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Role of Fibroblast Activation Protein Alpha in Fibroblast-like Synoviocytes of Rheumatoid Arthritis

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ABSTRACT

Fibroblast-like synoviocytes (FLSs) have been introduced in recent years as a key player in the pathogenesis of rheumatoid arthritis (RA), but the exact mechanisms of their transformation and intracellular pathways have not yet been determined. This study aimed to investigate the role of fibroblast activation protein-alpha (FAP-α) in the regulation of genes involved in the transformation and pathogenic activity of RA FLSs.

Synovial FLSs were isolated from RA patients and non-arthritic individuals (n=10 in both groups) and characterized; using immunocytochemistry and flow cytometry analysis. FLSs were divided into un-treated and Talabostat-treated groups to evaluate the FAP-α effect on the selected genes involved in cell cycle regulation (p21, p53, CCND1), apoptosis (Bcl-2, PUMA), and inflammatory and destructive behavior of FLSs (IL-6, TGF-β1, MMP-2, MMP-9, P2RX7). Gene expression analysis was performed by quantitative real-time polymerase chain reaction (qRT-PCR), and immunoblotting was carried out to evaluate FAP-α protein levels.

The basal level of FAP-α protein in RA patients was significantly higher than non-arthritic

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control individuals. However, no differences were observed between RA and non-arthritic FLSs, at the baseline mRNA levels of all the genes. Talabostat treatment significantly reduced FAP-α protein levels in both RA and non-arthritic FLSs, however, had no effect on mRNA expressions except an upregulated TGF-β1 expression in non-arthritic FLSs.

A significantly higher protein level of FAP-α in FLSs of RA patients compared with that of healthy individuals may point to the pathogenic role of this protein in RA FLSs. However, more investigations are necessary to address the mechanisms mediating the FAP-α pathogenic role in RA FLSs.

Keywords: Fibroblast activation protein alpha; Rheumatoid arthritis; Fibroblast-like synoviocytes

INTRODUCTION

Rheumatoid arthritis (RA) is a progressive immune-related disease that primarily affects the joints and is associated with progressive destruction of cartilage and bone, due to chronic synovial inflammation.[1,2] Indeed, hyperplasia, hyperactivity, and invasion of fibroblast-like synoviocytes (FLSs) into articular cartilage and the underlying bone are the main clinical manifestation of RA.[3,4] In healthy individuals, FLSs play a chief role in nourishing the joint environment, regulating normal leukocyte trafficking, and chemical and immunological homeostasis of the joints. These cells are transformed in the toxic and inflammatory microenvironment of RA and take on an aggressive, invasive, and tumor-like phenotype. Genetic changes markedly contribute to the invasive behavior of rheumatoid arthritis fibroblast-like synoviocytes (RAFLSs).[4-6] On the other hand, the pathophysiological properties of RA FLSs are comparable to cancer-associated fibroblasts (CAFs) which contribute to tumor growth, invasion, and metastasis.[7]

Fibroblast activation protein (FAP) as one of the main markers of CAFs, plays a fundamental role in the pathogenesis of various malignancies, and its expression in RA has also been documented.[8-10] Recent studies have shown that the FAP expression is significantly correlated with tumor growth, tumor invasion depth, blood and lymph node metastasis, advanced pathological stages, immunosuppressive properties of CAFs, and ultimately poor prognosis.[11-15] In addition, it was observed that high expression of FAP in RA FLSs is associated with an invasive phenotype of FLSs accompanied by high proliferation and destruction of the extracellular matrix, and can be considered as a therapeutic target to inhibit the destructive potential of FLSs.[9] Talabostat (L-valinyl-L-boroproline; PubChem CID: 11522448) which is a non-selective inhibitor of the dipeptidyl peptidase (DPP) family, has been introduced as the first clinical inhibitor of FAP.[16,17]

Here, the central role of FAP-αin RA was investigated using cultured FLSs from RA, as well as the non-arthritic individuals, in untreated and treated FLSs with Talabostat. For this purpose, the mRNA expression of FAP-α, the genes involved in the cell cycle (p21, p53, CCND1), apoptosis regulatory genes (Bcl-2, PUMA), efficient mediators (IL6, TGF-β1, matrix metalloproteinases (MMP)-2/-9) and a representative of purinergic signaling (P2RX7) in RA FLSs vs. non-arthritic subjects (knee trauma cases) were evaluated.

MATERIALS AND METHODS

Study Subjects

This study was approved by the Human Research Ethics Committees of Tehran University of Medical Sciences, and informed consent was obtained from all participants (IR.TUMS.MEDICINE.REC.1397.949). Synovial tissues were obtained from 10 RA patients during knee replacement surgery and 10 non-arthritic individuals mostly due to sports trauma and during arthroscopy for ligament reconstructions or meniscus surgeries at the time of joint replacement surgery or synovectomy from Shariati Hospital Orthopedic ward, Tehran, Iran, 2018-2019. The diagnosis of RA conformed to the 2010 American College of Rheumatology (ACR) criteria.[18] RA patients and non-arthritic control group were randomly selected, and matched in terms of age, sex, and race/ethnicity. Six women and four men from RA patients and non-arthritic healthy individuals were included in the study. The mean age of the subjects in RA and non-arthritic control groups was 57.44±11.45 and 51.7±12.73, respectively. Smoker RA subjects were excluded from the study, and the non-arthritic control individuals had

no history of autoimmune, rheumatologic, and cancer diseases, or glucocorticoid medications.

FLS Isolation and Cell Culture Conditions

The extracted synovium fragments were washed sequentially with phosphate-buffered saline (PBS; Gibco Invitrogen, USA), 70% ethanol, and PBS solution containing 2% penicillin, streptomycin, and amphotericin B (Sigma-Aldrich, USA). The tissues were then minced and incubated in serum-free Dulbecco's modified Eagle's medium (DMEM; Gibco, Thermo Fisher Scientific, USA) for 1 hour and 20 minutes at 37°C with 0.5 mg/mL of type VIII collagenase (Sigma-Aldrich, USA).

The samples were then centrifuged at 1000g for ten minutes, the supernatant was carefully removed, and the cell pellet cultured in complete DMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco, BRL, USA), 1% penicillin, and streptomycin (pen-strep). At this stage, FLSs were incubated in a humidified atmosphere containing 5% CO2 so that the cells adhered overnight to the bottom of the flasks. The culture medium was replenished every 2 days, and cells were splitted at a 1:3 ratio and sub-cultured when they reached 70%–80% confluency. Finally, FLSs were used in the characterization assay after the third passage. After confirmation of the fibroblastic nature of the isolated cell, the cells were stored in a nitrogen tank for further experiments.

FLS Characterization; Using Immunocytochemistry (ICC) and Flowcytometry Analysis

The morphology of the FLSs was evaluated using an inverted microscope (Nikon Eclipse TS100, Japan) daily until they reached 70 to 80% confluence (Figure 1). Immunocytochemistry using a specific antibody against fibroblast surface protein (FSP; ab11333, Abcam, UK), 4′,6-diamidino-2-phenylindole (DAPI) dye for the cell nucleus staining and the fluorescence microscopy were done to confirm the high-purity isolation of FLSs[19] and to investigate possible cellular contamination (Figure 2A). For this purpose, 5×10^4 FLS cells were incubated for 24h in a 24-cell plate containing DMEM supplemented with 20% FBS. The supernatant was discarded, and the cultured FLSs were washed with PBS and then incubated for 5min with cold methanol to fix the cells. The cells were washed again with PBS and incubated for 1h with a solution of PBS/Triton-X100 containing 1% bovine serum albumin (BSA) as the blocking buffer. The cells were incubated with primary antibody (anti-FSP) overnight at 4°C and then with sheep anti-mouse Ig-FITC-conjugated secondary antibody (IbnSina, ARI2011F), for 1 hour at room temperature (RT). After counterstaining the nuclei with DAPI, an inverted fluorescence microscope was used to evaluate the stained FLSs.

In the following, FLSs were incubated for 1h at 37°C with fluorescein isothiocyanate (FITC)-conjugated anti-human CD90 (Thy-1) (ab225), CD68 (ab31630), CD44 (ab6124), and CD13 (ab227663) (All from BD bioscience, USA)[20] and appropriate isotype-matched antibodies for 30 min on ice, avoiding exposure to the light. The cells were analyzed using a CyFlow ML flow cytometer (Partec, GmbH, Germany) and data analysis was performed using the FlowJo software (Tree Star, Ashland, USA) (Figure 2B).

Cytotoxicity Analysis by MTT Assay

FLSs were seeded in 48-well plates (7.5×10^3 cells/well) containing 500 µL DMEM supplemented with 10% FBS and 1% pen-strep. The cells were treated with selected concentrations of Talabostat (ValboroPro; PT-100, MedChemExpress, USA) (0.25, 0.5, 1, 2, 4, and 8 µM). After 24 and 48 h of incubation at 37°C, 50 µL of the MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] solution (Sigma-Aldrich, USA) was added to each well and incubated for 4 h at 37°C with 5% CO2. At this point, formazan crystals were observed under an inverted microscope. The MTT containing medium was discarded and crystals were dissolved by adding 500 µL of dimethyl sulfoxide (DMSO; Sigma-Aldrich, USA) under agitation. Finally, the optical density (OD) was read at 570 nm wavelength using an enzyme-linked immunosorbent assay (ELISA) reader (BioTek-ELx800, USA).

FLS Cell Grouping

RA and non-arthritic FLSs with 70-80% confluence were trypsinized and harvested and seeded in T-25 culture flasks at a density of 5×10^3. After dividing the flasks into un-treated and Talabostat-treated (2 µM) groups, they were incubated in a 37°C, 5% CO2 incubator overnight (24 h). At last, the attached FLSs were harvested by trypsinization for next molecular evaluations such as gene expression and western blot analysis.

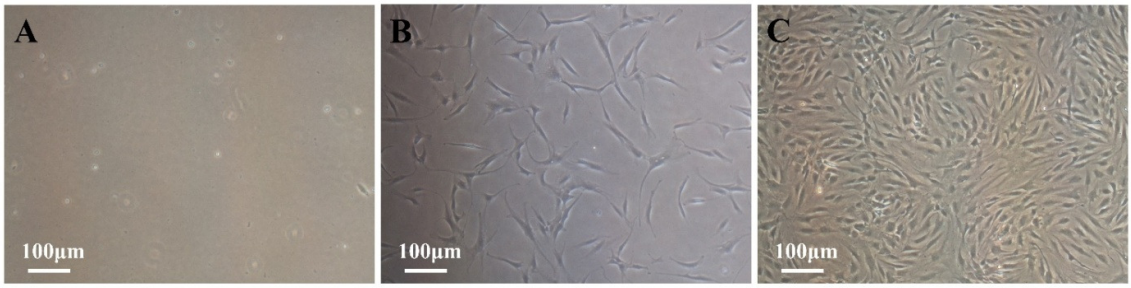

Figure 1. Morphology analysis of fibroblast-like synoviocytes (FLSs): using inverted microscopy. A. FLSs right after isolation. B. FLSs after 48 h and medium replenishments. C. FLSs with 70-80% confluence on culture flask. The magnification is 10× and the scale bar represents 100 micrometers (µm).

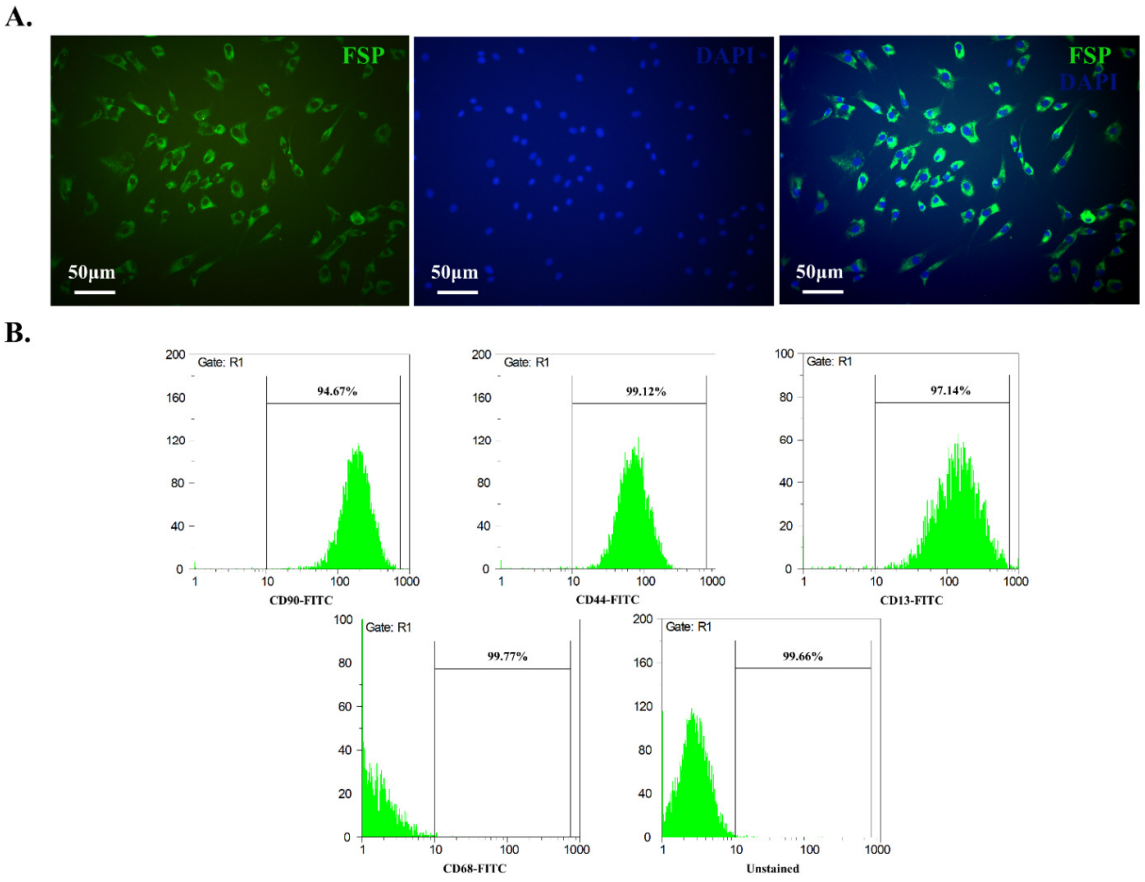

Figure 2. Immunocytochemistry staining and flow cytometry analysis of fibroblast-like synoviocytes (FLSs). A. Stained cultured cells with a specific antibody against fibroblast surface protein (FSP), and 4′,6-diamidino-2-phenylindole (DAPI) dye, to confirm the fibroblastic origin of the cells. The scale bar represents 50 µm. B. Flow cytometry analysis of CD90, CD68, CD44, and CD13 expression in rheumatoid arthritis (RA) FLSs.

Gene Expression Analysis; Using Quantitative Real-time PCR (RT-PCR)

Quantitative RT-PCR (qRT-PCR) was then carried out for gene expression analysis of FAP-α, p21, p53, CCND1, Bcl-2, PUMA, IL-6, TGF-β1, MMP-2, MMP-9, P2RX7, using the RealQ Plus Master Mix Green-high ROX (Ampliqon, Denmark) on a StepOne Real-Time PCR System (Applied Biosystems, USA).

After 24 h treatment of FLSs with 2 μM Talabostat, the total RNA was isolated; using SinaPure-RNA kit (SinaClonBioScience, Iran) according to the manufacturer's instructions. The quality of isolated RNA was evaluated by the NanoDrop ND-2000C spectrophotometer (Thermo Fisher Scientific, USA). Complementary DNA (cDNA) was synthesized from 300 ng of the template RNA; using the RT-ROSET kit (RojeTechnologies, Iran). The PCR conditions of cDNA synthesis and the qPCR reaction conditions were carried out based on the manufactures' protocols. The sequences of primer sets and the respective product sizes were provided in Table 1. The relative mRNA expression levels of target genes were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and the fold changes calculated; using the comparative Ct ($2^{-\Delta\Delta Ct}$) method.[21]

Western Blot Analysis

After 24 h treatment of FLSs with 2 μM Talabostat, RA and non-arthritic FLSs were lysed in ice-cold radio immunoprecipitation assay (RIPA) buffer (Cytomatingene, Iran). The protein concentrations were estimated with the Lowry protein assay.

Equal amounts of cell lysates (50 μg) were run on a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a polyvinylidene difluoride (PVDF) membrane (Thermo Scientific, USA) at 90V for 2.5 h. The membranes were washed thrice in TRIS-buffered saline (TBS).

Table 1. Primer sequences and the product size of the studied genes in a real-time quantitative polymerase chain reaction (qRT-PCR).

Gene name*	Primer sequences (5'→3')	Product size (bp)
FAP-α	F: ATGAGCTTCCTCGTCCAATTCA R: AGACCACCAGAGAGCATATTTTG	215
P21	F: CGATGGAACTTCGACTTTGTCA R: GCACAAGGGTACAAGACAGTG	220
P53	F: TCAACAAGATGTTTTGCCAACTG R: ATGTGCTGTGACTGCTTGTAGATG	118
CCND1	F: CTCGGTGTCCTACTTCAAATG R: TTCTGTTCCTCGCAGACCTC	96
Bcl-2	F: ATCGCCCTGTGGATGACTGAG R: CAGCCAGGAGAAATCAAACAGAGG	129
PUMA	F: GACCTCAACGCACAGTACGAG R: AGGAGTCCCATGATGAGATTGT	98
IL-6	F: AATCATCACTGGTCTTTTGGAG R: GGTTATTGCATCTAGATTCTTTGC	154
TGF-β1	F: CGACTACTACGCCAAGGA R: GAGAGCAACACGGGTTCA	150
MMP-2	F: GATGATGCCTTTGCTCGTGC R: GGTATCCATCGCCATGCTCC	127
MMP-9	F: ACCTCGAACTTTGACAGCGA R: GTTCAGGGCGAGGACCATAG	220
P2RX7	F: TATGAGACGAACAAAGTCACTCG R: GCAAAGCAAACGTAGGAAAAGAT	95
GAPDH	F: GAGTCAACGGATTTGGTCGT R: GACAAGCTTCCCGTTCTCAG	185

*FAP-α: Fibroblast activation protein alpha, CCND1: Cyclin D1, Bcl-2: B-cell lymphoma 2, PUMA: p53 upregulated modulator of apoptosis, IL-6: Interleukin 6, TGF-β1: Transforming growth factor-beta 1, MMP-2: matrix metalloproteinase-2, MMP-9: matrix metalloproteinase-9, P2RX7: purinergic receptor P2X7, GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

The membrane was blocked with 5% skim milk (Sigma- Aldrich, USA) in TBS with Tween (TBST) for 1 h at RT. Then, the membrane was incubated at 4˚C overnight with the anti-human fibroblast activation protein α-antibody (1:1000; ab28244, Abcam, USA) as the primary antibody and β-actin (1:1000; ab8226, Abcam, USA) as the loading control. After incubation for 2 h at RT with goat anti-rabbit HRP-conjugated secondary antibody (db9572, DNAbiotech, IRAN), the protein bands were visualized on X-ray film using an enhanced chemiluminescence (ECL) kit (GE Healthcare, USA), and the protein bands were analyzed using ImageJ software version 1.52 (NIH, USA).

Statistical Analysis

Statistical analysis was performed; using IBM SPSS software version 25 (SPSS Inc., Chicago, IL, USA). The normal distribution of variants was analyzed using Shapiro–Wilk test. The Mann-Whitney U test was used to compare the variables between RA and non-arthritis FLS groups. Besides, mean comparison analysis between un-treated and Talabostat treated samples was performed; using Wilcoxon signed-rank tests. *p* values<0.05 were considered to indicate significance statistically, and all data were represented as the mean±standard deviation. Finally, the GraphPad Prism software version 8.0.2 (GraphPad Software, La Jolla California USA) was used to draw the plots.

RESULTS

FLS Characterization

The fibroblastic phenotype of the isolated cells was first confirmed by inverted microscopy evaluation (Figure 1) and then by the ICC (Figure 2A). Flow cytometric analysis showed the high expression of CD90 (94.67%±3.7%), CD44 (99.12%±2.21%), and CD13 (97.14%±2.06%) surface markers (Figure 2B). Conversely, they were almost negative (99.77%±4.01%) for the CD68 marker (monocyte/macrophage marker).

Cytotoxicity Analysis with MTT Assay

MTT cytotoxicity assay was performed to evaluate the non-cytotoxicity of the selected concentration and time for treatment with Talabostat on FLSs. No different effect was observed on the survival of FLSs after Talabostat treatment with different concentrations and at different times (Figure 3). Although all concentrations studied in MTT assay can be used for cell treatment, the literature review[22,23] and our pilot evaluation (data are not shown) of the gene expression levels showed that 2µMand 24h was the most appropriate concentration and time for Talabostat treatment on FLSs.

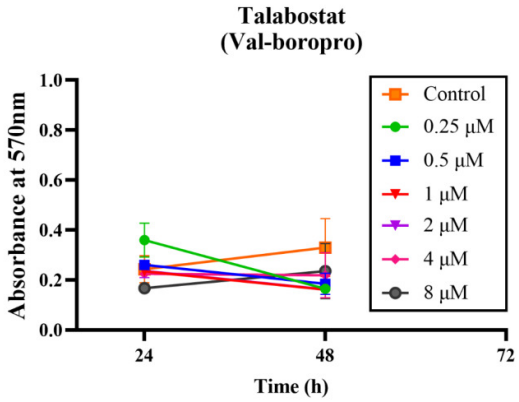

Figure 3. The effects of Talabostat on the viability of fibroblast-like synoviocytes (FLSs). The cultures were supplied with Talabostat, and an un-treated FLS group was cultured; using a pure culture medium. Cytotoxic effects were evaluated with MTT assay after treatment with different concentrations of Talabostat (0.25, 0.5, 1, 2, 4, and 8µM) after 24 as well as 48h. Data are presented as Mean ± SEM.

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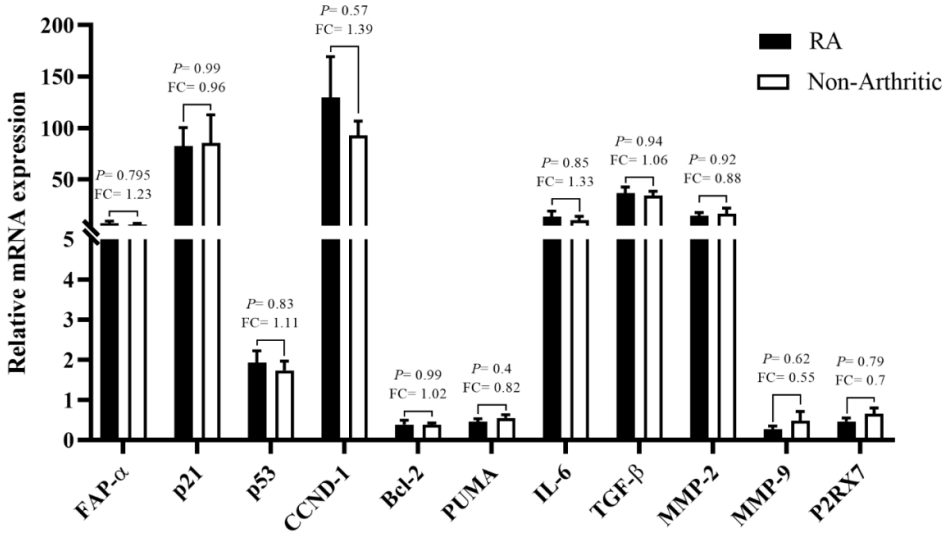

Figure 4. Baseline expression of the selected genes in rheumatoid arthritis(RA) and non-arthritic fibroblast-like synoviocytes (FLSs). Comparison of mRNA expression levels of studied genes in FLSs of RA patients versus the healthy non-arthritic control group did not show any significant differences. (FAP-α: Fibroblast activation protein alpha, CCND1: Cyclin D1, Bcl-2: B-cell lymphoma 2, PUMA: p53 upregulated modulator of apoptosis, IL-6: Interleukin 6, TGF-β1: Transforming growth factor-beta 1, MMP-2: matrix metalloproteinase-2, MMP-9: matrix metalloproteinase-9, P2RX7: purinergic receptor P2X7, FC: Fold Change).

Different Gene Expression Pattern in RA FLSs Compared to Non-arthritic FLSs

The mRNA expression levels of the selected genes were compared between the FLSs of RA patients and healthy non-arthritic control individuals. As shown in Figure 4, there were no significant differences in the mRNA levels of the studied genes between the FLSs extracted from RA patients and non-arthritic individuals.

Talabostat Treatment Reduced FAP-α Protein Levels but Had no Effect on mRNA Levels

There was no significant change in FAP-α mRNA expression in FLSs of RA patients and non-arthritic controls following Talabostat treatment (Figure 5A). The levels of FAP-α protein in RA patients (un-treated states) were significantly higher than non-arthritic control samples (p<0.001) (Figure 5B, C). Talabostat treatment significantly reduced the expression of FAP-α protein in the cell surface of RA FLSs and also non-arthritic cases (p<0.001).

Talabostat Had no Effect on the Expression of the Selected Genes Involved in Regulating Cell Cycle and Apoptosis

There were no statistically significant differences in the mRNA levels of the selected genes involved in the regulation of cell cycle (p21, p53, CCND1) and apoptosis (Bcl-2, PUMA) following treatment (2 μM Talabostat, 24 h) in both RA and non-arthritic FLS groups (Figure 6).

Talabostat Had no Effect on the Expression of the Selected Genes Associated with FLS Activity

In this section, the effect of treatment on P2RX7, a crucial player in inflammation, and the selected cytokine genes including IL-6, TGF-β1, and MMPs including MMP-2 and -9 were investigated to evaluate FLS cell activity. Elevated TGF-β1 level was observed only in the non-arthritic FLSs following treatment with 2 μM of Talabostat for 24 h (p=0.02) (Figure 7). There were no statistically significant differences in the other selected genes associated with FLS activity after treatment in the two FLS groups.

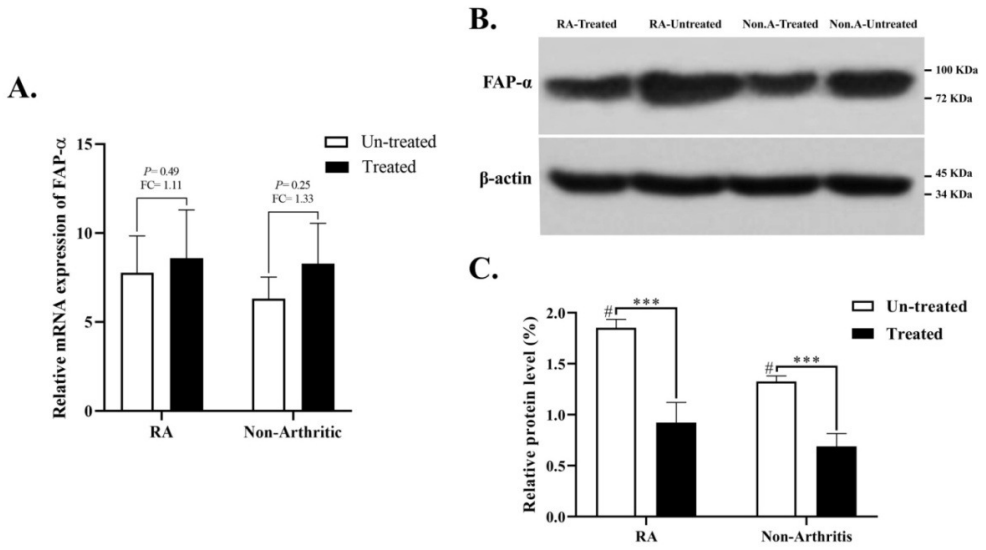

Figure 5. The mRNA and protein expression levels of fibroblast activation protein- alpha (FAP-α) following Talabostat treatment. A. FAP-α mRNA expression in rheumatoid arthritis (RA) and non-arthritic fibroblast-like synoviocytes (FLSs) B. FAP-α protein was detected by FAP-α specific antibody in treated and untreated groups and β-actin used as internal control C. Decreased FAP-α protein levels following Talabostat treatment (2 µM, 24 h) were observed in the FLSs of both RA and non-arthritic control groups (p<0.001). # Also, the basal (untreated) levels of FAP-α protein in RA patients was significantly higher than non-arthritic control samples (*; p<0.001). (Values are expressed as the Mean±SEM. FAP-α: Fibroblast activation protein alpha).**

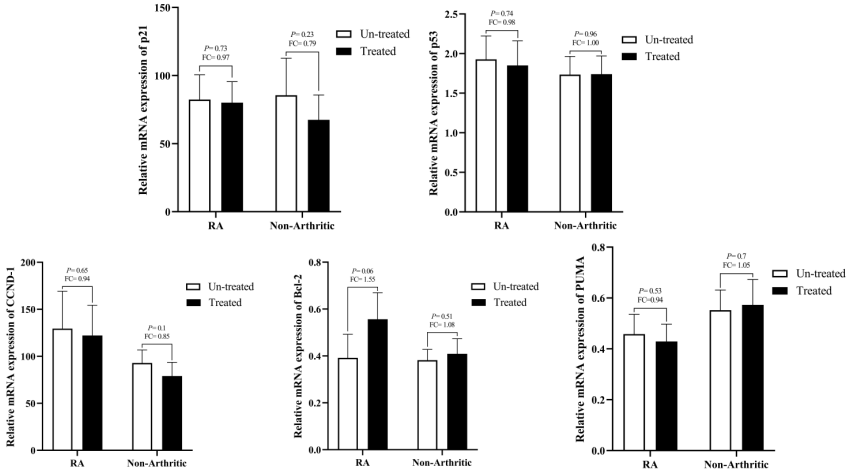

Figure 6. The effect of Talabostat on the expression of the key genes; involved in cell cycle and apoptosis regulation. Talabostat treatment had no significant effect on the expression of selected genes involved in the regulation of cell cycle (p21, p53, CCND1) and apoptosis (Bcl-2, PUMA) in fibroblast-like synoviocytes (FLSs) of rheumatoid arthritis (RA) patients and healthy non-arthritic control group. (Values are expressed as the Mean±Standard error of the mean (SEM). FAP-α: Fibroblast activation protein alpha, CCND1: Cyclin D1, Bcl-2: B-cell lymphoma 2, PUMA: p53 upregulated modulator of apoptosis, FC: Fold Change).

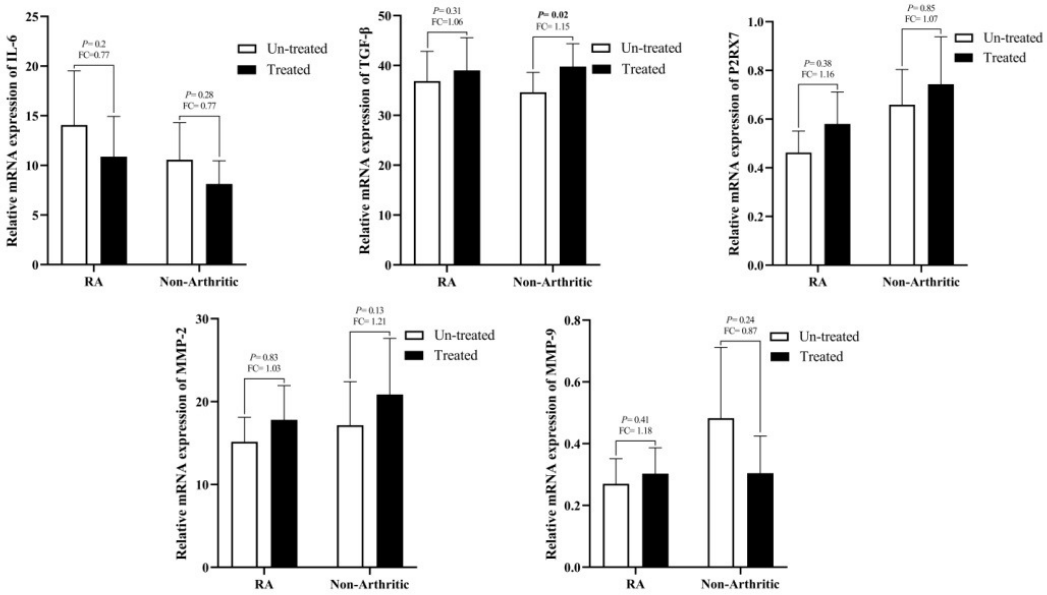

Figure 7. Effect of Talabostat on the expression of some key genes involved in FLS functions. Talabostat treatment had no significant effect on the expression of selected genes associated with fibroblast-like synoviocytes (FLS) activity (IL-6, TGF-β1, and MMP-2/9 along with P2RX7) in rheumatoid arthritis (RA) patients. Only a significant increase in TGF-β1 levels was observed in non-arthritic FLSs following treatment (p=0.02). (Values are expressed as the Mean±SEM. IL-6: Interleukin 6, TGF-β1: Transforming growth factor-beta 1, MMP-2: matrix metalloproteinase-2, MMP-9: matrix metalloproteinase-9, P2RX7: purinergic receptor P2X7, FC: Fold Change).

DISCUSSION

FLSs in rheumatoid arthritis show an altered aggressive phenotype that plays a key role in the disease progression, recruitment of immune cells into the inflamed site, and destruction of joint cartilage and bone.[4,24] On the other hand, the transformed FLSs in RA similar to cancer cells, by rapid proliferation, escape of apoptosis, and high production of growth factors and pro-angiogenic factors, cause the tumor-like expansion of the synovium and increase the local vascular network.[25,26] These pathophysiological characteristics of RA FLSs are reminiscent of the role of CAFs in various neoplasms.

Studies have shown that FAP expression is responsible for the tumor growth and immunosuppressive phenotype of the CAFs.[11,27,28] The FAP expression was significantly associated with cell growth, lymph node metastasis, depth of tumor invasion, and advanced pathological stages.[11-13] It has been further observed that the FAP expression was significantly correlated with the poor prognosis and survival in patients with cancer, and could be used as a predictor for the disease prognosis.[14, 15] In addition, a study shows that FAP acts as an oncogene and could be a potential therapeutic target in oral squamous cell carcinoma (OSCC). Wang et al. reported that downregulation of FAP could inactivate PTEN/ PI3K/ Akt and Ras-Erk signaling pathways in the OSCC, and inhibit tumor cells proliferation and metastasis.[12]

In general, FAP-α protein expression is undetectable in most normal tissues, while it is selectively expressed by cells and tissues under stress, active tissue remodeling, and repair.[29,30] Our present findings showed that the protein expression levels of FAP-α in RA FLSs were significantly higher than non-arthritic individuals. Our results share several similarities with Bauer et al findings that FLSs extracted from refractory RA patients had higher expression levels of FAP at the mRNA transcript and

surface protein compared to end-stage osteoarthritis (OA) patients.[9] Wäldele et al reported a very high expression of FAP on RA FLSs compared to OA FLSs. In addition, they observed that cartilage adhesion capacity and cartilage degradation were decreased in FLSs from a transgenic FAP-deficient mouse model.[10] In this regard, Bartok et al. suggested that targeting FLSs could improve clinical outcomes in RA without systemic immunosuppression.[4] Conversely, Ospelt et al reported increased FAP and CD26 surface expression on RA FLSs, however, inhibition of FAP function using L-glutamyl L-boroproline (PT-630) promotes FLSs invasion through increasing levels of stromal cell-derived factor-1 (SDF-1), MMP-1, and MMP-3 which are downstream effectors of SDF-1.[31]

Here, we investigated the expression of candidate genes related to cell cycle regulation, apoptosis, and cell activity in RA FLSs compared with a healthy non-arthritic control group. Furthermore, to evaluate the role of FAP in FLSs, these assays were performed in the presence of Talabostat (Val-boroPro; PT-100) as an inhibitor of DPPs, and the first clinical inhibitor of FAP.[16] Talabostat inhibits the enzymatic activity of FAP, by cleaving N-terminal Xaa-Pro or Xaa-Ala residues, and as an antineoplastic agent has been directly associated with reduced tumor growth.[13]

Despite the difference in FAP-α protein levels between RA FLSs and non-arthritic FLSs, this difference was not seen in FAP-α mRNA expression levels. In addition, there were no significant differences in mRNA levels of the other studied genes between RA and non-arthritic FLSs. This was probably because all studied RA patients were in the severe stage of the disease and underwent a variety of treatments and biological drugs. These anti-inflammatory and immunosuppressive therapies usually inhibit the expression of effective genes involved in the pathogenesis of the disease. In investigating the role of Talabostat treatment in FLS cell activity, the results showed that the treatment had a significant additive effect on TGF-β1 mRNA levels, only in FLSs of non-arthritic individuals. In the following, our results showed that Talabostat treatment significantly reduced FAP-α protein levels in both RA and non-arthritic FLSs but had no effect on FAP-α mRNA expression levels in both RA and non-arthritic groups. Our results do not support the findings of Egger et al that treatment with Talabostat reduced FAP gene expression in a murine model of pulmonary fibrosis.[32] Studies on mRNA-protein correspondence, which have shown a weak correlation between mRNA levels and protein expression, emphasize the strong contribution of post-transcriptional regulations in determining protein levels.[33]

The RA FLSs have been identified as the main source of proinflammatory cytokines and matrix-degrading enzymes, which are the main cause of inflammation and destruction in the joint.[3,34] Nonetheless, the expected differences in the expression of genes involved in pathogenesis were not well observed, probably because the RA patients studied received severe immunosuppressive as well as anti-inflammatory therapies. Therefore, Talabostat treatment probably did not have a noticeable effect on the expression of the studied genes, despite the effective reduction in FAP-α protein levels. Finally, based on observations at protein levels and our knowledge of FAP-α association with the invasive phenotype of CAFs, expression, and incidence of FAP-α in FLSs is probably a key tool in the transformation of these cells. Even so, it is suggested to evaluate its role in hyperplasia and the destructive activity of FLSs in RA patients with different stages and also new cases.

In conclusion, the results of our present study showed a hyperactive phenotype of RA FLSs correlated with high levels of FAP surface protein. By further elucidating the role of FAP and its downstream factors in the pathogenesis of RA, FAP could become a key therapeutic target in these patients.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

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