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Sustained Inhibition of Proliferative Response After Transient FGF Stimulation Is Mediated by Interleukin 1 Signaling

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Transient FGF stimulation of various cell types results in FGF memory—a sustained blockage of efficient proliferative response to FGF and other growth factors. FGF memory establishment requires HDAC activity, indicating its epigenetic character. FGF treatment stimulates proinflammatory NFκB signaling, which is also critical for FGF memory formation. The search for FGF-induced mediators of FGF memory revealed that FGF stimulates HDAC-dependent expression of the inflammatory cytokine IL1α. Similarly to FGF, transient cell treatment with recombinant IL1α inhibits the proliferative response to further FGF and EGF stimulation, but does not prevent FGF receptor-mediated signaling. Interestingly, like cells pretreated with FGF1, cells pretreated with IL1α exhibit enhanced restructuring of actin cytoskeleton and increased migration in response to FGF stimulation. IRAP, a specific inhibitor of IL 1 receptor, and a neutralizing anti-IL1α antibody prevent the formation of FGF memory and rescue an efficient proliferative response to FGF restimulation. A similar effect results following treatment with the anti-inflammatory agents aspirin and dexamethasone. Thus, FGF memory is mediated by proinflammatory IL1 signaling. It may play a role in the limitation of proliferative response to tissue damage and prevention of wound-induced hyperplasia.

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We recently determined that transient FGF stimulation results in a stable inhibition of proliferative response to repeated FGF treatment (Poole et al., 2014). This phenomenon termed "FGF memory" has been detected in fibroblasts, endothelial cells, mesenchymal stem cells, and adipose-derived stem cells. Cell stress occurring in damaged tissues can results in the nonclassical release of FGF1 (Prudovsky et al., 2008). We hypothesized that FGF memory may serve to moderate the proliferative response after tissue damage, and thus prevent hyperplasia and decrease scar formation. Establishment of FGF memory depends on NFκB signaling and requires histone deacetylase (HDAC) activity (Poole et al., 2014). Based on these results, a study was undertaken to understand the molecular mechanisms underlying FGF memory. We found that FGF enhances the expression of interleukin (IL) 1α, and this effect depends on HDAC activity. Similarly to FGF, transient treatment with IL1α drastically inhibits the proliferative response to subsequent stimulation with FGF but does not interfere with FGF-promoted signaling and cell migration. Moreover, cell pretreatment with both FGF1 and IL1α blocks the proliferative response to an unrelated growth factor, EGF. FGF memory is abolished by IRAP, the competitive inhibitor of IL1 receptor type I, and a specific neutralizing antibody against IL1α. In addition, anti-inflammatory agents aspirin and dexamethasone eliminate it. Collectively, these results show that FGF memory is mediated by IL1α production resulting in enhanced inflammatory signaling.

Materials and Methods

Cell cultures

Swiss 3T3 (ATCC, Manassas, VA) cells and mouse lung endothelial cells Le II (Friesel and Maciag, 1988) were maintained in DMEM (HyClone, Logan, UT) supplemented with 10% bovine calf serum (HyClone) and 1% antibiotic-antimycotic mixture (GIBCO, Grand Island, NY). Quiescence was induced by culturing cells in DMEM containing 0.2% bovine calf serum and 5 units/mL heparin (Sigma, St. Louis, MO).

Cell treatment

Prior to stimulation, cells were transferred for 48 h to quiescence (Q) medium: DMEM with 5 units/mL heparin and 0.2% bovine calf serum. This basic medium was used in all types of stimulation. Upon induction of quiescence as well as withdrawal of growth factors, the cells were washed twice with DMEM medium containing 5 units/mL heparin.

Recombinant human FGF1 was prepared as described (Forough et al., 1991) and applied at 10 ng/mL. Human recombinant EGF (Lonza, Portsmouth, NH) and IL1α (Roche, Nutley, NJ) were used

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at 10 ng/mL. Human recombinant IRAP (Roche) and neutralizing antibodies against mouse IL1 α (R&D, Minneapolis, MN) were used at 600 or 300 ng/mL.

The following chemical compounds were used: aspirin (acetylsalicylic acid) (Sigma), dexamethasone (Sigma), trichostatin A (TSA) (Sigma) and panabinostat (Selleck, Houston, TX).

Dna synthesis study

Throughout the final 36 h of each stimulation condition, the cells were exposed to 10 μg/mL bromodeoxyuridine (BrdU) (Sigma). Once stimulation schedules were completed, the cells were fixed for a minimum of 10 min in 100% ethanol, washed with PBS, and DNA was denatured by incubation in 1 N HCl at 55°C for 30 min. The residual acid was then washed with PBS. Non-specific binding of antibodies was prevented by a 30 min pre-exposure to blocking buffer (5% bovine albumin, 0.1% Triton X-100, 0.1% sodium azide in PBS), followed by an 1 h incubation in a 1:500 dilution of monoclonal mouse anti-BrdU antibody (Dako, Carpinteria, CA) in blocking buffer. The cells were then washed with PBS and incubated for 30 min in 1:500 dilution of Alexa 546-conjugated anti-mouse IgG antibodies (Invitrogen). Counting of BrdU-positive nuclei in cell populations was performed using an Olympus IX70 microscope with a combination of fluorescence and phase contrast. Two coverslips were studied per each experimental point. For each coverslip, 500 nuclei were counted; the number of fluorescent nuclei was recorded to acquire BrdU incorporation percentages.

Cell migration assay

Linear scratches in cell monolayers were made using a 1 mL pipette tip. Photographs of scratches were taken at 0 and 24 h after monolayer wounding. Per each experimental condition, three independent wells were studied, and in each of them fifteen microscopic fields were photographed using the 10 X objective. The mean distances covered by the migrating fronts of monolayers and corresponding SEM were calculated.

Confocal fluorescence microscopy

The effects of FGF stimulation on the actin skeleton of naïve and IL1α-pretreated Swiss 3T3 cells were studied using confocal fluorescence microscopy. The cells were fixed with 4% neutral formalin, pre-incubated in blocking permeabilizing buffer (PBS with 5% BSA and 0.1% Triton X100), and then stained with Oregon green-conjugated phalloidin and TOPRO3 (both from Invitrogen, Carlsbad, CA). Cell images were taken using Leica SP8 confocal microscope at the MMCRI confocal microscopy facility.

Immunoblot analysis

Lysates were prepared from cell monolayers after various schedules involving IL1α and FGF1 stimulation. The cells were washed and scraped in ice-chilled PBS and centrifuged for collection at 2,500 rpm for 10 min. NPB buffer (20 mM Tris-HCl (pH 7.4), 250 mM sucrose, 60 mM KCl, 20 mM EDTA, 1.5 M NaCl, 1% TritonX-100, 0.1% deoxycholic acid, and a 1:50 dilution of protease inhibitor cocktail from Sigma) was used to lyse the cells. Relative protein concentrations were determined with Coomassie Plus Protein Assay Kit (Pierce, Rockford, IL) using a DU 640 spectrophotometer (Beckman, Fullerton, CA) at an excitation wavelength of 595 nm. The lysate was then mixed with an equal volume of SDS–PAGE sample buffer and incubated at 95°C for 10 min. Equivalent sample amounts were resolved by 12% PAGE and transferred to membranes Hybond-P (GE Healthcare, Little Chalfont, UK). The membranes were blocked in 5% fat-free dry milk diluted in TBS-Tween buffer at 42°C for 2 h, and then blotted with the appropriate primary mouse or rabbit antibody overnight at 4°C.

Membranes blotted with mouse anti-β-actin antibodies (Sigma) served as loading controls. Antibodies against the following proteins were used: rabbit antibodies against phosphorylated Erk1/2 (Sigma) and against cyclin D1 (Santa Cruz Biotechnologies, Santa Cruz, CA), and mouse monoclonal antibodies recognizing cyclin A (Millipore, Temecula, CA). The bound primary antibodies were visualized using horseradish peroxidase-conjugated goat antibodies against rabbit or mouse IgG (BioRad, Hercules, CA) and the ECL detection system (Amersham, Piscataway, NJ).

RT–PCR

RNA was prepared from Swiss 3T3 cells using the RNeasy kit (Qiagen, Hilden, Germany). Expression of IL1β and IL1α was assessed by RT-PCR using the SuperScript kit (Invitrogen). The following pairs of primers were utilized:

IL1α: as. -5′-GTC TCA TGA AGT GAG CCA TAG C-3′, s. -5′-CAA GAT GGC CAA AGT TCG TGA C-3′.

IL1β: as. -5′-CAG GAC AGG TAT AGA TTC TTT CTT TT-3′, s. -5′-ATG GCA ACT GTT CCT GAA CTC AAC T-3′.

β-actin: as. -5′-GTC TCA AAC ATG ATC TGG G-3′, s. 5′-AGA AAA TCT GGC ACC ACA CC-3′.

For qRT-PCR, we used the same IL1α primers and the following cyclophilin primers:

as. -5′-CAG TGC TCA GAG CTC GAA AG-3′, s. -5′-CCA CCG TGT TCT TCG ACA T-3′.

Elisa

IL1α concentration in cell lysates was determined using an ELISA kit from eBioscience (San Diego, CA) according to the instructions of the manufacturer.

Statistical analysis

Each experiment was repeated at least three times with consistent results. In the studies analyzing DNA synthesis, percentage of BrdU-labeled cells with 95% confidence interval was calculated for each condition. In qRT-PCR and ELISA studies of IL1α expression and in the studies of cell growth and migration, Student's *t* test was used to assess the significance of observed effects.

Results

FGF induces the expression of IL1α

The observations that FGF stimulation activates NFκB signaling, and establishment of FGF memory depends on NFκB pathway (Poole et al., 2014) indicated that FGF may induce the expression of a proinflammatory cytokine. Interestingly, IL1α, a potent inducer of inflammation and activator of NFκB signaling, represses cell proliferation (Ohmori et al., 1988; Ikeda et al., 1991) and plays a key role in some cases of cell senescence (Maier et al., 1990; McCarthy et al., 2013). We used RT-PCR to assess the effect of FGF1 stimulation on the expression of proinflammatory cytokines and inducers of NFκB signaling, IL1α, and IL1β, in Swiss 3T3 cells (Fig. 1A). While IL1β expression remained unchanged, FGF1 stimulated the expression of IL1α. qRT-PCR study has shown a 20-fold induction of IL1α by FGF (Fig. 1B). Interestingly, the induction of IL1α expression by FGF1 was abolished by the HDAC inhibitor panabinostat (Fig. 1C). The RT-PCR results were confirmed by determining the IL1α content in the lysates of Swiss 3T3 cells stimulated with FGF1 in presence or absence of HDAC inhibitors (Fig. 1D). It is noteworthy that the

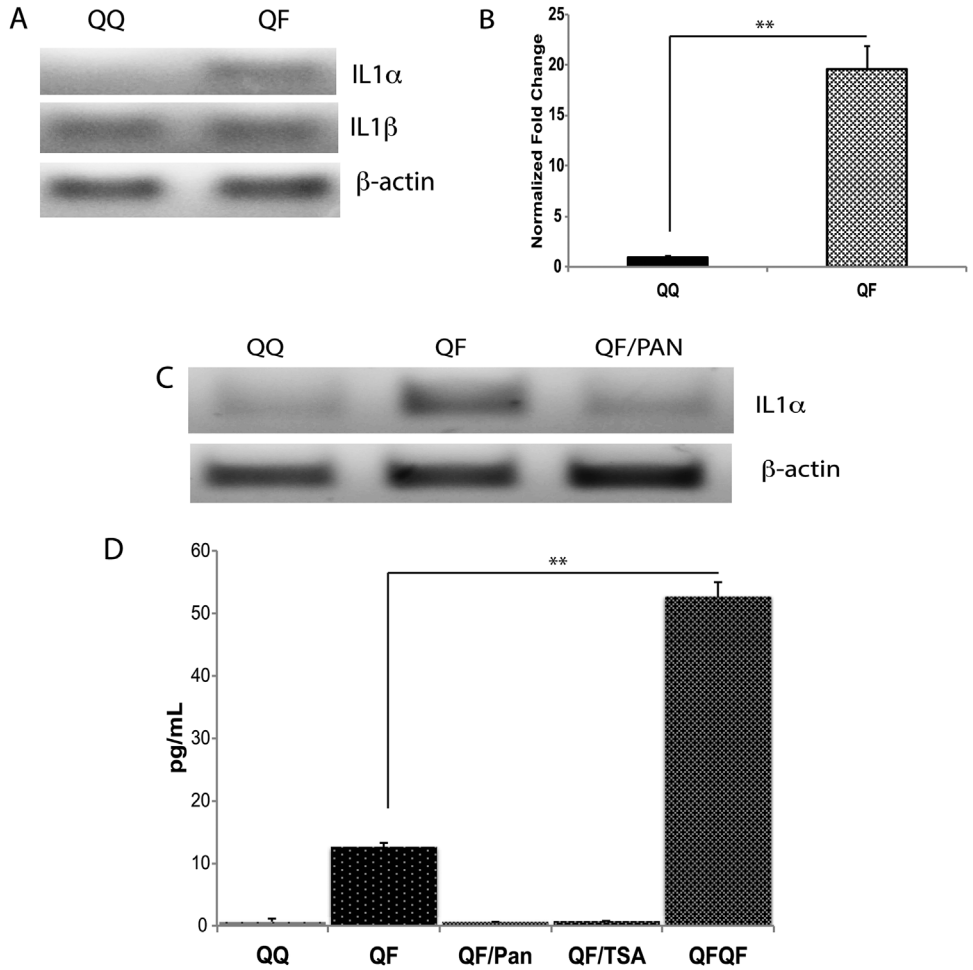

Fig. 1. FGF1 induces IL1α expression in an HDAC-dependent manner. A: Confluent Swiss 3T3 cell cultures were incubated for 48 h in quiescence medium (0.2% serum) and then transferred for 36 h to quiescence medium with (QF) or without (QQ) 10 ng/mL FGF1. Expression of IL1β and IL1α was determined by RT-PCR. B: Cells were treated similarly to A and IL1α expression was determined by qRT-PCR. **Indicates $P < 0.01$. C: Cells were treated similarly to A with an additional condition, when FGF stimulation was performed in the presence of 200 nM panabinostat (QF/PAN). Expression of IL1α was determined by RT-PCR. D: Cells were treated similarly to C except that quiescence was extended to 168 h in QQ, and to 132 h in QF and QF/PAN, and two additional schedules were added: (i) repeated FGF1 stimulation (QFQF), when following the primary FGF1 stimulation cells were transferred for 48 h to quiescence medium and after that restimulated for 36 h with FGF1; (ii) FGF1 stimulation of naïve cells in presence of 300 nM trichostatin A (QF/TSA). Cell lysates were prepared and equalized based on total protein concentration. The concentration of IL1α in the equalized lysates was determined using an ELISA kit. **Indicates $P < 0.01$.

concentration of IL1α in the cell lysates after repeated FGF1 stimulation was approximately five times higher than after the primary stimulation. Thus, FGF induces the expression of IL1α in an HDAC-dependent manner.

Transient pretreatment with IL1α prevents proliferative response to FGF and EGF

Because FGF1 induces the expression of IL1α, we assessed whether similar to transient FGF1 stimulation, transient application of IL1α to Swiss 3T3 cells prevents proliferative response to further FGF stimulation. The following schedules of cell treatment were applied: QQ – 168 h of quiescence; QF – 132 h quiescence, 36 h FGF1; QIQQ – 48 h quiescence, 36 h IL1α, 84 h quiescence; QIQF – 48 h quiescence, 36 h IL1α, 48 h quiescence, 36 h FGF1. BrdU incorporation during the last 36 h of each schedule was determined (Fig. 2A). IL1α pretreatment (QIQF) resulted in a 10-fold decrease of DNA synthesis in comparison to FGF1 stimulation of naive quiescent cells (QF) (Fig. 2B). In a series of similar experiments, we found that transient FGF1 and IL1α stimulations inhibited the proliferative response to epidermal growth factor (EGF) (Fig. 2C,D).

Transient pretreatment with IL1α does not interfere with the activation of Erk1/2 and induction of cyclin D in response to FGF stimulation but prevents the induction of cyclin A

To understand the mechanisms through which the pretreatment of FGF1 inhibits growth factor-induced proliferation, we used immunoblotting to assess the

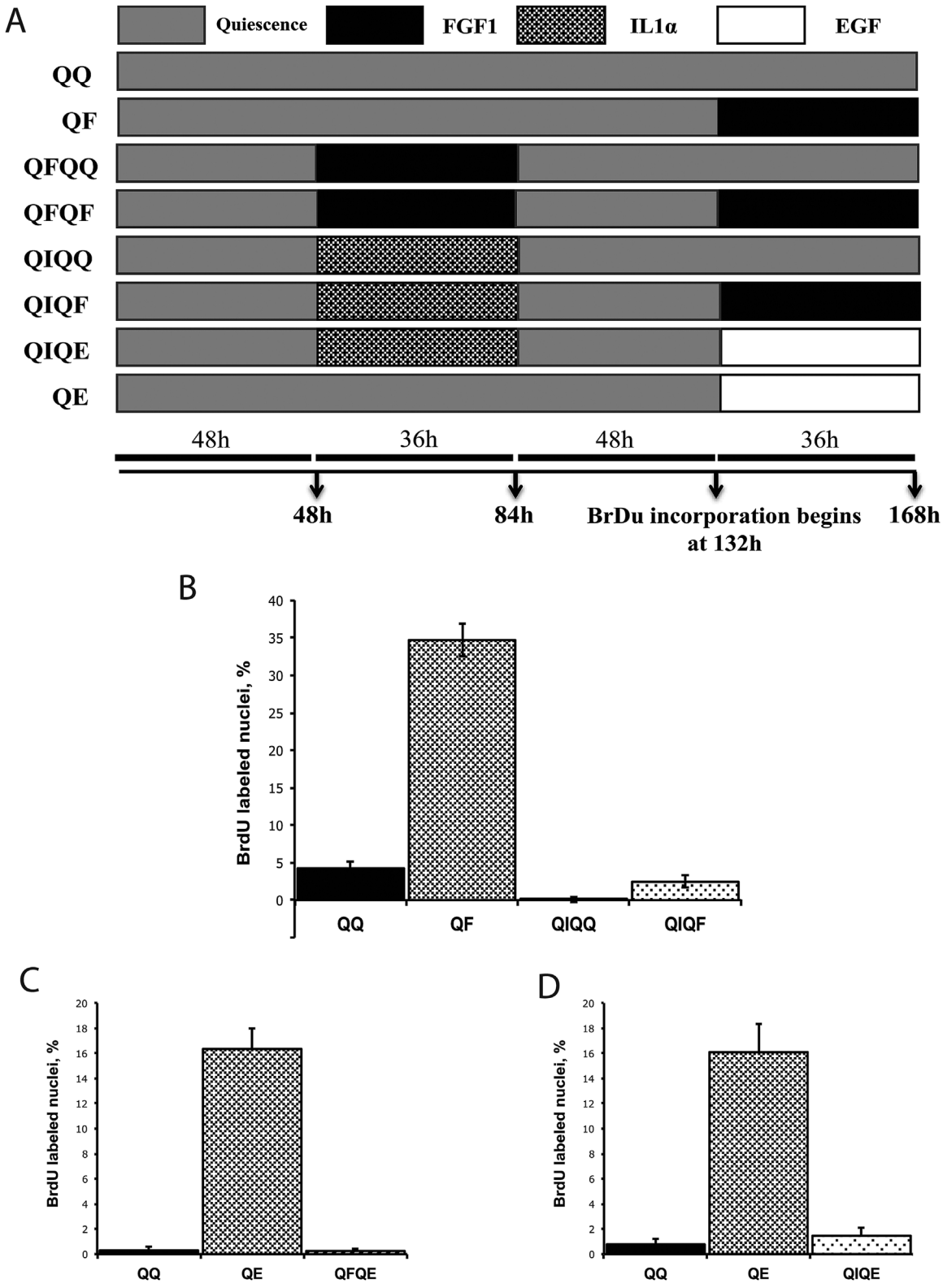

Fig. 2. Transient treatment with IL1α results in a stable repression of proliferative response to growth factors. A: Schedules of cell stimulations with IL1α, FGF1, and EGF (explanation in the text). BrdU labeling of Swiss 3T3 cells was performed during the final 36 h of each stimulation schedule. B: Acute repression of proliferative response to FGF1 (10 ng/mL) after transient pretreatment with IL1α (10 ng/mL) and the intermediate 48 h period of quiescence. C,D: Transient pretreatment with FGF1 (C) or IL1α (D) inhibits the proliferative response to EGF (10 ng/mL). In B, C, and D, the percentage of BrdU labeled cells with 95% confidence interval for each condition is shown. The experiments were repeated at least three times and the results of representative experiments are presented.

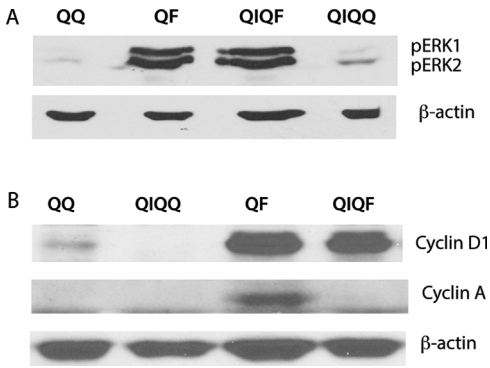

Fig. 3. FGF-induced proliferative signaling in Swiss 3T3 cells pretreated with IL1α. Phosphorylation of Erk1/2 (A) and expression of cyclins D1 and A (B) in naïve (QF) and IL1α-pretreated (QIQF) Swiss 3T3 cells. The duration of final FGF1 stimulation was 30 min (A) or 24 h (B). Total cell lysates were resolved by SDS–PAGE and immunoblotted for phospho-Erk1/2, cyclin D1, cyclin A, or β-actin.

signaling events and expression of key cell cycle proteins in response to FGF1 stimulation of IL1α-pretreated (QIQF) and naïve (QF) Swiss 3T3 cells. Thirty minutes after final FGF1 stimulation, the phosphorylation of Erk1/2 reflecting the early signaling downstream of FGFR was identical in both QIQF and QF cells (Fig. 3A). The same was true for the expression of cyclin D1 24 h after stimulation, a period corresponding to late G1/early S phase after the exit from quiescence (Fig. 3B). In contrast, unlike naive cultures, cells with IL1α history failed to express cyclin A, the key regulator of the S-phase initiation and transition, in response to 24 h FGF stimulation (Fig. 3B).

IL1α pretreatment enhances the restructuring of actin cytoskeleton and cell migration in response to FGF stimulation

Cells transiently treated with FGF respond to FGF restimulation by enhanced actin cytoskeleton reorganization and migration (Poole et al., 2014). We used phalloidin staining to compare the status of the actin cytoskeleton after FGF treatment of naïve (QF) and IL1α-prestimulated Swiss 3T3 (QIQF) cells. Confocal fluorescence study demonstrated a stronger formation of actin fibers in cells with the history of IL1α treatment (Fig. 4A). In agreement with these results, the study of wounded monolayers showed that QIQF cells exhibit a faster migration than cells in QF populations (Fig. 4B).

FGF memory depends on IL1 receptor signaling

The induction of IL1α expression by FGF1 and the similarity between the effects of FGF1 and IL1α pretreatment on proliferative and migratory response to FGF stimulation indicate that FGF memory could be dependent on IL1 signaling. To assess this hypothesis, we determined the effect of IRAP, a specific competitive antagonist of IL1 receptor type I, on FGF memory. When IRAP was applied throughout the QFQF schedule of a typical FGF memory experiment, it completely rescued the proliferative response of Swiss 3T3 cells after repeated FGF1 stimulation (Fig. 5A).

Neutralization of IL1α rescues the proliferative response to the secondary FGF stimulation

To assess the role of IL1α in FGF memory, we evaluated the effect of a specific neutralizing anti-IL1α antibody upon the proliferative response of Swiss 3T3 cells to secondary FGF stimulation. We found that DNA synthesis after repeated FGF treatment was rescued when anti-IL1α antibodies were present throughout the QFQF experimental schedule (Fig. 5B).

Anti-inflammatory drugs rescue the proliferative response to the secondary FGF stimulation

IL1α is a potent proinflammatory cytokine. To elucidate whether inflammatory signaling is required for FGF memory, we used two anti-inflammatory drugs: aspirin and dexamethasone. Both of them restored the proliferative response of Swiss 3T3 cells to the secondary FGF stimulation (Fig. 6A,B). Decreasing the concentrations of aspirin and IL1α resulted in weaker rescuing effects (Supplementary Figure 1).

The rescuing effects of aspirin and dexamethasone on DNA synthesis were confirmed in FGF restimulation experiments using Le II mouse lung endothelial cells (Fig. 7A). Moreover, in special experiments where the time of secondary FGF stimulation was extended to 2 days, we detected a significant increase of LeII cell numbers upon the treatment with aspirin and dexamethasone (Fig. 7B,C).

Discussion

The earlier observation that transient stimulation with FGF results in a stable and potent inhibition of proliferative response to repeated growth factor stimulation (Poole et al., 2014) encouraged us to elucidate the key molecular determinant(s) of this phenomenon that had been termed FGF memory. The present study shows that IL1α plays a critical role in FGF memory. The following results underlie this conclusion: (i) FGF treatment results in a HDAC-dependent IL1α expression; (ii) IL1α stimulation generates a cell phenotype similar to that observed in FGF-pretreated cells, i.e., strongly inhibited proliferative and enhanced migratory response to growth factor stimulation; (iii) IRAP and specific neutralizing anti-IL1α antibodies inhibit FGF memory.

It has long been known that IL1α exhibits anti-proliferative effects in non-transformed cells (Ohmori et al., 1988; Ikeda et al., 1991). In particular, it was found that senescence of human endothelial cells can be significantly delayed by the inhibition of IL1α expression (Maier et al., 1990). In recent years, IL1α was identified as a key determinant of the senescence-associated secretory cell phenotype, which is characterized by the inhibition of proliferation and production of several proinflammatory cytokines in addition to IL1α (McCarthy et al., 2013).

We have earlier reported that transient FGF stimulation results in a sustained activation of NFκB signaling, which is critical for FGF memory establishment (Poole et al., 2014). IL1α (Niu et al., 2004; Melisi et al., 2009) and FGF (Muddasani et al., 2007; Salazar et al., 2014) have been demonstrated to activate the proinflammatory NFκB pathway. Moreover, IL1α expression and NFκB signaling constitute an autoregulatory feedback loop that maintains IL1α production (Niu et al., 2004). Proinflammatory signaling can result in the inhibition of DNA synthesis (Vlahos and Stewart, 1999). Concurrently, we have shown that the anti-inflammatory drugs, aspirin and dexamethasone, abolish IL1α-mediated FGF memory. Thus, FGF-induced IL1α expression and

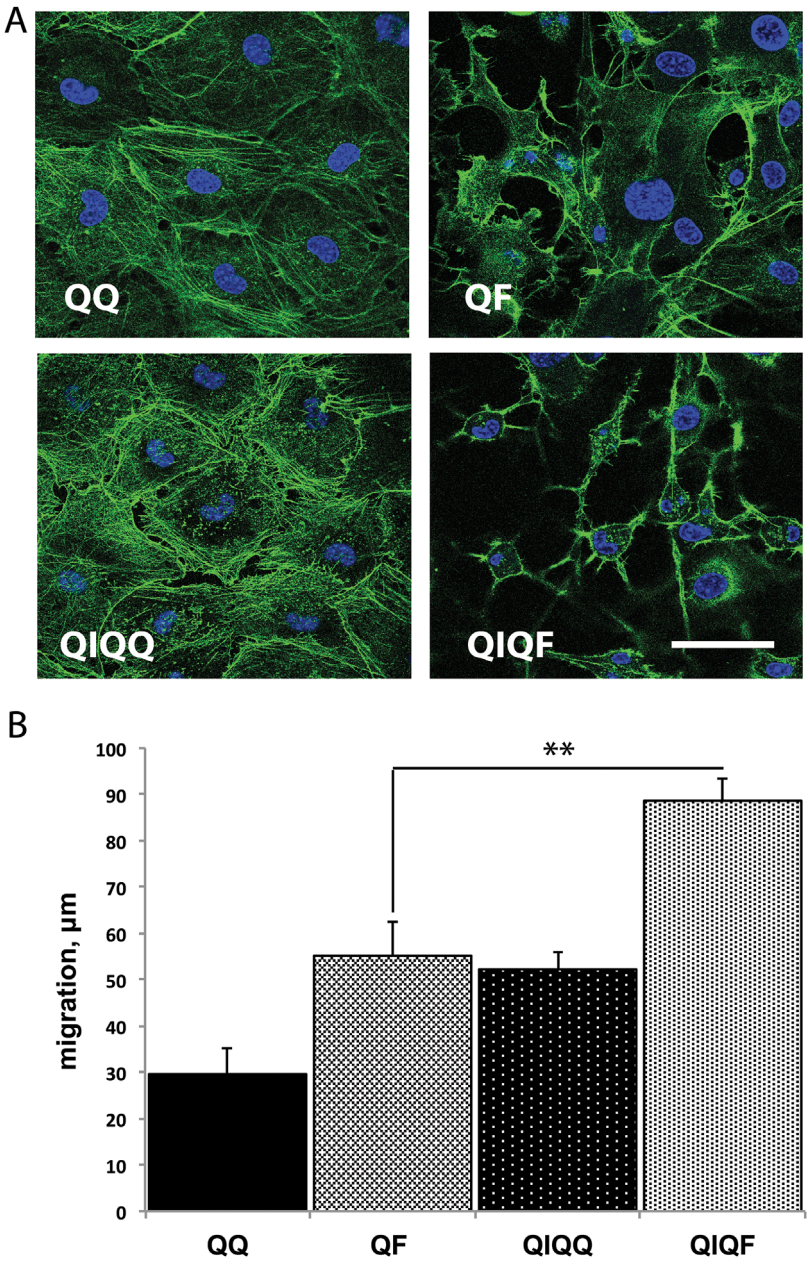

Fig. 4. Cell pretreated with IL1α respond to FGF with an enhanced restructuring of the actin cytoskeleton and accelerated migration. A: IL1α pretreatment results in an exaggerated restructuring of the actin cytoskeleton in response to FGF stimulation of Swiss 3T3 cells. Following formalin fixation (36 h after final FGF stimulation), cells were co-stained with Oregon green-phalloidin for F-actin (green) and DAPI for DNA (blue), and studied using a confocal microscope. Bar −25 µm. B: A standard experiment with FGF1 stimulation after IL1α pretreatment was performed. At the end of the intermediate quiescence period, linear scratches in cell monolayers were made as described in Experimental Procedures and then the usual stimulation schedules were followed. Photographs of scratches were taken at 0 and 24 h after monolayer wounding. Mean distances (with corresponding SEM) covered by migrating monolayer fronts by 24 h are presented with ** indicating $P < 0.01$.

proinflammatory signaling limit the proliferative response to continuous FGF stimulation. The experiments involving transient cell treatment with IL1α demonstrated a sustained inhibition of proliferative response to FGF and EGF stimulation. Similar to earlier reported experiments with FGF restimulation (Poole et al., 2014), cells pretreated with IL1α respond to FGF by Erk1/2 phosphorylation and cyclin D1 expression. Moreover, their cytoskeletal and migratory responses to FGF are significantly stronger than in naïve cells. These results indicate that FGFR-mediated signaling, progression from quiescence to G1 phase as well as morphological and migratory changes are resistant to IL1α or increased by it. Indeed, IL1 signaling enhances cell migration in the course of inflammation (Mitchell et al.,

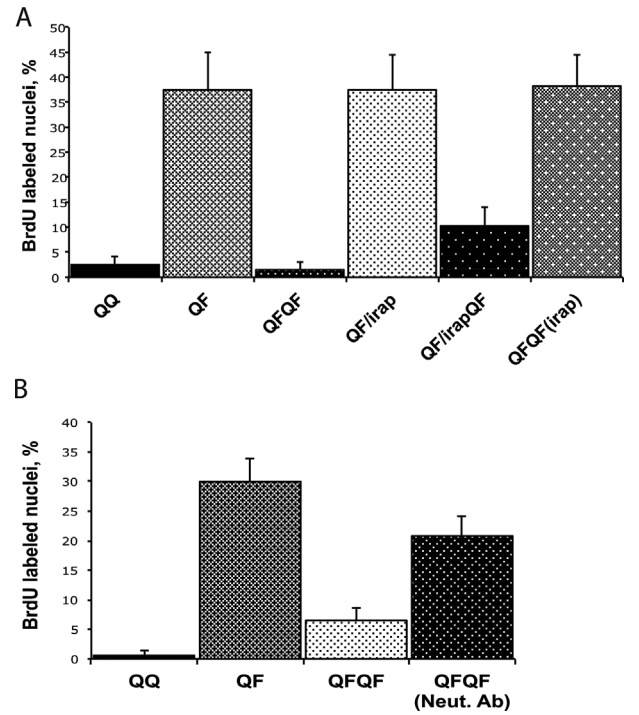

Fig. 5. Blockage of IL1 signaling inhibits FGF memory. A: IRAP, the inhibitor of IL1 receptor type I rescues the proliferative response of Swiss 3T3 cells to repeated FGF1 stimulation. QQ – quiescent cells, QF – naïve cells stimulated with FGF1, QFQF – cells restimulated with FGF1 (for details see Fig. 2A). Additional schedules: (i) QF/irap – 600 ng IRAP present during the stimulation of naïve cells; (ii) QFirapQF – 600 ng/mL IRAP during the primary FGF1 stimulation; (iii) QFQF(irap) – 600 ng/mL IRAP present starting from the first FGF1 stimulation to the end of restimulation. B: Neutralizing antibodies against mouse IL1α rescue the proliferation of Swiss 3T3 cells restimulated with FGF1. QFQF (neut. Ab) –300 ng/mL neutralizing antibody present starting from the first FGF1 stimulation to the end of restimulation. In A and B, the percentage of BrdU labeled cells with 95% confidence interval is shown for each condition. In each case, the experiments were triplicated, and the results of a representative experiment are shown.

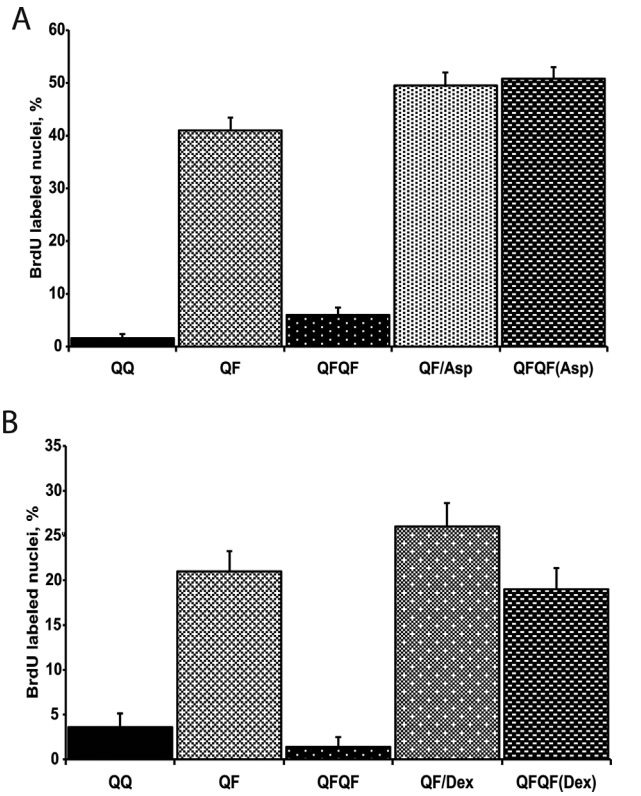

Fig. 6. Anti-inflammatory agents aspirin (A) and dexamethasone (B) suppress FGF memory in Swiss 3T3 cells. QQ – quiescent cells, QF – naïve cells stimulated with FGF1, QFQF – cells restimulated with FGF1 (for details see Fig. 2A). Additional schedules in A: (i) QF/asp – 1 mM aspirin present during the FGF1 stimulation of naïve cells; (ii) QFQF(asp) – 1 mM aspirin present starting from the first FGF1 stimulation to the end of restimulation. Additional schedules in B: (i) QF/dex – 1 μM dexamethasone present during the FGF1 stimulation of naïve cells; (ii) QFQF(Dex) – 1 μM dexamethasone present starting from the first FGF1 stimulation to the end of restimulation. In A and B, the percentage of BrdU labeled cells with 95% confidence interval is shown for each condition. In each case, the experiments were triplicated, and the results of a representative experiment are shown.

2007). On the other hand, FGF-induced IL1α prevents DNA replication. The mechanisms of the sustained inhibition of proliferative response in cells transiently treated with IL1α and FGF1 require further studies. The inability of such cells to express cyclin A in response to FGF indicates the blockage of cell cycle events immediately preceding the onset of the S phase.

FGF memory and induction of IL1α expression require not only NFκB signaling but also HDAC activity. Deacetylation of NFκB p65 is required for its nuclear translocation and retention and thus for efficient NFκB signaling (Grabiec et al., 2012; Liu and McCall, 2013; Ziesche et al., 2013; Zhang et al., 2015). FGF memory could be dependent on HDAC-mediated histone deacetylation or on HDAC-dependent activation of NFκB signaling or on both. As we have reported (Poole et al., 2014), FGF does not change the expression of major HDACs. Thus, the search for a specific HDAC involved in FGF1 memory followed by the identification of its mechanism of action requires a comprehensive siRNA knockdown screen study involving the assessment of FGF memory establishment, NFκB signaling activity and IL1α expression.

The significance of FGF memory for in vivo processes remains to be elucidated. This phenomenon could play a role in the regulation of the proliferative response during repair of tissue damage. It is noteworthy that liver regeneration after partial hepatectomy is accompanied by the increase of IL1 expression, which limits cell proliferation and prevents hyperplasia (Boulton et al., 1997; Sgroi et al., 2011). It is also interesting that endothelial-specific overexpression of FGF1 in transgenic mice paradoxically results not in the improvement of post-ischemic kidney repair, but in the inhibition of reparative processes and increased inflammation (Kirov et al., 2012). We hypothesize that FGF1 memory mediated by IL1α production may regulate the repair of damaged tissues through the limitation of cell proliferation and enhancement of cell migration, which decrease scarring and help restore the normal tissue structure.

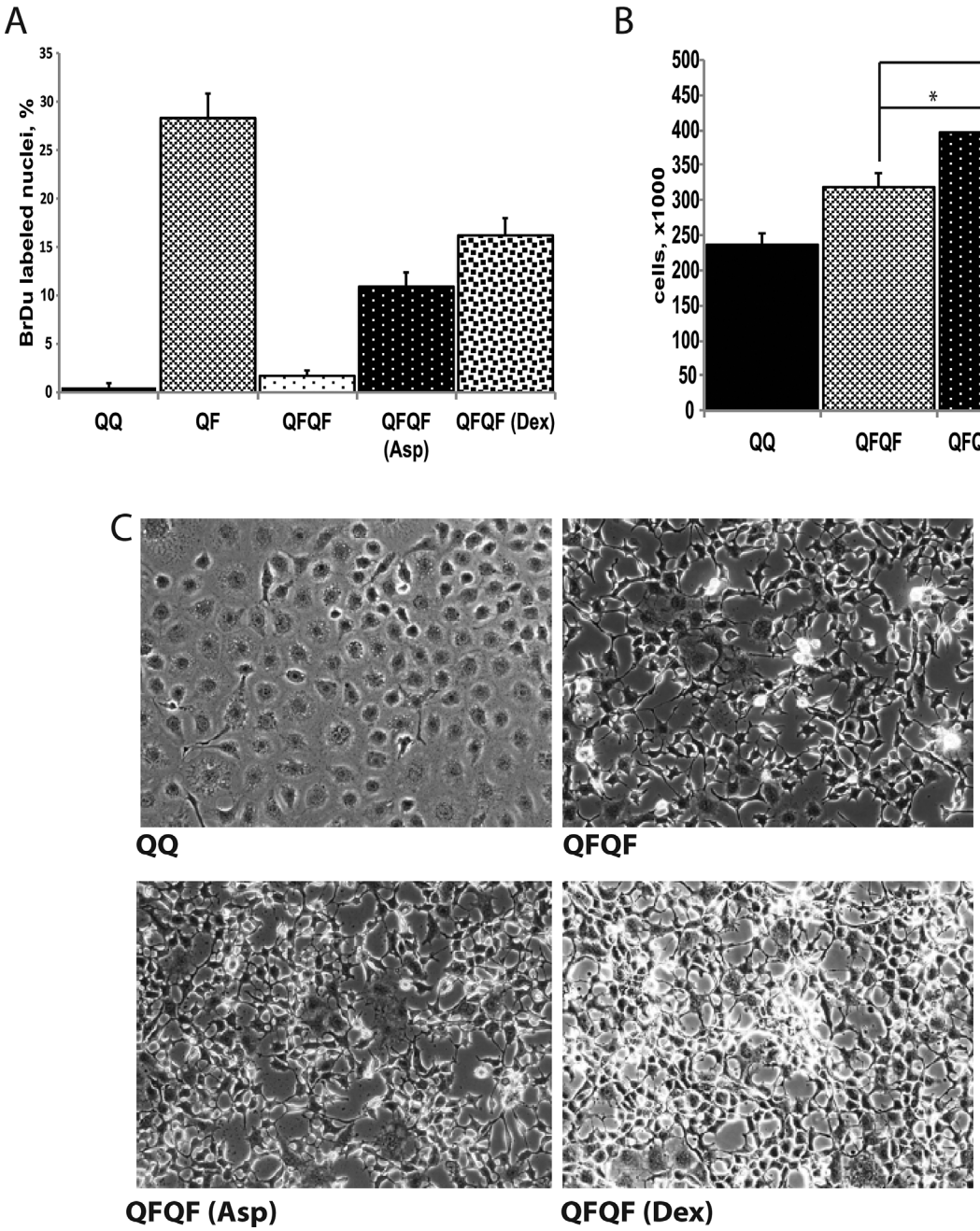

Fig. 7. FGF memory in endothelial cells is suppressed by anti-inflammatory agents. A: Aspirin and dexamethasone rescue DNA replication in Le II cells in response to FGF restimulation. QQ – quiescent cells, QF – naïve cells stimulated with FGF1, QFQF – cells restimulated with FGF1 (for details see Fig. 2A). Additional schedules: QFQF (Asp) – 1 mM aspirin present starting from the first FGF1 stimulation to the end of restimulation; (ii) QFQF(Dex) – 1 μM dexamethasone present starting from the first FGF1 stimulation to the end of restimulation. The percentage of BrdU labeled cells with 95% confidence interval is shown for each condition. B: Aspirin and dexamethasone enhance the growth of Le II cells restimulated with FGF. The standard FGF restimulation experiment (see Fig. 2A) was modified by increasing the secondary FGF stimulation period to 48 h. At the end of the experiment, cells were trypsinized and cell numbers per well of 12-well plates were counted using hemocytometer. QQ – quiescent cells. QFQF – cells restimulated with FGF1. QFQF (Asp) – 1 mM aspirin present starting from the first FGF1 stimulation to the end of restimulation. QFQF(Dex) – 1 μM dexamethasone present starting from the first FGF1 stimulation to the end of restimulation. *Indicates $P < 0.05$. **Indicates $P < 0.01$. C: Representatives phase contrast photographs of LeII cell growth under conditions described in B. Bar – 100 μM.

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Literature Cited

Boulton R, Woodman A, Calnan D, Selden C, Tam F, Hodgson H. 1997. Nonparenchymal cells from regenerating rat liver generate interleukin-1alpha and -1beta: A mechanism of negative regulation of hepatocyte proliferation. Hepatology 26:49–58.

Forough R, Engleka K, Thompson JA, Jackson A, Imamura T, Maciag T. 1991. Differential expression in Escherichia coli of the alpha and beta forms of heparin-binding acidic fibroblast growth factor-1: Potential role of RNA secondary structure. Biochim Biophys Acta 1090:293–298.

Friesel R, Maciag T. 1988. Internalization and degradation of heparin binding growth factor-I by endothelial cells. Biochem Biophys Res Commun 151:957–964.

Grabiec AM, Korchynskyi O, Tak PP, Reedquist KA. 2012. Histone deacetylase inhibitors suppress rheumatoid arthritis fibroblast-like synoviocyte and macrophage IL-6 production by accelerating mRNA decay. Ann Rheum Dis 71:424–431.

Ikeda M, Ikeda U, Oohara T, Takeda K, Kano S. 1991. Recombinant interleukin-1 alpha inhibits the growth of rat mesangial cells in culture. Clin Exp Immunol 83:149–153.

Kirov A, Duarte M, Guay J, Karolak M, Yan C, Oxburgh L, Prudovsky I, . 2012. Transgenic expression of nonclassically secreted FGF suppresses kidney repair. PLoS One 7:e36485.

Liu TF, McCall CE. 2013. Deacetylation by SIRT1 reprograms inflammation and cancer. Genes cancer 4:135–147.

Maier JA, Voulalas P, Roeder D, Maciag T. 1990. Extension of the life-span of human endothelial cells by an interleukin-1 alpha antisense oligomer. Science 249:1570–1574.

McCarthy DA, Clark RR, Bartling TR, Trebak M, Melendez JA. 2013. Redox control of the senescence regulator interleukin-1alpha and the secretory phenotype. J Biol Chem 288:32149–32159.

Melisi D, Niu J, Chang Z, Xia Q, Peng B, Ishiyama S, Evans DB, Chiao PJ. 2009. Secreted interleukin-1alpha induces a metastatic phenotype in pancreatic cancer by sustaining a constitutive activation of nuclear factor-kappaB. Mol Cancer Res 7:624–633.

Mitchell MD, Laird RE, Brown RD, Long CS. 2007. IL-1beta stimulates rat cardiac fibroblast migration via MAP kinase pathways. Am J Physiol Heart Circ Physiol 292:H1139–H1147.

Muddasani P, Norman JC, Ellman M, van Wijnen AJ, Im HJ. 2007. Basic fibroblast growth factor activates the MAPK and NFkappaB pathways that converge on Elk-1 to control production of matrix metalloproteinase-13 by human adult articular chondrocytes. J Biol Chem 282:31409–31421.

Niu J, Li Z, Peng B, Chiao PJ. 2004. Identification of an autoregulatory feedback pathway involving interleukin-1alpha in induction of constitutive NF-kappaB activation in pancreatic cancer cells. J Biol Chem 279:16452–16462.

Ohmori Y, Hanazawa S, Amano S, Hirose K, Kumegawa M, Kitano S. 1988. Effects of recombinant human interleukin 1 alpha and interleukin 1 beta on cell growth and alkaline phosphatase of the mouse osteoblastic cell line MC3 T3– E1. Biochim Biophys Acta 970:22–30.

Poole A, Knowland N, Cooper E, Cole R, Wang H, Booth L, Kacer D, Tarantini F, Friesel R, Prudovsky I, . 2014. Transitory FGF treatment results in the long-lasting suppression of the proliferative response to repeated FGF stimulation. J Cell Biochem 115:874–888.

Prudovsky I, Tarantini F, Landriscina M, Neivandt D, Soldi R, Kirov A, Small D, Kathir KM, Rajalingam D, Kumar TK. 2008. Secretion without Golgi. J Cell Biochem 103:1327–1343.

Salazar L, Kashiwada T, Krejci P, Meyer AN, Casale M, Hallowell M, Wilcox WR, Donoghue DJ, Thompson LM. 2014. Fibroblast growth factor receptor 3 interacts with and activates TGFbeta-activated kinase 1 tyrosine phosphorylation and NFkappaB signaling in multiple myeloma and bladder cancer. PLoS One 9:e86470.

Sgroi A, Gonelle-Gispert C, Morel P, Baertschiger RM, Niclauss N, Mentha G, Majno P, Serre-Beinier V, Buhler L. 2011. Interleukin-1 receptor antagonist modulates the early phase of liver regeneration after partial hepatectomy in mice. PLoS One 6:e25442.

Vlahos R, Stewart AG. 1999. Interleukin-1alpha and tumour necrosis factor-alpha modulate airway smooth muscle DNA synthesis by induction of cyclo-oxygenase-2: Inhibition by dexamethasone and fluticasone propionate. Br J Pharmacol 126:1315–1324.

Zhang Q, Yang F, Li X, Wang LW, Chu XG, Zhang H, Gong ZJ. 2015. Trichostatin A Protects Against Experimental Acute-on-Chronic Liver Failure in Rats Through Regulating the Acetylation of Nuclear Factor-kappaB. Inflammation 38:1364–1373.

Ziesche E, Kettner-Buhrow D, Weber A, Wittwer T, Jurida L, Soelch J, Muller H, Newel D, Kronich P, Schneider H, Dittrich-Breiholz O, Bhaskara S, Hiebert SW, Hottiger MO, Li H, Burstein E, Schmitz ML, Kracht M. 2013. The coactivator role of histone deacetylase 3 in IL-1-signaling involves deacetylation of p65 NF-kappaB. Nucleic Acids Res 41:90–109.

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