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Research Article

Tumor-produced, active Interleukin-1 β regulates gene expression in carcinoma-associated fibroblasts

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ABSTRACT

Recently we described a co-culture model of periodontal ligament (PDL) fibroblasts and SCC-25 lingual squamous carcinoma cells, which resulted in conversion of normal fibroblasts into carcinoma-associated fibroblasts (CAFs), and in epithelial–mesenchymal transition (EMT) of SCC-25 cells. We have found a constitutive high interleukin-1 β (IL1- β) expression in SCC-25 cells in normal and in co-cultured conditions. In our hypothesis a constitutive IL1- β expression in SCC-25 regulates gene expression in fibroblasts during co-culture. Co-cultures were performed between PDL fibroblasts and SCC-25 cells with and without dexamethasone (DEX) treatment; IL1- β processing was investigated in SCC-25 cells, tumor cells and PDL fibroblasts were treated with IL1- β . IL1- β signaling was investigated by western blot and immunocytochemistry. IL1- β -regulated genes were analyzed by real-time qPCR.

SCC-25 cells produced 16 kD active IL1- β , its receptor was upregulated in PDL fibroblasts during co-culture, which induced phosphorylation of interleukin-1 receptor-associated kinase-1 (IRAK-1), and nuclear translocation of NF κ B α . Several genes, including interferon regulatory factor 1 (IRF1) interleukin-6 (IL-6) and prostaglandin-endoperoxide synthase 2 (COX-2) were induced in CAFs during co-culture. The most enhanced induction was found for IL-6 and COX-2. Treatment of PDL fibroblasts with IL1- β reproduced a time- and dose-dependent upregulation of IL1-receptor, IL-6 and COX-2. A further proof was achieved by DEX inhibition for IL1- β -stimulated IL-6 and COX-2 gene expression. Constitutive expression of IL1- β in the tumor cells leads to IL1- β -stimulated gene expression changes in tumor-associated fibroblasts, which are involved in tumor progression.

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Abbreviations: BDNF, brain-derived neurotrophic factor; CAFs, carcinoma-associated fibroblasts; DEX, dexamethasone; EMT, epithelial–mesenchymal transition; HNSCC, head and neck squamous cell carcinoma; IL1- β , interleukin-1 beta; IRAK-1, interleukin-1 receptor-associated kinase-1; IRF1, interferon regulatory factor 1; NF κ B α , nuclear factor kappa beta; IL-6, interleukin-6; PAI1, Plasminogen-activator inhibitor-1; PDLs, periodontal ligament fibroblasts; COX-2, prostaglandin-endoperoxide synthase 2; SDF1, stromal-derived factor 1; TrkB, tropomyosin-like kinase B receptor; TNF- α , tumor necrosis factor alpha.

Introduction

Carcinoma-associated fibroblasts (CAFs), have been extracted from a number of invasive human carcinomas, which are competent to promote the growth of carcinoma cells [1]. A functional property of CAFs is the sustained expression of stromal derived factor 1 (SDF-1) [2,3], which plays a central role in the local invasion of cancer [4]. In tumor cells, stroma microenvironment induces an epithelial–mesenchymal transition (EMT), which is considered as a major biological process in epithelial tumor invasion [5], progression and metastasis. During this process invasive tumor cells tend to lose their epithelial antigens [6], their epithelial cell polarity and morphology, and acquire mesenchymal and stemness-related features [7–9]. EMT is implicated in the progression of primary tumors toward metastases [3]. In our recent report we described a co-culture model of periodontal ligament (PDL) fibroblasts and SCC-25 oral squamous carcinoma cells, which resulted in conversion of normal fibroblasts into CAFs. In the same model EMT occurred in SCC-25 cells, representing its key-events: detection of snail-expression, increase of vimentin production and significant reduction of E-cadherin expression [3]. We have identified CAFs as a major source of BDNF (brain-derived neurotrophic factor) [3], which specifically binds to tropomyosin-like kinase B receptor (TrkB) and drives EMT in the tumor cells [10]. This finding described a novel mechanism for the involvement of the BDNF-TrkB-axis in tumor progression, as it was the first clear demonstration, which showed that conversion of oral fibroblasts into CAFs and EMT in oral carcinoma cells are simultaneous, coordinated events [3]. There are scarce reports about the regulation of BDNF-gene-expression. The role of the inflammatory cytokines in induction of BDNF-expression was described in astrocytes [11]. TNF- α represented a potential factor for regulating the induction of CAFs. TNF- α -expression was also significant in both oral fibroblasts and in carcinoma cells; moreover, in EMT-carcinoma cells its expression significantly increased [3]. A role of TNF- α in regulating EMT has been extensively reported recently [12,13]. In addition, in our previous report we described a constitutive high interleukin-1 β (IL1- β) expression in SCC-25 cells in normal and in co-cultured conditions [3].

Interleukin 1 (IL1- β) is a protein with several biological activities regulating host defense and immune responses. Its' cDNA encodes a precursor polypeptide of 269 amino acids (30.747 kD), which is initially translated as a precursor molecule and subsequently processed into the 15–20 kD protein associated with IL-1 activity [14]. Several genes are regulated by IL1- β including interferon regulatory factor 1 (IRF1), interleukin-6 (IL-6), plasminogen-activator inhibitor-1 (PAI1) and prostaglandin-endoperoxide synthase 2 (COX-2) [15].

In our hypothesis human oral SCC cells produce processed, active IL1- β ; CAFs react on this cytokine and produce IL1- β -regulated gene products. In this study we prove this hypothesis and provide evidences that oral squamous carcinoma cells, via IL1- β -gene expression, protein synthesis and procession induce the expression of IL1- β -regulated genes in CAFs, and initiate the release of major tumor prognostic factors of head and neck oncology into the stroma.

Materials and methods

Cell lines

PDL fibroblasts were routinely cultured in DMEM-low glucose (PAA, Linz, Austria) supplemented with 10% fetal bovine serum (FBS) (PAA), 2 mM L-glutamine, 100 units/ml penicillin, and 100 μ g/ml streptomycin. SCC-25 cells were purchased from the German Collection of Microorganisms and cell cultures (Braunschweig, Germany), and were routinely cultured in DMEM/F12 (PAA, Linz, Austria) supplemented with 10% FBS (PAA), 2 mM L-glutamine, 1 mM sodium pyruvate, 100 units/ml penicillin, and 100 μ g/ml streptomycin [3].

Co-culture

Co-culture between SCC-25 cells and PDL fibroblasts was detailed described before [3].

Briefly, PDL fibroblasts were plated in DMEM-low-glucose/10% FBS at 10^4 /ml on cell culture inserts containing a 0.45 μ m plastic membrane filter, and SCC-25 cells in DMEM/F12/10% FBS at 4×10^4 /ml in wells of six-well plate (Greiner, Kremsmünster, Austria). Each experimental system contained controls, which were plated in the same way. After 48 h the cell culture inserts with the fibroblasts were put together with the wells containing SCC-25 cells. The direct contact of the cells was prevented by a 0.45 μ m plastic membrane, but there was a continuous communication between the upper and the lower cells. The cells in the filter received DMEM-low-glucose/10% FBS, the cells underneath in the wells received DMEM/F12/10% FBS. In the controls the fibroblasts or SCC-25 cells were omitted, but the corresponding medium was always given to the inserts or to the wells. The medium in the co-cultures and in the controls was changed after 3 days. After 7 days co-culture the experiment was finished, the cells in the inserts and in the wells were used for RNA isolation, or for protein fractionation [3]. In some experiments the co-cultured cells were treated with 10^{-6} mol/L dexamethasone (Sigma, Vienna, Austria) as previously reported [15]. In the co-culture system in some cases 1.5 cm in diameter iBidi Dishes (Geradsdorf, Austria) were placed into the 6-well plates under the inserts and PDL fibroblasts were plated into them in 10% fetal bovine serum- (FBS-) supplemented-DMEM-low glucose (PAA, Pasching, Austria) at 10^4 /ml [3]. SCC-25 cells were plated into the cell culture inserts (Greiner) in DMEM/F12/ – 10% FBS (PAA) at 4×10^4 /ml [3]. The co-culture was timely performed as referenced [3]. The SCC-25 cells in the inserts were prepared for protein isolation, the iBidi Dishes were used for NF κ B α immunocytochemistry.

Treatment of PDLs with IL1- β

2×10^5 /ml PDLs were plated in 10% FBS-supplemented-DMEM-low-glucose (PAA, Pasching, Austria) in 10 cm culture plates (Unilab, Innsbruck, Austria). After 48 h, medium was replaced by 0.3% FBS-containing-DMEM. Cells were treated with IL1- β at 0.015–1.5 ng/ml concentration range for 4, 8 and 24 h as described before [16].

RNA extraction, reverse transcription and PCR

Total RNA was isolated and reverse transcribed from control and co-cultured cells as described before [3,17]. All used PCR primers

were published previously: β -actin [18], IL1- β [3], Interleukin-6 (IL-6) [19], Interferon regulatory factor 1 (IRF1) [20], Plasminogen-activator inhibitor-1 (PAI1) [21], and Prostaglandin-endoperoxide synthase 2 (COX-2) [22]. β -actin functioned well as housekeeping gene [3], and did not show significant changes in co-culture conditions compared to controls. The relative gene expression was calculated as previously reported [17].

Protein fractionation, immunoprecipitation and western blot

Cells after co-culture and controls were scraped into 500 μ l extraction buffer/well or/insert, non-nuclear and nuclear protein fractionation were performed as described previously [23]. Non-nuclear protein fractions were subjected to protein concentration measurement [23], and 16 μ g proteins were used for western blots with mouse monoclonal antibodies for NF κ B α (Cat. No: sc-8008) or for Interleukin-1 receptor-associated kinase-1 (IRAK-1) (Cat. No: sc-5288) or with rabbit polyclonal antibody for phospho-IRAK (Cat No: sc-130197). All these primary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and used at 1 μ g/ml final concentration. Nuclear protein fractions were also subjected to protein concentration measurement, and 16 μ g proteins were used for western blot of NF κ B α (Santa Cruz). Blots were processed in a western blot protocol previously detailed described [3].

Serum-reduced (0.3%) DMEM/F12 medium was used for 4 h incubation of SCC-25 cells plated at 10^5 cells/plate in 10 cm in diameter tissue culture dishes (Unilab) 48 h after plating. The conditioned medium was collected and the cells were lysed in an immunoprecipitation buffer (25 mM Tris, 150 mM NaCl, pH:7.5, proteinase inhibitors added as one tablet of Complete Mini/10 ml buffer (Roche, Mannheim, Germany)) following the instructions of the provider of Protein A/G agarose (Cat. No: 20421, Pierce, Rockford, IL, USA). In the conditioned cell supernatants and in the cell lysates immunoprecipitation was performed using a rabbit polyclonal antibody for IL1- β (Cat. No. PAI-28380, Pierce) according to the instruction of the provider of Protein A/G agarose. Immunoprecipitated fractions of the conditioned cell supernatants and cell lysates were taken into 50 μ l $1 \times$ Laemmli SDS-containing gel loading buffer, were subjected to vertical polyacrylamide gel electrophoresis (18% gels) [24], and were trans-blotted to nitrocellulose membranes. The membranes were reacted with polyclonal antibody for IL1- β (Pierce) (1:200 dilution), and were further processed in the western blot protocol as described before [3]. Non-binding fractions and 1 μ g pure rabbit IgG (Pierce) were used as controls for the immunoprecipitation.

If quantitative analysis was required, density of the detected bands was measured by the Image J software [3,17].

Immunocytochemistry and confocal microscopy

iBidi Dishes with control and co-cultured PDLs were used for NF κ B α immunocytochemistry after fixation with methanol for 20 mins at -20°C . Blocking was performed with PBS-buffer containing 5% bovine serum albumin (BSA) (Roth, Karlsruhe, Germany) and 1% normal donkey serum (Jackson Immunosciences, West Grove, PA, USA) for 2 h at room temperature, the primary antibody of mouse monoclonal anti-NF κ B α (Santa Cruz) was used at 1:40 dilution (5 μ g/ml) at 4°C , overnight.

Isotype matching control IgG (Santa Cruz) was used in control reactions; this IgG did not show detectable reactivity. Anti-mouse Alexa Fluor 647- (Invitrogen, Carlsbad, CA, USA) coupled secondary donkey immunoglobulin was used at 1:1200 final dilution in PBS-buffer containing 1% BSA for signal detection by 2 h at room temperature incubation. Finally, iBidi Dishes were covered with DAPI-containing Vectashield (Vector Laboratories, Burlingame, CA, USA) and observed in a Zeiss LSM 510 (Jena, Germany) confocal microscope.

Statistical analysis

Each experiment was performed in three independent sets containing at least three biological repeats/sets, altogether 9 repeats were performed. The relative gene-expression results were tested for normal distribution by D'Agostino & Pearson omnibus normality test using the Graphpad Prism 4.03 (Graphpad Software Inc). Significance of changes in co-culture vs. controls was tested by non-parametric tests (Mann-Whitney) and Students' t-tests depending on the distribution of the data, using Graphpad Prism. The independent experimental sets were then compared for reproducibility. Only reproducible significant changes were considered as "significant". Significance was declared by the standard $p < 0.05$ level.

Results

Synthesis and procession of IL1- β in SCC-25 oral squamous carcinoma cells

Recently we reported a constitutive IL1- β mRNA expression not only in SCC-25 oral squamous carcinoma cells in co-culture with PDL fibroblasts, but also in control cells [3]. In the next step, we investigated the IL1- β protein synthesis and processing in the conditioned supernatant and cell lysate of SCC-25 cells by immunoprecipitation. The active IL1- β protein was expected by 15–20 kD [14], which was found both in the conditioned supernatant and the cell lysate of SCC-25 (Fig. 1A arrow).

IL1- β signaling in co-cultured fibroblasts

At first, the IL1 receptor gene expression was investigated in control and co-cultured SCC-25 cells and fibroblasts, in order to elucidate, which cells might react on the high expressed and processed IL1- β protein of SCC-25 cells. SCC-25 possess low levels of IL1 receptor, which does not change during co-culture ($p = 0.18$; Fig. 1B). PDL fibroblasts express 4.56-times higher levels of IL1 receptor mRNA, which significantly ($p < 10^{-4}$) further increases 3.37-times in co-culture with SCC-25 cells (Fig. 1B).

Following binding of IL1- β protein to the receptor a key event is the phosphorylation of Interleukin-1 receptor-associated kinase 1 (IRAK1). Phosphorylated IRAK1 was also detected in control PDL fibroblasts, but IRAK phosphorylation was significantly induced ($p = 0.02$) in co-cultured fibroblasts (Figs. 2A and C).

After several steps and activation of multiple pathways a further, non-exclusive, but major event in IL1- β signaling is the nuclear translocation of the p65 c-Rel fragment of the transcription factor NF κ B α . In co-cultured fibroblasts a significant increase of nuclear p65 was observed ($p < 0.05$) (Figs. 2B and D).

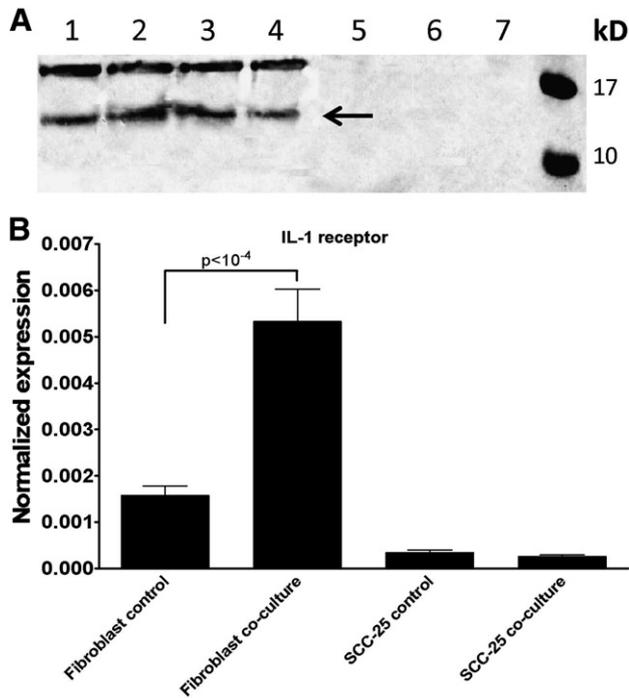


Fig. 1 – A) Detection of IL1- β protein in cell lysate and supernatant of SCC-25 cells by combined immunoprecipitation and western blot. 1–2: supernatant of SCC-25 cells, 3–4: cell lysate of SCC-25 cells, non-antibody-binding fraction of SCC-25 supernatant (5) and cell lysate (6), and 1 μ g anti-IL1- β IgG (7). Arrow represents the active, processed 16 kD IL1- β detectable both in the supernatant and cell lysate of SCC-25 cells. **B)** Normalized gene expression of IL-1 receptor in control and co-cultured fibroblasts and SCC-25 cells. In co-cultured fibroblasts IL-1R gene expression significantly increases.

Using confocal microscopy a further proof was provided for the nuclear localization of p65 NF κ B α (Figs. 3 A-B).

Analysis of IL1- β -regulated genes in co-cultured fibroblasts

Following the investigation of the IL1- β signaling, the expression of known IL1- β – regulated genes was analyzed in co-cultured fibroblasts vs. control cells. IRF1, IL-6 and COX-2 showed significant upregulation in co-cultured fibroblasts compared to control cells ($p < 10^{-4}$), while PAI gene expression did not change significantly ($p = 0.1$). IRF1 increased 3-times, IL-6 60-times and COX-2: 222-times. The most prominent responsive genes for the presence of SCC-25 tumor cells in fibroblasts were: IL-6 and COX-2.

IL1- β -treatment of fibroblasts and SCC-25 cells

PDL fibroblasts were treated with IL1- β following a previously described treatment schedule [16]. The gene expression of IL-1 receptor and of the previously found induced genes: IRF1, IL-6 and COX-2 was investigated. IL-1 receptor, IL-6 and COX-2 showed time- and dose-dependent upregulation, which was statistically significant ($p < 0.05$; Fig. 4) in the case of IL-6 and COX-2. In the same treatment schedule 24 h treatment of IL1- β at 1.5 ng/ml

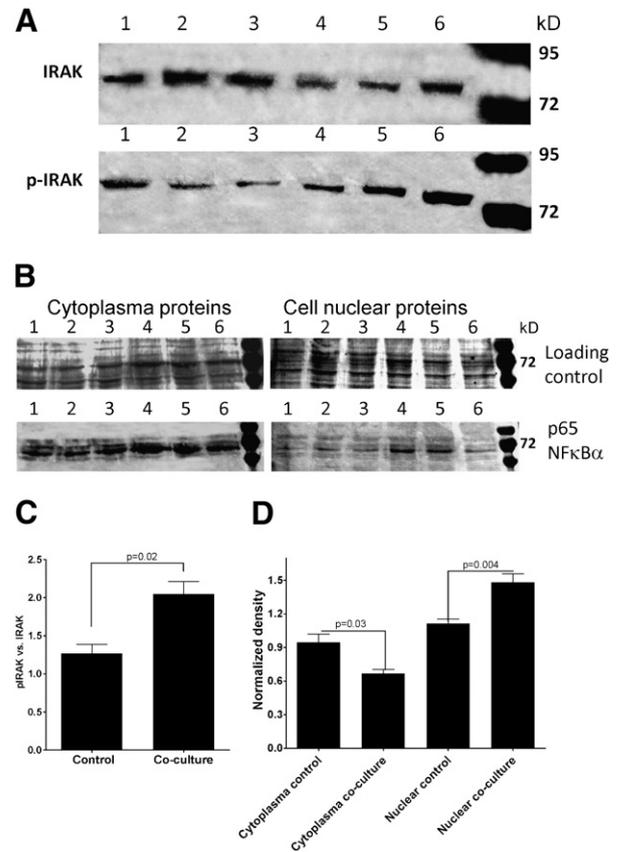


Fig. 2 – IL1- β signaling in co-cultured fibroblasts. A) Detection of IRAK and phosphorylated-IRAK (p-IRAK) protein in cytoplasmic extract of control (1–3) and co-cultured (4–6) fibroblasts. **B)** Detection of NF κ B α protein in cytoplasmic and nuclear extracts of control (1–3) and co-cultured (4–6) fibroblasts. **C)** Densitometry of p-IRAK bands normalized to IRAK bands in cytoplasmic extracts of control and co-cultured fibroblasts. **D)** Densitometry of NF κ B α protein in cytoplasmic and nuclear extracts of control and co-cultured fibroblasts normalized to protein loading controls.

induced a significant upregulation ($p < 10^{-3}$) of COX-2 and of IL-1 receptor ($p = 0.023$) in SCC-25 cells. IL-6 was not significantly regulated by IL1- β ($p > 0.05$) in SCC-25 cells.

Dexamethasone treatment of the co-culture

IL-6 and COX-2 two extensively regulated genes in PDL fibroblasts upon IL1- β -treatment and in co-culture with SCC-25 were investigated in the same co-culture conditions as described before [3], and some of the co-cultures were treated with 10^{-6} mol/L dexamethasone (DEX) [15]. The gene expression of IL-6 decreased to 30.85% (Fig. 5A), of COX-2 (Fig. 5B) to 0.3% in DEX-treated co-cultured fibroblasts of that of only co-cultured fibroblasts.

Discussion

Inflammation is commonly associated with cancer, and the upregulation of proinflammatory mediators has been observed

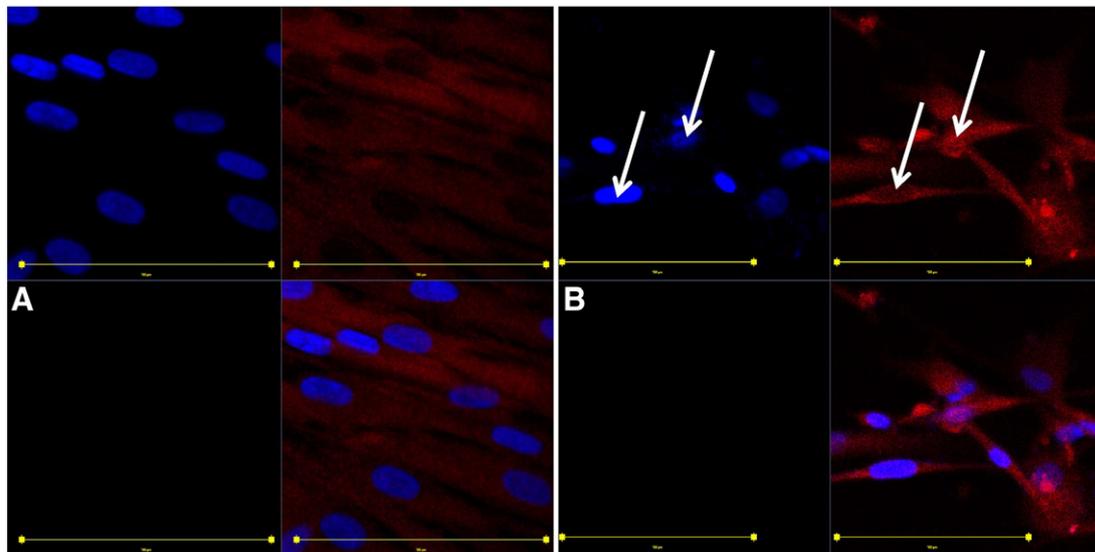


Fig. 3 – NFκBα (red) immunocytochemistry in control (A) and co-cultured fibroblasts (B). Arrows indicate nuclear localization (blue DAPI-detection) of NFκBα (red) reaction. Panels are represented separately and merged. Bars: 100 μm.

in HNSCCs [25]. A previous study reported that, IL-1β enhances activation of a cascade of proinflammatory cytokines and functions as an important autocrine and/or paracrine factor in coordinating expression of a repertoire of cytokines in HNSCC [26]. Authors found that IL-1β caused the downregulation of E-cadherin expression and upregulation of COX-2 expression, showing a direct relationship between EMT occurring in HNSCC tumor cells and inflammatory cytokines [26]. In our recent report we described a co-culture model of periodontal ligament (PDL) fibroblasts and SCC-25 oral squamous carcinoma cells, which resulted in conversion of CAFs from normal fibroblasts, and in EMT in SCC-25 cells [3]. In addition, we have identified CAFs as a major source of BDNF (brain-derived neurotrophic factor) [3], which specifically binds to tropomyosin-like kinase B receptor (TrkB) and drives EMT in the tumor cells [10]. The role of the inflammatory cytokines in induction of BDNF-expression was described before [11]. We also reported a constitutive interleukin-1β (IL1-β) expression in SCC-25 cells in normal and in co-cultured conditions [3]. In our hypothesis a constitutive IL1-β expression in SCC-25 regulates genes in fibroblasts during co-culture. We have proven this regulation step by step. SCC-25 cells produce active, processed IL1-β. PDL fibroblasts possess receptor for IL1-β, and its expression is increased 4.56-times in the presence of SCC-25 tumor cells. IL1-β receptor expression in fibroblasts, especially in CAFs represents a major option in coordination of fibroblast and tumor behavior. A key event in IL1-β signaling, the phosphorylation of IRAK, occurred in co-cultured fibroblasts, which has led to nuclear translocation of NFκBα, and finally to induction of several genes, including BDNF, IRF1, IL-6 and COX-2. The most enhanced induction was found for IL-6 and COX-2 (Fig. 6). Treatment of PDL fibroblasts with IL1-β reproduced a time- and dose-dependent upregulation of IL1-receptor, IL-6 and COX-2. A further proof was achieved by DEX inhibition of IL1-β stimulated gene expression, which was an adaptation of a previously published experimental system of human fibroblast-like synoviocytes [15]. Interestingly,

not only the IL1-β stimulated IL-6 expression, but also the stimulation of COX-2 was inhibited by DEX. All these proofs underline the main finding of this work that, HNSCC-tumor cells produce active IL1-β and stimulate extensive gene expression of IL-6 and of COX-2 in carcinoma-associated fibroblasts. All findings in this study were achieved by complementary methodology using RNA methods, western blots, immunoprecipitation and immunocytochemistry. IL1-β treatment and DEX-inhibition of IL1-β-stimulated gene expression were based on peer-reviewed and published references [15,16].

Both of the extensively regulated genes in CAFs, IL-6 and COX-2, play major roles in the progression of HNSCC. Interestingly, SCC-25 tumor cells might also respond to external IL1-β with increased COX-2 expression. Nevertheless, IL1-β treated tumor cells have no significant contribution to IL-6 levels.

Evidences were reported that IL-6 can directly influence cell proliferation and the invasion potential as the first step of tumor metastasis, in fact, IL-6 increased the invasion potential of several HNSCC cell lines [27]. Sensitive detection technologies have been developed [28] in order to measure serum IL-6, which is the only serum marker that significantly improved outcome prediction. Higher levels of IL-6 were associated with a higher second primary cancer incidence [29]. Further studies revealed that serum IL-6 could be a valuable biomarker for predicting recurrence and overall survival among HNSCC patients. Using IL-6 as a biomarker for recurrence and survival may allow for earlier identification and treatment of disease relapse [30]. From a clinical point of view it is highly important that increased IL-6 levels were found in cachectic patients, whose quality of life was substantially reduced [31]. Based on experimental results in IL-6 gene knock-out mice it was suggested that, IL-6 secretion and cancer cachexia syndrome may be involved in the defense mechanism against tumor progression [32].

Similarly to IL-6 also COX-2 plays a major role in the progression of HNSCC. The overexpression of COX-2 is a frequent

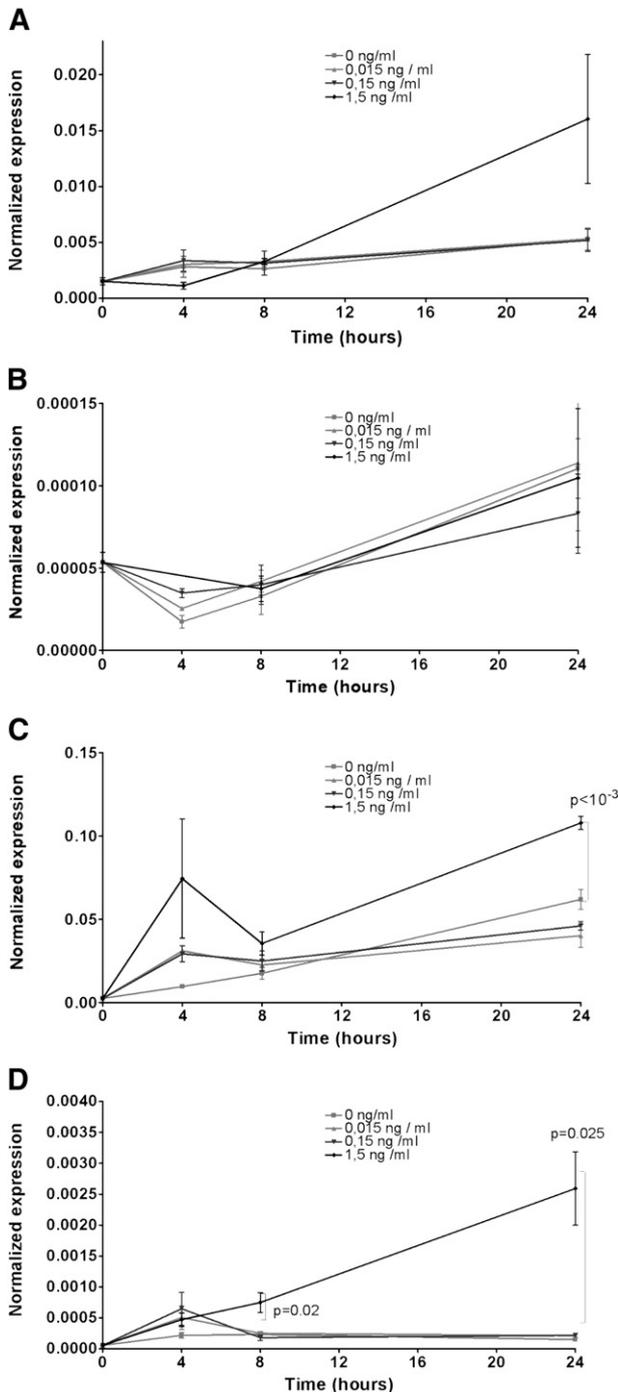


Fig. 4 – Expression of IL1- β -regulated genes in PDL fibroblasts after IL1- β -treatment (IL1 receptor: A, IRF1: B, IL-6: C, COX-2: D). IL1 receptor: (A), IL-6 (C) and COX-2 (D), showed time- and dose-dependent regulation.

event in squamous cell carcinomas of the head and neck (HNSCC), and non-steroidal anti-inflammatory drugs, which are potent inhibitors of COX-1 and COX-2, exert chemopreventive effects on HNSCC cancer development. COX-2 promotes the release of the pro-inflammatory mediator prostaglandin E2 (PGE2), which acts on its cell surface G protein-coupled receptors EP1, EP2, EP3, and EP4. PGE2 produced in the tumor microenvironment by the

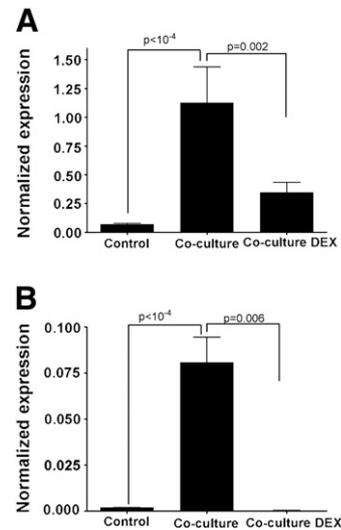


Fig. 5 – Expression of IL-6: (A) and COX-2 (B), in control, co-cultured and Dexamethason (DEX)-treated, co-cultured fibroblasts. IL-6 decreased to 30.85% (A), COX-2 (B) to 0.3% in DEX-treated co-cultured fibroblasts of that of only co-cultured fibroblasts.

overexpression of COX-2 in tumor and stroma cells may promote the growth of HNSCC cells in an autocrine and paracrine fashion by acting on PGE2 receptors that are widely expressed in most HNSCC cancer cells [33].

COX-2 expression is closely correlated to VEGF expression and to tumor vascularisation. Patients with COX-2 tumor overexpression and with higher PgE (2) tumor levels have significantly shorter overall survival estimates [34].

Conclusion

In our work we have provided further evidences that production of proinflammatory cytokines in HNSCC, which is associated with increased tumor growth or metastasis, is promoted by the interaction of neoplastic cells with factors and cells in the host environment. As it was previously reported, the expression of these cytokines by HNSCC cells occurs independently of T and B lymphocyte-mediated immunity [35]. The expression of some proinflammatory cytokines is detectable in the tumor cells and the one of other cytokines in the tumor-associated fibroblasts, and is promoted by tumor–host interactions [35]. Inflammatory regulatory pathways are represented as a major therapeutic target in the HNSCC oncology.

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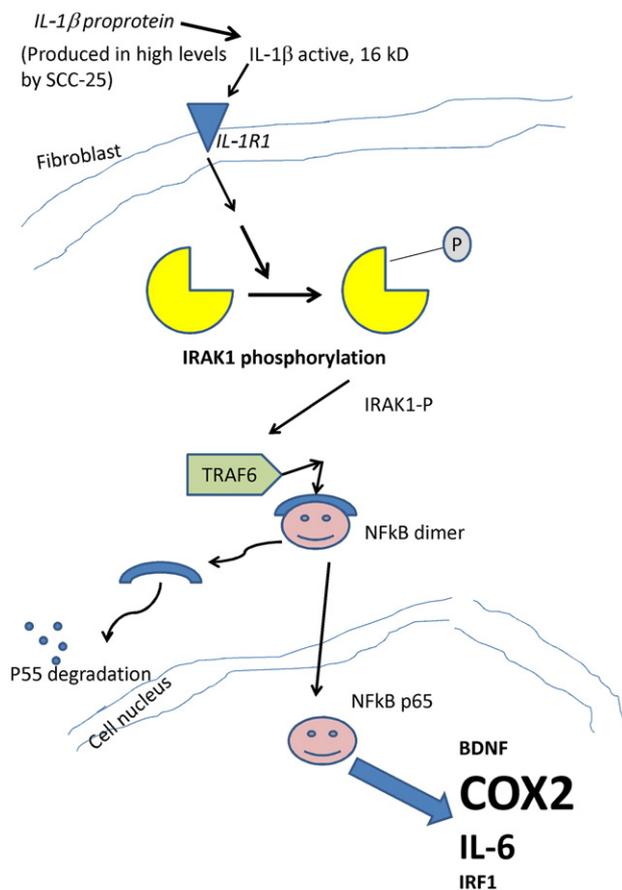


Fig. 6 – Schematic summary of the main regulation mechanism of IL1 β in CAFs. SCC-25 cells produce active, processed 16 kD IL1 β . PDL fibroblasts possess receptor for IL1 β , which leads to the phosphorylation of IRAK, and to subsequent nuclear translocation of NF κ B α , and finally to induction of several genes, including BDNF, IRF1, IL-6 and COX-2.

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