Conclusion:

stimulated neutrophils. GM-CSF. Type-I IFN enhanced AKT phosphorylation in TNF by GM-CSF, and altered the activation kinetics of ERK and AKT by perpetuate systemic inflammation, may explain why some patients TNFi. The complexity and heterogeneity of inflammatory diseases such consequences sustained the TNF

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233. BIOLOGICAL ROLES OF C5orf30 IN RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS

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Background: A recent genome wide association study identified the variant rs26232 in the first intron of an uncharacterized gene C5orf30 as a RA susceptibility variant1. In addition, it has been associated with severity of radiological joint damage suggesting a role in tissue breakdown2. To date there is no function assigned for C5orf30 and neither the gene or protein show homology to any known functional sequences. However, C5orf30 is highly conserved in chimpanzee, dog, cow, mouse, chicken, and zebrafish (orthologs). The aim of this study is to determine the biological role of C5orf30 in RA synovial fibroblast.

Methods: Immunohistochemistry on synovial samples was used to determine expression of C5orf30 including co-localization using antibodies to macrophages (CD68), fibroblasts (5B5), T (CD3) & B (CD19) cells. Real time PCR and western blotting were used to examine C5orf30 transcript and protein levels in fibroblast-like synovial cells (FLS stimulated with TNF & hypoxia). To investigate gene function siRNA was used to knockdown (KD) either C5orf30 or a non-targeting control (NTC) in synovial FLS in vitro. After knockdown cell viability was assessed by annexin V/propidium iodide staining. Cell invasion and migration were assessed using matrigel invasion and scratch assays and gene expression changes were assessed using an Illumina BeadChip Array.

Results: Confocal microscopy revealed C5orf30 to be strongly expressed in both the nuclear and cytoplasmic compartment of RA synovial lining cells including macrophages and fibroblasts, but not T & B cells. C5orf30 was undetectable in arthroscopy sections obtained from OA or control synovium. C5orf30 was expressed in FLS and was found to be up-regulated by hypoxia (8-fold) and down-regulated by TNF (0.5-fold). We found that C5orf30KD compared with the NTC increased the number of invading FLS using the Matrigel invasion assay (P = 0.01) and increased FLS migration using a scratch assay (P = 0.02) (n = 6). Gene profiling studies suggest that multiple gene sets involved in cell migration, adhesion, angiogenesis, and immune and inflammatory pathways were significantly modified following C5orf30KD.

Conclusion: C5orf30 knockdown increased FLS migration and invasion into matrigel suggesting C5orf30 is negatively regulating cellular invasion. Together this identifies a potentially novel pathway mediating tissue damage in RA. We are currently performing proteomic analysis and animal studies in order to work out the biology of this important marker in the pathogenesis and severity of RA.

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