

Supplementary Materials: Discrepancy between Jun/Fos Proto-Oncogene mRNA and Protein Expression in the Rheumatoid Arthritis Synovial Membrane

René Huber, Bruno Stuhlmüller, Elke Kunisch and Raimund W. Kinne

Table 1. Clinical characteristics of the patients at the time of synovectomy/sampling.

Patients (total)	Gender (m/f)	Age (yrs)	Disease Duration (yrs)	RF (+/-)	ESR (mm/1h)	CRP (mg/l)	# of ARA-Criteria (RA)	Concomitant medication (n)
Rheumatoid Arthritis								
24	6/18	63.3±2.4	16.2±3.3	23/1	46.1±5.2	30.2±8.0	5.2±0.2	MTX (11)
-	-	-	-	-	-	-	-	Prednis. (16)
-	-	-	-	-	-	-	-	Sulfas. (1)
-	-	-	-	-	-	-	-	Leflunomide (2)
-	-	-	-	-	-	-	-	NSAIDs (17)
Osteoarthritis								
24	6/18	70.7±1.6	3.8±0.5	1/19 (n.d. = 4)	21.3±4.8	7.9±4.2	0.2±0.1	MTX (1)
-	-	-	-	-	-	-	-	Prednis. (1)
-	-	-	-	-	-	-	-	NSAIDs (12)
-	-	-	-	-	-	-	-	None (10)
Joint trauma								
9	6/3	32.3±8.9	0.4±0.3	n.d.	n.d.	n.d.	0.0±0.0	L-Thyroxin (1)
-	-	-	-	-	-	-	-	Nifedipin (1)
-	-	-	-	-	-	-	-	Propanolol (1)
-	-	-	-	-	-	-	-	None (5)
Post-mortem								
5	3/2	54.0±11.8	0.0±0.0	n.d.	n.d.	n.d.	0.0±0.0	None (5)

m = male; f = female; yrs = years; RF = rheumatoid factor; +/- = positive/negative; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein, *normal range: < 5 mg/l; ARA = American Rheumatism Association (now American College of Rheumatology); n.d. = not determined; RA = rheumatoid arthritis; MTX = Methotrexate; Prednis. = Prednisolone; Sulfas. = Sulfasalazine; NSAIDs = non-steroidal anti-inflammatory drugs; For the parameters age, disease duration, ESR, CRP, and # of ARA-Criteria (RA) means ± SEM are given, for the remaining parameters numbers (n) are provided.

Table 2. Primer sequences and gene-specific qPCR conditions.

Gene	Primer upstream	Primer downstream	Product (in bp)	Amplification protocol (50 cycles)	Melting curve (1 cycle)
GAPDH	5'- CGGAGTCAA CGGATTTGG - 3'	5'- AGCCTTCTCCA TGGTGGTG -3'	307	denaturation: 5 s, 95°C, primer annealing: 10 s, 53°C, amplification: 20 s, 72°C, additional heating step*: 5 s, 77°C	cooling: 15 s, 58°C
<i>cjun</i>	5'- GCAACTTCA ACCCAGGCG CGCTGA -3'	5'- CGTCTTCAAA ATGTTTGCAAC TG -3'	478	denaturation: 15 s, 95°C, primer annealing: 10 s, 55°C, amplification: 30 s, 72°C, additional heating step*: 5 s, 82°C	cooling: 15 s, 60°C
<i>junB</i>	5'- GAGCTCGTA CCCGACGAC CAC -3	5'- TTCCGCAGCC GCTTGCGCTCC AC -3'	218	denaturation: 15 s, 95°C, primer annealing: 10 s, 60°C, amplification: 13 s, 72°C, additional heating step*: 5 s, 87°C	cooling: 15 s, 72°C
<i>junD</i>	5'- GCCGCCTCC AAGTGCCGC AAG -3'	5'- CGGACTCAGT ACGCGGGCAC CTG -3'	208	denaturation: 10 s, 95°C, primer annealing: 10 s, 63°C, amplification: 15 s, 72°C, additional heating step*: 5 s, 85°C	cooling: 15 s, 68°C
<i>cfos</i>	5' - AGACATGGA CCTATCTGGG TCCT - 3'	5'- TGTAATGCAC CAGCTCGG- GCAGTG -3'	336	denaturation: 5 s, 95°C, primer annealing: 10 s, 58°C, amplification: 25 s, 72°C, additional heating step*: 5 s, 83°C	cooling: 15 s, 68°C

* additional heating step to melt potential primer dimers.

Table 3. Spearman-Rank correlation among the mRNA expression of different proto-onco-genes in the SM of post-mortem/joint trauma controls (n = 10), RA-patients (n = 18), and OA-patients (n = 18).

A: normal controls/joint trauma				
	<i>cfos</i>	<i>cjun</i>	<i>junB</i>	<i>junD</i>
		$r = 0.782$		$r = 0.745$
<i>cfos</i>	-	$P = 0.01$	-	$P = 0.01$
		n = 10		n = 10
	$r = 0.782$			$r = 0.915$
<i>cjun</i>	$P = 0.01$	-	-	$P < 0.01$
	n = 10			n = 10
<i>junB</i>	-	-	-	-
	$r = 0.745$	$r = 0.915$		
<i>junD</i>	$P = 0.01$	$P < 0.01$	-	-
	n = 10	n = 10		
B: OA				
	<i>cfos</i>	<i>cjun</i>	<i>junB</i>	<i>junD</i>
		$r = 0.693$		$r = 0.594$
<i>cfos</i>	-	$P < 0.01$	-	$P = 0.01$

	n = 18		n = 18	
	$r = 0.693$		$r = 0.756$	
<i>cjun</i>	$P < 0.01$	-	$P < 0.01$	
	N = 18		n = 18	
<i>junB</i>	-	-	-	-
	$r = 0.594$	$r = 0.756$		
<i>junD</i>	$P = 0.01$	$P < 0.01$	-	-
	N = 18	n = 18		
C: RA				
	<i>cfos</i>	<i>cjun</i>	<i>junB</i>	<i>junD</i>
			$r = 0.747$	$r = 0.740$
<i>cfos</i>	-	-	$P < 0.01$	$P < 0.01$
			n = 18	n = 18
<i>cjun</i>	-	-	-	-
	$r = 0.747$			$r = 0.588$
<i>junB</i>	$P < 0.01$	-	-	$P = 0.01$
	N = 18			n = 18
	$r = 0.740$		$r = 0.588$	
<i>junD</i>	$P < 0.01$	-	$P = 0.01$	-
	N = 18		n = 18	

r = correlation coefficient, P = P -value (significance), - = no correlation detected.



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