NEW ARTHRITIC PANNUS-SPECIFIC PROTEIN PROMOTES FIBROBLAST MOTILITY AND POLARIZATION

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Introduction. Rheumatoid arthritis (RA) is chronic inflammatory disease characterized by the development of hypercellular pannus tissue in the affected joints of patients. Pannus invasiveness and activation correlates with stronger tissue destruction and worse clinical prognosis. Using murine arthritis model, we recently discovered that synovial concentration of Collagen Triple Helix Repeat-containing 1 (CTHRC1) message and protein is directly correlated with arthritis severity. In carcinogenesis, overexpression of CTHRC1 is associated with enhanced metastatic potential of solid tumors and increased cell motility. Our goal is to investigate the mechanism of synovial cell motility and invasiveness and the role of non-canonical WNT signaling in pannus development.

Materials and methods. NIH 3T3 fibroblasts were grown in DMEM supplemented with 10% FBS, 10 U/ml penicillin, 10 |ig/ml streptomycin and 2.5 |ig/ml amphotericin B at 37°C and 5% CO_2. Fibroblasts were seeded into micro chemotaxis chambers (ц-Slides Chemotaxis 2D from IBIDI); surface was coated with collagen I. ц-Slides constitutes of two reservoirs connected with a thin bridge, where cells were monitored. Cells were treated with human recombinant CTHRC1 (Sino Biological Inc.) at 33 nM concentration. Time-lapse cell imaging was performed for up to 24 hours with Axio Observer Z1 inverted microscope (Zeiss). Resulting images were analyzed using ImageJ (NIH) and MTrackJ (Biomedical Imaging Group) and Chemotaxis Tool (IBIDI). Cells were manually tracked using MtrackJ plugin for ImageJ and resulting coordinates of at least 20 cell paths were analyzed automatically by Chemotaxis Tool according to parameters like directionality of cell movement, Euclidean distance and accumulated distance travelled by the cells, forward migration index (FMI) and velocity.

Results and discussion. First, we have studied temporal and spatial cell migration conditions to test uniformity of the gradient formation in ц-Slides. We found that initial cell position in FBS gradient influenced cell migration. Maximum effect of the protein upon fibroblast motility was observed after 4 h incubation with CTHRC1 and lasted for 12-24 h. Treatment with recombinant protein had no observable effect upon cell morphology and viability. CTHRC1 significantly induced cell migration by increasing cell movement velocity (30%), Euclidean distance travelled by the cells (%), and directionality of cell movement (%). Assessment of cell polarization measured as longest cell dimension showed significant increase upon CTHRC1 treatment when compared to untreated reference NIH 3T3 fibroblasts (XXXXX% or folds, $p < 0.0000001$).

Conclusions. We established cell motility assay for testing synoviocytes properties. In normal fibroblasts, CTHRC1 promoted cell polarization and motility.

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