Cancer Metastasis Rev (2007) 26:359–371
DOI 10.1007/s10555-007-9092-9

Inflammation and melanoma growth and metastasis: The role of platelet-activating factor (PAF) and its receptor

Vladislava Melnikova · Menashe Bar-Eli

Published online: 25 August 2007

Abstract An inflammatory tumor microenvironment fosters tumor growth, angiogenesis and metastatic progression. Platelet-activating factor (PAF) is an inflammatory biolipid produced from membrane glycerophospholipids. Through the activity of its G-protein coupled receptor, PAF triggers a variety of pathological reactions including tumor neoangiogenesis. Several groups have demonstrated that inhibiting PAF-PAF receptor pathway at the level of a ligand or receptor results in an effective inhibition of experimental tumor growth and metastasis. In particular, our group has recently demonstrated that PAF receptor antagonists can effectively inhibit the metastatic potential of human melanoma cells in nude mice. Furthermore, we showed that PAF stimulated the phosphorylation of CREB and ATF-1 in metastatic melanoma cells, which resulted in overexpression of MMP-2 and MT1-MMP. Our data indicate that PAF acts as a promoter of melanoma metastasis *in vivo*. Since only metastatic melanoma cells overexpress CREB/ATF-1, we propose that these cells are better equipped to respond to PAF within the tumor microenvironment when compared to their non-metastatic counterparts.

Keywords Platelet-activating factor · CREB · Melanoma · Metastasis · MMP-2 · MT1-MMP

V. Melnikova · M. Bar-Eli (✉)
Department of Cancer Biology,
The University of Texas M. D. Anderson Cancer Center,
P.O. Box 173, Houston, TX 77030, USA
e-mail: mbareli@mdanderson.org

1 Introduction

An expending amount of data reveals the importance of an inflammatory microenvironment in cancer initiation and progression, which brings new directions and approaches to cancer treatment. Genetic and functional experiments indicated that inflammatory cells such as tumor-infiltrating monocytes/macrophages, neutrophils, mast cells, eosinophils and activated T lymphocytes contribute to malignancies by releasing growth and survival factors, as well as extracellular proteases, proangiogenic factors and chemokines [1–4]. Furthermore, the formation of cancer cell emboli surrounded by platelets in the circulation is thought to protect tumor cells from the rapid clearance and cell death due to mechanical forces and immune surveillance. This promotes an adherence and penetration of tumor cells through the blood vessel endothelial cell barrier at potential metastatic sites [5–8]. Activated platelets also play a major role in tumor neoangiogenesis [9]. Initiating a pathological circle, cancer cells promote the recruitment of the inflammatory cells and produce inflammatory mediators and angiogenic factors through the activity of NF-κB and STAT3 transcription factors, or the COX-2 enzyme [2, 4, 10–12].

Melanoma is strongly associated with the inflammatory process due to high levels of cytokine secretion, including that of NFκB-dependent interleukin 8 (IL-8), GROα, GROβ, GROγ, and vascular endothelial growth factor (VEGF) [13–16]. These factors exert an autocrine control over melanoma tumor growth and promote tumor angiogenesis [13–16]. In addition, we have demonstrated that the expression of the proinflammatory protease-activated receptor-1 (PAR-1, thrombin receptor) directly correlates with the metastatic phenotype of melanoma [17, 18]. PAR-1 appears to play a central role in tumor growth and metastasis of many cancers, modulating expression of IL-8, matrix metalloproteinase-2, VEGF, platelet-derived growth factor, and integrins [18, 19].

Among proinflammatory mediators, platelet activating factor (PAF, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phoshocholine) is assuming a major role as a primary and secondary messenger involved in homotypic and heterotypic cell-to-cell communication that results in the activation of platelets, neutrophils, macrophages, lymphocytes, and endothelial cells [20–28]. All these cells can produce PAF, and can be activated by PAF (Fig. 1). In particular, PAF promotes the migration of granulocytes to sites of inflammation [29], mediates the adhesion of neutrophils to endothelial cells [20, 21], and is mitotic for fibroblasts [23, 24] and regulates lymphocyte functions [30]. In addition, it induces *in vitro* migration of human endothelial cells, and promotes angiogenesis *in vivo* [26–28]. In physiological conditions, PAF synthesis and degradation is tightly regulated. PAF is typically produced from the glycerophosphocholines, the most abundant lipids in biomembranes, and is rapidly degraded [31]. The reaction that releases PAF precursor is catalyzed by phospholipase A_2 (PLA_2), and yields an additional product, arachidonic acid, a substrate for the COX-2-mediated eprostanoid production. In pathological situations, PAF and PAF-like oxidized phospholipids [25, 31, 32] are produced in an unregulated fashion resulting in stimulation of a seven membrane-spanning domain G-protein coupled PAF receptor (PAFR) and the induction of various intracellular signaling cascades and gene transcription [22, 33, 34].

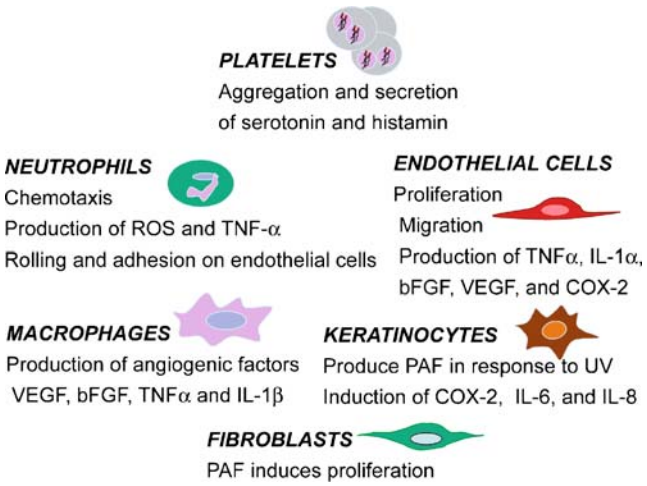

Fig. 1 PAF in the biology of various cells. Platelets, inflammatory cells, vascular endothelial cells and keratinocytes produce PAF and respond to PAF. In response to thrombin, platelets secrete the prestored PAF. In neutrophils, PAF mediates chemotaxis, rolling and adhesion on endothelial cells, and production of reactive oxygen species and TNF-α. In macrophages, it mediates production of angiogenic factors. In endothelial cells, PAF can directly induce or be a second messenger in growth factor- or cytokine-induced proliferation and migration. It also mediates the production of TNF-α, IL-1α, bFGF, VEGF and COX-2. Keratinocytes produce PAF when irradiated with UV, and respond to PAF by induction of COX-2, IL-6 and IL-8

PAF is a mediator of diverse pathological and physiological effects including wound healing, inflammation, apoptosis, angiogenesis, and reproduction [31]. Recent evidence suggests that PAF receptor-mediated signaling may be also involved in tumorigenesis and metastatic progression. The present review summarizes current data on the role of PAF in tumor growth and metastasis, with a special emphasis on its role in melanoma.

2 PAF and PAF receptor

2.1 PAF and PAF-like substances

Platelet-activating factor is a potent proinflammatory mediator and the first bioactive lipid ever identified [35–37]. PAF was initially discovered as a substance responsible for the aggregation of platelets that is released from IgE-sensitized rabbit basophils after antigen challenge [38]. PAF is produced via the *de novo* and remodeling pathways, the latter being regulated by extracellular signals [39–41]. In the remodeling pathway, PAF synthesis occurs from the 1-*O*-alkyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine via enzymatic hydrolysis catalyzed by phospholypase A_2, and further conversion of lysophosphatidylcholine into PAF by an acetyl-CoA:lysoPAF acetyltransferase [42–45], the latter enzyme being cloned most recently [46]. In a parallel process, the arachidonic acid released in the first reaction is further converted by COX-2 enzyme to form the precursor of prostanoids. Normally, the life-span of PAF is short due to a rapid and tightly regulated degradation by a unique class of enzymes, the PAF acetylhydrolases [47]. Although PAF can be metabolized to potentially biologically active neutral lipid or phosphatidic acid species [48, 49], the majority of PAF molecules will act through the specific seven transmembrane spanning G-protein-coupled receptor [50–52]. In addition to enzymatic routes, oxidation of unsaturated phosphatidylcholine produces fragmented phospholipids with agonistic PAF receptor activity [25, 31, 32]. Other products are known to possess PAF-like activity [53, 54]. Their list includes oxidized low density lipoprotein (LDL) with its major component implicated in atherosclerosis—lysophosphatidylcholine [42, 53, 54]. Furthermore, Nakamura and colleagues showed that bacterial lipopolysaccharide (LPS) can also directly activate PAF receptors [55]. Lipotechoic acid moieties on *Streptococcus* species can also directly stimulate PAF receptors [56].

2.2 PAF receptor

The PAF receptor (PAFR) gene has been localized to chromosome 1p35–p34.3 [53]. It generates two distinct mRNA species (PAF receptor transcripts 1 and 2), and

produces single PAFR protein [34, 53]. A variety of tissue and cell types can synthesize PAF and express functional PAF receptor [53]. Transcription factors NF-κB, SP-1 and TGF-β2 positively regulate PAFR expression through the consensus elements on promoter 1, while estrogen, retinoic acid and thyroid hormone T_3 demonstrate activity toward promoter of the PAFR transcript 2 [53]. A single nucleotide polymorphism (A224D) of PAFR that impairs its G-protein binding and function has been detected in Japanese populations [57]. Its significance vis-à-vis phenotypic variations in inflammatory response and sensitivity among the individuals remains to be elucidated.

Following stimulation with PAF, PAFR becomes rapidly desensitized due to phosphorylation-dependent internalization, and consequent proteosomal as well as lysosomal ubiquitin-dependent degradation [58]. Intracellular PAFR localized to endosomes has been detected and is thought to be a consequence of a ligand-driven receptor internalization [59, 60]. Similar to specific G-protein coupled receptors for other lipid mediators such as prostaglandin E2 and lysophosphatidic acid (LPA), functional PAF receptors have been identified on the nuclear envelope of endothelial cells [61, 62]. Using confocal microscopy and immunogold electron microscopy, Marache and colleagues found that PAFR is localized to the nuclear envelope and inside the nucleus of the isolated piglet brain microvascular endothelial cells *in vitro* and *in situ* [61]. Radiolabeled ligand binding studies and gene expression analysis in the freshly isolated nuclei further confirmed that PAFR was functional [61]. Furthermore, stimulation of nuclei with methylcarbamate-PAF evoked a decrease in cAMP production and a pertussis toxin-sensitive rise in nuclear calcium, unlike observations in plasma membrane, which exhibited a pertussis toxin-insensitive elevation in inositol phosphates [61]. Moreover, on isolated nuclei, methylcarbamate-PAF induced the expression of proinflammatory iNOS and COX-2 and was associated with an increase in ERK1/2 MAPK phosphorylation and activation of NF-κB binding to the DNA consensus sequence [61]. It is proposed that intracellular PAFR mediates the effects of PAF acting as a second messenger.

3 PAF and PAFR-mediated signal transduction pathways

Through different G-protein species and second messengers, Ca^{2+}, cAMP, inositol 1,4,5-triphosphate (IP_3), and diacylglycerol (DAG), PAF receptor activates MAP kinase, PI3 kinase, PLA_2, PKC and Src pathways [54, 63]. In Chinese hamster ovarian cells (CHO), PAF has been shown to trigger phosphorylation of ERK1/2 MAPK in a PKC-dependent, RAS-independent manner [64, 65]. ERK1/2 MAPK and p38 MAPK activation, as well as increased proliferation was induced by PAF in human epidermal KB cells transduced with PAFR gene [66]. These studies suggested that PAF-induced activation of ERK1/2 MAPK occurred via matrix metalloproteinase (MMP)-dependent cleavage of heparin-binding epidermal growth factor (HB-EGF) and subsequent activation of EGF receptor [66]. In contrast, in human melanoma cells, PAF (at nano-molar concentrations) does not stimulate ERK1/2 MAPK phosphorylation (our unpublished data). Several groups, including ours demonstrated that PAF can stimulate the activity of p38 MAP kinase in human neutrophils, metastatic melanoma cells and human epidermal cells [67–69]. PAF has also been shown to activate the PI3K/AKT pathway in human eosinophils [70]. In addition, PAF receptor stimulation can induce activation of the non-receptor tyrosine kinase Src [53]. Activation of these signal transduction cascades by PAF results in upregulation of important effectors of tumor growth, angiogenesis and malignant progression, such as NF-κB, STAT-3 and MMPs.

The ability of PAF to activate NF-κB was first demonstrated by Kravchenko et al. in epidermal KB cells transduced with PAFR gene, in P388D murine macrophages and in human ASK.0 cells [71]. Through PAFR on the nuclear envelop, PAF has been shown to induce the expression of proinflammatory iNOS and COX-2 in endothelial cells via the ERK1/2 MAPK/NF-κB cascade [61].

Deo et al. have demonstrated that in human umbilical vein endothelial cells (HUVECs), basic fibroblast growth factor (bFGF) activates STAT-3 phosphorylation via a PAF-PAFR-mediated mechanism [72, 73]. Their analysis revealed that the first rapid phase of STAT-3 phosphorylation requires activation of $G\alpha_q$ pertussis toxin-insensitive protein, adenylyl cyclase, increase in cAMP levels and activation of PKA, which is mediated by Src. On the other hand, a delayed phase of PAF-induced phosphorylation of STAT-3 occurs independently from these intermediates and proceeds through activation of Janus kinase-2 (JAK-2) [72, 73]. In addition, the same authors demonstrated that PAF induced phosphorylation of focal adhesion kinase (FAK) in HUVECs in a cAMP/PKA/Src independent manner [72, 74].

Several reports suggest that PAF regulates the activity of various matrix metalloproteinases in different cell types, including corneal epithelial cells and corneal myofibroblast, as well as human uterine cervical fibroblasts, human leukocytes, and endothelial cells of different origins [73, 75–80]. Axelrad et al. reported that 100 nM PAF induced transactivation of MT1-MMP and TIMP-2 genes, which resulted in proteolytic activation of MMP-2 in HUVECs, although no increase in proMMP-2 expression was detected [75]. The absence of PAF's effect on the MMP-2 gene could be attributed to the high constitutive levels of proMMP-2 expression in the endothelial cells [75]. The

PAF-induced expression of MT1-MMP and TIMP-2 in HUVECs was found to occur through a mechanism involving $G\alpha_q$ protein, JAK-2 and Src kinases [73]. Importantly, Axelrad et al. reported that PAFR antagonists inhibited the migration and invasion of HUVECs induced by medium conditioned by a prostatic carcinoma cells, suggesting the involvement of PAF in prostate cancer cell-mediated neoangiogenesis [75]. Ottino *et al.* showed that in corneal myofibroblasts, 100 nM cPAF also stimulated the expression of MMP-9, but not MMP-2 [78]. In corneal cultures, cPAF upregulated levels of urokinase plasminogen activator (uPA) and MMP-1 and -9 through the COX-2-mediated production of prostaglandins [77]. In endothelial ECV304 cells, PAF induced expression of MMP-9 through a mechanism involving activation of NF-κB [76]. Alternatively to the reports showing positive regulation of MMPs by PAF, Barletta et al. showed that 300 nM PAF donw-regulated the mRNA levels of MT1-MMP and MMP-2 and reduced levels of MMP-2 activation in isolated neuroblastoma cell clones [81]. These authors further reported that downregulation of MMPs inversely correlated with PAF-induced differentiation in these cells [81].

4 PAF and angiogenesis

PAF is a potent mediator of tumor neoangiogenesis [26, 27, 82–86]. Numerous reports show that PAF can activate endothelial cells directly, as well as mediate angiogenesis induced by other angiogenic factors [26, 28]. Camussi's group demonstrated that PAF produced by breast or Kaposi's sarcoma cancer cells induces and sustains the *in vivo* neo-angiogenesis in experimental tumor models [28, 87]. Hunt's group has demonstrated the effectiveness of PAF antagonists in the inhibition of angiogenesis in prostate cancer xenografts [27]. The same group showed that PAF induces activation of matrix metalloproteinase 2 activity and vascular endothelial cell invasion and migration [75]. They further found that bFGF stimulated PAF-dependent proliferation in human umbilical vein endothelial cells [72]. HGF and TNF-α both induce angiogenesis through mechanisms that involve the production of PAF [83, 85, 86]. Montrucchio et al. demonstrated that Nitric Oxide (NO) mediates the angiogenesis induced by PAF or TNF, the latter itself being dependent on the production of PAF [85]. Brizzi et al. showed that HUVECs express the Thrombopoietin receptor, which activates cell migration *in vitro* and angiogenesis *in vivo*; these effects were mediated by PAF- and interleukin-8 (IL-8)-dependent phosphorylation of the STAT1 and STAT5B [82]. Furthermore, it was found that vascular permeability induced by VEGF was mediated by PAF [88]. In HUVECs, both VEGF-induced P-selectin translocation and subsequent neutrophil adhesion requires PAF synthesis [89]. Angiopoietin-1, and -2 stimulate PAF synthesis [90]. Russo and colleagues have shown that PAF production, induced by stimulation of endothelial CD40 by CD154, is instrumental for the *in vitro* migration and vessel-like organization of endothelial cells, as well as the interaction of endothelial cells with smooth muscle cells [91]. In addition, it has been shown that in human endometrial adenocarcinoma cells HEC-1A, estrogen induces production of angiogenic factors TNF-α, IL-1, bFGF and VEGF, and this induction occurs through the PLA_2- PAF-PAFR-mediated activation of NF-κB [92].

5 The role of PAF in various cancers

In breast, prostate, and the Kaposi's sarcoma, interference with PAF-PAFR pathway inhibits tumor growth. These effects were associated with the inhibition of tumor angiogenesis. Bussolati and colleagues demonstrated that breast cancer cells express PAFR and produce PAF *in vitro* in response to stimulation with VEGF, bFGF, HGF, TNFα and thrombin [87]. They further showed that PAF promotes motility specifically in highly metastatic human MDA-MB231 cells, but not in non-metastatic breast cancer cells, suggesting for the first time that metastatic cells might be more responsive to PAF [87]. In addition, PAF receptor antagonists significantly reduced tumor angiogenesis. This effect was more pronounced in MDA-MB231 tumors as compared to tumors derived from non-metastatic cells [87]. Moreover, PAFR antagonist WEB-2086 induces growth inhibition and differentiation of human breast cancer cells [93], while the PAFR antagonist BN-50730 inhibited human prostatic carcinoma xenografts in nude mice [27]. Bocceline et al. have found that PAF acts as a potent inducer of tumor cell motility in CHO cells *in vitro* [94]. They demonstrated that in the renal carcinoma cell lines, the blockade of PAF-R by WEB-2170, a PAF-R antagonist, abolished the CD154-dependent motility [95].

A complex approach involving simultaneous detection of PAF, PAF precursor lyso-PAF, PAFR, PAF-AH and PLA_2 transcripts was used to examine the role of this pathway in the progression of tumors of different origins [96–102]. In colon cancer patients' blood, levels of both PAF-AH and PLA_2 were found to be elevated as compared to those in healthy individuals, while no difference was found in PAF levels in the plasma [96]. Intriguingly, while PAF levels were lower in tumor tissues of colon cancer patients Dukes' stage C than those of Dukes' A and B, a remarkable increase in PAF levels was observed in colon cancer liver metastases [96–98]. In addition, a new longer splice variants of PAFR mRNA transcript was detected in colon cancer specimens by Kotelevets et al. [103]. This group also found that PAF inhibited the invasion in v-*src*-transformed intestinal and

kidney epithelial cells [103]. Higher amounts of PAF and PAFR transcripts 1 and 2 were found in hepatocellular carcinoma specimens as compared with non-tumor tissues, while no difference was observed in PLA_2, lyso-PAF and PAF-AH levels [99]. No changes in the levels of these molecules were detected in papillary thyroid cancer [100]. PAFR transcripts as well as PAF, PLA_2 and PAF-AH were detected in human meningiomas, although no correlation was observed between their levels and tumor grade or tumor vascularization [101]. Finally, PAF receptor was detected by flow cytometry on the surface of B cells of several types of chronic (mature) B cell leukemia patients [102].

Clearly more needs to be done in order to establish whether the levels of PAF or its receptor expression has some prognostic value in cancer. Significant progress has been made in PAF detection methods and with the development of screening strategies. Notably, Kita and colleagues described a new multiplex quantification method for eicosanoids and PAF, using column-switching reversed-phase liquid chromatography-tandem mass spectrometry, which allows rapid and reliable analysis of these lipid mediators [104].

6 PAF and PAF receptor in melanoma

The first demonstration of the role of PAF in melanoma metastasis was made in 1996 by Im et al. [105]. These authors found that IL-1α and TNF-α-induced increase in experimental pulmonary metastasis produced by the B16F10 murine melanoma cells in C57Bl/6 mice was augmented by a single intraperitoneal injection of PAF [105]. Furthermore, they showed that several repeated injections of PAFR antagonist BN50739 (day 0 through day 2) decreased both IL-1α and TNF-α-induced metastasis as well as control lung metastasis, suggesting the role of endogenous PAF in tumor cell lung colonization. PAF caused an increase in the retention of radiolabeled B16F10 cells in the lungs, suggesting that stimulation of endothelial cell adhesion was the primary mechanism for the observed pro-metastatic effect of PAF [105].

The investigation on the role of PAF in skin pathologies was further incited by the studies in PAFR-overexpressing transgenic mice generated by Takao Shimizu and his group [106, 107]. PAFR transgenic mice exhibited progressive hyperproliferative changes in the epidermis as soon as 2 weeks after birth. The keratinocyte hyperplasia was accompanied by hyperpigmentation and increase in the number of dermal melanocytes in the ear and tail, and consequent development of melanocytic tumors late in life [106, 107]. The PAFR transgene expression was detected in keratinocytes but not in melanocytes, suggesting that the progressive recruitment of melanocytes to the dermis was driven by keratinocytes, and possibly by the accumulating fibroblasts and mast cells. In human skin, all these types of cells are indeed known to play a significant role in regulating skin homeostasis and behavior of resident melanocytes, as well as melanoma growth and local malignant invasion [108].

Although it is unclear how closely pathological characteristics of the melanocytic tumors developed by PAFR transgenic mice resembled those of human lesions, further support for the role of PAF receptor in melanoma growth and metastatic dissemination came from the experimental systems utilizing *in vitro* and *in vivo* murine melanoma models. Indeed, it was reported that inhibition of PAF activity by means of overexpressing PAF-acetyl-hydrolase in B16F10 murine melanoma cells led to a significant decrease in tumor vascularization and growth, allowing longer animal survival [109]. Most recently, Fallani et al. have demonstrated that PAF is being synthesized by B16F10 cells in response to INF-γ treatment, and that PAF promoted the invasion of these cells through the Matrigel-coated filters [110]. Moreover, it has been shown that a single intraperitoneal injection of PAF induced expression of MMP-9 and MMP-2 in the mouse lungs, and significantly enhanced B16F10 pulmonary lung metastasis, suggesting an addition, non-tumor-cell mechanism of PAF action [111]. Finally, in the human melanoma cell line Hs294T, PAFR antagonists were able to prevent adhesion to IL-1-stimulated endothelial cells [112].

In human skin, the melanocyte homeostasis and number is tightly controlled by neighboring keratinocytes through an E-cadherin-mediated adhesion [108]. Essential for melanoma tumorigenesis, keratinocytes and corneal stromal cells secrete PAF in response to UV exposure [113–115]. Keratinocytes express PAF receptors on their surface [116], and PAF upregulates their COX-2, IL-6, and IL-8 mRNA expression and PGE_2 secretion [117, 118]. Although the incidence of severe sunburns in childhood have been linked to melanoma development later in life, the precise mechanism by which UV contributes to melanomagenesis has not been discovered. This mechanism may involve, at least in part, UV-induced immunosuppression [119, 120]. Intriguingly, Walterscheid et al. have found that the UVB-induced inflammation in mouse skin and the consequent systemic immunosuppression was abolished by PAFR antagonists [118]. Indeed, Travers's group has demonstrated UVB-induced generation of PAF-like substances in human epidermal keratinocytes [121].

7 PAF induces MMP-2 and MT1-MMP via activation of CREB/ATF-1 in melanoma

Recently, we have examined the role of PAF in human melanoma metastasis and found that PAF receptor was

expressed in all cultured melanoma cell lines regardless of their metastatic potential [68]. Cells cultured *in vitro* and cells from tumor xenografts express PAFR [68] and Fig. 2). When used in nanomolar concentrations, cPAF stimulated melanoma cell growth in confluent cultures [68]. Similarly, Bennett et al. have demonstrated that PAF-stimulated primary rat embryonic cells were able to reach a higher saturation density in complete cell culture medium [122]. We showed that cell exposure to PAF receptor antagonist PCA4248 caused a rapid inhibition of cell growth in all melanoma cell lines examined [68]. This effect correlated with the observed inhibition of cellular expression of Cyclin D1 by two chemically unrelated PAF receptor antagonists, PCA4248 and (±)trans-2,5-Bis(3,4,5-trimethoxyphenyl)-1,3-dioxolane (Fig. 3(a)). This Cyclin D downregulation was further confirmed in a model of small interfering RNA-mediated transient knockdown of the PAFR expression in A375SM melanoma cells (Fig. 3(b)). This suggested that PAFR is constitutively active in human melanoma cells and mediates gene expression. A weak stimulatory effect of PAF on proliferation has been previously observed in different cell types, whereas potent mitogenic response has been achieved in vascular endothelial cells [24, 28, 74, 122–124].

We further hypothesized that PAF through the activity of PAFR can phsophorylate and activate CREB and ATF-1 transcription factors. CREB and ATF-1 expression levels directly correlate with the transition of human melanoma cells from the radial to the vertical growth phase and with their metastatic potential in nude mice [125, 126]. We previously showed that quenching CREB and ATF-1 activities in metastatic melanoma cells by means of over-expression of a dominant-negative form of CREB, KCREB, which has a single-base pair substitution in the DNA-binding domain [127], decreased melanoma tumorigenicity and metastatic potential in nude mice [128]. Furthermore, we have shown that intracellular expression of an inhibitory anti-ATF-1 single-chain antibody fragment in MeWo human melanoma cells suppressed their tumorigenicity and metastatic potential *in vivo* [129]. At least two

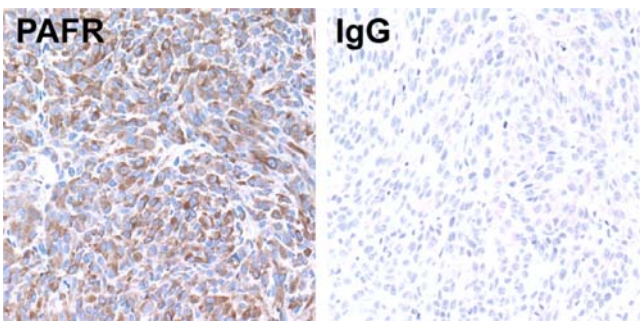

Fig. 2 Immunohistochemical detection of PAF receptor in A375SM human melanoma xenografts in nude mice. Staining with anti-PAFR antibody reveals PAF receptor expression in tumor cells

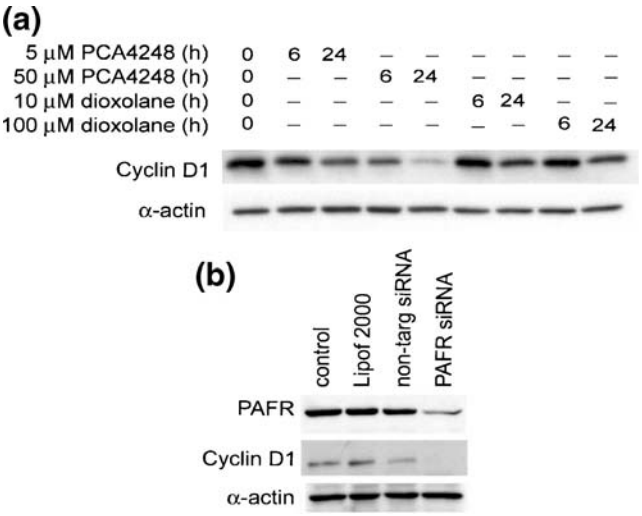

Fig. 3 PAFR mediates constitutive expression of Cyclin D1 in A375SM melanoma cells. The expression of Cyclin D1 was measured by Western blot (**a**) after A375SM cells were treated with PAFR antagonists PCA4248 or (±)trans-2,5-Bis(3,4,5-trimethoxyphenyl)-1,3-dioxolane, or (**b**) after 72 h-transfection with small interfering RNA oligonucleotides targeting PAFR expression

mechanisms explain how CREB and ATF-1 overexpression contributes to the metastatic phenotype: (1) CREB and ATF-1 play essential roles in regulating the expression of the type IV matrix metalloproteinase 2 (MMP-2; gelatinase A), a key enzyme in melanoma invasion and metastasis, and of the adhesion molecule MCAM/MUC18 genes [128], (2) CREB and ATF-1 act as survival factors that promote melanoma cell survival *in vitro* in response to apoptosis-inducing agents (*e.g.,* thapsigargin and ionizing radiation) and in *in vivo* experimental tumors [129, 130].

Although it has been shown that melanocyte proliferation and differentiation can be positively regulated by agents that increase cAMP levels [126, 131], little is known about factors that induce CREB and ATF-1 activation in melanoma. As demonstrated by Halaban's group, in normal melanocytes, the HGF, mast/stem-cell growth factor, bFGF, and endothelin-1 induce CREB phosphorylation on Ser133, probably via p90RSK and p70S6K cascades [126]. We have shown that in metastatic melanoma cell lines, which are known to overexpress CREB and ATF-1 transcription factors, PAF induces CREB and ATF-1 phosphorylation via a PAFR-mediated signal transduction mechanism that required pertussis toxin-insensitive $G\alpha_q$ protein and adenylate cyclase activity and was antagonized by a cAMP-dependent protein kinase A (PKA) and p38 MAPK inhibitors (Fig. 4 and [68]). Addition of PAF to metastatic A375SM cells stimulated CRE-dependent transcription. Furthermore, PAF activated MMP-2 expression (Fig. 5) and stimulated the gelatinase activity of MMP-2 [68]. MMP-2 activation correlated with the PAF-induced increase in the expression of an MMP-2 activator, membrane type 1-MMP (Fig. 5). PAF-induced activation of pro-MMP-

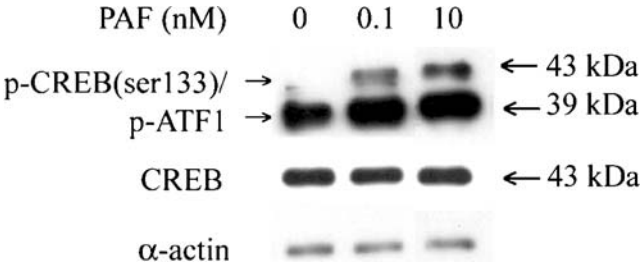

Fig. 4 PAF induces phosphorylation of CREB(ser133) and ATF-1 in A375SM melanoma cells. A375SM cells were exposed to PAF, protein extracts were generated and analyzed by Western blot for the expression of phospho-CREB/ATF-1. Blots were stripped and re-probed with anti-total-CREB antibody, and further stripped and re-probed with antibody against actin

2 was causally related to PAF-induced CREB and ATF-1 phosphorylation; it was prevented by PAFR antagonist and inhibitors of p38 MAPK and PKA and was abrogated upon quenching of CREB and ATF-1 activities by forced over-expression of a dominant-negative form of CREB [68]. Based on our results, we propose that all melanoma cells, regardless of their metastatic potential, express PAFR and secrete basal levels of MMP-2 and MT1-MMP (Fig. 6). However, within the melanoma tumor microenvironment, where melanoma cells come in contact with cells such as PAF-secreting platelets, endothelial cells, and inflammatory cells, PAF will phosphorylate CREB and ATF1 through the activity of its receptor and a signaling cascade involving p38 MAPK and PKA. This will further result in overexpression and secretion of MMP-2 and MT1-MMP. However, since only metastatic melanoma cells overexpress CREB and ATF-1, they are, therefore, better equipped to respond to the effect of PAF within the tumor microenvironment.

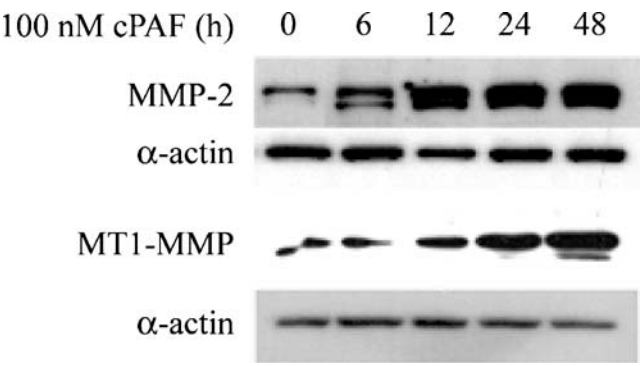

Fig. 5 PAF induces overexpression of MMP-2 and MT1-MMP proteins. A375SM cells were exposed to PAF, protein extracts were generated and analyzed by Western blot for the expression of MMP-2 and MT1-MMP. Blots were stripped and re-probed with antibody against actin

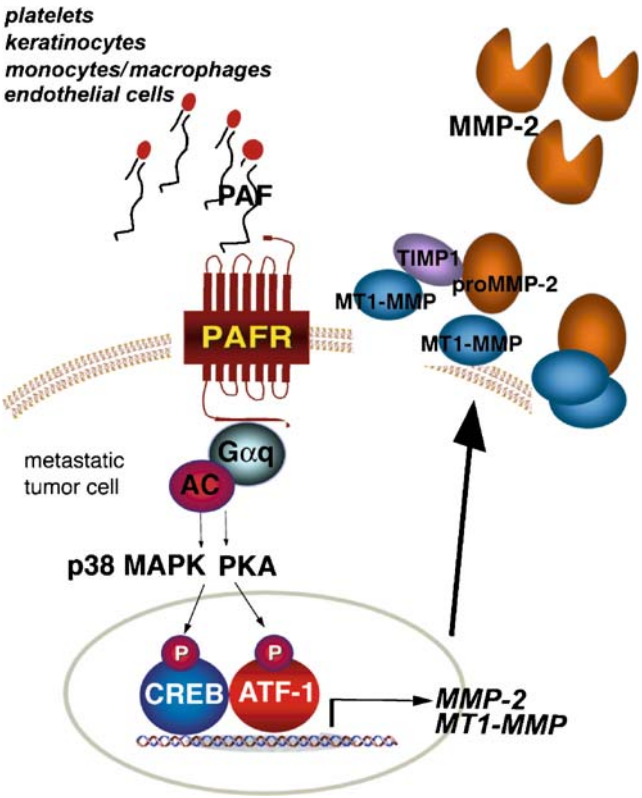

Fig. 6 A model for the stimulation of MMP-2 and MT1-MMP by PAF via activation of CREB/ATF-1. We propose that melanoma cells, regardless of their metastatic potential, express PAFR and secrete basal levels of MMP-2 and MT1-MMP. However, within the melanoma tumor microenvironment, when melanoma cells come in contact with cells such as platelets, endothelial cells, and inflammatory cells that secrete PAF. PAF, through the activity of its receptor and a signaling cascade involving pertussis-toxin insensitive $G\alpha_q$ protein, adenylate cyclase, p38 MAPK and PKA, phosphorylates CREB and ATF1. This results in overexpression and secretion of MMP-2 and MT1-MMP. However, since only metastatic melanoma cells over-express CREB and ATF-1, they are better equipped to respond to the stimulatory effect of PAF within the tumor microenvironment

8 PAF receptor antagonist (PCA4248) inhibits experimental human melanoma lung metastasis

Our *in vivo* experiments showed that the PAF receptor antagonist PCA4248 acts as a potent inhibitor of experimental melanoma lung metastasis when delivered intravenously before melanoma cells inoculation (Table 1). This effect could be attributed to its action as an inhibitor of platelet aggregation. Indeed, various pharmacological anti-platelet/anti-coagulant agents have an inhibitory effect on tumor metastases [5–7]. Also, as mentioned previously, Im et al. found that PAF receptor antagonists interfere with murine melanoma cell adhesion to the endothelial wall [105]. Furthermore, we found that daily PCA4248 injections initiated 7 days after melanoma cell injection, also drastically downregulated melanoma lung metastasis, sug-

Table 1 Experimental A375SM melanoma lung metastasis in nude mice treated with PCA4248

Number of metastasis			
Treatment	Median	Range	Incidence (number of mice)
Control[a]	12	5–53	9 out of 9
20 mg/kg PCA4248 i.v. before cell injection	1	0–1	2 out of 5
40 mg/kg PCA4248 i.p. daily from day 7	1	0–8	6 out of 9

[a] A375SM cells (0.5×10^6) were injected i.v. into nude mice; the number of lung metastasis was determined 31 days post-injection.

gesting that antagonizing PAF-PAFR signaling has an inhibitory effect on the growth of established microscopic tumor cell colonies in the lungs. At present, we cannot exclude the possibility that the inhibitory effect of PCA4248 *in vivo* was directly related to its ability to inhibit activation of CREB and MMPs in the tumor cells. Clearly however, tumor cells are not the only targets of PCA4248. Owing to its higher concentration in the circulation, PAFR antagonist may have primarily affected the process of recruitment of the inflammatory cells to the tumor site, which is instrumental for successful tumor angiogenesis. Nevertheless, our data indicate that PAF has a strong tumor-promoting effect in melanoma [68].

9 PAF modulates chemotherapeutic and cytokine treatment responses

The therapeutic modalities to control tumor growth and metastasis of human melanoma are very limited. Recently, studies by our group and others have shown that practical chemotherapeutic drugs such as dacarbazine (DTIC) or cisplatin, while being toxic, induce a stress response in melanoma cells that resulted in the activation of MAPK cascade and stimulation of IL-8 and VEGF production [132–134]. These are potent survival, angiogenic, and invasion-associated factors. We found that *in vitro*, long-term cell treatment with DTIC selects for the resistant cells with very high levels of IL-8 and VEGF production, which exhibited increased tumor growth and metastatic behavior in nude mice as compared to control parental cells. This implies that treatment of melanoma patients with DTIC may produce a considerable hazard by selecting cells with a more aggressive melanoma phenotype [132]. We have proposed that the combination treatment of DTIC with anti-VEGF/IL-8 or MEK inhibitors may potentiate the therapeutic effects of the cytotoxic drug. Furthermore, based on our observations that IL-8 plays a major role in the acquisition of the metastatic phenotype in human melanoma, we have developed fully human antibodies targeting IL-8, which effectively inhibited tumor growth, invasion, angiogenesis, and metastasis of melanoma in animal models [135].

Intriguingly, Darst and colleagues have found that reconstitution of PAFR expression in PAFR-negative epidermal KB cells resulted in an enhanced, NF-κB-mediated, production of IL-8 and TNF-α in response to the chemotherapeutic drugs etoposide and mitomycin C [136]. It is therefore highly possible that DTIC-induced IL-8 production by melanoma, observed in our experiments, is also mediated by PAF. If so, PAF receptor antagonists may be effective in suppressing undesirable cytokine production during chemotherapeutic treatment. Meanwhile, the same group of investigators showed that PAF receptor re-expression protected epidermal KB cells from TNF-α and Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL)-induced apoptosis, and this protection was mediated through NF-κB [137]. Furthermore, they demonstrated that *in vitro* cell death in response to cell treatment with etoposide and mitomycin C, but not to TRAIL or C_2 ceramide, was potentiated by PAF receptor in an NF-κB-dependent manner [138]. This convergence of cytotoxic (apoptosis) and survival-promoting (IL-8, TNF-α) signals at the level of PAF-induced NF-κB activation in response to cytostatic drugs is an interesting phenomenon and perhaps suggests that PAFR acts upstream in signal transductions pathways activated by chemotherapeutic drugs. Similarly, it was demonstrated that PAF potentiates UV-induced death in human corneal epithelial cells [139].

The involvement of PAF in the anti-tumor activity of the natural killer (NK) and activated dendritic cells has also been proposed. Indeed, it was found that glycoprotein 170 (MDR1) induces the expression of PAFR in K562 and Tera-2 cells, and PAFR expression serves as a prerequisite for the NK cells-mediated tumor cell lysis [140]. It was also suggested that the impaired ability of the engineered monocyte-derived dendritic cells (moDCs) to mount an effective anti-tumor response, including their ability to migrate, induce activation of NK cells, and to induce type 2 T helper cell differentiation, could be linked to a single defect. In particular, dendritic cells generated in the presence of GM-CSF and IL-4, fail to produce PAF and other eicosanoids due to suppression of PLA_2 by IL-4 [141]. Consequently, the authors argue for the importance of the preservation of lipid metabolism in order to yield effective anti-tumor activities with DCs [141]. Clearly, further *in vivo* studies should be conducted in order to determine the therapeutic outcome of combination therapies involving various chemotherapeutic drugs, cytokine or immune therapies with PAF receptor antagonists.

10 Concluding remarks

Metastatic melanoma is virtually incurable and new treatment modalities are urgently needed. The gold standard for metastatic melanoma therapy, treatment with DTIC, leads to undesirable generation of the angiogenic factors IL-8 and VEGF. Among other proinflammatory mediators, PAF has been shown to play an essential role in tumor progression, serving as a primary and second messenger that mediates tumor angiogenesis and metastatic dissemination in murine tumor models, including that of melanoma. Meanwhile, we have demonstrated that in human metastatic melanoma cells, PAF induced phosphorylation of CREB and ATF-1 in a p38 MAPK and PKA-dependent manner. Furthermore, PAF stimulated CREB-dependent expression and activation of MMP-2 and MT1-MMP in melanoma cells. *In vivo*, PAF receptor antagonist PCA4248 inhibited experimental human melanoma lung metastasis in nude mice. We propose to further assess the applicability of PAF receptor antagonists for preventing or treating metastatic melanoma, alone, in combination with DTIC or with other targeted therapies such as fully humanized anti-IL-8 antibodies.

References

1. Balkwill, F., & Mantovani, A. (2001). Inflammation and cancer: back to Virchow? *Lancet, 357*(9255), 539–545.
2. Coussens, L. M., & Werb, Z. (2002). Inflammation and cancer. *Nature, 420*(6917), 860–867.
3. Mantovani, A. (2005). Cancer: Inflammation by remote control. *Nature, 435*(7043), 752–753.
4. Pikarsky, E., Porat, R. M., Stein, I., Abramovitch, R., Amit, S., Kasem, S., et al. (2004). NF-kappaB functions as a tumour promoter in inflammation-associated cancer. *Nature, 431*(7007), 461–466.
5. Chen, Y. Q., Liu, B., Tang, D. G., & Honn, K. V. (1992). Fatty acid modulation of tumor cell-platelet-vessel wall interaction. *Cancer and Metastasis Reviews, 11*(3–4), 389–409.
6. Karpatkin, S., & Pearlstein, E. (1981). Role of platelets in tumor cell metastases. *Annals of Internal Medicine, 95*(5), 636–641.
7. Honn, K. V., Tang, D. G., & Crissman, J. D. (1992). Platelets and cancer metastasis: A causal relationship? *Cancer and Metastasis Reviews, 11*(3–4), 325–351.
8. Nieswandt, B., Hafner, M., Echtenacher, B., & Mannel, D. N. (1999). Lysis of tumor cells by natural killer cells in mice is impeded by platelets. *Cancer Research, 59*(6), 1295–1300.
9. Pinedo, H. M., Verheul, H. M., D'Amato, R. J., & Folkman, J. (1998). Involvement of platelets in tumour angiogenesis? *Lancet, 352*(9142), 1775–1777.
10. Robinson, S. C., & Coussens, L. M. (2005). Soluble mediators of inflammation during tumor development. *Advances in Cancer Research, 93*, 159–187.
11. Dauer, D. J., Ferraro, B., Song, L., Yu, B., Mora, L., Buettner, R., et al. (2005). Stat3 regulates genes common to both wound healing and cancer. *Oncogene, 24*(21), 3397–3408.
12. Brown, J. R., & DuBois, R. N. (2004). Cyclooxygenase as a target in lung cancer. *Clinical Cancer Research, 10*(12 Pt 2), 4266s–4269s.
13. Leslie, M. C., & Bar-Eli, M. (2005). Regulation of gene expression in melanoma: New approaches for treatment. *Journal of Cell Biochemistry, 94*(1), 25–38.
14. Amiri, K. I., & Richmond, A. (2005). Role of nuclear factor-kappa B in melanoma. *Cancer and Metastasis Reviews, 24*(2), 301–313.
15. Richmond, A., Balentien, E., Thomas, H. G., Flaggs, G., Barton, D. E., Spiess, J., et al. (1988). Molecular characterization and chromosomal mapping of melanoma growth stimulatory activity, a growth factor structurally related to beta-thromboglobulin. *EMBO Journal, 7*(7), 2025–2033.
16. Claffey, K. P., Brown, L. F., del Aguila, L. F., Tognazzi, K., Yeo, K. T., Manseau, E. J., et al. (1996). Expression of vascular permeability factor/vascular endothelial growth factor by melanoma cells increases tumor growth, angiogenesis, and experimental metastasis. *Cancer Research, 56*(1), 172–181.
17. Tellez, C., McCarty, M., Ruiz, M., & Bar-Eli, M. (2003). Loss of activator protein-2alpha results in overexpression of protease-activated receptor-1 and correlates with the malignant phenotype of human melanoma. *Journal of Biological Chemistry, 278*(47), 46632–46642.
18. Tellez, C. S., Davis, D. W., Prieto, V. G., Gershenwald, J. E., Johnson, M. M., McCarty, M. F., et al. (2007). Quantitative analysis of melanocytic tissue array reveals inverse correlation between activator protein-2alpha and protease-activated receptor-1 expression during melanoma progression. *Journal of Investigative Dermatology, 127*(2), 387–393.
19. Tellez, C., & Bar-Eli, M. (2003). Role and regulation of the thrombin receptor (PAR-1) in human melanoma. *Oncogene, 22*(20), 3130–3137.
20. Lorant, D. E., Patel, K. D., McIntyre, T. M., McEver, R. P., Prescott, S. M., & Zimmerman, G. A. (1991). Coexpression of GMP-140 and PAF by endothelium stimulated by histamine or thrombin: A juxtacrine system for adhesion and activation of neutrophils. *Journal of Cell Biology, 115*(1), 223–234.
21. Zimmerman, G. A., McIntyre, T. M., Mehra, M., & Prescott, S. M. (1990). Endothelial cell-associated platelet-activating factor: A novel mechanism for signaling intercellular adhesion. *Journal of Cell Biology, 110*(2), 529–540.
22. Zimmerman, G. A., McIntyre, T. M., Prescott, S. M., & Stafforini, D. M. (2002) The platelet-activating factor signaling system and its regulators in syndromes of inflammation and thrombosis. *Critical Care Medicine, 30*(5 Suppl), S294–S301.
23. Bennett, S. A., & Birnboim, H. C. (1997). Receptor-mediated and protein kinase-dependent growth enhancement of primary human fibroblasts by platelet activating factor. *Molecular Carcinogenesis, 20*(4), 366–375.
24. Roth, M., Nauck, M., Yousefi, S., Tamm, M., Blaser, K., Perruchoud, A. P., et al. (1996). Platelet-activating factor exerts mitogenic activity and stimulates expression of interleukin 6 and interleukin 8 in human lung fibroblasts via binding to its functional receptor. *Journal of Experimental Medicine, 184*(1), 191–201.
25. Prescott, S. M., Zimmerman, G. A., Stafforini, D. M., & McIntyre, T. M. (2000). Platelet-activating factor and related lipid mediators. *Annual Reviews of Biochemical, 69*, 419–445.
26. Camussi, G., Montrucchio, G., Lupia, E., De Martino, A., Perona, L., Arese, M., et al. (1995). Platelet-activating factor directly stimulates *in vitro* migration of endothelial cells and promotes *in vivo* angiogenesis by a heparin-dependent mechanism. *Journal of Immunology, 154*(12), 6492–6501.
27. Robert, E. G., & Hunt, J. D. (2001). Lipid messengers as targets for antiangiogenic therapy. *Current Pharmaceutical Design, 7*(16), 1615–1626.
28. Bussolino, F., Arese, M., Montrucchio, G., Barra, L., Primo, L., Benelli, R., et al. (1995). Platelet activating factor produced *in vitro* by Kaposi's sarcoma cells induces and sustains *in vivo* angiogenesis. *Journal of Clinical Investigation, 96*(2), 940–952.

29. Shaw, J. O., Pinckard, R. N., Ferrigni, K. S., McManus, L. M., & Hanahan, D. J. (1981). Activation of human neutrophils with 1-O-hexadecyl/octadecyl-2-acetyl-snglycerol-3-phosphorylcholine (platelet activating factor). *Journal of Immunology, 127*(3), 1250–1255.
30. Rola-Pleszczynski, M., Pouliot, C., Turcotte, S., Pignol, B., Braquet, P., & Bouvrette, L. (1988). Immune regulation by platelet-activating factor. I. Induction of suppressor cell activity in human monocytes and CD8+ T cells and of helper cell activity in CD4+ T cells. *Journal of Immunology, 140*(10), 3547–3552.
31. Stafforini, D. M., McIntyre, T. M., Zimmerman, G. A., & Prescott, S. M. (2003). Platelet-activating factor, a pleiotrophic mediator of physiological and pathological processes. *Critical Reviews in Clinical Laboratory Sciences, 40*(6), 643–672.
32. Travers, J. B. (1999). Oxidative stress can activate the epidermal platelet-activating factor receptor. *Journal of Investigative Dermatology, 112*(3), 279–283.
33. Shimizu, T., Mutoh, H., & Kato, S. (1996). Platelet-activating factor receptor. Gene structure and tissue-specific regulation. *Advances in Experimental Medicine and Biology, 416*, 79–84.
34. Mutoh, H., Bito, H., Minami, M., Nakamura, M., Honda, Z., Izumi, T., et al. (1993). Two different promoters direct expression of two distinct forms of mRNAs of human platelet-activating factor receptor. *FEBS Letters, 322*(2), 129–134.
35. Benveniste, J., Tence, M., Varenne, P., Bidault, J., Boullet, C., & Polonsky, J. (1979). [Semi-synthesis and proposed structure of platelet-activating factor (P.A.F.): PAF-acether an alkyl ether analog of lysophosphatidylcholine]. *Comptes Rendus des Seances de Academie Sciences De Roumanie, 289*(14), 1037–1040.
36. Blank, M. L., Snyder, F., Byers, L. W., Brooks, B., & Muirhead, E. E. (1979). Antihypertensive activity of an alkyl ether analog of phosphatidylcholine. *Biochemical and Biophysical Research Communications, 90*(4), 1194–1200.
37. Demopoulos, C. A., Pinckard, R. N., & Hanahan, D. J. (1979). Platelet-activating factor. Evidence for 1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine as the active component (a new class of lipid chemical mediators). *Journal of Biological Chemistry, 254*(19), 9355–9358.
38. Benveniste, J., Henson, P. M., & Cochrane, C. G. (1972). Leukocyte-dependent histamine release from rabbit platelets. The role of IgE, basophils, and a platelet-activating factor. *Journal of Experimental Medicine, 136*(6), 1356–1377.
39. Snyder, F. (1995). Platelet-activating factor and its analogs: Metabolic pathways and related intracellular processes. *Biochimica Biophysica Acta, 1254*(3), 231–249.
40. Prescott, S. M., Zimmerman, G. A., & McIntyre, T. M. (1990). Platelet-activating factor. *Journal of Biological Chemistry, 265*(29), 17381–17384.
41. Serhan, C. N., Haeggstrom, J. Z., & Leslie, C. C. (1996). Lipid mediator networks in cell signaling: Update and impact of cytokines. *FASEB Journal, 10*(10), 1147–1158.
42. Marathe, G. K., Davies, S. S., Harrison, K. A., Silva, A. R., Murphy, R. C., Castro-Faria-Neto, H., et al. (1999). Inflammatory platelet-activating factor-like phospholipids in oxidized low density lipoproteins are fragmented alkyl phosphatidylcholines. *Journal of Biological Chemistry, 274*(40), 28395–28404.
43. Uemura, Y., Lee, T. C., & Snyder, F. (1991). A coenzyme A-independent transacylase is linked to the formation of platelet-activating factor (PAF) by generating the lyso-PAF intermediate in the remodeling pathway. *Journal of Biological Chemistry, 266*(13), 8268–8272.
44. Montrucchio, G., Alloatti, G., & Camussi, G. (2000). Role of platelet-activating factor in cardiovascular pathophysiology. *Physiological Reviews, 80*(4), 1669–1699.
45. Blank, M. L., Lee, Y. J., Cress, E. A., & Snyder, F. (1988). Stimulation of the *de novo* pathway for the biosynthesis of platelet-activating factor (PAF) via cytidylyltransferase activation in cells with minimal endogenous PAF production. *Journal of Biological Chemistry, 263*(12), 5656–5661.
46. Shindou, H., Hishikawa, D., Nakanishi, H., Harayama, T., Ishii, S., Taguchi, R., et al. (2007). A single enzyme catalyzes both platelet-activating factor production and membrane biogenesis of inflammatory cells. Cloning and characterization of acetyl-CoA: LYSO-PAF acetyltransferase. *Journal of Biological Chemistry, 282*(9), 6532–6539.
47. Stafforini, D. M., Prescott, S. M., & McIntyre, T. M. (1987). Human plasma platelet-activating factor acetylhydrolase. Purification and properties. *Journal of Biological Chemistry, 262*(9), 4223–4230.
48. Travers, J. B., Sprecher, H., & Fertel, R. H. (1990). The metabolism of platelet-activating factor in human T-lymphocytes. *Biochimica et biophysica acta, 1042(2), 193–197.*
49. Wilcox, R. W., Wykle, R. L., Schmitt, J. D., & Daniel, L. W. (1987). The degradation of platelet-activating factor and related lipids: Susceptibility to phospholipases C and D. *Lipids, 22*(11), 800–807.
50. Bito, H., Honda, Z., Nakamura, M., & Shimizu, T. (1994). Cloning, expression and tissue distribution of rat platelet-activating-factor-receptor cDNA. *European Journal of Biochemistry, 221*(1), 211–218.
51. Nakamura, M., Honda, Z., Izumi, T., Sakanaka, C., Mutoh, H., Minami, M., et al. (1991). Molecular cloning and expression of platelet-activating factor receptor from human leukocytes. *Journal of Biological Chemistry, 266*(30), 20400–20405.
52. Ye, R. D., Prossnitz, E. R., Zou, A. H., and Cochrane, C. G. (1991). Characterization of a human cDNA that encodes a functional receptor for platelet activating factor. *Biochemical and Biophysical Research Communications, 180*(1), 105–111.
53. Ishii, S., Nagase, T., & Shimizu, T. (2002). Platelet-activating factor receptor. *Prostaglandins Other Lipid Mediators 68–69,* 599–609.
54. Ishii, S., & Shimizu, T. (2000). Platelet-activating factor (PAF) receptor and genetically engineered PAF receptor mutant mice. *Progress in Lipid Research, 39*(1), 41–82.
55. Nakamura, M., Honda, Z., Waga, I., Matsumoto, T., Noma, M., & Shimizu, T. (1992). Endotoxin transduces Ca2+ signaling via platelet-activating factor receptor. *FEBS Letters, 314*(2), 125–129.
56. Uozumi, N., Kume, K., Nagase, T., Nakatani, N., Ishii, S., Tashiro, F., et al. (1997). Role of cytosolic phospholipase A2 in allergic response and parturition. *Nature, 390*(6660), 618–622.
57. Fukunaga, K., Ishii, S., Asano, K., Yokomizo, T., Shiomi, T., Shimizu, T., et al. (2001). Single nucleotide polymorphism of human platelet-activating factor receptor impairs G-protein activation. *Journal of Biological Chemistry, 276*(46), 43025–43030.
58. Dupre, D. J., Chen, Z., Le Gouill, C., Theriault, C., Parent, J. L., Rola-Pleszczynski, M., et al. (2003). Trafficking, ubiquitination, and down-regulation of the human platelet-activating factor receptor. *Journal of Biological Chemistry, 278*(48), 48228–48235.
59. Ihida, K., Predescu, D., Czekay, R. P., & Palade, G. E. (1999). Platelet activating factor receptor (PAF-R) is found in a large endosomal compartment in human umbilical vein endothelial cells. *Journal of Cell Science, 112*(Pt 3), 285–295.
60. Marcheselli, V. L., Rossowska, M. J., Domingo, M. T., Braquet, P., & Bazan, N. G. (1990). Distinct platelet-activating factor binding sites in synaptic endings and in intracellular membranes of rat cerebral cortex. *Journal of Biological Chemistry, 265*(16), 9140–9145.
61. Marrache, A. M., Gobeil, F., Jr., Bernier, S. G., Stankova, J., Rola-Pleszczynski, M., Choufani, S., et al. (2002). Proinflammatory gene induction by platelet-activating factor mediated via its cognate nuclear receptor. *Journal of Immunology, 169*(11), 6474–6481.

62. Zhu, T., Gobeil, F., Vazquez-Tello, A., Leduc, M., Rihakova, L., Bossolasco, M., et al. (2006). Intracrine signaling through lipid mediators and their cognate nuclear G-protein-coupled receptors: A paradigm based on PGE2, PAF, and LPA1 receptors. *Canadian Journal of Physiology and Pharmacology, 84*(3–4), 377–391.
63. Chao, W., & Olson, M. S. (1993). Platelet-activating factor: Receptors and signal transduction. *Biochemical Journal, 292*(Pt 3), 617–629.
64. Franklin, R. A., Mazer, B., Sawami, H., Mills, G. B., Terada, N., Lucas, J. J., et al. (1993). Platelet-activating factor triggers the phosphorylation and activation of MAP-2 kinase and S6 peptide kinase activity in human B cell lines. *Journal of Immunology, 151*(4), 1802–1810.
65. Honda, Z., Takano, T., Gotoh, Y., Nishida, E., Ito, K., & Shimizu, T. (1994). Transfected platelet-activating factor receptor activates mitogen-activated protein (MAP) kinase and MAP kinase kinase in Chinese hamster ovary cells. *Journal of Biological Chemistry, 269*(3), 2307–2315.
66. Marques, S. A., Dy, L. C., Southall, M. D., Yi, Q., Smietana, E., Kapur, R., et al. (2002). The platelet-activating factor receptor activates the extracellular signal-regulated kinase mitogen-activated protein kinase and induces proliferation of epidermal cells through an epidermal growth factor-receptor-dependent pathway. *Journal of Pharmacology and Experimental Therapeutics, 300*(3), 1026–1035.
67. Landis, M., Yi, Q., Hyatt, A. M., Travers, A. R., Lewis, D. A., & Travers, J. B. (2007). Involvement of P38 MAP kinase in the augmentation of UVB-mediated apoptosis via the epidermal platelet-activating factor receptor. *Archives of Dermatological Research, 299,* 263–266.
68. Melnikova, V. O., Mourad-Zeidan, A. A., Lev, D. C., & Bar-Eli, M. (2006). Platelet-activating factor mediates MMP-2 expression and activation via phosphorylation of cAMP-response element-binding protein and contributes to melanoma metastasis. *Journal of Biological Chemistry, 281*(5), 2911–2922.
69. Nick, J. A., Avdi, N. J., Young, S. K., Knall, C., Gerwins, P., Johnson, G. L., et al. (1997). Common and distinct intracellular signaling pathways in human neutrophils utilized by platelet activating factor and FMLP. *Journal of Clinical Investigation, 99*(5), 975–986.
70. Coffer, P. J., Schweizer, R. C., Dubois, G. R., Maikoe, T., Lammers, J. W., & Koenderman, L. (1998). Analysis of signal transduction pathways in human eosinophils activated by chemoattractants and the T-helper 2-derived cytokines interleukin-4 and interleukin-5. *Blood, 91*(7), 2547–2557.
71. Kravchenko, V. V., Pan, Z., Han, J., Herbert, J. M., Ulevitch, R. J., & Ye, R. D. (1995). Platelet-activating factor induces NF-kappa B activation through a G protein-coupled pathway. *Journal of Biological Chemistry, 270*(25), 14928–14934.
72. Deo, D. D., Axelrad, T. W., Robert, E. G., Marcheselli, V., Bazan, N. G., & Hunt, J. D. (2002). Phosphorylation of STAT-3 in response to basic fibroblast growth factor occurs through a mechanism involving platelet-activating factor, JAK-2, and Src in human umbilical vein endothelial cells. Evidence for a dual kinase mechanism. *Journal of Biological Chemistry, 277*(24), 21237–21245.
73. Deo, D. D., Bazan, N. G., & Hunt, J. D. (2004). Activation of platelet-activating factor receptor-coupled G alpha q leads to stimulation of Src and focal adhesion kinase via two separate pathways in human umbilical vein endothelial cells. *Journal of Biological Chemistry, 279*(5), 3497–3508.
74. Kume, K., & Shimizu, T. (1997). Platelet-activating factor (PAF) induces growth stimulation, inhibition, and suppression of oncogenic transformation in NRK cells overexpressing the PAF receptor. *Journal of Biological Chemistry, 272*(36), 22898–22904.
75. Axelrad, T. W., Deo, D. D., Ottino, P., Van Kirk, J., Bazan, N. G., Bazan, H. E., et al. (2004). Platelet-activating factor (PAF) induces activation of matrix metalloproteinase 2 activity and vascular endothelial cell invasion and migration. *FASEB Journal, 18*(3), 568–570.
76. Ko, H. M., Park, Y. M., Jung, B., Kim, H. A., Choi, J. H., Park, S. J., et al. (2005). Involvement of matrix metalloproteinase-9 in platelet-activating factor-induced angiogenesis. *FEBS Letters, 579*(11), 2369–2375.
77. Ottino, P., & Bazan, H. E. (2001). Corneal stimulation of MMP-1, -9 and uPA by platelet-activating factor is mediated by cyclooxygenase-2 metabolites. *Current Eye Research, 23*(2), 77–85.
78. Ottino, P., He, J., Axelrad, T. W., & Bazan, H. E. (2005). PAF-induced furin and MT1-MMP expression is independent of MMP-2 activation in corneal myofibroblasts. *Investigative Ophthalmology and Visual Science, 46*(2), 487–496.
79. Sugano, T., Nasu, K., Narahara, H., Kawano, Y., Nishida, Y., & Miyakawa, I. (2000). Platelet-activating factor induces an imbalance between matrix metalloproteinase-1 and tissue inhibitor of metalloproteinases-1 expression in human uterine cervical fibroblasts. *Biology of Reproduction, 62*(3), 540–546.
80. Takafuji, S., Ishida, A., Miyakuni, Y., & Nakagawa, T. (2003). Matrix metalloproteinase-9 release from human leukocytes. *Journal of Investigational Allergology & Clinical Immunology, 13*(1), 50–55.
81. Barletta, E., Mugnai, G., & Ruggieri, S. (2002). Platelet activating factor inhibits the expression of matrix metalloproteinases and affects invasiveness and differentiation in a system of human neuroblastoma clones. *Biological Chemistry, 383*(1), 189–197.
82. Brizzi, M. F., Battaglia, E., Montrucchio, G., Dentelli, P., Del Sorbo, L., Garbarino, G., et al. (1999) Thrombopoietin stimulates endothelial cell motility and neoangiogenesis by a platelet-activating factor-dependent mechanism. *Circulation Research, 84*(7), 785–796.
83. Camussi, G., Montrucchio, G., Lupia, E., Soldi, R., Comoglio, P. M., & Bussolino, F. (1997). Angiogenesis induced *in vivo* by hepatocyte growth factor is mediated by platelet-activating factor synthesis from macrophages. *Journal of Immunology, 158*(3), 1302–1309.
84. Montrucchio, G., Lupia, E., Battaglia, E., Passerini, G., Bussolino, F., Emanuelli, G., et al. (1994). Tumor necrosis factor alpha-induced angiogenesis depends on *in situ* platelet-activating factor biosynthesis. *Journal of Experimental Medicine, 180*(1), 377–382.
85. Montrucchio, G., Lupia, E., de Martino, A., Battaglia, E., Arese, M., Tizzani, A., et al. (1997). Nitric oxide mediates angiogenesis induced *in vivo* by platelet-activating factor and tumor necrosis factor-alpha. *American Journal of Pathology, 151*(2), 557–563.
86. Montrucchio, G., Sapino, A., Bussolati, B., Ghisolfi, G., Rizea-Savu, S., Silvestro, L., et al. (1998). Potential angiogenic role of platelet-activating factor in human breast cancer. *American Journal of Pathology, 153*(5), 1589–1596.
87. Bussolati, B., Biancone, L., Cassoni, P., Russo, S., Rola-Pleszczynski, M., Montrucchio, G., et al. (2000). PAF produced by human breast cancer cells promotes migration and proliferation of tumor cells and neo-angiogenesis. *American Journal of Pathology, 157*(5), 1713–1725.
88. Sirois, M. G., & Edelman, E. R. (1997). VEGF effect on vascular permeability is mediated by synthesis of platelet-activating factor. *American Journal of Physiology, 272*(6 Pt 2), H2746–2756.
89. Rollin, S., Lemieux, C., Maliba, R., Favier, J., Villeneuve, L. R., Allen, B. G., et al. (2004). VEGF-mediated endothelial P-selectin translocation: Role of VEGF receptors and endogenous PAF synthesis. *Blood, 103*(10), 3789–3797.
90. Maliba, R., Lapointe, S., Neagoe, P. E., Brkovic, A., & Sirois, M. G. (2006). Angiopoietins-1 and -2 are both capable of mediating endothelial PAF synthesis: Intracellular signalling pathways. *Cell Signal, 18*(11), 1947–1957.

91. Russo, S., Bussolati, B., Deambrosis, I., Mariano, F., & Camussi, G. (2003). Platelet-activating factor mediates CD40-dependent angiogenesis and endothelial-smooth muscle cell interaction. *Journal of Immunology, 171*(10), 5489–5497.
92. Seo, K. H., Lee, H. S., Jung, B., Ko, H. M., Choi, J. H., Park, S. J., et al. (2004). Estrogen enhances angiogenesis through a pathway involving platelet-activating factor-mediated nuclear factor-kappaB activation. *Cancer Research, 64*(18), 6482–6488.
93. Cellai, C., Laurenzana, A., Vannucchi, A. M., Caporale, R., Paglierani, M., Di Lollo, S., et al. (2006). Growth inhibition and differentiation of human breast cancer cells by the PAFR antagonist WEB-2086. *British Journal of Cancer, 94*(11), 1637–1642.
94. Boccellino, M., Biancone, L., Cantaluppi, V., Ye, R. D., & Camussi, G. (2000). Effect of platelet-activating factor receptor expression on CHO cell motility. *Journal of Cellular Physiology, 183*(2), 254–264.
95. Bussolati, B., Russo, S., Deambrosis, I., Cantaluppi, V., Volpe, A., Ferrando, U., et al. (2002). Expression of CD154 on renal cell carcinomas and effect on cell proliferation, motility and platelet-activating factor synthesis. *International Journal of Cancer, 100* (6), 654–661.
96. Denizot, Y., Truffinet, V., Bouvier, S., Gainant, A., Cubertafond, P., & Mathonnet, M. (2004). Elevated plasma phospholipase A2 and platelet-activating factor acetylhydrolase activity in colorectal cancer. *Mediators of Inflammation, 13*(1), 53–54.
97. Denizot, Y., Descottes, B., Truffinet, V., Valleix, D., Labrousse, F., & Mathonnet, M. (2005). Platelet-activating factor and liver metastasis of colorectal cancer. *International Journal of Cancer, 113*(3), 503–505.
98. Denizot, Y., Gainant, A., Guglielmi, L., Bouvier, S., Cubertafond, P., & Mathonnet, M. (2003). Tissue concentrations of platelet-activating factor in colorectal carcinoma: Inverse relationships with Dukes' stage of patients. *Oncogene, 22*(46), 7222–7224.
99. Mathonnet, M., Descottes, B., Valleix, D., Truffinet, V., Labrousse, F., & Denizot, Y. (2006). Platelet-activating factor in cirrhotic liver and hepatocellular carcinoma. *World Journal of Gastroenterology, 12*(17), 2773–2778.
100. Denizot, Y., Chianea, T., Labrousse, F., Truffinet, V., Delage, M., & Mathonnet, M. (2005). Platelet-activating factor and human thyroid cancer. *European Journal of Endocrinology, 153*(1), 31–40.
101. Denizot, Y., De Armas, R., Caire, F., Pommepuy, I., Truffinet, V., & Labrousse, F. (2006). Platelet-activating factor and human meningiomas. *Neuropathology & Applied Neurobiology 32*(6), 674–678.
102. Guglielmi, L., Trimoreau, F., Donnard, M., Jaccard, A., Bordessoule, D., & Denizot, Y. (2003). Presence of membrane platelet-activating factor receptors on B cells of chronic B cell leukaemia patients. *Leukemia & Lymphoma, 44*(6), 1087–1088.
103. Kotelevets, L., Noe, V., Bruyneel, E., Myssiakine, E., Chastre, E., Mareel, M., et al. (1998). Inhibition by platelet-activating factor of Src- and hepatocyte growth factor-dependent invasiveness of intestinal and kidney epithelial cells. Phosphatidylinositol 3′-kinase is a critical mediator of tumor invasion. *Journal of Biological Chemistry, 273*(23), 14138–14145.
104. Kita, Y., Takahashi, T., Uozumi, N., & Shimizu, T. (2005). A multiplex quantitation method for eicosanoids and platelet-activating factor using column-switching reversed-phase liquid chromatography-tandem mass spectrometry. *Analytical Biochemistry, 342*(1), 134–143.
105. Im, S. Y., Ko, H. M., Kim, J. W., Lee, H. K., Ha, T. Y., Lee, H. B., et al. (1996). Augmentation of tumor metastasis by platelet-activating factor. *Cancer Research, 56*(11), 2662–2665.
106. Sato, S., Kume, K., Ito, C., Ishii, S., & Shimizu, T. (1999). Accelerated proliferation of epidermal keratinocytes by the transgenic expression of the platelet-activating factor receptor. *Archives of Dermatological Research, 291*(11), 614–621.
107. Ishii, S., Nagase, T., Tashiro, F., Ikuta, K., Sato, S., Waga, I., et al. (1997). Bronchial hyperreactivity, increased endotoxin lethality and melanocytic tumorigenesis in transgenic mice overexpressing platelet-activating factor receptor. *EMBO Journal, 16*(1), 133–142.
108. Lee, J. T., & Herlyn, M. (2006). Microenvironmental influences in melanoma progression. *Journal of Cellular Biochemistry.*
109. Biancone, L., Cantaluppi, V., Del Sorbo, L., Russo, S., Tjoelker, L. W., & Camussi, G. (2003). Platelet-activating factor inactivation by local expression of platelet-activating factor acetyl-hydrolase modifies tumor vascularization and growth. *Clinical Cancer Research, 9*(11), 4214–4220.
110. Fallani, A., Calorini, L., Mannini, A., Gabellieri, S., Mugnai, G., and Ruggieri, S. (2006). Platelet-activating factor (PAF) is the effector of IFN gamma-stimulated invasiveness and motility in a B16 melanoma line. *Prostaglandins Other Lipid Mediators, 81* (3–4), 171–177.
111. Ko, H. M., Kang, J. H., Jung, B., Kim, H. A., Park, S. J., Kim, K. J., et al. (2007). Critical role for matrix metalloproteinase-9 in platelet-activating factor-induced experimental tumor metastasis. *International Journal of Cancer, 120*(6), 1277–1283.
112. Mannori, G., Barletta, E., Mugnai, G., & Ruggieri, S. (2000). Interaction of tumor cells with vascular endothelia: Role of platelet-activating factor. *Clinical & Experimental Metastasis, 18* (1), 89–96.
113. Barber, L. A., Spandau, D. F., Rathman, S. C., Murphy, R. C., Johnson, C. A., Kelley, S. W., et al. (1998). Expression of the platelet-activating factor receptor results in enhanced ultraviolet B radiation-induced apoptosis in a human epidermal cell line. *Journal of Biological Chemistry, 273*(30), 18891–18897.
114. Sheng, Y., & Birkle, D. L. (1995). Release of platelet activating factor (PAF) and eicosanoids in UVC-irradiated corneal stromal cells. *Current Eye Research, 14*(5), 341–347.
115. Calignano, A., Cirino, G., Meli, R., & Persico, P. (1988). Isolation and identification of platelet-activating factor in UV-irradiated guinea pig skin. *Journal of Pharmacological Methods, 19*(1), 89–91.
116. Travers, J. B., Huff, J. C., Rola-Pleszczynski, M., Gelfand, E. W., Morelli, J. G., & Murphy, R. C. (1995). Identification of functional platelet-activating factor receptors on human keratinocytes. *Journal of Investigative Dermatology, 105*(6), 816–823.
117. Pei, Y., Barber, L. A., Murphy, R. C., Johnson, C. A., Kelley, S. W., Dy, L. C., et al. (1998). Activation of the epidermal platelet-activating factor receptor results in cytokine and cyclooxygenase-2 biosynthesis. *Journal of Immunology, 161*(4), 1954–1961.
118. Walterscheid, J. P., Ullrich, S. E., & Nghiem, D. X. (2002). Platelet-activating factor, a molecular sensor for cellular damage, activates systemic immune suppression. *Journal of Experimental Medicine, 195*(2), 171–179.
119. De Fabo, E. C., Noonan, F. P., Fears, T., & Merlino, G. (2004). Ultraviolet B but not ultraviolet A radiation initiates melanoma. *Cancer Research, 64*(18), 6372–6376.
120. Noonan, F. P., Recio, J. A., Takayama, H., Duray, P., Anver, M. R., Rush, W. L., et al. (2001). Neonatal sunburn and melanoma in mice. *Nature, 413*(6853), 271–272.
121. Marathe, G. K., Johnson, C., Billings, S. D., Southall, M. D., Pei, Y., Spandau, D., et al. (2005). Ultraviolet B radiation generates platelet-activating factor-like phospholipids underlying cutaneous damage. *Journal of Biological Chemistry, 280*(42), 35448–35457.
122. Bennett, S. A., Leite, L. C., & Birnboim, H. C. (1993). Platelet activating factor, an endogenous mediator of inflammation, induces phenotypic transformation of rat embryo cells. *Carcinogenesis, 14* (7), 1289–1296.
123. Behrens, T. W., & Goodwin, J. S. (1990). Control of human T cell proliferation by platelet-activating factor. *International Journal of Immunopharmacology, 12*(2), 175–184.

124. Maggi, M., Bonaccorsi, L., Finetti, G., Carloni, V., Muratori, M., Laffi, G., et al. (1994). Platelet-activating factor mediates an autocrine proliferative loop in the endometrial adenocarcinoma cell line HEC-1A. *Cancer Research, 54*(17), 4777–4784.
125. Rutberg, S. E., Goldstein, I. M., Yang, Y. M., Stackpole, C. W., & Ronai, Z. (1994). Expression and transcriptional activity of AP-1, CRE, and URE binding proteins in B16 mouse melanoma subclones. *Molecular Carcinogenesis, 10*(2), 82–87.
126. Bohm, M., Moellmann, G., Cheng, E., Alvarez-Franco, M., Wagner, S., Sassone-Corsi, P., et al. (1995). Identification of p90RSK as the probable CREB-Ser133 kinase in human melanocytes. *Cell Growth & Differentiation, 6*(3), 291–302.
127. Walton, K. M., Rehfuss, R. P., Chrivia, J. C., Lochner, J. E., & Goodman, R. H. (1992). A dominant repressor of cyclic adenosine 3′,5′-monophosphate (cAMP)-regulated enhancer-binding protein activity inhibits the cAMP-mediated induction of the somatostatin promoter *in vivo*. *Molecular Endocrinology, 6*(4), 647–655.
128. Xie, S., Price, J. E., Luca, M., Jean, D., Ronai, Z., & Bar-Eli, M. (1997). Dominant-negative CREB inhibits tumor growth and metastasis of human melanoma cells. *Oncogene, 15*(17), 2069–2075.
129. Jean, D., Tellez, C., Huang, S., Davis, D. W., Bruns, C. J., McConkey, D. J., et al. (2000). Inhibition of tumor growth and metastasis of human melanoma by intracellular anti-ATF-1 single chain Fv fragment. *Oncogene, 19*(22), 2721–2730.
130. Jean, D., Harbison, M., McConkey, D. J., Ronai, Z., & Bar-Eli, M. (1998). CREB and its associated proteins act as survival factors for human melanoma cells. *Journal of Biological Chemistry, 273*(38), 24884–24890.
131. Halaban, R., Ghosh, S., & Baird, A. (1987). bFGF is the putative natural growth factor for human melanocytes. *In Vitro Cellular & Development Biology, 23*(1), 47–52.
132. Lev, D. C., Onn, A., Melinkova, V. O., Miller, C., Stone, V., Ruiz, M., et al. (2004). Exposure of melanoma cells to dacarbazine results in enhanced tumor growth and metastasis *in vivo*. *Journal of Clinical Oncology, 22*(11), 2092–2100.
133. Lev, D. C., Ruiz, M., Mills, L., McGary, E. C., Price, J. E., & Bar-Eli, M. (2003). Dacarbazine causes transcriptional up-regulation of interleukin 8 and vascular endothelial growth factor in melanoma cells: A possible escape mechanism from chemotherapy. *Molecular Cancer Therapeutics, 2*(8), 753–763.
134. Mandic, A., Viktorsson, K., Heiden, T., Hansson, J., & Shoshan, M. C. (2001). The MEK1 inhibitor PD98059 sensitizes C8161 melanoma cells to cisplatin-induced apoptosis. *Melanoma Research, 11*(1), 11–19.
135. Huang, S., Mills, L., Mian, B., Tellez, C., McCarty, M., Yang, X. D., et al. (2002). Fully humanized neutralizing antibodies to interleukin-8 (ABX-IL8) inhibit angiogenesis, tumor growth, and metastasis of human melanoma. *American Journal of Pathology, 161*(1), 125–134.
136. Darst, M., Al-Hassani, M., Li, T., Yi, Q., Travers, J. M., Lewis, D. A., et al. (2004). Augmentation of chemotherapy-induced cytokine production by expression of the platelet-activating factor receptor in a human epithelial carcinoma cell line. *Journal of Immunology, 172*(10), 6330–6335.
137. Southall, M. D., Isenberg, J. S., Nakshatri, H., Yi, Q., Pei, Y., Spandau, D. F., et al. (2001). The platelet-activating factor receptor protects epidermal cells from tumor necrosis factor (TNF) alpha and TNF-related apoptosis-inducing ligand-induced apoptosis through an NF-kappa B-dependent process. *Journal of Biological Chemistry, 276*(49), 45548–45554.
138. Li, T., Southall, M. D., Yi, Q., Pei, Y., Lewis, D., Al-Hassani, M., et al. (2003). The epidermal platelet-activating factor receptor augments chemotherapy-induced apoptosis in human carcinoma cell lines. *Journal of Biological Chemistry, 278*(19), 16614–16621.
139. Ma, X., & Bazan, H. E. (2001). Platelet-activating factor (PAF) enhances apoptosis induced by ultraviolet radiation in corneal epithelial cells through cytochrome c-caspase activation. *Current Eye Research, 23*(5), 326–335.
140. Geromin, D., Bourge, J. F., Soulie, A., Pawliuk, R., Fleet, C., Michel, E., et al. (2004). Glycoprotein 170 induces platelet-activating factor receptor membrane expression and confers tumor cell hypersensitivity to NK-dependent cell lysis. *Journal of Immunology, 172*(6), 3604–3611.
141. Thurnher, M., Zelle-Rieser, C., Ramoner, R., Bartsch, G., & Holtl, L. (2001). The disabled dendritic cell. *FASEB Journal, 15*(6), 1054–1061.