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Expression Profile of Human Gingival Fibroblasts Induced by Interleukin-1 β Reveals Central Role of Nuclear Factor-Kappa B in Stabilizing Human Gingival Fibroblasts During Inflammation

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Abstract

Background—Interleukin (IL)-1 β is a key cytokine in the pathogenesis of periodontitis, and it induces inflammatory mediators in periodontal diseases. We developed immortalized human gingival fibroblasts (HGFs), investigated the effects of IL-1 β on the gene expression using expression arrays containing ~40,000 genes, and tested the role of nuclear factor-kappa B (NF- κ B) in maintaining an activated HGF population.

Methods—Total RNA was isolated from IL-1 β -induced and mock-induced control cells. Gene expression analyses were performed using expression arrays and confirmed by quantitative real-time polymerase chain reaction. Western blot analysis to show inhibitor of kappa B- α (I κ B α) phosphorylation and immunostaining of cells for NF- κ B nuclear translocation were performed. Apoptosis was confirmed by assay of poly ADP-ribose polymerase (PARP) cleavage.

Results—A total of 382 probe sets corresponding to 254 genes were differentially expressed in IL-1 β -induced cells ($P < 0.001$). A total of 215 genes were upregulated, and 39 genes were downregulated. Most notable NF- κ B pathway members (NF κ B1, NF κ B2, I κ B α , I κ B ϵ , I κ B ζ , REL, RELB, and TA-NFKBH) were upregulated. I κ B α was phosphorylated, and NF- κ B accumulated in the nucleus. An IL-1 β -induced set of 27 genes was downregulated by an NF- κ B inhibitor, leading to a decreased number of viable cells and suggesting an antiapoptotic role for NF- κ B.

Conclusions—IL-1 β leads to a large number of significant expression changes consistent with a pathologic role in periodontitis, including enhancement of inflammatory cytokines, chemokines, transcription factors, matrix metalloproteinases, adhesion molecules, and especially NF- κ B-dependent antiapoptotic genes. NF- κ B activation blocks apoptosis, thereby stabilizing the HGF population in inflammation.

Keywords

Fibroblasts; gene expression; interleukin-1 beta; microarrays; nuclear factor-kappa B; real-time polymerase chain reaction

Periodontal diseases are chronic inflammatory conditions that result in the degradation of supporting connective tissue structures surrounding teeth. Periodontal inflammation is driven by the overexpression of proinflammatory cytokines, mainly interleukin (IL)-1 β and tumor necrosis factor- α (TNF- α). IL-1 is one of the major cytokines produced at inflamed sites and is involved in the initiation and progression of connective tissue degradation.^{1,2} IL-1 production is mainly upregulated by microbial products, such as lipopoly-saccharide (LPS).¹ Gingival and periodontal ligament fibroblasts secrete high levels of cytokines, chemokines, matrix metalloproteinases, and adhesion molecules during the progression of periodontal inflammation.² The synthesis, secretion, and biologic activity of IL-1 cytokines have been identified as therapeutic targets for the treatment of periodontitis.³

LPS induces macrophages and fibroblasts to produce inflammatory cytokines, including IL-1 β and -6 and TNF- α , which, in turn, promote the destruction of periodontal tissues, aggravate inflammation, and initiate alveolar bone resorption.⁴ IL-1 β is prominent in the periodontal tissue and gingival crevicular fluid of patients with periodontitis.⁵ Numerous genes downstream of IL-1 β have been identified in gingival fibroblasts and implicated in periodontitis. However, there are only limited data showing the important target genes of nuclear factor-kappa B (NF- κ B) in gingival inflammation because these studies^{6,7} focused on only a single gene or a few genes. Thus, the full spectrum of expression changes induced by IL-1 β has not been fully characterized. Targeting IL-1–signaling pathways is the subject of intense research, and a number of promising compounds have emerged that may modulate IL-1 signaling for therapeutic advantage. Understanding the immunobiology of IL-1 β in human gingival fibroblasts (HGFs) by gene expression arrays will provide broader insight into the pathogenesis of periodontal diseases and may help to identify novel therapeutic targets.

Microarray techniques have been developed recently that may facilitate comprehensive expression analyses of large numbers of defined genes. Microarray analysis is an efficient method for exploring the functions of uncharacterized genes and can be used to find differentially expressed genes that may have diagnostic and therapeutic target potential.⁸ We previously developed and characterized an immortalized line of HGFs that provide a stable model for systematic studies (unpublished observations). In the present study, we identified gene-expression patterns of IL-1 β –stimulated HGFs by using pangenomic oligonucleotide microarrays and identified many important inflammatory intermediates responsive to IL-1 β that might be important targets for novel therapeutic strategies in the treatment of periodontal diseases. IL-1 β seems to play a central role in the regulation of inflammatory genes, such as interleukins, chemokines, and adhesion molecules. Further, we identified the NF- κ B transcription factor family and downstream targets as potential intermediates. Functional studies, using an inhibitor of NF- κ B prior to IL-1 β induction, support a role for NF- κ B in blocking apoptosis and stabilizing activated HGFs during inflammation. Inhibition of NF- κ B may be an important treatment strategy in periodontal inflammation.

MATERIALS AND METHODS

Cell Culture

Primary HGFs[†] were immortalized by human telomerase reverse transcriptase (hTERT) infection and selected with G418.^{§9} Cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 4 mM L-glutamine, and 100 µg/ml penicillin-streptomycin. Cultures were kept at 37°C in a humidified incubator in an atmosphere of 95% air and 5% CO₂. Cells were subcultured at a ratio of 1:4. Cells with a low passage number (<25) after immortalization were used for all experiments. We stimulated cells with 5 ng/ml recombinant human IL-1 β^{||} for 2 hours; this duration allowed us to identify early and intermediate genes and minimize the number of secondary gene inductions.

Gene Expression Analysis

All microarray hybridization analyses were performed in replicates using expression arrays[¶] including 54,675 probe sets to analyze >47,000 transcripts, including 38,500 well-characterized human genes. Isolated total RNA samples were processed as recommended by the manufacturer. In brief, total RNA was initially isolated using reagent[#] and passed through a spin column^{**} for further purification. Eluted total RNAs were quantified with a portion of the recovered total RNA adjusted to a final concentration of 1.25 µg/µl. All total RNA samples were assessed for quality prior to beginning target preparation/processing steps by the application of a small amount of each sample (typically 25 to 250 ng/well) onto an RNA device^{††} that was evaluated on a bioanalyzer.^{‡‡} Single-stranded and then double-stranded (ds) complement (c) DNA was synthesized from the poly(A)+ mRNA present in the isolated total RNA (typically 10 mg total RNA starting material each sample reaction) using a kit^{§§} and poly (T)-nucleotide primers that contained a sequence recognized by T7 RNA polymerase. A portion of the resulting ds-cDNA was used as a template to generate biotin-tagged complement cRNA from an in vitro transcription reaction, using a kit.^{|||} Fifteen micrograms of the resulting biotin-tagged cRNA was fragmented to an average strand length of 100 bases (range, 35 to 200 bases) following prescribed protocols.^{¶¶} Subsequently, 10 mg this fragmented target cRNA was hybridized at 45°C with rotation for 16 hours^{##} to probe sets present on expression arrays.^{***} The arrays were washed and then stained (streptavidin-phycoerythrin),^{†††} followed by scanning on a scanner.^{‡‡‡} The results were quantified and analyzed using software^{§§§} set at default values (scaling, target signal

[†]American Type Culture Collection CRL-2014, Manassas, VA.

[§]Calbiochem, San Diego, CA.

^{||}Calbiochem.

[¶]GeneChip U133 plus 2.0 arrays, Affymetrix, Santa Clara, CA.

[#]TRIzol, Gibco BRL Life Technologies, Rockville, MD.

^{**}RNeasy spin column, Qiagen, Chatsworth, CA.

^{††}Lab-On-A-Chip, Caliper Technologies, Mountain View, CA.

^{‡‡}Agilent Bioanalyzer 2100, Agilent Technologies, Palo Alto, CA.

^{§§}SuperScript Double-Stranded cDNA Synthesis Kit, Invitrogen, Carlsbad, CA.

^{|||}Affymetrix GeneChip IVT Labeling Kit, Affymetrix.

^{¶¶}Affymetrix GeneChip Expression Analysis Technical Manual, Affymetrix.

^{##}Affymetrix GeneChip Hybridization Oven 640, Affymetrix.

^{***}GeneChip U133 Plus 2.0 arrays, Affymetrix.

^{†††}Affymetrix Fluidics Station 450, Affymetrix.

^{‡‡‡}GeneChip Scanner 3000, Affymetrix.

intensity = 500; normalization, all probe sets; and parameters, all set at default values). All arrays analyzed here exhibited >60% present call intensities. Data were analyzed with a statistical software program.^{||||} The analysis module computes regularized *t* tests using a Bayesian estimate of the variance among the gene measurements to infer significant gene changes. $P < 0.001$ genes were accepted as differentially expressed.

Quantitative Real-Time Polymerase Chain Reaction (PCR)

Thirty-one genes (29 were upregulated and two showed no change), including NF κ B1, NF κ B2, NF κ BIA, REL, TRAF1, IRAK2, CSF1, CSF2, ICAM1, VCAM1, IRF1, IL-6, IL-8, PTGS2, CCL2, CCL5, CCL20, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL10, CXCL11, BDKRB1, BDKRB2, CD44, ETS1, STAT5A, TNFRSF6, and TNFAIP2 were analyzed by quantitative real-time PCR. Total RNA was isolated using reagent^{||||} and passed through an RNeasy spin column^{###} for further purification. cDNA was synthesized from 2 mg total RNA using a kit.^{****} mRNA from cDNA samples was amplified with specific primers. The sequence detection system^{††††} was used for quantitative assessment of gene expression. This was done using green dye. Reaction mixtures contained cDNA reverse-transcribed from 2 μ g total-RNA from each sample, 20 μ M each primer, and 12.5 μ l 2 $\times$ SYBR green PCR master mix.^{††††} Thermal cycling conditions were 50°C for 2 minutes (for uracil-DNA N-glycosylase enzyme activity), 95°C for 10 minutes, and 40 cycles at 95°C for 15 seconds followed by 60°C for 1 minute. Experiments were performed in triplicate for each sample in the same reaction and repeated when a coefficient of variation >5% was observed. The GAPDH gene was used as an internal control. The relative quantification was given by the cycle threshold (Ct) values determined for triplicate reactions for test and reference samples for each target and for the internal control gene (GAPDH). The relative expression level was determined as 2^{-Ct} . Evaluation of 2^{-Ct} indicates the fold change in gene expression relative to the reference sample.^{10,11} Values are expressed as n-fold change in target gene expression.

Western Blot Analyses

Immunoblotting was performed by resolving whole-cell lysates on 10% Tris-acetate gel^{§§§§} and blotting onto membranes.^{||||} The membranes were blocked for 1 hour in 5% skim milk, rinsed, and incubated with primary antibodies for phospho-I κ B α , phospho-c-Jun N terminal kinase (JNK), phospho-c-Jun, and poly (ADP-ribose) polymerase (PARP)-1^{||||} in phosphate buffered saline Tween-20 overnight at 4°C. After washings, the membrane was incubated with 0.1 mg/ml peroxidase-labeled secondary antibodies for 45 minutes. Antibody-antigen complexes were detected with a kit.^{####}

§§§§CGOS 1.2 software, Affymetrix.

||||Cyber-t, University of California, Irvine, CA.

||||TRIzol, Gibco BRL Life Technologies.

###RNeasy spin column, Qiagen.

****SuperScript III First-Strand Synthesis SuperMix Kit, Invitrogen.

††††ABI Prism 7900 Sequence Detection System, PE Applied Biosystems, Foster City, CA.

††††2 $\times$ SYBR green PCR Master Mix, PE Applied Biosystems, Warrington, U.K.

§§§§NuPAGE 10% Tris-Acetate Gel, Invitrogen.

||||Immobilon-P membranes, Millipore, Billerica, MA.

||||Santa Cruz Biotechnology, Santa Cruz, CA.

####ECL Kit, Amersham Biosciences, Piscataway, NJ.

Immunostaining of Cells in Culture

A major role of NF- κ B in mediating the response to IL-1 β predicts that cytoplasmic NF- κ B is activated, leading to localization in the nucleus, chromatin binding, and gene activation.¹² Therefore, we tested whether inhibitor of kappa B-alpha (I κ B α) is inactivated by phosphorylation, leading to the accumulation of NF- κ B in the nucleus after IL-1 β stimulation of HGFs. Cells treated with IL-1 β and untreated cells were fixed with 4% formaldehyde for 20 minutes, permeabilized with 0.1% Triton X-100, and incubated with 3% hydrogen peroxide to block endogenous peroxidase activity. Cells were incubated with 5% goat serum for 1 hour to block non-specific binding. Cells were rinsed with phosphate buffered saline and incubated with rabbit anti-NF- κ B p65 primary antibody^{*****} (1:250) overnight at 4°C, secondary biotin-conjugated goat anti-rabbit antibody (1:200) for 20 minutes, and tertiary antibody (peroxidase-conjugated streptavidin)^{†††††} (1:1,000) for 45 minutes. Staining was visualized with a peroxidase substrate kit^{§§§§§} using diaminobenzidine as chromogen.

Cell Proliferation Assays

NF- κ B is believed to mediate apoptotic responses in many settings;^{12,13} therefore, we tested whether IL-1 β is involved in cell proliferation. The cell proliferation rate was analyzed by WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate)-based colorimetric assay.^{§§§§§} Briefly, cells were seeded at a concentration of 1,000 cells/well in a 96-well plate in 100 μ l culture medium and treated with IL-1 β and/or NF- κ B inhibitor (10 μ M).^{|||||||} After the incubation of cells for 24, 48, and 72 hours, 10 μ l WST-1 reagent was added to the cultured cells (1:10 final dilution), and they were shaken for 5 minutes on a shaker. Cells were incubated for 2 hours in the cell culture incubator. Absorbances of samples were measured against the background control using a microplate (enzyme-linked immunosorbent assay) reader at 450 nm wavelength.

Apoptosis Assays

We performed an apoptosis assay using a PARP cleavage detection kit^{¶¶¶¶¶} to test whether the inhibition of cell growth by the NF- κ B inhibitor in this study was due to apoptosis. The kit is designed to detect PARP cleavage by Western blot. PARP is an enzyme implicated in DNA damage-and-repair mechanisms. During apoptosis, PARP is cleaved by the protease caspase-3, an important downstream apoptotic caspase. Cleavage of PARP from the native 116 kDa to 85 kDa is a hall-mark of apoptosis. Cell lysate preparation and the detection of 85-kDa cleaved PARP was described above in the section on Western blot analysis.

***** Santa Cruz Biotechnology.

††††† Jackson ImmunoResearch Laboratories, West Grove, PA.

‡‡‡‡‡ Vector Laboratories, Burlingame, CA.

§§§§§ Roche Applied Bioscience, Mannheim, Germany.

||||||| BAY 11-7082, Calbiochem.

¶¶¶¶¶ Calbiochem.

RESULTS

In the present study, we stimulated cells with IL-1 β for 2 hours, which would allow us to identify genes of early and intermediate expression change, thereby minimizing the number of secondary gene-regulating events. Our preliminary studies indicated that genes of the NF- κ B and mitogen-activated protein kinase pathways and known IL-1 β -responsive genes, including IL-6 and -8 and cyclooxygenase-2, exhibited expression changes within 1 hour after IL-1 β stimulation (data not shown).

Gene Expression Analyses

A total of 382 probe sets corresponding to 254 genes were differentially expressed in IL-1 β -induced cells and compared to control cells. Of 254 genes, 215 genes were upregulated (Table 1) and 39 genes were downregulated (Table 2) by IL-1 β . For these microarray gene expression data, a stringent cutoff P value of 0.001 was chosen. Thus, the false positive rate (FPR) was $54,675 \times 0.001 = 54.7$ probe sets or $\sim 40,000 \times 0.001 = 40$ genes. The FPR corresponds to $54.7 / 382 = 14.3\%$ for probe sets and $40 / 254 = 15.7\%$ for genes, indicating that 214 of 254 genes are real differentially expressed genes in this experiment. In our quantitative real-time PCR validation analyses, we validated 29 of 31 genes (93.5%), indicating that significantly differentially expressed genes identified here accurately characterize mRNA changes.

A large number of transcription factors were significantly altered by IL-1 β stimulation. Potentially more cytokine-regulated genes would be identified at later time points. The literature-supported pathway-analysis program^{#####} showed that, of the 254 significant genes, 126 genes were related to each other in the literature (Tables 1 and 2; bold genes). This means that when we put the differentially expressed genes into a pathway-analysis program that draws the pathways of these genes according to the current literature, 126 genes seemed to be related to each other in one of the pathways. No connectivity in the literature is apparent for 128 genes, suggesting that up to 128 IL-1 β -induced genes may represent new regulatory relationships of IL-1 β , one of the most valuable findings of the present study (Tables 1 and 2). In particular, we noted that one of the largest functionally related gene groups included NF κ B and NF κ B pathway-related genes (e.g., NF κ B1, NF κ B2, I κ B α , I κ B ϵ , I κ B ζ , REL, and RELB). This observation suggests that the NF- κ B pathway may play an important role in the IL-1 β response in HGFs during inflammation.

Experimental Confirmation and Role of NF- κ B

Thirty-one genes were analyzed by quantitative realtime PCR, and 29 genes were validated (93.5%). Of the 29 upregulated genes, 27 genes, including NF- κ B family members (NF κ B1, NF κ B2, NF κ BIA, and REL), TRAF1, IRAK2, CSF1, CSF2, ICAM1, VCAM1, IRF1, IL-6, IL-8, PTGS2, several chemokines (CCL2, CCL5, CCL20, CXCL1, CXCL2, CXCL3, CXCL6, and CXCL10), BDKRB1, BDKRB2, CD44, ETS1, and STAT5A, were downregulated by NF κ B inhibitor plus IL1 β , whereas the addition of the inhibitor alone

#####Pathway Studio, Ariadne Genomics, Rockville, MD.

had no regular effect (Table 3). These results indicate that NF- κ B is involved in mediating the IL-1 β -induced expression changes of this cohort (Table 3).

IL-1 β Activates the NF- κ B Pathway and Promotes Nuclear Translocation in HGFs

Western blot analyses, using whole-cell lysates of HGFs treated with IL-1 β , revealed phosphorylation of I κ B α starting at 30 minutes after treatment with IL-1 β ; it reached a maximum at 1 hour, followed by downregulation at 2 hours (Fig. 1A). One of the mitogen-activated protein kinases (JNK) and its downstream target (c-Jun transcription factor) were phosphorylated after IL-1 β treatment at 15 minutes (Fig. 1A), indicating that at 2 hours, NF- κ B and activating protein (AP)-1 transcription factors might play important roles in the transcription of inflammatory genes by IL-1 β . Immunostaining with anti-NF- κ B p65 antibody showed nuclear translocation of NF- κ B in IL-1 β -treated cells (Fig. 1B).

Cell Growth Rate

Cell proliferation assays did not show any difference in cell growth with IL-1 β treatment compared to non-treated cells, but treatment with the NF- κ B inhibitor plus IL-1 β completely inhibited cell proliferation (Fig. 2A). Treatment of HGFs with IL-1 β alone did not induce apoptosis of cultured cells; however, NF- κ B suppression augmented apoptosis. Initially, cells treated with NF- κ B inhibitor (10 μ M) plus IL-1 β (5 ng/ml) showed little morphologic change; however, at 6 hours, approximately half of the cells exhibited signs of apoptosis, and, by 24 hours, numerous detached and floating cells and extracellular debris were apparent, suggesting apoptosis (Fig. 2B). Western blot analysis of cell extracts with anti-PARP revealed a high proportion of PARP degradation, confirming apoptosis, whereas no degradation was apparent in untreated and IL-1 β -treated cells (Fig. 2B). These results are consistent with a role for NF- κ B in stabilizing HGFs by impeding apoptosis.

Therefore, the results indicate that IL-1 β is a potent inducer of numerous significant expression changes, many of which are associated with the NF- κ B pathway, as judged by specific inhibition studies, and contribute a survival advantage to HGFs via the inhibition of apoptosis.

DISCUSSION

In this study, we used a new model of immortalized HGFs that is particularly relevant to the properties of a major cell mediator of gingivitis, HGFs. We previously developed an immortalized line of HGFs and characterized them with microarray analysis and functional studies. Low-passage immortalized cells provide a stable model for systematic studies. We used these immortalized cells for the present study. It was reported that telomerase-rescued human cells retained all biologic features of their normal progenitors, and hTERT-immortalized cells have normal growth potential.^{14,15}

We observed in the present study that IL-1 β could significantly alter the expression of genes in HGFs involved in inflammation. These genes include transcription factors, cytokines, chemokines, extracellular matrix molecules and enzymes, antiapoptotic genes, growth factors, and adhesion molecules (Table 4). The involvement of IL-1 β in inflammation has been examined in a large number of studies^{4,5,7,16} aimed at developing efficacious biologic

therapies targeting IL-1 β in inflammatory diseases, such as periodontitis and rheumatoid arthritis. Despite the fact that numerous IL-1 β target genes have been identified, the broad spectrum of transcriptional effects of IL-1 β has not been characterized. We performed gene profiling using expression arrays on IL-1 β -stimulated HGFs in an attempt to fully understand the role of proinflammatory cytokine IL-1 β in gingival inflammation. This study reveals new IL-1 β downstream targets. To the best of our knowledge, this is the first study analyzing IL-1 β regulation in HGFs using pangenomic gene expression arrays.

NF- κ B and AP-1 Transcription Factors Are Activated by IL-1 β

IL-1 β was used as stimulus in this study because it activates NF- κ B and AP-1 transcription factors in HGFs, and the expression of these factors is highly correlated with the severity of periodontitis.^{7,17} Binding of IL-1 β to its receptor initiates a signaling cascade leading to the activation of NF- κ B and AP-1.¹⁸ Ambili et al.¹⁹ recently showed that nuclear factor p65 was present in the gingival tissue of 75% of the patients with chronic periodontitis compared to 5% of patients with healthy gingiva. NF- κ B activation is mainly regulated by I κ B proteins. I κ B α is the only inhibitor that dissociates from the NF- κ B complex in response to stimulation, such as by LPS and IL-1 β .²⁰ In the present study, we showed I κ B α phosphorylation and subsequent NF- κ B p65 nuclear translocation, suggesting that NF- κ B activation is regulated by NF- κ B/I κ B α dissociation.

Most of the biologic effects take place in cells after nuclear translocation of NF- κ B and AP-1, two nuclear factors common to many IL-1-induced genes.^{12,21} IL-1 β increases the nuclear binding of c-Jun and c-fos,²¹ the two components of AP-1 complex as confirmed in the present study (Table 1; Fig. 1A). Similar to NF- κ B, AP-1 sites are present in the promoter regions of many IL-1 β -inducible genes. It was shown that NF- κ B and AP-1 work together in regulating the transcription of inflammatory genes.⁷ In the present study, inhibition of NF- κ B controlled the expression of a cohort of many important inflammatory genes.

NF- κ B Inhibition Induces Apoptosis

It has been suggested that NF- κ B activation protects against apoptotic signals.²² Considerable evidence has been presented that NF- κ B induces the expression of antiapoptotic gene products,^{23,24} among them the antiapoptotic regulator Bcl-x_L, which is a known NF- κ B target gene.²⁵ We observed that inhibition of NF- κ B induces apoptosis in cells stimulated by IL-1 β . GADD45A and GADD45B are antiapoptotic genes that increase the survival of hematopoietic cells after exposure to ultraviolet irradiation and certain anticancer drugs.²⁶ In the present study, IL-1 β induced the expression of BCL2A1, BIRC5, and GADD45A genes (Table 1); thereby it might increase the survival of IL-1 β -stimulated cells in inflammation. However, addition of the NF- κ B inhibitor induced apoptosis of HGFs, suggesting a potential therapeutic effect of NF- κ B inhibitors by eliminating IL-1 β -affected cells through programmed cell death from inflammatory sites of periodontitis. This study provides a mechanism by which NF- κ B inhibitors act in IL-1 β -induced inflammation. Similar to our findings, the treatment of synovial cells with TNF- α and IL-1 β alone did not induce apoptosis of cultured synovial cells. However, NF- κ B suppression augmented

apoptosis and cas-pase-3 activity in TNF- α - and IL-1 β -stimulated synovial cells compared to unstimulated cells.²²

IL-1 β Receptor Antagonists as Potential Therapeutic Agents in Periodontal Inflammation

In inflammation, first neutrophils and later mono-cytes/macrophages appear and control aspects of the inflammatory reaction; however, if they cannot limit the immune reaction, they release cytokines to recruit resident cells and immune cells at sites where inflammation is becoming chronic.²⁷ Resident cells play a crucial role in the transition from the acute phase to the chronic phase of inflammation. Our findings reveal many potentially important IL-1 β - stimulated genes in gingival resident cells that lead us to consider preventing IL-1 β binding to the receptor on HGFs as a possible point of intervention. Research into the use of anticytokine therapies in the treatment of periodontitis is at an early stage, although exogenous soluble IL-1 receptor type I and soluble TNF receptor type II applied to the gingival tissue of primates with experimental periodontitis resulted in the inhibition of inflammatory cell infiltration, alveolar bone loss, and connective tissue break-down.²⁸⁻³⁰ Based on the number of proinflammatory gene-expression changes observed here, it might be a good strategy to use IL-1 β receptor antagonists to prevent IL-1 β binding.

NF- κ B Inhibition Can Attenuate the Actions of IL-1 β

The actions of IL-1 β could be attenuated by the addition of an NF- κ B inhibitor to cell culture before activation by blocking of I κ B α phosphorylation.³¹ In agreement with that study, we showed I κ B α phosphorylation within 1 hour of induction by IL-1 β , and we repressed gene expression in a cohort of IL-1 β -induced inflammatory genes with I κ B α phosphorylation inhibitor (BAY-11 7082), indicating that I κ B α is the main inhibitor of NF- κ B activation by IL-1 β in HGFs. Thus, the NF- κ B pathway in HGFs may be an important, if not dominant, pathway that regulates inflammatory gene expression. NF- κ B activation by IL-1 β in the present study protected against apoptotic signals,³² suggesting a strong involvement of NF- κ B in the maintenance of IL-1 β -affected cells in the inflammatory environment, thereby promoting the continued release of inflammatory factors that maintain the inflammatory reaction. Thus, NF- κ B inhibitors induce apoptosis and clear the inflammatory area. These data strongly support the notion that NF- κ B is a potential target for the treatment of inflammatory diseases, such as periodontitis. Further studies are required to confirm these conjectures.

Chemokines are considered key players in the diapedesis of leukocytes from the vasculature into tissues in inflammatory diseases.³³⁻³⁵ Many chemokines were upregulated by IL-1 β in HGFs in the present study. Chemokines produced by fibroblasts stimulate the chemotaxis of neutrophils, macrophages, and T lymphocytes; these inflammatory cells produce inflammatory cytokines and matrix-degradative enzymes. The released enzymes and oxidative metabolites cause degradation of the extracellular matrix; in turn, the inflammatory cytokines stimulate fibro-blasts to produce more chemokines.¹⁶ Upon IL-1 β stimulation, HGFs expressed high levels of several chemokines (CCL2, CCL5, CCL20, CXCL1, CXCL2, CXCL3, CXCL6, and CXCL10) (Table 3), suggesting that IL-1 β promotes the recruitment of leukocytes to the inflammation sites and, therefore, may play a significant role in the progression to the chronic phase of gingival inflammation.

Metallothioneins (MTs), zinc-binding proteins, as a regulator for intracellular zinc signaling increase in aging and chronic inflammation, thereby allowing the continuous sequestration of intracellular zinc with subsequent low zinc ion availability against inflammation. This phenomenon leads to an impaired inflammatory/immune response.^{36,37} It was suggested that the major target of zinc is NF- κ B, and the effects of zinc on the translocation of NF- κ B have been attributed to the suppression of phosphorylation of inhibitory proteins.³⁶ Therefore, zinc deficiency, via MT homeostasis, causes dysregulation of the inflammatory/immune response in chronic inflammation. To the best of our knowledge, this was the first study in which microarray data showed that MTs are upregulated by IL-1 β in HGFs, which might be through NF- κ B, and might contribute to the process of gingival inflammation. This phenomenon needs to be clarified in future studies to understand the role of MTs and zinc in the pathogenesis of periodontal disease.

The development and application of genome-scale technologies for studying the IL-1 β response may help us to understand inflammation and develop new treatment strategies. Targeting the NF- κ B family proteins with inhibitors might suggest a significant role for this transcription factor in regulating gingival inflammation; this needs to be verified in future studies.

CONCLUSIONS

IL-1 β leads to a large number of significant expression changes consistent with a pathologic role in periodontitis, including enhancement of inflammatory cytokines, chemokines, transcription factors, matrix metalloproteinases, adhesion molecules, and, especially, NF- κ B-dependent antiapoptotic genes. NF- κ B activation blocks apoptosis, thereby stabilizing the HGF population in inflammation.

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Figure 1.

A) Western blot analyses of whole-cell lysates using phosphoantibodies of IκB α , c-Jun, and JNK. **B)** Immunostaining of HGFs in culture with NF-κB p65 antibody showing nuclear translocation of NF-κB p65 after IL-1 β treatment compared to untreated control cells (original magnification $\times 10$). IL-1 β -5ng/ml = test; C = control; m = minutes; h = hours; IL-1 β -5ng/ml-1h = test-1 hour.

Figure 2.

A) Growth rates of HGFs after treatment with IL-1 β and IL-1 β plus NF- κ B inhibitor in different concentrations. **B)** Apoptosis assay. OD = optical density; h = hours; test 1 = IL1 β –1 ng/ml; test 2 = IL1 β –5 ng/ml; test 3 = IL1 β –10 ng/ml; INH1 = NF- κ B inhibitor (10 μ M) plus 1 ng/ml IL-1 β ; INH2 = NF- κ B inhibitor plus 5 ng/ml IL-1 β ; INH3 = NF- κ B inhibitor plus 10 ng/ml IL-1 β ; C = control; INH = NF- κ B inhibitor plus 5 ng/ml IL-1 β .

Table 1Genes Upregulated by IL-1 β ($P < 0.001$)

ID	Gene Title	Gene Symbol	Fold Change
209239_at	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)	NFKB1	4.86E+00
207535_s_at	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100)	NFKB2	6.67E+00
201502_s_at	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (I κ B- α)	NFKBIA	1.49E+01
203927_at	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, epsilon (I κ B- ϵ)	NFKBIE	8.61E+00
223218_s_at	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta (I κ B-zeta)	NFKBIZ	2.67E+01
206036_s_at	V-rel reticuloendotheliosis viral oncogene homolog (avian)	REL	5.95E+00
205205_at	V-rel reticuloendotheliosis viral oncogene homolog B, nuclear factor of kappa light polypeptide gene enhancer in B-cells 3 (avian)	RELB	9.58E+00
230052_s_at	T-cell activation NFKB-like protein	TA-NFKBH	1.83E+01
202672_s_at	Activating transcription factor 3	ATF3	4.51E+00
204420_at	FOS-like antigen 1	FOSL1	2.90E+00
201464_x_at	V-jun sarcoma virus 17 oncogene homolog (avian)	JUN	2.46E+00
201473_at	Jun B proto-oncogene	JUNB	3.89E+00
206115_at	Early growth response 3	EGR3	6.81E+00
224833_at	V-ets erythroblastosis virus E26 oncogene homolog 1 (avian)	ETS1	3.09E+00
203010_at	Signal transducer and activator of transcription 5A	STAT5A	4.09E+00
202531_at	Interferon regulatory factor 1	IRF1	2.23E+01
210162_s_at	Nuclear factor of activated t-cells, cytoplasmic, calcineurin-dependent 1	NFATC1	7.02E+00
203973_s_at	CCAAT/enhancer binding protein (c/ebp), delta	CEBPD	4.09E+00
221249_s_at	C/EBP-induced protein /// C/EBP-induced protein	LOC81558	4.79E+00
209706_at	NK3 transcription factor related, locus 1 (<i>Drosophila</i>)	NKX3-1	1.51E+01
203554_x_at	Pituitary tumor-transforming 1	PTTG1	3.33E+00
236982_at	Pituitary tumor-transforming 1 interacting protein	PTTG1IP	6.91E+00
205193_at	V-maf musculoaponeurotic fibrosarcoma oncogene homolog f (avian)	MAFF	3.75E+00
216598_s_at	Chemokine (C-C motif) ligand 2	CCL2	4.61E+02
205476_at	Chemokine (C-C motif) ligand 20 (MIP-3)	CCL20	3.79E+04
1405_i_at	Chemokine (C-C motif) ligand 5	CCL5	1.65E+01
204470_at	Chemokine (C-X-C motif) ligand 1	CXCL1	1.92E+03
204533_at	Chemokine (C-X-C motif) ligand 10	CXCL10	8.23E+00
1569203_at	Chemokine (C-X-C motif) ligand 2	CXCL2	4.40E+01
207850_at	Chemokine (C-X-C motif) ligand 3	CXCL3	1.13E+03
206336_at	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)	CXCL6	1.55E+02
1555464_at	Interferon induced with helicase C domain 1	IFIH1	5.48E+00
226757_at	Interferon-induced protein with tetratricopeptide repeats 2	IFIT2	5.00E+00
229450_at	Interferon-induced protein with tetratricopeptide repeats 3	IFIT3	5.20E+00

ID	Gene Title	Gene Symbol	Fold Change
227125_at	Interferon (alpha, β and omega) receptor 2	IFNAR2	1.18E+01
201642_at	Interferon gamma receptor 2 (interferon gamma transducer 1)	IFNGR2	3.06E+00
219684_at	28 kDa interferon responsive protein	IFRG28	8.80E+00
202270_at	Guanylate binding protein 1, interferon-inducible, 67 kDa /// guanylate binding protein 1, interferon-inducible, 67kDa	GBP1	3.56E+00
242907_at	Guanylate binding protein 2, interferon-inducible	GBP2	9.60E+00
211506_s_at	Interleukin-8	IL-8	1.52E+04
222974_at	Interleukin-22	IL-22	2.00E+02
205207_at	Interleukin-6 (interferon, β 2)	IL-6	3.07E+01
1553740_a_at	Interleukin-1 receptor-associated kinase 2	IRAK2	1.62E+01
227697_at	Suppressor of cytokine signaling 3	SOCS3	4.98E+00
206157_at	Pentraxin-related gene, rapidly induced by IL-1 β	PTX3	2.08E+00
202638_s_at	Intercellular adhesion molecule 1 (CD54), human rhinovirus receptor	ICAM1	5.24E+02
203868_s_at	Vascular cell adhesion molecule 1	VCAM1	1.14E+02
217523_at	CD44 antigen (homing function and Indian blood group system)	CD44	4.99E+00
205173_x_at	CD58 antigen, (lymphocyte function-associated antigen 3)	CD58	3.03E+00
204440_at	CD83 antigen (activated B lymphocytes, immunoglobulin superfamily)	CD83	1.48E+01
204170_s_at	CDC28 protein kinase regulatory subunit 2	CKS2	2.91E+00
227458_at	CD274 antigen	PDCD1LG1	1.21E+01
215078_at	Superoxide dismutase 2, mitochondrial	SOD2	3.15E+04
205027_s_at	Mitogen-activated protein kinase kinase kinase 8	MAP3K8	4.02E+01
201631_s_at	Immediate early response 3	IER3	1.18E+01
224341_x_at	Toll-like receptor 4	TLR4	3.13E+02
242982_x_at	Integrin, β 8	ITGB8	4.73E+00
204580_at	Matrix metalloproteinase 12 (macrophage elastase)	MMP12	5.07E+01
205828_at	Matrix metalloproteinase 3 (stromelysin 1, progelatinase)	MMP3	7.29E+00
209716_at	Colony stimulating factor 1 (macrophage)	CSF1	8.41E+00
210229_s_at	Colony stimulating factor 2 (granulocyte-macrophage)	CSF2	4.88E+01
206432_at	Hyaluronan synthase 2	HAS2	5.85E+00
223541_at	Hyaluronan synthase 3	HAS3	6.01E+00
232458_at	Collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)	COL3A1	3.77E+00
205479_s_at	Plasminogen activator, urokinase	PLAU	3.37E+00
201939_at	Polo-like kinase 2 (<i>Drosophila</i>)	PLK2	2.30E+00
213506_at	Coagulation factor II (thrombin) receptor-like 1	F2RL1	4.80E+00
204363_at	Coagulation factor III (thromboplastin, tissue factor)	F3	5.73E+00
229830_at	Platelet-derived growth factor alpha polypeptide	PDGFA	2.45E+00
231382_at	Fibroblast growth factor 18	FGF18	3.37E+00
210310_s_at	Fibroblast growth factor 5	FGF5	2.42E+00
38037_at	Heparin-binding EGF-like growth factor	HBEGF	5.94E+00
223689_at	IGF-II mRNA-binding protein 1	IMP-1	1.98E+02
207113_s_at	Tumor necrosis factor (TNF superfamily, member 2)	TNF	4.22E+01

ID	Gene Title	Gene Symbol	Fold Change
202510_s_at	Tumor necrosis factor, alpha-induced protein 2	TNFAIP2	6.19E+01
202644_s_at	Tumor necrosis factor, alpha-induced protein 3 (A20)	TNFAIP3	7.06E+01
206025_s_at	Tumor necrosis factor, alpha-induced protein 6	TNFAIP6	1.96E+01
208296_x_at	Tumor necrosis factor, alpha-induced protein 8	TNFAIP8	6.34E+00
221085_at	Tumor necrosis factor (ligand) superfamily, member 15	TNFSF15	5.70E+00
215719_x_at	Fas (TNF receptor superfamily, member 6)	FAS	2.53E+00
207196_s_at	TNFAIP3 interacting protein 1	TNIP1	2.72E+00
205599_at	TNF receptor-associated factor 1	TRAF1	2.06E+01
202870_s_at	TNF receptor-associated factor 4	TRAF4	3.50E+00
235971_at	TRAF-interacting protein with a forkhead-associated domain	TIFA	8.40E+00
220975_s_at	C1q and tumor necrosis factor related protein 1 /// C1q and tumor necrosis factor related protein 1	C1QTNF1	6.13E+00
205681_at	BCL2-related protein A1	BCL2A1	1.19E+01
204908_s_at	B-cell CLL/lymphoma 3	BCL3	6.27E+00
223566_s_at	BCL6 co-repressor	BCOR	5.16E+00
202076_at	Baculoviral IAP repeat-containing 2	BIRC2	1.89E+00
210538_s_at	Baculoviral IAP repeat-containing 3	BIRC3	9.25E+01
202095_s_at	Baculoviral IAP repeat-containing 5 (survivin)	BIRC5	3.18E+00
221436_s_at	Cell division cycle associated 3 /// cell division cycle associated 3	CDCA3	3.61E+00
209714_s_at	Cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)	CDKN3	3.31E+00
210563_x_at	CASP8 and FADD-like apoptosis regulator (anti-apoptosis protein)	CFLAR	2.82E+00
214710_s_at	Cyclin B1	CCNB1	3.74E+00
202705_at	Cyclin B2	CCNB2	2.94E+00
203725_at	Growth arrest and DNA-damage-inducible, alpha	GADD45A	2.12E+00
204641_at	NIMA (never in mitosis gene a)-related kinase 2	NEK2	3.57E+00
202954_at	Ubiquitin-conjugating enzyme E2C	UBE2C	3.32E+00
202779_s_at	Ubiquitin-conjugating enzyme E2S	UBE2S	1.95E+00
227489_at	SMAD specific E3 ubiquitin protein ligase 2	SMURF2	2.08E+00
207510_at	Bradykinin receptor B1	BDKRB1	2.75E+01
205870_at	Bradykinin receptor B2	BDKRB2	4.92E+00
229055_at	G protein-coupled receptor 68	GPR68	3.76E+00
223767_at	G protein-coupled receptor 84	GPR84	5.64E+00
209324_s_at	Regulator of G-protein signaling 16	RGS16	1.27E+01
202388_at	Regulator of G-protein signaling 2, 24kDa	RGS2	3.93E+00
203823_at	Regulator of G-protein signaling 3	RGS3	5.70E+00
228176_at	Endothelial differentiation, sphingolipid G-protein-coupled receptor, 3	EDG3	3.08E+00
208394_x_at	Endothelial cell-specific molecule 1	ESM1	1.51E+01
232017_at	Tight junction protein 2 (zona occludens 2)	TJP2	5.72E+00
218113_at	Transmembrane protein 2	TMEM2	4.03E+00
216005_at	Tenascin C (hexabrachion)	TNC	1.52E+01

ID	Gene Title	Gene Symbol	Fold Change
210190_at	Syntaxin 11	STX11	6.24E+00
214520_at	Forkhead box C2 (MFH-1, mesenchyme forkhead 1)	FOXC2	7.35E+00
205659_at	Histone deacetylase 9	HDAC9	4.82E+00
218755_at	Kinesin family member 20A	KIF20A	3.21E+00
209212_s_at	Kruppel-like factor 5 (intestinal)	KLF5	8.60E+00
230636_s_at	Kruppel-like factor 9	KLF9	6.45E+00
214470_at	Killer cell lectin-like receptor subfamily B, member 1 /// killer cell lectin-like receptor subfamily B, member 1	KLRB1	5.23E+00
220091_at	Solute carrier family 2 (facilitated glucose transporter), member 6	SLC2A6	3.63E+00
212110_at	Solute carrier family 39 (zinc transporter), member 14	SLC39A14	3.21E+00
202357_s_at	B-factor, properdin	BF	7.89E+00
205013_s_at	Adenosine A2a receptor	ADORA2A	2.55E+01
201272_at	Aldo-keto reductase family 1, member B1 (aldose reductase)	AKR1B1	1.66E+00
207992_s_at	Adenosine monophosphate deaminase (isoform E)	AMPD3	1.02E+01
1552619_a_at	Anillin, actin binding protein (scraps homolog, <i>Drosophila</i>)	ANLN	3.45E+00
203586_s_at	ADP-ribosylation factor 4-like	ARF4L	2.93E+00
233620_at	Rho guanine nucleotide exchange factor (GEF) 12	ARHGEF12	7.35E+02
221485_at	UDP-Gal:βGlcNAc β 1,4- galactosyltransferase, polypeptide 5	B4GALT5	2.73E+00
221530_s_at	Basic helix-loop-helix domain containing, class B, 3	BHLHB3	8.14E+00
229437_at	BIC transcript	BIC	1.16E+02
227143_s_at	BH3 interacting domain death agonist	BID	3.41E+00
220987_s_at	Chromosome 11 open reading frame 17 /// chromosome 11 open reading frame 17 /// NUA family, SNF1-like kinase, 2 /// NUA family, SNF1-like kinase, 2	C11orf17 /// NUA	AK217E+01
221182_at	Chromosome 1 open reading frame 129	C1orf129	4.14E+02
226936_at	Chromosome 6 open reading frame 173	C6orf173	3.02E+00
228695_at	Chromosome 8 open reading frame 46	C8orf46	7.70E+00
203921_at	Carbohydrate (N-acetylglucosamine-6-O) sulfotransferase 2	CHST2	3.42E+00
1554862_at	Cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMP-N-acetylneuraminate monooxygenase)	CMAH	1.37E+01
221903_s_at	Cylindromatosis (turban tumor syndrome)	CYLD	3.48E+00
228915_at	Dachshund homolog 1 (<i>Drosophila</i>)	DACH1	6.77E+00
235545_at	DEP domain containing 1	DEPDC1	3.57E+00
203764_at	Discs, large homolog 7 (<i>Drosophila</i>)	DLG7	3.56E+00
204135_at	Downregulated in ovarian cancer 1	DOC1	6.07E+00
209039_x_at	EH-domain containing 1	EHD1	2.45E+00
227180_at	ELOVL family member 7, elongation of long chain fatty acids (yeast)	ELOVL7	5.63E+02
201341_at	Ectodermal-neural cortex (with BTB-like domain)	ENC1	4.12E+00
227844_at	Formin-like 3	FMNL3	4.86E+00
234830_at	Similar to FSHD region Gene 2 protein	FRG2	5.09E+02
204224_s_at	GTP cyclohydrolase 1 (dopa-responsive dystonia)	GCH1	6.02E+03
204472_at	GTP binding protein overexpressed in skeletal muscle	GEM	7.53E+00
232080_at	HECT, C2 and WW domain containing E3 ubiquitin protein ligase 2	HECW2	2.15E+00

ID	Gene Title	Gene Symbol	Fold Change
212642_s_at	Human immunodeficiency virus type I enhancer binding protein 2	HIVEP2	5.95E+00
221458_at	5-hydroxytryptamine (serotonin) receptor 1F	HTR1F	6.41E+00
230640_at	Huntingtin interacting protein C	HYPIC	5.49E+00
207826_s_at	Inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	ID3	3.16E+00
202503_s_at	KIAA0101	KIAA0101	2.14E+00
228325_at	KIAA0146 protein	KIAA0146	5.92E+00
202859_x_at	KIAA0476	KIAA0476	4.66E+05
219218_at	KIAA1447 protein	KIAA1447	4.62E+00
211762_s_at	Karyopherin alpha 2 (RAG cohort 1, importin alpha 1) /// karyopherin alpha 2 (RAG cohort 1, importin alpha 1)	KPNA2	2.04E+00
205266_at	Leukemia inhibitory factor (cholinergic differentiation factor)	LIF	2.04E+01
211596_s_at	Leucine-rich repeats and immunoglobulin-like domains 1 /// leucine-rich repeats and immunoglobulin-like domains 1	LRIG1	1.52E+01
205381_at	Leucine rich repeat containing 17	LRRC17	4.02E+00
209841_s_at	Leucine rich repeat neuronal 3	LRRN3	5.28E+00
228885_at	MAM domain containing 2	MAMDC2	2.99E+00
237849_at	Mannosidase, alpha, class 1A, member 1	MAN1A1	5.12E+00
225316_at	Major facilitator superfamily domain containing 2	MFSD2	9.63E+00
212859_x_at	Metallothionein 1E (functional)	MT1E	2.32E+00
217165_x_at	Metallothionein 1F (functional)	MT1F	2.69E+00
206461_x_at	Metallothionein 1H	MT1H	2.14E+00
216336_x_at	Metallothionein 1M	MT1K	2.33E+00
204326_x_at	Metallothionein 1X	MT1X	2.15E+00
212185_x_at	Metallothionein 2A	MT2A	1.75E+00
213906_at	V-myb myeloblastosis viral oncogene homolog (avian)-like 1	MYBL1	3.63E+00
201976_s_at	Myosin X	MYO10	2.46E+00
203045_at	Ninjurin 1	NINJ1	7.92E+00
223484_at	Normal mucosa of esophagus specific 1	NMES1	1.49E+01
222877_at	Neuropilin 2	NRP2	6.63E+00
218706_s_at	HCV NS3-transactivated protein 2	NS3TP2	2.03E+00
232666_at	2'-5'-oligoadenylate synthetase 3, 100kDa	OAS3	4.84E+00
1565111_x_at	Orofacial cleft 1 candidate 1	OFCC1	4.90E+01
219582_at	Opioid growth factor receptor-like 1	OGFRL1	3.51E+00
243905_at	Organic solute transporter alpha	OSTalpha	2.19E+01
202759_s_at	PALM2-AKAP2 protein	PALM2-AKAP2	2.03E+00
219148_at	PDZ binding kinase	PBK	3.31E+00
218691_s_at	PDZ and LIM domain 4	PDLIM4	5.06E+00
212094_at	Paternally expressed 10	PEG10	3.45E+00
217997_at	Pleckstrin homology-like domain, family A, member 1	PHLDA1	2.31E+00
209193_at	Pim-1 oncogene	PIM1	4.37E+00
230673_at	Polycystic kidney and hepatic disease 1 (autosomal recessive)-like 1	PKHD1L1	1.17E+02
228343_at	POU domain, class 2, transcription factor 2	POU2F2	6.78E+00

ID	Gene Title	Gene Symbol	Fold Change
201490_s_at	Peptidylprolyl isomerase F (cyclophilin F)	PPIF	3.07E+00
239488_at	Protein phosphatase 1 (formerly 2C)-like	PPM1L	1.84E+01
218009_s_at	Protein regulator of cytokinesis 1	PRC1	3.04E+00
201762_s_at	Proteasome (prosome, macropain) activator subunit 2 (PA28 β)	PSME2	2.15E+00
221872_at	Retinoic acid receptor responder (tazarotene induced) 1	RARRES1	5.80E+00
1554834_a_at	Ras association (RalGDS/AF-6) domain family 5	RASSF5	1.43E+01
209545_s_at	Receptor-interacting serine-threonine kinase 2	RIPK2	4.65E+00
211456_x_at	Similar to 60S ribosomal protein L35	RPL35	1.97E+00
204803_s_at	Ras-related associated with diabetes	RRAD	7.41E+00
206027_at	S100 calcium binding protein A3	S100A3	5.70E+00
1556472_s_at	Sex comb on midleg-like 4 (<i>Drosophila</i>)	SCML4	1.61E+01
236599_at	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2	SERPINE2	3.29E+00
211767_at	SLD5 homolog /// SLD5 homolog	SLD5	3.51E+00
210357_s_at	Spermine oxidase	SMOX	3.92E+00
1562287_at	Syntrophin, gamma 1	SNTG1	4.18E+02
226075_at	SplA/ryanodine receptor domain and SOCS box containing 1	SPSB1	4.61E+00
1565579_at	TatD DNase domain containing 2	TATDN2	6.26E+00
225868_at	Tripartite motif-containing 47	TRIM47	4.44E+00
202589_at	Thymidylate synthetase	TYMS	2.22E+00
205480_s_at	UDP-glucose pyrophosphorylase 2	UGP2	1.58E+00
203137_at	Wilms tumor 1 associated protein	WTAP	3.95E+00
218810_at	Zinc finger CCCH-type containing 12A	ZC3H12A	3.78E+01

Bold indicates genes that are related to each other in the literature.

Table 2Genes Downregulated by IL-1 β ($P<0.001$)

ID	Gene Title	Gene Symbol	Fold Change
202912_at	Adrenomedullin	ADM	-2.33E+00
1564699_at	Chromosome 5 open reading frame 4	C5orf4	-3.77E+00
229218_at	Collagen, type I, alpha 2	COL1A2	-2.42E+00
209101_at	Connective tissue growth factor	CTGF	-2.09E+00
219179_at	Dapper, antagonist of β -catenin, homolog 1 (<i>Xenopus laevis</i>)	DACT1	-4.73E+00
209383_at	DNA-damage-inducible transcript 3	DDIT3	-4.01E+00
208215_x_at	Dopamine receptor D4	DRD4	-7.43E+02
225275_at	EGF-like repeats and discoidin I-like domains 3	EDIL3	-1.66E+00
205782_at	Fibroblast growth factor 7 (keratinocyte growth factor)	FGF7	-3.07E+00
206377_at	Forkhead box F2	FOXF2	-2.01E+00
218665_at	Frizzled homolog 4 (<i>Drosophila</i>)	FZD4	-2.34E+00
204602 at	Dickkopf homolog 1 (<i>Xenopus laevis</i>)	DKK1	-3.98E+00
204457_s_at	Growth arrest-specific 1	GAS1	-2.74E+00
208937_s_at	Inhibitor of DNA binding 1, dominant negative helix-loop-helix protein	ID1	-4.29E+00
201565_s_at	Inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	ID2	-3.39E+00
209184_s_at	Insulin receptor substrate 2	IRS2	-4.92E+00
205175_s_at	Ketohexokinase (fructokinase)	KHK	-4.36E+02
205051_s_at	V-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	KIT	-3.75E+00
234880_x_at	Keratin associated protein 1-3	KRTAP1-3	-5.99E+00
233533_at	Keratin associated protein 1-5	KRTAP1-5	-4.74E+00
206163_at	Mab-21-like 1 (<i>C. elegans</i>)	MAB21L1	-4.20E+00
213568 at	Odd-skipped related 2 (<i>Drosophila</i>)	OSR2	-5.23E+00
225975_at	Protocadherin 18	PCDH18	-2.39E+00
1559341_at	Protocadherin 9	PCDH9	-2.14E+01
225816_at	PHD finger protein 17	PHF17	-4.40E+00
228481_at	Periostin, osteoblast specific factor	POSTN	-5.52E+00
203083 at	Thrombospondin 2	THBS2	-1.71E+00
204284_at	Protein phosphatase 1, regulatory (inhibitor) subunit 3C	PPP1R3C	-4.53E+00
226069_at	Prickle-like 1 (<i>Drosophila</i>)	PRICKLE1	-3.50E+00
237719_x_at	R7 binding protein	R7BP	-1.91E+01
226492_at	Sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6D	SEMA6D	-5.30E+00
222258_s_at	SH3-domain binding protein 4	SH3BP4	-2.53E+00
231145_at	Solute carrier family 25 (mitochondrial oxodicarboxylate carrier), member 21	SLC25A21	-3.31E+00
220924_s_at	Solute carrier family 38, member 2	SLC38A2	-2.09E+00
232481_s_at	SLIT and NTRK-like family, member 6	SLITRK6	-5.05E+00
201416_at	SRY (sex determining region Y)-box 4	SOX4	-5.17E+00

ID	Gene Title	Gene Symbol	Fold Change
234989_at	Trophoblast-derived noncoding RNA	TncRNA	-2.64E+00
201010_s_at	Thioredoxin interacting protein	TXNIP	-2.87E+00
210599_at	Zinc finger protein 614	ZNF614	-1.17E+03

Bold indicates genes that are related to each other in the literature.

Table 3

Gene Expression Changes (fold change) of HGFs After Treatment With IL-1 β and/or NF- κ B Inhibitor Relative to Untreated Control Cells Quantified by Quantitative Real-Time PCR

Treatment	Genes (fold change)										
	NFKB1	NFKB2	NFKBIA	REL	TRAF1	CSF1	CSF2	ICAM1	IRF1	BDKRB1	
No treatment	1	1	1	1	1	1	1	1	1	1	
NF- κ B-I	0.92	0.99	0.88	1.03	1.03	1.32	0.45	1.06	1.18	1.07	
IL-1 β	4.4	8.95	26.2	6.92	9.33	5.83	482.8	739.7	33.9	10.45	
NF- κ B-I + IL-1 β	0.97	1.0991	1.90678	2.08	0.5	1.07	1.86	5.27	1.76	1.7	

Treatment	Genes (fold change)										
	CCL2	CCL5	CCL20	CXCL1	CXCL2	CXCL3	CXCL5	CXCL6	CXCL10	CXCL11	PTGS2
No treatment	1	1	1	1	1	1	1	1	1	1	1
NF- κ B-I	1.03	0.46	0.76	1.88	0.08	0.23	0.3	6.12	0.39	0.3	2.53
IL-1 β	397.6	66.4	173.3	17.77	566.9	67.3	0.6	38.5	302	0.6	21.23
NF- κ B-I + IL-1 β	0.6	2.15	1.12	0.01	26.8	1.6	0.2	1.12	13.8	0.1	13.8

Treatment	Genes (fold change)							
	IL-6	IL-8	CD44	ETS1	IRAK2	BDKRB2	VCAM1	STAT5A
No treatment	1	1	1	1	1	1	1	1
IL-1 β	559.6	713.1	5.91	3.7	8.99	8.84	34.96	5.93
NF- κ B-I + IL-1 β	12.7	2.95	3.39	2.34	1.18	0.37	0.15	0.79

Note upregulation of gene expressions by IL-1 β and downregulation by addition of NF- κ B inhibitor to IL-1 β . CXCL5 and CXCL11 were analyzed as unchanged gene expressions and confirmed by quantitative real-time PCR.

NFKB-I = NF- κ B inhibitor (10 μ M).

Table 4

Summary of Significantly Altered Gene Expressions in Groups

Group	Genes Differentially Expressed by IL-1 β
NF- κ B pathway members	($P < 0.001$) NF κ B1, NF κ B2, I κ B α , I κ B ϵ , I κ B ζ , Rel, RELB, TA-NF κ BH, MAP3K8
Other transcription factors	ATF3, FOSL1, JUN, JUNB, EGR3, ETS1, STAT5A, IRF1, NFATC1, CEBP, PTTG1
Chemokines	CCL2, CCL5, CCL20, CXCL1, CXCL2, CXCL3, CXCL6, CXCL10
Interferon-induced proteins and receptors	Interferon (INF)- α receptor 2 (INF α R2), INF γ R2, IFIH1, IFIT2, IFIT3, GBP1, GBP2
Cytokines, interleukins	IL-6, IL-8, IL-22, IRAK2, CSF1, CSF2
Adhesion molecules and receptors	ICAM1, VCAM1, CD44, CD58, CD83, integrin B8, bradykinin receptor 1 and 2,
Extracellular matrix proteins and enzymes	MMP3, MMP12, collagen 3, collagen 1, * HAS2, HAS3, CTGF, * thrombospondin 2, * periostin, * plasminogen activator
TNF family members	TNF, TNFAIP2, 3, 6, 8, TNFSF15, FAS, TRAF1, TRAF4, TIFA, C1QTNF1
Antiapoptotic genes and cell cycle regulators	BCL2A1, BCL3, BCOR, BIRC2, BIRC3, BIRC5, CASP8, GADD45A, CCNB1, CCNB1, NEK2, UBE2C, CDCA3
Coagulation factors	Coagulation factor 2 receptor, coagulation factor 3,
Growth factors	PDGFA, FGF5, FGF18, HB-EGF
Metallothioneins	MT1E, MT1F, MT1H, MT1K, MT1X, MT2A

* Downregulated after IL-1 β treatment. All other genes were upregulated by IL-1 β .