ORIGINAL ARTICLE

See related commentary on pg 2911

Platelet-Activating Factor Induces Epigenetic Modifications in Human Mast Cells

Elisabetta Damiani[1,2,4], Nahum Puebla-Osorio[2,4], Enrique Gorbea[2] and Stephen E. Ullrich[2,3]

UV radiation-induced systemic immune suppression is a major risk factor for skin cancer induction. The migration of dermal mast cells from the skin to the draining lymph nodes has a prominent role in activating systemic immune suppression. UV-induced keratinocyte-derived platelet-activating factor (PAF) activates mast cell migration, in part by upregulating the expression of CXCR4 on the surface of mast cells. Others have indicated that epigenetic mechanisms regulate CXCR4 expression; therefore, we asked whether PAF activates epigenetic mechanisms in mast cells. Human mast cells were treated with PAF, and the effect on DNA methylation and/or acetylation was measured. PAF suppressed the expression of DNA methyltransferase (DNMT) 1 and 3b. On the other hand, PAF increased p300 histone acetyltransferase expression, and the acetylation of histone H3, which coincided with a decreased expression of the histone deacetylase HDAC2. Chromatin immunoprecipitation assays indicated that PAF treatment activated the acetylation of the *CXCR4* promoter. Finally, inhibiting histone acetylation blocked p300 upregulation and suppressed PAF-induced surface expression of CXCR4. Our findings suggest a novel molecular mechanism for PAF, activation of epigenetic modifications. We suggest that PAF may serve as an endogenous molecular mediator that links the environment (UV radiation) with the epigenome.

Journal of Investigative Dermatology (2015) **135,** 3034–3040; doi:10.1038/jid.2015.336; published online 24 September 2015

INTRODUCTION

Platelet-activating factor (PAF; 1-*O*-alkyl-2-acetyl-sn-glycero-3-phosphocholine) is a naturally occurring phospholipid mediator of inflammation (Prescott *et al.*, 2000). PAF is produced by epidermal keratinocytes in response to UVB irradiation (Marathe *et al.*, 2005; Travers *et al.*, 2010) and is linked to UVB-induced systemic immune suppression (Walterscheid *et al.*, 2002), a major risk factor for skin cancer induction (Yoshikawa *et al.*, 1990). Because most UVB radiation is absorbed within the upper layers of the skin, indirect mechanisms must be involved in transmitting the suppressive signal from the skin to the immune system. Migration of mast cells from the irradiated skin to the draining lymph nodes has a critical role (Byrne *et al.*, 2008). Mast cell–deficient mice are resistant to the immunosuppressive effects of UV radiation (Hart *et al.*, 1998), and inhibiting mast cell migration in UVB-irradiated mice treated with a CXCR4 antagonist blocks the induction of immune suppression (Byrne *et al.*, 2008). PAF activates the upregulation of the chemokine CXCR4 on the mast cell surface and promotes the migration of mast cells from the skin to the lymph nodes (Chacón-Salinas *et al.*, 2014). PAF also upregulates the expression of the CXCR4 ligand (CXCL12) on lymph node cells (Byrne *et al.*, 2008), thus setting the chemokine gradient for directing mast cell migration from the skin to the draining lymph node, where they secrete IL-10 and suppress the immune response (Chacón-Salinas *et al.*, 2011). In addition to skin cancer (Sreevidya *et al.*, 2008; Sahu *et al.*, 2012), PAF is involved in other types of cancer (Bussolati *et al.*, 2000; Denizot *et al.*, 2005; Denizot *et al.*, 2006; Aponte *et al.*, 2008), and likely has an important role in inflammation-related carcinogenesis. Hence, a better understanding of the mechanism(s) by which inflammatory mediators such as PAF contribute to inflammation, carcinogenesis and immune suppression is needed.

Evidence suggests that epigenetic mechanisms may be involved in modulating the expression of pro-inflammatory signals (Shuto *et al.*, 2006; Adcock *et al.*, 2007; Sullivan *et al.*, 2007; Medzhitov and Horng, 2009). Epigenetic alterations have also been linked to tumor development, including skin

[1]*Dipartimento di Scienze delle Vita e dell'Ambiente, Università Politecnica delle Marche, Ancona, Italy;* [2]*Department of Immunology, Unit 902, The Center for Cancer Immunology Research, The University of Texas, MD Anderson Cancer Center, Houston, Texas, USA and* [3]*The Graduate School for Biomedical Sciences, Houston, Texas, USA*

Correspondence: Stephen E. Ullrich, Department of Immunology, Unit 902, The Center for Cancer Immunology Research, The University of Texas, MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030, USA. E-mail: sullrich@mdanderson.org

[4]*These authors contributed equally to this work.*

Abbreviations: ChIP, Chromatin immunoprecipitation; cPAF, carbamyl-platelet-activating factor; CXCR4, C-X-C chemokine receptor type 4; CXCL12, Chemokine C-X-C motif ligand 12; DNMT, DNA methyltransferase; FcεRI, Fc epsilon receptor 1; H3, histone H3; HDAC, histone deacetylase; PAF, platelet activating factor; RT–qPCR, real-time quantitative polymerase chain reaction; UVB, UV radiation 290–320 nm

Received 9 July 2015; revised 27 July 2015; accepted 11 August 2015; accepted article preview online 28 August 2015; published online 24 September 2015

cancer (Counts and Goodman, 1995; Jones, 2002; Jones and Baylin, 2007). The upregulation of CXCR4 expression on melanoma cells treated with DNA-demethylating agents suggests that epigenetic mechanisms regulate the expression of CXCR4 (Mori *et al.*, 2005). Sato *et al.* (2005) reported hyperacetylation of the *CXCR4* promoter in pancreatic cancer. Similarly, epigenetic regulation of CXCR4 in cutaneous and uveal melanoma has been reported (Mori *et al.*, 2005; Li *et al.*, 2013). Furthermore, the absence of methylation on the *CXCR4* promoter is associated with poorer overall survival in breast cancer patients (Ramos *et al.*, 2011). Inhibition of histone deacetylase by valproic acid increased CXCR4 expression in hematopoietic stem/progenitor cells (Gul *et al.*, 2009). These studies support the concept that *CXCR4* may be regulated at the transcriptional level by both DNA methylation and chromatin remodeling.

It is now recognized that environmental stimuli can modify the epigenetic profile of a gene, and some suggest that epigenetic mechanisms connect the genome with the external environment (Suarez-Alvarez *et al.*, 2013). Chromatin structure is unraveled by the acetylation of lysine tails of the nucleosomal core histones H3- and H4-activating gene expression, whereas histone deacetylation is associated with gene silencing (Pazin and Kadonaga, 1997; Cheung *et al.*, 2000). Gene silencing may also be controlled by DNA methylation in which the addition of cytosines to the promoter region of target genes is mediated by DNA methyltransferase enzymes (Rhee *et al.*, 2002). Because PAF is an important inducer of CXCR4 on mast cells, we asked whether PAF activates epigenetic mechanisms that affect the expression of CXCR4.

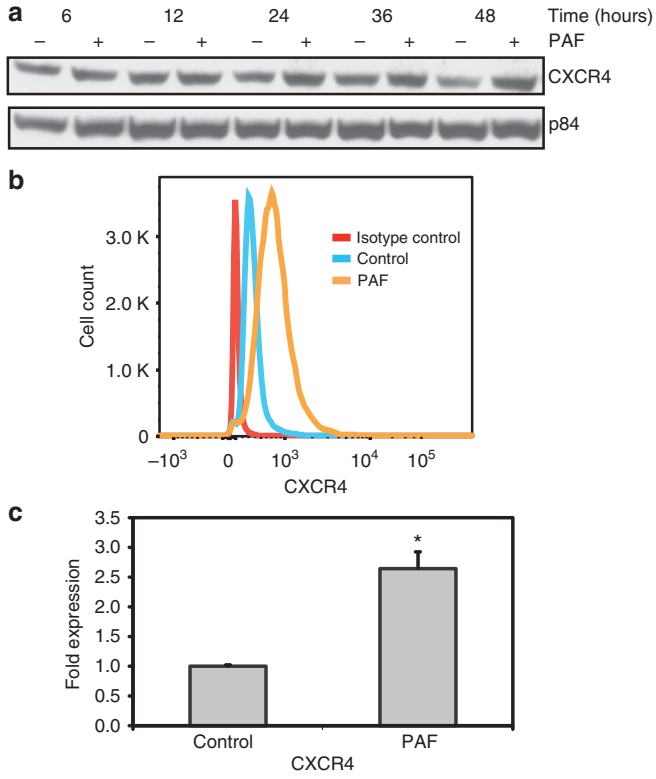

Figure 1. CXCR4 expression in platelet-activating factor (PAF)-treated HMC-1 cells. (**a**) Immunoblotting analysis of CXCR4 protein expression 6–24 hours after carbamyl-PAF (cPAF; 10 μM) treatment. p84 was the loading control. (**b**) Flow cytometry was used to measure CXCR4 surface expression 24 hours post-cPAF treatment. (**c**) CXCR4 mRNA levels were increased 24 hours post-cPAF treatment. Data represent the mean ± SEM ($N = 3$). *$P < 0.05$ vs. control (Student's *t*-test).

RESULTS

PAF upregulates CXCR4 expression on human mast cells

Because all of our previous data demonstrating that PAF upregulates CXCR4 expression were obtained with murine mast cells, we asked whether PAF similarly affects human mast cells. We used the human mast cell line HMC-1 (Butterfield *et al.*, 1988; Juremalm *et al.*, 2000; Ma *et al.*, 2013). On the basis of previous studies, we used concentrations of PAF that activated significant physiological changes *in vitro* (Pei *et al.*, 1998; Dy *et al.*, 1999; Walterscheid *et al.*, 2002; Feuerherm *et al.*, 2013; Chacón-Salinas *et al.*, 2014), without affecting cell viability (Puebla-Osorio *et al.*, 2015). The metabolically stable PAF analog carbamyl-PAF (cPAF) was used. Immunoblotting demonstrated the upregulation of CXCR4 in HMC-1 cells 24, 36 and 48 hours after cPAF treatment (Figure 1a). Using flow cytometry we confirmed that cPAF induced the expression of CXCR4 on the surface of HMC-1 cells (Figure 1b), and real-time quantitative polymerase chain reaction (RT–qPCR) was used to document that cPAF induced close to a 3-fold increase in CXCR4 mRNA expression (Figure 1c). These results demonstrate that cPAF upregulates human mast cell CXCR4 expression.

PAF depresses DNA methyltrasferase expression in HMC-1 cells

To determine whether PAF affected methylation patterns in mast cells, we first addressed its effect on common DNA methyltransferases. cPAF induced a concentration- and time-dependent reduction of DNMT1 in HMC-1 cells (Figure 2a). cPAF induced a reduction in DNMT1 as early as 6 hours, and a dramatic decrease in DNMT1 expression was observed 24 hours after the cPAF treatment (Figure 2b). The effect was sustained for up to 48 hours post exposure. To confirm the effect on DNMT1 expression was attributed to cPAF binding to its receptor on mast cells, we used a PAF receptor antagonist ABT-491 (Albert *et al.*, 1997). Failure to see a downregulation of DNMT1 expression in cells treated with the PAF receptor antagonist indicates that the cPAF-induced reduction in DNMT1 is via cPAF binding to its receptor (Figure 2c). Next, we assessed the effect of cPAF on DNMT3b expression and observed a similar effect. cPAF induced a decrease in DNMT3b protein expression 36 and 48 hours post treatment (Figure 2d). These results were confirmed using RT–qPCR, which indicated that cPAF induced a significant reduction in mRNA levels, including a 5-fold decrease in DNMT3b and a 3-fold decrease in DNMT1 (Figure 2e). However, when we measured the methylation pattern of CpG islands on the promoter region of *CXCR4*, by bisulfite modification coupled with pyrosequencing, we found no statistical differences between the control group and the

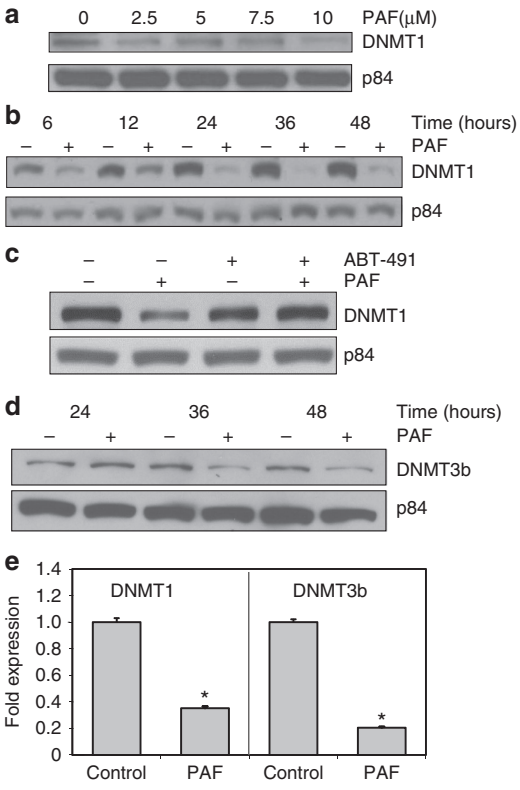

Figure 2. Platelet-activating factor (PAF) decreases the expression of DNMT1 and 3b. (**a**) DNMT1 protein expression in carbamyl-PAF (cPAF)-treated HMC-1 cells was analyzed by immunoblotting; p84 is the loading control. (**b**) Time course of cPAF (10 μM)-induced DNMT1 depression. (**c**) Treating the cells with a PAF receptor antagonist (AMT-491) blocks the cPAF-induced depression of DNMT1. (**d**) Time course of cPAF-induced depression of DNMT3b expression. (**e**) Effect of cPAF on DNMT1 and 3b mRNA expression as measured with real-time quantitative polymerase chain reaction (RT–qPCR). Data represent the mean ± SEM ($N = 3$). *$P < 0.05$ vs. control (Student's *t*-test).

cPAF-treated group (Supplementary Figure 1 online). This observation led us to focus on the effects of cPAF on histone acetylation.

PAF effects acetylation of the *CXCR4* promoter

First we asked whether cPAF affected the expression of known acetylating and deacetylating enzymes, which could be indicators of active gene expression (Gong and Miller, 2013). cPAF induced a time-dependent increase in the expression of p300, which has an intrinsic histone acetyltransferase activity (Counts and Goodman, 1995). Increased protein expression of p300 was observed starting at 12 hours and high expression continued for the duration of the experiment (Figure 3a). This coincided with decreased expression of the deacetylating enzyme, HDAC2 (Figure 3b).

Next, we determined whether cPAF affected the protein expression of acetylated H3 (H3K9/14/18/23/27) and H4 (H4K5/8/12/16; Figure 4). As shown in Figure 4a, the protein expression of acetyl-H3 was increased by cPAF. On the other hand, we were unable to reproducibly document acetylation of H4 (data not shown).

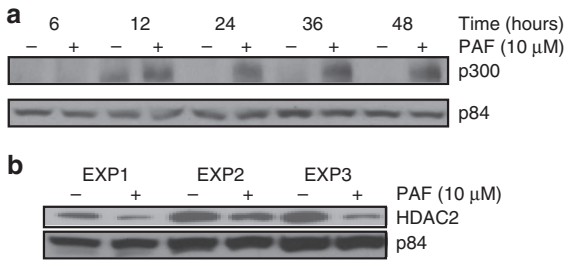

Figure 3. Platelet-activating factor (PAF) affects the expression of p300 and HDAC2 in HMC-1 cells. (**a**) Time course expression of p300 after incubation with 10-μM carbamyl-PAF (cPAF). p84 is the loading control. (**b**) HDAC2 expression was depressed 24 hours post-cPAF (10 μM) treatment. Data from three independent experiments are shown.

To confirm that an increase in acetyl-H3 expression affected the acetylation status of the *CXCR4* promoter, we performed chromatin immunoprecipitation assay (ChIP). Our results showed that cPAF induced an almost 3-fold increase in the acetylation status of H3 (H3K9/14/18/23/27) on the promoter region of the *CXCR4* gene (Figure 4b).

Next, we tested whether cPAF had a similar effect on non-transformed cells. We isolated buffy coat mast cells, as described previously (Puebla-Osorio *et al.*, 2015), and treated them with different concentrations of cPAF. Our results indicate that cPAF upregulates the expression of Acetyl-H3 (H3K9/14/18/23/27) in non-transformed mast cells, in a concentration dependent manner (Figure 4c), similar to that found in HMC-1 cells.

Inhibiting acetylation blocks cPAF-induced upregulation of CXCR4

Because our findings to this point indicate that cPAF upregulates mast cell surface expression of CXCR4 and histone acetylation of the *CXCR4* promoter, we wanted to determine whether the cPAF-induced upregulation of mast cell CXCR4 surface expression was linked to increased acetylation. We used curcumin, a specific inhibitor of histone acetylation (Balasubramanyam *et al.*, 2004) to block acetylation in cPAF-treated cells. The data presented in Figure 5a indicate that curcumin effectively reduced mast cell expression of CXCR4. Furthermore, curcumin depressed cPAF-induced upregulation of p300 (Figure 5b). These data support the hypothesis that cPAF upregulates CXCR4 expression on mast cells by activating epigenetic mechanisms.

DISCUSSION

Here we show that CXCR4 expression is upregulated in human mast cells by cPAF and that this is associated with changes in epigenetic marks. As mentioned above, the dose of cPAF used here was chosen on the basis of studies in the literature in which cPAF was used to activate cells *in vitro* (Feuerherm *et al.*, 2013; Puebla-Osorio *et al.*, 2015). Generally, tissue concentrations of PAF are reported to be in the picomolar range (Marathe *et al.*, 2005; Travers *et al.*, 2010); however, under certain conditions, such as inflammation and cancer, serum levels as high as 10^{-7} molar, which

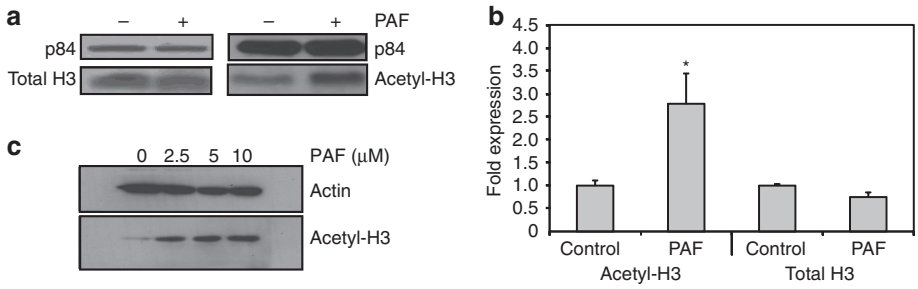

Figure 4. Effect of platelet-activating factor (PAF) on histone acetylation. (**a**) Protein levels of total H3 and acetylated H3 in HMC-1 cells 24 hours post-carbamyl-PAF (cPAF; 10 μM) treatment. p84 is the loading control. (**b**) Acetylation of the CXCR4 promoter using chromatin immunoprecipitation assay (ChIP) analysis. HMC-1 cells were treated with 10-μM cPAF and harvested 24 hours later. Total H3 was used as positive control, and data were normalized against input DNA. Data represent the mean ± SEM ($N = 4$). *$P < 0.05$ vs. control (Mann–Whitney *U*-test,). (**c**) PAF upregulates the expression of acetyl-H3 mRNA in normal mast cells. Buffy coat–derived mast cells were treated with increasing concentrations of cPAF, and immunoblotting was used to determine the expression of acetyl-H3 (K9/14/18/23/27). Actin is the loading control.

approach the concentrations used here, have been reported (Pitton *et al.*, 1989; Lehr *et al.*, 1997). One must also keep in mind that PAF in the serum has a limited half-life (minutes) because of the action of PAF acetylhydrolase (Stafforini *et al.*, 1996). Furthermore, platelets and endothelial cells are known to produce PAF but not secrete it, rather the cell-associated form of PAF is active (Lorant *et al.*, 1991). This suggests that the local concentration of PAF may be very high in inflammed tissues.

The downregulation of DNA methyltransferase DNMT1 and DNMT3b is suggestive of reduced promoter methylation, which is associated with gene reactivation. These results are in line with the findings of Przybylski *et al.* (2010) who showed that the reduction of both DNMT1 and DNMT3b results in an increased demethylation of the *CXCR4* promoter, leading to an increased expression of CXCR4. Although our results on the methylation status, as assessed by bisulphite sequencing, showed no significant difference between cPAF-treated and the control samples, we showed that DNMTs are downregulated upon PAF exposure. Because cPAF promotes rather than depresses the expression of CXCR4, rather than pursuing methylation, we focused our attention on acetylation. We observed a significant upregulation of the acetylation of H3 (H3K9/14/18/23/27) on the promoter region of *CXCR4* in cPAF-treated mast cells. Further, we observed increased expression of p300 acetyltransferase, which promotes chromatin relaxation on transcriptionally active genes (Liu *et al.*, 2008), and a decrease in HDAC2 histone deacetylase, a key factor in deacetylation of lysine residues on core histones. These changes are generally associated with gene activation (Hildmann *et al.*, 2007). Moreover, when we inhibited acetylation in cPAF-treated cells with curcumin, we were able to decrease both the expression of p300 and the cPAF-induced expression of CXCR4 on the surface of mast cells. These findings demonstrate that cPAF-induced expression of CXCR4 is associated with increased acetylation of the promoter region of *CXCR4*, which presumably activates its transcription. cPAF also upregulated histone acetylation in normal mast cells, suggesting that its effect was not exclusive to transformed cells. Interestingly, these findings are

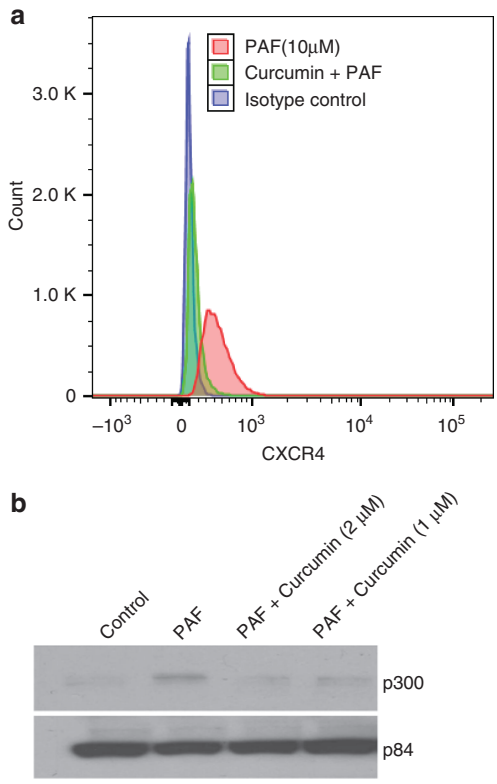

Figure 5. Blocking acetylation inhibits platelet-activating factor (PAF)-induced cell surface expression of CXCR4. (**a**) Mast cells treated with carbamyl-PAF (cPAF; red) or cPAF in the presence of curcumin (green) were analyzed using FACS 24 hours post-cPAF treatment. The isotype control is shown in blue. (**b**) cPAF-induced upregulation of p300 is inhibited by curcumin. An aliquot of the same cells analyzed using FACS was removed and lysed and p300 expression determined by immunoblotting. p84 is the loading control.

supported by the work of Conte *et al.* (2014) who recently reported that HDAC2 reduces gene expression by repressing areas of chromatin that do not allow p300 binding and consequent acetylation, whereas silencing of HDAC2 activates p300 recruitment and H3K9-14 acetylation. Others

have suggested that there is synergy between demethylation and histone deacetylase inhibition in re-expression of genes (Cameron *et al.*, 1999; Gray and Teh, 2001). In this respect, cPAF appears to share similarities in its action with both HDAC inhibitors and methylation inhibitors used in the development of drugs that target epigenetic regulators, such as Trichostatin A and 5-Aza 2-deoxycytidine (Mori *et al.*, 2005; Przybylski *et al.*, 2010; Ramos *et al.*, 2011; Li *et al.*, 2013).

Our findings provide evidence for a novel molecular mechanism for PAF, in which it may affect physiological and pathological processes via epigenetic modifications in human mast cells, and likely other cell types. Our findings allow us to speculate that a possible mechanism by which PAF mediates photoimmune suppression is through epigenetic modulation of the *CXCR4* gene. Therefore, PAF may serve as a critical molecular mediator that links the environment (UVB radiation) with the epigenome.

MATERIALS AND METHODS

Antibodies and reagents

cPAF, a non-hydrolyzable bioactive analog of PAF, was obtained from Sigma-Aldrich (St Louis, MO). A 10-mM stock solution was prepared in water, aliquoted, and stored at − 20 °C until use. The PAF receptor antagonist, ABT-491, was purchased from Sigma-Aldrich and prepared as a 24-mM stock solution in water. Antibodies specific for DNMT1 (sc-135886) and HDAC2 (sc-9959) were purchased from Santa Cruz Biotechnology (Dallas, TX). Antibodies specific for DNMT3b (ab13604), CXCR4 (ab2074), Acetyl-H3 (ab47915), and Total H3 (ab1791) were purchased from Abcam (Cambridge, MA). Anti-p84 (GTX70220) was purchased from Genetex (Kennesaw, GA). Anti-mouse (7076S), anti-rabbit horseradish peroxidase (7074S) and anti-p300 (K1499) were obtained from Cell Signaling Technology (Danvers, MA). Anti-rabbit Alexa488 was purchased from Molecular Probes (Eugene, OR). All other chemicals were purchased from Sigma-Aldrich.

Cell cultures

The human mast cell line, HMC-1 (kindly provided by Dr J.H. Butterfield, May Clinic, Rochester, MN; Butterfield *et al.*, 1988), was cultured in complete RPMI-1640 medium containing 10% heat-inactivated fetal calf serum under standard culture conditions (37 °C, 5% CO_2, humidified atmosphere). The cells were passaged every 3–4 days. Normal mast cells were isolated from a buffy coat obtained from an undisclosed healthy donor from the Gulf Coast Regional Blood Center (MDACC IRB LAB-03-0390), as described previously (Puebla-Osorio *et al.*, 2015). Briefly, CD34+ cells were cultured in complete medium with human IL-6, IL-3, and recombinant human Stem Cell factor. After 4–6 weeks in culture all the viable cells stained positive for CD117 (cKit), tryptase, and toluidine blue.

Treatment with cPAF

HMC-1 cells were seeded at a density of 5×10^5 cells ml^{-1} in 60 × 15-mm Petri dishes and treated with 10 μM cPAF for various time points (6-48 hours). When the PAF receptor antagonist was used the cells were pre-treated with 100 μM ABT-491, 1 hour before treatment with cPAF.

Western blot analysis

Cells were harvested, centrifuged, and washed with cold PBS, and the cell pellet was then lysed with 200 μl RIPA buffer. Protein concentration was determined using the Pierce BCA Protein Assay Kit (Thermo Scientific, Rockford, IL). The proteins were separated on 6, 8, or 15% SDS-PAGE gels. Proteins were transferred onto polyvinylidene difluoride membranes overnight at 4 °C. Nonspecific binding sites were blocked in 5% non-fat dry milk/Superblock blocking buffer in PBS (Thermo Scientific) for 1 hour at room temperature, followed by 2-hour incubation at room temperature with primary antibodies, appropriately diluted, against DNMT1 (1:300), DNMT3b (1:300), CXCR4 (1:300), HDAC2 (1:250), p300 (1:250), Acetyl-H3 (1:800), Total H3 (1:2,000), and p84 (1:1,000). After washing in PBS/0.1% Tween 20, the membranes were incubated with horseradish peroxidase–conjugated secondary antibody (mouse, 1:3,000; rabbit 1:1,000) for 1 hour at room temperature. Band detection was carried out using an enhanced chemiluminescent substrate (Supersignal West Dura; Thermo Scientific) and captured on X-ray films. p84 was used as the loading control because it is contained in the nucleus where most of the proteins that were assessed in this study reside, and it is not affected by cPAF treatment.

Quantitative real-time PCR

Cells were harvested, washed with cold PBS, and 500 μl of Trizol reagent (Invitrogen, Grand Island, NY) was added to the resulting pellet. Total RNA was extracted according to the manufacturer's instructions for Trizol reagent and stored at − 80 °C. The concentration and purity of RNA obtained was determined by UV spectrophotometry, and 0.2 μg from each sample was then reverse-transcribed using iScript Reverse Transcription Supermix (Bio-Rad, Hercules, CA) according to the manufacturer's instructions. All complementary DNA samples were diluted five times, and 3 μl of each dilution was used as template in RT–qPCR reactions (total volume 15 μl). Manufacturer-supplied standardized primer pairs from Bio-Rad were used to measure DNMT1 and DNMT3b while the following primer pairs for CXCR4 amplification were used: 5′-GGAA GCTGTTGGCTGAAAAG-3′ (forward) and 5′-CTCACTGACGTTGGC AAAGA-3′ (reverse). As an internal reference, beta-2-microglobulin was used to normalize the amount of mRNA in each qPCR reaction, using the following primers: 5′-CATTCCTGAAGCTGACAGCATTC -3′ (forward), 5′-TGCTGGATGACGTGAGTAAACC-3′ (reverse). The reactions were run on a CFX96 Real-Time PCR Detection System (Bio-Rad) under the following PCR conditions: 95 °C for 2 minutes, 40 cycles at 95 °C for 5 seconds, and 60 °C for 30 seconds. The results obtained were analyzed using the CFX Manager Software (Bio-Rad). The fold changes in relative mRNA expression levels were normalized to the expression level of the beta-2-microglobulin mRNA in each sample using the cycle threshold (C_t) method and using the $2^{-\Delta\Delta Ct}$ formula according to Arya *et al.* (2005).

Flow cytometry analysis

Cells were harvested, washed twice with PBS, and maintained in 1 ml Superblock blocking buffer in PBS (Thermo Scientific) for 20 minutes. Cells were then incubated with anti-human CXCR4-specific antibody diluted to 1:200 in PBS/0.2% FBS for 45 minutes at room temperature. Cells were washed twice with PBS and incubated with anti-rabbit Alexa488 antibody diluted to

1:750 in PBS/0.2% FBS for 45 minutes at room temperature. Cells were then washed twice with PBS, fixed in 2% paraformaldehyde for 10 minutes at room temperature in the dark, washed twice with PBS, resuspended in 0.2 ml PBS, and fluorescence measured using an LSRII (Becton Dickinson, San Jose, CA). The data were analyzed using the FlowJo Software (Ashland, OR).

Chromatin immunoprecipitation

ChIP was conducted according to the manufacturer's protocol (EMD Millipore, Billerica, MA). After a 24-hour incubation with 10 μM cPAF, the treated mast cells were exposed to 1% formaldehyde for 10 minutes at room temperature to crosslink chromatin. Cells were harvested and washed twice with ice-cold PBS. The cell pellet was resuspended in SDS lysis buffer on ice for 10 minutes and then sonicated five times for 10 seconds, each followed by centrifugation for 10 minutes at 4 °C. Before immunoprecipitation, the supernatants were diluted 10× with ChIP dilution buffer and precleared by the addition of protein A agarose/salmon sperm DNA (50% slurry) for 2 hours at 4 °C. After centrifugation, the precleared chromatin supernatant fraction was immunoprecipitated overnight at 4 °C with 15 μl of specific antibodies or with control rabbit-IgG. After immunoprecipitation, protein A agarose/salmon sperm DNA (50% slurry) was added and the samples were incubated for 1 hour at 4 °C to collect the antibody/histone complex. After centrifugation, the precipitates were washed sequentially before elution by 15-minute incubation in 250 μl of freshly prepared elution buffer (1% SDS, 0.1 M $NaHCO_3$). Eluates were heated at 65 °C for 6 hours in the presence of 5 M NaCl to reverse crosslinking. The samples were then treated with proteinase K (10 mg ml^{-1}) for 1 hour at 45 °C before purification using the QIAquick PCR purification kit (QIAGEN, Valencia, CA) and collected in 50 μl of elution buffer (10 mM Tris-HCl, pH 8.5). Purified DNA was subjected to RT–qPCR analysis with appropriate primer pairs for human *CXCR4* promoter region, which spanned from 812 to 898 bp: 5′-GAGAGACGCGTTCCTAGCC-3′ (forward) and 5′-GGACCTCCCAGAGGCATTTC-3′ (reverse). The primer sequences used in our experiments were blasted against the human genome database and generated a 100% alignment with the promoter region of *CXCR4*. The reactions were run and analyzed as reported above using the input samples for normalization.

Statistical analysis

Each experiment was repeated three times, with the exception of the data generated with normal mast cells, which was repeated twice. Statistical differences between the control and experimental groups were analyzed using either the Mann–Whitney *U*-test or the Student's *t*-test (GraphPad Prism, La Jolla, CA). Significant differences were defined as $P < 0.05$.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Cancer Institute (CA131207) and the Cancer Prevention and Research Institute of Texas (RP120777). The histology, characterized cell line core, DNA methylation analysis core, and flow cytometry facilities at the MDACC are supported in part by a core grant from the NCI (CA16672). We thank Nasser Kazimi and Francesca Brugè for technical support, and Dr Haiyan Li for help with the ChIP assays.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

REFERENCES

Adcock IM, Tsaprouni L, Bhavsar P *et al.* (2007) Epigenetic regulation of airway inflammation. *Curr Opin Immunol* 19:694–700

Albert DH, Magoc TJ, Tapang P *et al.* (1997) Pharmacology of ABT-491, a highly potent platelet-activating factor receptor antagonist. *Eur J Pharmacol* 325:69–80

Aponte M, Jiang W, Lakkis M *et al.* (2008) Activation of platelet-activating factor receptor and pleiotropic effects on tyrosine phospho-EGFR/Src/FAK/paxillin in ovarian cancer. *Cancer Res* 68:5839–48

Arya M, Shergill IS, Williamson M *et al.* (2005) Basic principles of real-time quantitative PCR. *Expert Rev Mol Diagn* 5:209–19

Balasubramanyam K, Varier RA, Altaf M *et al.* (2004) Curcumin, a novel p300/CREB-binding protein-specific inhibitor of acetyltransferase, represses the acetylation of histone/nonhistone proteins and histone acetyltransferase-dependent chromatin transcription. *J Biol Chem* 279:51163–71

Bussolati B, Biancone L, Cassoni P *et al.* (2000) PAF produced by human breast cancer cells promotes migration and proliferation of tumor cells and neo-angiogenesis. *Am J Pathol* 157:1713–25

Butterfield JH, Weiler D, Dewald G *et al.* (1988) Establishment of an immature mast cell line from a patient with mast cell leukemia. *Leuk Res* 12: 345–55

Byrne SN, Limón-Flores AY, Ullrich SE (2008) Mast cell migration from the skin to the draining lymph nodes upon ultraviolet irradiation represents a key step in the induction of immune suppression. *J Immunol* 180: 4648–55

Cameron EE, Bachman KE, Myohanen S *et al.* (1999) Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. *Nat Genet* 21:103–7

Chacón-Salinas R, Chen L, Chávez-Blanco AD *et al.* (2014) An essential role for platelet-activating factor in activating mast cell migration following ultraviolet irradiation. *J Leukoc Biol* 95:139–48

Chacón-Salinas R, Limón-Flores AY, Chávez-Blanco AD *et al.* (2011) Mast cell-derived IL-10 suppresses germinal center formation by affecting T follicular helper cell function. *J Immunol* 186:25–31

Cheung P, Allis CD, Sassone-Corsi P (2000) Signaling to chromatin through histone modifications. *Cell* 103:263–71

Conte M, Dell'Aversana C, Benedetti R *et al.* (2014) HDAC2 deregulation in tumorigenesis is causally connected to repression of immune modulation and defense escape. *Oncotarget* 6:886–901

Counts JL, Goodman JI (1995) Alterations in DNA methylation may play a variety of roles in carcinogenesis. *Cell* 83:13–5

Denizot Y, De Armas R, Caire F *et al.* (2006) Platelet-activating factor and human meningiomas. *Neuropathol Appl Neurobiol* 32:674–8

Denizot Y, Descottes B, Truffinet V *et al.* (2005) Platelet-activating factor and liver metastasis of colorectal cancer. *Int J Cancer* 113:503–5

Dy LC, Pei Y, Travers JB (1999) Augmentation of ultraviolet B radiation-induced tumor necrosis factor production by the epidermal platelet-activating factor receptor. *J Biol Chem* 274:26917–1

Feuerherm AJ, Jorgensen KM, Sommerfelt RM *et al.* (2013) Platelet-activating factor induces proliferation in differentiated keratinocytes. *Mol Cell Biochem* 384:83–94

Gong F, Miller KM (2013) Mammalian DNA repair: HATs and HDACs make their mark through histone acetylation. *Mutat Res* 750:23–30

Gray SG, Teh BT (2001) Histone acetylation/deacetylation and cancer: an "open" and "shut" case? *Curr Mol Med* 1:401–29

Gul H, Marquez-Curtis LA, Jahroudi N *et al.* (2009) Valproic acid exerts differential effects on CXCR4 expression in leukemic cells. *Leuk Res* 34: 235–42

Hart PH, Grimbaldeston MA, Swift GJ *et al.* (1998) Dermal mast cells determine susceptibility to Ultraviolet B-induced systemic suppression of contact hypersensitivity responses in mice. *J Exp Med* 187:2045–53

Hildmann C, Riester D, Schwienhorst A (2007) Histone deacetylases–an important class of cellular regulators with a variety of functions. *Appl Microbiol Biotechnol* 75:487–97

Jones PA (2002) DNA methylation and cancer. *Oncogene* 21:5358–60

Jones PA, Baylin SB (2007) The epigenomics of cancer. *Cell* 128:683–92

Juremalm M, Hjertson M, Olsson N *et al.* (2000) The chemokine receptor CXCR4 is expressed within the mast cell lineage and its ligand stromal cell-derived factor-1alpha acts as a mast cell chemotaxin. *Eur J Immunol* 30: 3614–22

Lehr HA, Weyrich AS, Saetzler RK *et al.* (1997) Vitamin C blocks inflammatory platelet-activating factor mimetics created by cigarette smoking. *J Clin Invest* 99:2358–64

Li H, Niederkorn JY, Sadegh L *et al.* (2013) Epigenetic regulation of CXCR4 expression by the ocular microenvironment. *Invest Ophthalmo Vis Sci* 54: 234–43

Liu X, Wang L, Zhao K *et al.* (2008) The structural basis of protein acetylation by the p300/CBP transcriptional coactivator. *Nature* 451:846–50

Lorant DE, Patel KD, McIntyre TM *et al.* (1991) Coexpression of GMP-140 and PAF by endothelium stimulated by histamine or thrombin: a juxtacrine system for adhesion and activation of neutrophils. *J Cell Biol* 115: 223–34

Ma Y, Hwang RF, Logsdon CD *et al.* (2013) Dynamic mast cell-stromal cell interactions promote growth of pancreatic cancer. *Cancer Res* 73: 3927–7

Marathe GK, Johnson C, Billings SD *et al.* (2005) Ultraviolet B radiation generates platelet-activating factor-like phospholipids underlying cutaneous damage. *J Biol Chem* 280:35448–57

Medzhitov R, Horng T (2009) Transcriptional control of the inflammatory response. *Nat Rev Immunol* 9:692–703

Mori T, Kim J, Yamano T *et al.* (2005) Epigenetic up-regulation of C-C chemokine receptor 7 and C-X-C chemokine receptor 4 expression in melanoma cells. *Cancer Res* 65:1800–7

Pazin MJ, Kadonaga JT (1997) What's up and down with histone deacetylation and transcription? *Cell* 89:325–8

Pei Y, Barber LA, Murphy RC *et al.* (1998) Activation of the epidermal platelet-activating factor receptor results in cytokine and cyclooxygenase-2 biosynthesis. *J Immunol* 161:1954–61

Pitton C, Lanson M, Besson P *et al.* (1989) Presence of PAF-acether in human breast carcinoma: relation to axillary lymph node metastasis. *J Natl Cancer Inst* 81:1298–302

Prescott SM, Zimmerman GA, Stafforini DM *et al.* (2000) Platelet-activating factor and related lipid mediators. *Annu Rev Biochem* 69:419–5

Przybylski M, Kozlowska A, Pietkiewicz PP *et al.* (2010) Increased CXCR4 expression in AsPC1 pancreatic carcinoma cells with RNA interference-mediated knockdown of DNMT1 and DNMT3B. *Biomed Pharmacother* 64:254–8

Puebla-Osorio N, Damiani E, Bover L *et al.* (2015) Platelet-activating factor induces cell cycle arrest and disrupts the DNA damage response in mast cells. *Cell Death Dis* 6:e1745

Ramos EA, Grochoski M, Braun-Prado K *et al.* (2011) Epigenetic changes of CXCR4 and its ligand CXCL12 as prognostic factors for sporadic breast cancer. *PLoS One* 6:e29461

Rhee I, Bachman KE, Park BH *et al.* (2002) DNMT1 and DNMT3b cooperate to silence genes in human cancer cells. *Nature* 416:552–6

Sahu RP, Turner MJ, DaSilva SC *et al.* (2012) The environmental stressor ultraviolet B radiation inhibits murine antitumor immunity through its ability to generate platelet-activating factor agonists. *Carcinogenesis* 33: 1360–7

Sato N, Matsubayashi H, Fukushima N *et al.* (2005) The chemokine receptor CXCR4 is regulated by DNA methylation in pancreatic cancer. *Cancer Biol Ther* 4:70–6

Shuto T, Furuta T, Oba M *et al.* (2006) Promoter hypomethylation of Toll-like receptor-2 gene is associated with increased proinflammatory response toward bacterial peptidoglycan in cystic fibrosis bronchial epithelial cells. *FASEB J* 20:782–4

Sreevidya CS, Khaskhely NM, Fukunaga A *et al.* (2008) Inhibition of photocarcinogenesis by platelet-activating factor or serotonin receptor antagonists. *Cancer Res* 68:3978–84

Stafforini DM, Prescott SM, Zimmerman GA *et al.* (1996) Mammalian platelet-activating factor acetylhydrolases. *Biochim Biophys Acta* 1301:161–73

Suarez-Alvarez B, Baragano Raneros A, Ortega F *et al.* (2013) Epigenetic modulation of the immune function: a potential target for tolerance. *Epigenetics* 8:694–702

Sullivan KE, Reddy AB, Dietzmann K *et al.* (2007) Epigenetic regulation of tumor necrosis factor alpha. *Mol Cell Biol* 27:5147–60

Travers JB, Berry D, Yao Y *et al.* (2010) Ultraviolet B radiation of human skin generates platelet-activating factor receptor agonists. *Photochem Photobiol* 86:949–54

Walterscheid JP, Ullrich SE, Nghiem DX (2002) Platelet-activating factor, a molecular sensor for cellular damage, activates systemic immune suppression. *J Exp Med* 195:171–9

Yoshikawa T, Rae V, Bruins-Slot W *et al.* (1990) Susceptibility to effects of UVB radiation on induction of contact hypersensitivity as a risk factor for skin cancer in humans. *J Invest Dermatol* 95:530–6