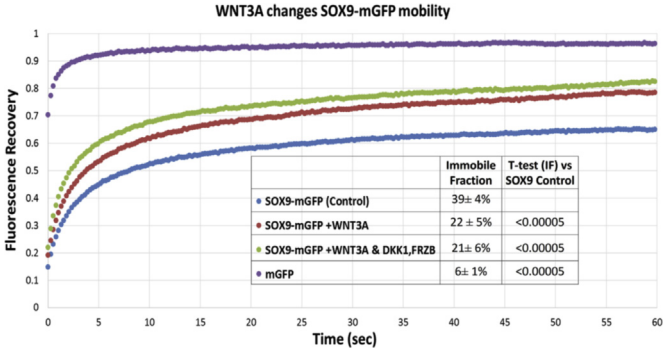

Figure 2. FRAP curve showing change of SOX9-mGFP mobility in response to WNT3A.

Conclusions: We present the application of FRAP for determining the direct effect of perturbation of signaling pathways on SOX9 mobility and show that this mobility change correlates to the activity of SOX9. As a single cell technique, FRAP offers the possibility for investigating cellular responses to external signals in small samples from for example biopsies. We show that FRAP can be used as a quick read-out of SOX9 function without the need for long-term experiments. We therefore propose to use FRAP to help understand the signaling interplay in osteoarthritis pathophysiology and to test potential drug treatments for individual patients.