Ras family GTPase signaling contributions to inflammation and joint destruction in rheumatoid arthritis

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**Figure 1** / Detection of RasGRF1 protein expression in RA and non-RA synovial tissue.
(a) Representative stainings of RA synovial tissue with control and anti-RasGRF1 antibodies. (b) Representative stainings of RA and OA synovial tissue with anti-RasGRF1 antibodies. Stainings were developed with AEC (red), and counterstained with Mayer’s hematoxyline. Magnification x 100. (c) Quantitative analysis of Ras signaling protein expression in RA and non-RA (OA and ReA) synovial tissue. Integrated optical densities (IOD)/mm², corrected for nucleated cells, for staining of synovial sublining (sub) and intimal lining (lin) layer of 10 RA and 11-non-RA (4 inflammatory OA, 7 ReA) patients with anti-RasGRF1 antibodies. IOD were calculated by computer-assisted image analysis. Box plots represent the 25th to 75th percentiles, the lines within each box the median, and lines outside the boxes designate the 10th and 90th percentiles. Bars indicate statistically significant differences in protein expression between sublining and intimal lining layer tissues within diagnostic groups and between diagnostic groups. * p < 0.05, ** p < 0.01, *** p < 0.005.

![Figure 1](image)

**Figure 2** / Representative double stainings of RA synovial tissue with antibodies against RasGRF1 and cell-specific markers.
Synovial tissue sections were stained overnight with antibodies against RasGRF1, followed by antibodies against CD3, CD55, and CD68. After biotin tyramide enhancement, staining was developed with AEC (red, RasGRF1) and Fast blue (blue, cell-specific markers). Magnification x 100.

![Figure 2](image)
Figure 7 / Association of RasGRF1 expression with MMP production in RA synovial tissue. 
(a) Representative stainings of RA synovial tissue with control and anti-MMP-1, MMP-3, and IL-6 antibodies (magnification x 100).

![Stainings of RA synovial tissue](image)

Figure 8 / Double immunofluorescence labeling of RasGRF1, MMP-1 and MMP-3 in RA synovial tissue. 
RA synovial tissue was stained with combinations of anti-RasGRF1 and either anti-MMP1 (upper panels) or anti-MMP-3 (lower panels). Sections were then stained with fluorochrome-conjugated anti-rabbit Ig (red) and anti-mouse IgG (green) antibodies to visualize RasGRF1 and MMP expression, respectively. Colocalization of RasGRF1 with MMPs is visualized by yellow staining in merged images (right panels).

![Double immunofluorescence labeling](image)
chapter 3

Figure 1 / Ras family homologues are expressed in the synovial tissue of patients with inflammatory arthritis.
(a) Jurkat cells were transfected with control cDNA (mock) or increasing concentrations (μg) of cDNA encoding active H-, K-, and N-RasV12 cDNA as indicated. The specificity of pan-Ras and homologue-specific Ras antibodies was examined by immunoblotting of cell lysates. (b) Expression of Ras proteins in synovial tissue sections of patients with RA and inflammatory OA was examined with pan-, H-, K-, and N-Ras antibodies by IHC (n=5, a representative picture is shown for each staining). (c) RA patient synovial tissue was stained by IHC with control mouse IgG. Magnification: x100. (d) Ras homologues are widely expressed in synovial tissue cells. Immunohistochemical double staining was performed on RA synovial tissue sections to detect T lymphocytes (CD3), FLS (CD55), and macrophages (CD68) (all blue) expressing H-Ras, K-Ras and N-Ras (red). Arrows indicate regions shown in insets. Magnification: x100; x400 (Inset).
Figure 8 / Pan-Ras LNA decrease synovial infiltration, cartilage destruction, and bone erosion in CIA.

(a) Knee joints of arthritic mice treated with scrambled control LNA and pan-Ras LNA were stained with HE and safranin O.
chapter 4

Figure 1 / Expression and phosphorylation of MAPKs in RA and PsA synovial tissue. Representative stainings of RA and PsA synovial tissue with antibodies recognizing phosphorylated (p-) and total p38, ERK and JNK. Stainings were developed with biotin tyramide enhancement, horseradish peroxidase and aminoethylcarbazole, followed by counterstaining with Mayer’s hematoxylin.

Figure 4 / Quantitative analysis of Tie2 expression in RA and PsA. (a) Representative stainings of RA synovial tissue with anti-Tie2 (upper panel) and control rabbit IgG (lower panel) antibodies. (b) Expression of Tie2 in the intimal lining layer (gray bars) and synovial sublining (white bars) of RA and PsA synovial tissue as determined by digital image analysis. Statistically significant differences between data sets are indicated. *** p< 0.005.
Figure 7 / Colocalization of phosphorylated MAPKs with Tie2 in RA and PsA synovial tissue.
RA and PsA synovial tissue sections were stained with primary antibodies and fluorochrome-conjugated secondary antibodies to detect phosphorylated (p-) p38, ERK, or JNK (all green) and Tie2 (red). Colocalization of proteins is visualized by yellow labeling in merged images.
**Figure 8** Cellular distribution of Tie2 expression in RA and PsA synovial tissue. RA and PsA synovial tissue sections were stained with primary antibodies and fluorochrome-conjugated secondary antibodies to detect Tie2 (red) and T lymphocytes (CD3), FLS (CD55), macrophages (CD68 and CD163) or endothelial cells (vWF) (all in green). Localization of Tie2 in specific cell populations is visualized by yellowing labeling in merged images.
Figure 9 | Cellular distribution of phosphorylated ERK in RA and PsA synovial tissue. RA and PsA synovial tissue sections were stained with primary antibodies recognizing phosphorylated (p-) ERK and secondary fluorochrome-conjugated anti-mouse IgG antibodies (red), followed by fluorochrome-conjugated antibodies recognizing T lymphocytes (CD3), FLS (CD55), macrophages (CD163), or endothelial cells (vWF) (all in green). Localization of p-ERK in specific cell populations is visualized by yellow labeling in merged images.
chapter 6

Figure 1 / FoxO family members are expressed and phosphorylated in RA synovial tissue. RA synovial tissue sections were stained with control irrelevant rabbit or goat IgG, or antibodies against phospho (p) PKB, pFoxO1, FoxO1, pFoxO3a, FoxO3a, pFoxO4, and FoxO4 as indicated. Stainings were developed with biotin tyramide enhancement horsera-dish peroxidase and AEC, followed by Mayer’s hematoxyline counterstaining.
Figure 2 / Phosphorylation of FoxO family members in specific cell-types in RA synovial tissue. 
(a) Representative double-staining of RA synovial tissue with phospho-specific anti-FoxO family antibodies and cell-specific markers. Synovial tissue sections were stained overnight at 4°C with anti-phospho (p)FoxO antibodies, followed by antibodies to detect CD3-positive T lymphocytes, CD55-positive FLS, and CD68 and CD163-positive macrophages. After biotin thyramide enhancement, staining was developed with AEC (red, pFoxO protein) and Fast blue (blue, cell-specific markers). Arrowheads show representative double-stained cells, except for pFoxO3a staining in which colocalization with CD3-positive T cells is readily evident. (b) Representative merged micrographs of immunofluorescent double-staining of RA synovial tissue with phosphospecific anti-FoxO antibodies (green) and CD markers (red).
chapter 7

Figure 1 / Expression of angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) in rheumatoid arthritis (RA) and psoriatic arthritis (PsA) synovial tissue.
RA tissue sections were stained with negative control goat antibodies (a) and RA and PsA tissue sections stained with goat anti-Ang1 and anti-Ang-2 antibodies. (b) Stainings were developed with biotin tyramide enhancement, horseradish peroxidase and aminoethylcarbazole, followed by counterstaining with Mayer’s hematoxylin.

Figure 3 / Expression and phosphorylation of Tie2 in RA and PsA synovial tissue. Representative stainings of RA synovial tissue with negative control (a) rabbit antibodies diluted in normal rabbit serum.
(b) Representative stainings of synovial tissue from 3 RA patients with anti-phospho (p)-Tie2 antibodies. Quantitative analysis of Tie2 expression and phosphorylation (p-Tie2) (c) and relative Tie phosphorylation (IOD p-Tie2/IOD Tie2, arbitrary units) (d) in RA and PsA. Data is presented as box plots, where the boxes represent the 25th to 75th percentiles, the lines within the box mark the median value, and lines outside the boxes denote the 10th and 90th percentiles. * p < 0.05.