

The Implication of Platelet Activating Factor in Cancer Growth and Metastasis: Potent Beneficial Role of PAF-Inhibitors and Antioxidants

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Abstract: Cancer is one of the leading causes of death in Europe and United States. New blood vessel formation penetrating into solid tumors seems to be required for their growth and metastasis. Several protein growth factors can induce endothelial cell proliferation and angiogenesis, through signal transduction cascades that result in the production of several inflammatory mediators and lipid second messengers such as prostaglandins and Platelet Activating Factor (PAF).

PAF is a potent mediator of inflammation that is implicated in several inflammatory pathological conditions such as atherosclerosis, cardiovascular and renal diseases, allergy, AIDS, cancer etc. It exerts its biological activities through G-protein-coupled receptors.

The presence of PAF in the microenvironment of tumors may be due to its synthesis from circulating and / or cancer cells. Moreover, cancer cells and activated endothelial cells expose PAF-receptor on their membrane surface. PAF binding on its receptor induces several pathways that result in the onset and development of tumor induced angiogenesis and metastasis.

PAF-receptor antagonists have exhibited promising results *in vitro* and *in vivo* as anti-angiogenic molecules in several cancer cells and tumors. A dietary profile rich in antioxidants and PAF-inhibitors (such as the Mediterranean Diet) may provide beneficial preventive and protective effects against development, growth and metastatic manifestations of cancer cells, through either their inhibition of PAF activity and / or its biosynthesis.

The clarification of factors that may down regulate pathologically increased PAF-levels in a tumor microenvironment may also contribute to the planning of a potent nontoxic preventive and therapeutic approach against cancer.

Keywords: PAF, cancer, angiogenesis, metastasis, PAF-receptor, PAF-inhibitors, antioxidants.

1. INTRODUCTION

1.1. Platelet Activating Factor

Platelet Activating Factor (PAF) [1] is a potent phospholipid mediator of inflammation implicated in several inflammatory diseases [2-5] such as cardiovascular, renal and periodontal diseases [6-9], allergy [10], diabetes [11], AIDS [12-14], cancer [15-20], etc (Fig. 1).

The structure of the classical PAF molecule has been characterized as 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine [1] (Fig. 1). Nevertheless, the term PAF embodies a family of molecules with PAF-like biological activities that includes: a) phospholipid molecules with structure similar to that of the classical PAF molecule (PAF-like lipids) such as 1-O-alkyl/acyl/alkenyl-2-acetyl/acyl-*sn*-glycero-3-phosphocholine [9, 21] and b) molecules with similar biological activities to PAF but with semi-similar and non-similar

structures to that of the classical PAF (PAF-like activity molecules)¹ [22].

The members of this PAF super-family can be found in a great variety of bacterial and eukaryotic mono/multi-cellular organisms as well as in natural products such as foods, etc. The main factor that embodies them to the PAF super family is that they exhibit similar biological activities to those of PAF, resulting in the same or different pathways according to cell-types, tissues and mono/multi-cellular organisms.

PAF and PAF-like molecules exhibit their biological activities through specific membrane receptors coupled with G-proteins (PAF-receptor) [2]. Binding of such lipid molecules on the PAF-receptor induce several intracellular signaling pathways that leads to auto/endo/para/juxta-crine cellular activation. Inappropriate activation of PAF-receptor pathways is implicated in inflammation and inflammatory pathological situations [2, 6].

In multicellular eukaryotic organisms PAF-levels are strictly regulated by a group of PAF-metabolic enzymes. De-

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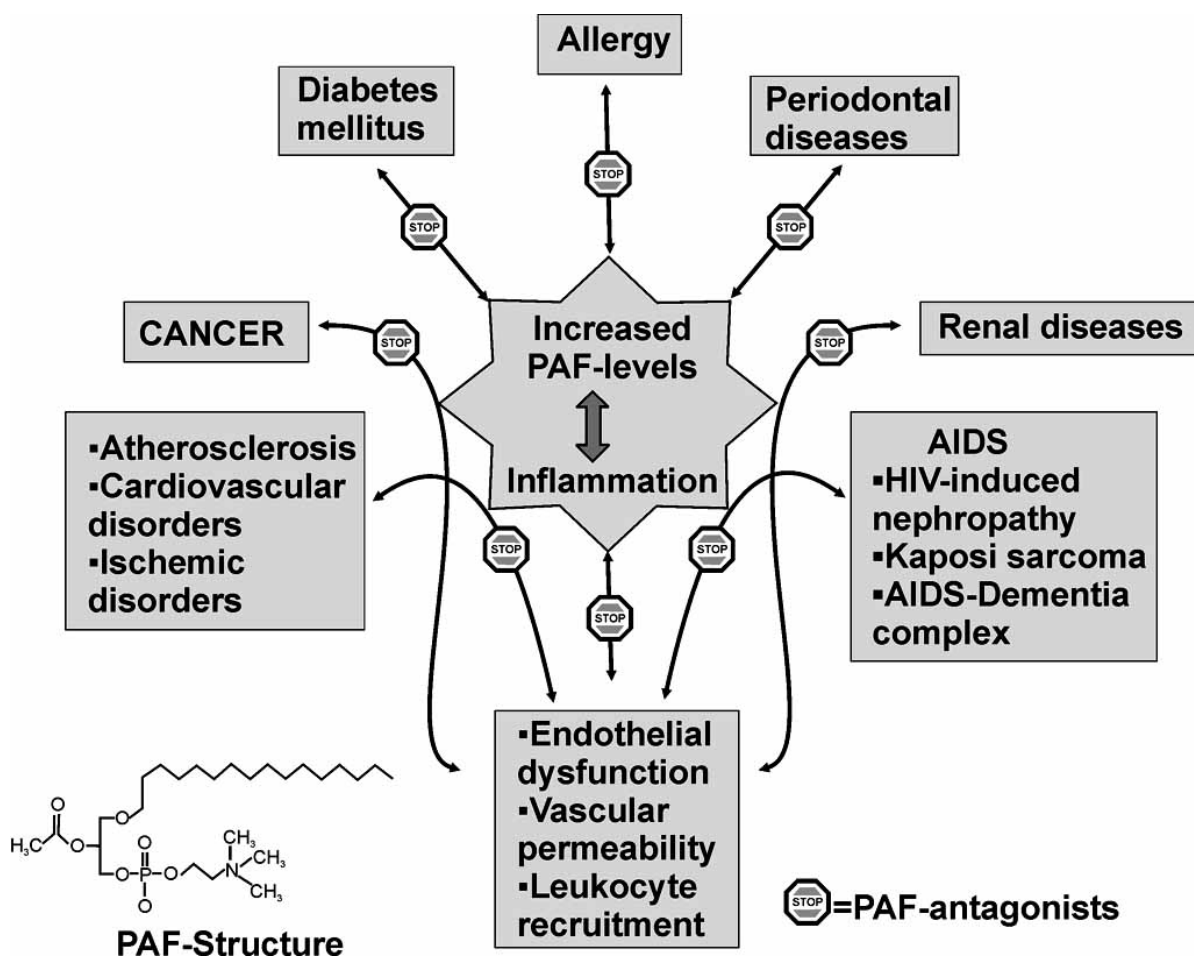


Fig. (1). The implication of the potent inflammatory mediator Platelet Activating Factor (PAF) in several diseases;

- The structure of the classical PAF molecule has been characterized as 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine.
- Increased PAF-levels in blood can initiate rapid inflammatory response in endothelial cells that results to increased endothelium permeability. Vascular inflammation that is characterized by leukocyte and platelet recruitment, aggregation and migration to the sub-endothelium through cell junctions of the dysfunctional endothelium, consists and results in common molecular mechanisms and morphological alterations (activated by elevated PAF levels and Oxidized-LDLs rich in PAF-like lipids) that take place in these diseases.
- Increased PAF-levels and the subsequent inflammatory disorders may serve as a significant common junction in the induction and progression of these diseases and vice versa. The presence of early and later phases of several of these diseases may provoke through this common junction the induction of each one of all the other diseases.
- Inhibition of PAF-biological activities and/or its biosynthesis by PAF-antagonists may provide beneficial effects in these pathological inflammatory manifestations.

regulation of PAF-metabolism induces an increase of PAF-levels that through PAF-receptor concludes in inflammatory manifestations.

In addition, in oxidative stress situations, such as smoking, PAF and several PAF-like molecules can be unregulated and excessively produced by non-specific oxidation of *sn*-2-acyl-position of the classical molecule of phosphatidylcholine. The oxidative and unregulated increase of PAF and PAF-like molecules are also implicated in several of the above mentioned inflammatory diseases such as atherosclerosis, cardiovascular diseases and cancer [2, 6, 7, 15-25].

Increased PAF activity in blood can initiate a rapid inflammatory response in endothelial cells that results in increased permeability of the endothelium which is a crucial

event in the initiation of such diseases. Vascular inflammation that is characterized by leukocyte and platelet recruitment, aggregation and migration to the sub-endothelium through cell junctions of the dysfunctional endothelium, consists of common molecular mechanisms and morphological alterations (activated by elevated PAF levels and Ox-LDLs rich in PAF-like lipids) that take place in the induction and progression of these diseases (Fig. 1) [6-12, 15-20].

1.2. PAF-Metabolism

Direct inactivation of PAF and PAF-structure-like lipids is achieved by the removal of the acetyl-group of *sn*-2 position of these phospholipid molecules, through the enzymatic activity of a special class of enzymes, PAF-acetyl-

hydrolases (PAF-AHs) that have been characterized at a molecular level.

These enzymes can indirectly terminate PAF-induced signaling pathways, as they directly reduce either enzymatic or oxidative upregulation of increased PAF-levels and/or PAF-like agents. Thus, in several pathological situations the administration of recombinant PAF-AH has shown several beneficial effects [2, 26].

Several cell types such as leukocytes, platelets, macrophages, endothelial cells, renal cells, etc. can produce PAF under appropriate stimuli [27]. Two distinctive enzymatic biosynthetic routes have been extensively described, the *de novo* and the remodelling pathways [28, 29]. The enzymes that catalyses the final step in each biosynthetic route, DTT-insensitive Cholinephosphotransferase of PAF (PAF-CPT) and Lyso-PAF-Acetyltransferase (Lyso-PAF-AT) respectively, exhibit a basic regulatory role in PAF-production.

In general, it has been proposed that the *de novo* route seems to contribute to the production of basic PAF-levels while the remodeling route seems to be implicated in the production of PAF in inflammatory responses [28-31]. Nevertheless, the information collected so far regarding these two biosynthetic routes of PAF, suggest that the contribution of the enzymes PAF-CPT and Lyso-PAF-AT in PAF-production depends on several factors in both physiological and pathological situations [14, 31-37] and that the above general hypothesis or assumption should be re-evaluated and studied further.

In particular, PAF-CPT activity seems to contribute to several systemic and chronic inflammatory pathological situations such as age-related malfunctions of the central nervous system [16] and cancer [17]. It seems that a slightly long-term induction of PAF-CPT activity concludes in stably increased PAF-levels that are implicated in such systemic conditions.

1.3. PAF-Inhibitors

A great variety of molecules that exhibit an inhibitory effect on PAF-induced biological activities have been found (Fig. 1). These molecules act either through their direct antagonistic / competitive effect in relation to PAF by binding on PAF-receptor, or through other indirect mechanisms. This has not been fully clarified but seems to correlate with changes in the membrane microenvironment of PAF-receptor and/or the general antioxidant activities of these molecules.

Such molecules have been found in several natural sources such as foods [25] (especially in foods that constitute a Mediterranean Diet such as olive oil [38]), plants (such as Ginkgo Biloba [39]), fish² [40] etc. Other studies have been focused on synthetically-produced PAF-inhibitors [39] that have been tested in several significant pathological situations [39, 41]. Several of these molecules, either from natural origin such as ginkgolides A and B or synthetic ones such as

roupatadine have already been used for the treatment of inflammatory pathological manifestations where PAF is implicated [39].

2. PAF AND CANCER

2.1. Introduction

Cancer is one of the major leading causes of death in developed countries. In spite of sequential investigations over the decades, little progress has been made in the context of treatment of several types of cancer. Modern Biochemical and Molecular Biology's techniques have contributed to the enlightenment of several signaling pathways that take part in the transformation of normal cells to cancer cells. The understanding of the deregulation of cell-cycle that results in such transformations has resulted in relative planning of specific inhibitors of this process. However, the expectations for treatment that target specifically and exclusively on cancer cells have declined, while they also bring on unfortunate side effects of drugs.

Several established tumors can increase their diameter by 1-2 mm, before their growth is retarded by an insufficient supply of oxygen and nutrients. In these cases the proliferation of cancer cells is counter-balanced by their apoptosis and thus tumors remain undetected even for years (dormant tumors).

These tumors acquire the ability of further extension and intrusion in neighbouring tissues where they are capable of inducing the production of small neoplastic blood vessels. The development, growth and metastatic extension of tumours precede the process of neoangiogenesis [15, 16, 42, 43].

This conclusion has caused the transposition of interest in the study of molecular mechanisms of tumour-induced angiogenesis, and the possibilities that the process of neoangiogenesis can offer as a target for anticancer therapy [15, 16, 42, 43].

Angiogenesis can be divided into four different phases: 1) establishment of vessel activation 2) proliferation of endothelial cells 3) migration of endothelial cells and 4) structural reorganisation.

The first phase includes the activation of endothelial cells on pre-existing vessels by cancer cells. Several cytokines and growth factors produced by cancer cells, when linked to their receptors in the endothelium, induce membrane signalling pathways that activate the vessel. Then, the activated endothelial cells produce serine-proteases and metallo-proteases that degrade the extra cellular matrix (ECM). A microenvironment has been constructed where the activated endothelial cells express specific adhesion molecules, proliferate and migrate from the pre-existing parental vessels and construct microtubules that finally join the parental vessels and blood circulation, establishing functional new vessels in the tumor microenvironment [16, 42].

Specific adhesion molecules on the endothelium surface and in membranes of cancer cells provide the cancer cells with the ability to attach to endothelial cells and platelets of the newly synthesized vessels in the tumor microenviron-

² Nasopoulou, C.; Nomikos, T.; Rementzis, J.; Demopoulos, C.A.; Zabetakis, I. Biologically active lipid fractions in fish and cephalopods of the Mediterranean diet. 45th International Conference on the Bioscience of Lipids, 2004, Ioannina, Greece. Abstract in *Chem. Phys. Lipids*, **2004**, *130*, 63-63

ment. This specific attachment of cancer cells on the endothelium constitutes a crucial step in the process of metastasis and colonization of other tissues / organs [15, 16, 42, 43].

2.2. Implication of PAF in Tumor Neoangiogenesis and Metastasis

2.2.1 Cytokines and Growth Factors Induce PAF-Production from Activated Endothelium in the Tumor Microenvironment and Vice-Versa

Several studies have been focused on angiogenic factors like cytokines, such as Interleukin-1 (IL-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Interleukin-13 (IL-13), Tumor Necrosis Factor alpha (TNF α), Interferon-gamma (IFN γ), etc and growth factors such as Vascular Endothelial Growth Factor (VEGF), acidic or basic Fibroblast Growth Factor (aFGF and bFGF, respectively), Epidermal Growth Factor (EGF), Insulin-like Growth Factor 1 (IGF-1), Hepatocyte Growth Factor (HGF), Platelet-Derived Growth Factor (PDGF), Transforming Growth Factor (TGF), colony stimulating factor (CSF), as well as their specific receptors, that are implicated in the induction of tumor neoangiogenesis [15-20, 43].

However, in an effort to inhibit this process through specific targeting of one growth factor such as VEGF and/or its receptor on the endothelium, unfortunately tumors continue to induce angiogenesis; as cancer cells can reprogram the production of other growth factors such as FGF that induce the proliferation and migration of endothelial cells of capillary vessels [15].

Binding of FGF molecules to their receptors induce intracellular pathways such as phosphatidylinositol (PIP₃) pathways and the rennin-angiotensin system (Ras) cascades that conclude in activation of the endothelium. Several similar growth factors share common pathways in the process of endothelium activation. The interesting feature of these factors is that through these cascades of signaling pathways they induce the production and release of significant lipid mediators such as arachidonate and PAF [15].

PAF, when released in the tumour microenvironment, can affect these endothelial cells either in an autocrine or paracrine mode including the neighbouring endothelial cells as well as inflammatory (leukocytes and / or platelets) and cancer cells (Fig. 2).

In particular, the production and secretion of PAF by activated endothelial cells can induce a panel of significant biological responses such as: a) an additional production of PAF and expression of its receptor in the membrane of these activated endothelial and / or neighbouring endothelial cells, inflammatory cells (leukocytes and / or platelets) and cancer cells (Fig. 2), b) induction of cellular proliferation, c) induction of prostaglandin production through direct Cyclooxygenase-2 (COX₂) activation, d) expression of both matrix metallo-proteases (MMP) and serine protease urokinase-type plasminogen activator (uPA), through activation of Janus tyrosine kinases (JAKs) signalling cascades and signal transducers and activators of transcription (STATs), leading to degradation of ECM.

The production of PAF by the activated endothelium seems to play a significant role in the induction and process of angiogenesis as it seems to be a common junction step of a panel of signalling pathways induced by the binding of several growth factors and / or cytokines on their receptors (Fig. 2, II).

In addition, PAF can induce the expression of several of the above mentioned angiogenic factors such as TNF α , IL-1 α , FGF, VEGF, etc in endothelial cells through the transcription Nuclear Factor Kappa B (NF-kB). PAF-induced angiogenesis seems to be depended on NF-kB [44]. Estrogens also enhance angiogenesis through a pathway involving PAF-mediated NF-kB activation [45]. PAF-inhibitors as well as NF-kB inhibitors (p65-antisense oligo-nucleotides and antioxidants such as α -tocopherol and N-acetyl-L-cystein) significantly reduce the PAF-induced (through NF-kB) increase in angiogenic factors and subsequent angiogenesis. It seems that PAF is not only a simple mediator but it also has the potential of provoking and enhancing angiogenesis through its ability to induce the production of angiogenic factors (cytokines and growth factors).

2.2.2. Cancer Cells may Trigger Malignant Manifestations also through their Autonomous Abilities to Produce PAF and Express its Receptor on their Membrane Surfaces

The presence of PAF in the tumour microenvironment may be due to the activated endothelium and/or cancer cells themselves (Fig. 2, I, C), since several studies have pointed out the ability of several cancer cell types to produce PAF and express PAF-receptor on their membrane [17-19, 46-58].

Several growth factors and cytokines such as VEGF, FGF, TNF α , can induce the production of PAF in tumour cells. Recent studies have correlated the malignancy of cancer cells with their PAF-production and PAF-receptor expression [16, 57].

For example, MDA-MB231 breast cancer cells that show more malignant activity than MCF-7 or T-47D breast cancer cells exhibited significantly higher ability to produce and release PAF than the latent ones, while MCF-10A breast non-cancer cells lack the ability to produce and release PAF [16]. In addition, PAF-receptor expression was also higher in MDA-MB231 cells than in MCF-7 and T-47D cells, while it was absent in MCF-10A cells [16]. In addition, the B77-A66 cancer cells with high metastatic ability presented with increased PAF-levels than their homolog cancer cells with very low metastatic ability [57].

Synthesised and cell-linked PAF affects these cancer cells in an autocrine way, while in the extracellular tumour microenvironment it affects neighboring cancer and/or endothelial cells in a paracrine way (Fig. 2, II); therefore it promotes further, the effects of cytokines and growth factors in all these cells.

Adhesion of cancer cells on the activated endothelium and /or platelets through adhesion molecules (such as VCAM-1 and E-Selectin) is also induced by the presence of PAF in the tumor microenvironment [19]. Binding of PAF on its receptor can induce cellular activities such as proliferation and motility through G-proteins and tyrosine

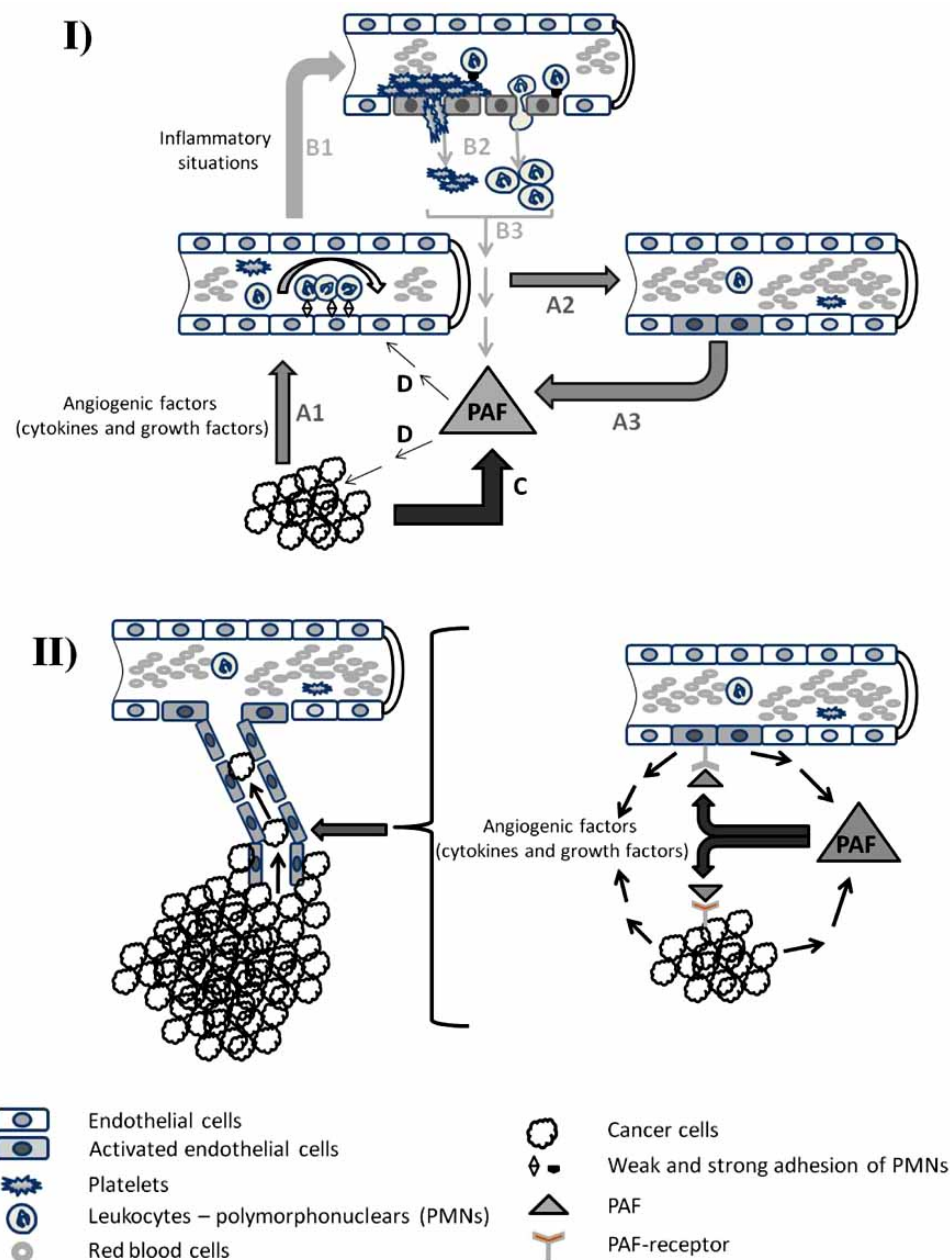


Fig. (2). Mechanism of PAF production and implication in metastatic angiogenesis of cancer cells

I) Production of PAF in the tumor microenvironment

- A1-A3: Activation of endothelial cells to produce PAF by angiogenic factors (cytokines and growth factors) from cancer cells
- B1-B3: Inflammatory situations can also lead to PAF-production by infused inflammatory cells (leukocytes and platelets)
- C: Cancer cells have the ability to produce PAF

II) PAF-effects

- Binding of PAF (produced either by each one of the above ways or by simultaneously all of them) on its receptor on the membrane of both endothelial and cancer cells (I, D) induces signaling pathway cascades that results in further activation of these cells in the direction of further production of both PAF and angiogenic factors, enhancing thus the initial signal
- In endothelial cells PAF induce mechanisms of proliferation, motility, expression of adhesion molecules, ECM breakdown, migration and endothelium reorder that conclude in the formation of distinct neoplastic vessels on the tumor microenvironment.
- In cancer cells PAF induces mechanisms of tumor development, growth proliferation, motility, expression of adhesion molecules and migration that concludes in metastatic angiogenesis.

kinases cascades; this correlates with the growth, development, migration and infusion of tumors.

The fact that several PAF-specific antagonists can inhibit the above malignant processes of cancer cells *in vitro* and *in*

in vivo [16, 19, 43, 55, 59, 60] indicate the implication of PAF in the neoangiogenic metastasis of tumors. The presence of PAF in a tumor microenvironment seems to promote and enhance the role of cytokines, growth factors and adhesion molecules through processes such as neoangiogenesis and metastasis. The ability of cancer cells to produce PAF and to express PAF-receptor on their membrane provides them with the autonomy to trigger such processes through increased PAF-levels.

2.2.3. The Presence of PAF in Tumor Microenvironment - a Triggering Effect of Tumor-Induced Angiogenesis

When and how PAF becomes present in the tumor microenvironment for the first time has not been yet clarified. Its initial levels may be derived from:

- a) activated endothelial (Fig. 2, I, A1-A3) and /or infused inflammatory cells (leukocytes and platelets) (Fig. 2, I, B1-B3) - This way initial levels of synthesized PAF can further activate these cells in an autocrine mode or in a paracrine/juxtacrine mode of neighboring endothelial and cancer cells -, and/or
- b) cancer cells (Fig. 2, I, C) – In this way the initial levels of synthesized PAF can further activate these cells in an autocrine way or in a paracrine/juxtacrine way of neighboring endothelial and inflammatory cells (leukocytes and platelets).

The first version (a) leads to the conclusion that malignant processes may be triggered by harboring inflammatory situations while the second one (b) leads to the conclusion that the cancer cells may have the autonomy to trigger malignant processes, through their ability to produce inflammatory mediators such as PAF. It is also possible that both cases may simultaneously take part in the production of initial levels of PAF in the tumor microenvironment.

Regardless of the origin of the initial PAF-levels (a, b, both a and b), it should be mentioned that when PAF is presented in the tumor microenvironment even in low levels, it has the ability to activate cancer cells and endothelial cells to amplify PAF-production and PAF-receptor expression on their membranes [53], a process that in coordination with cytokines and growth factors induces molecular mechanisms and morphological alterations that take place in the induction and progression of metastatic angiogenesis (Fig. 2, II). It is of great importance to clarify the conditions and factors that trigger and enhance PAF-biosynthesis in these processes.

It should be also mentioned that during cancer metastasis, the formation of platelet-tumor cell aggregates in the circulation facilitates immune evasion and the microvascular arrest of tumor cells at distant sites [61]. Other effects of activated platelets on cancer progression are associated with a release of platelet-derived factors that stimulate tumor growth and angiogenesis. Any interference in platelet-tumor cell interactions results in attenuation of cancer metastasis. It is also well established that PAF can induce the expression of such adhesion molecules in platelets that facilitate both atherosclerosis and cancer-metastasis, situations that share common mechanistic features.

PAF seems to act as a common mediator of several angiogenic and migrating factors that are implicated in the

triggering of tumor growth, development and metastasis, as well as in the requisition of endothelium and inflammatory cells in metastatic angiogenesis. While the specific inhibition of one angiogenic factor may not provide desirable results (since tumors can induce angiogenesis through the production of other angiogenic factors), it seems that the inhibition of junction points such as PAF involved in angiogenic signaling or the inhibition of PAF-biosynthesis may provide an alternative approach to the inhibition of metastatic angiogenesis of tumors.

2.3. PAF-Dual Action/Beneficial Effects in Cancer

PAF is a potent inflammatory mediator of angiogenesis. Binding of PAF on its receptor induces several signaling pathways that promote such processes. One of these pathways is the induction of the expression of angiogenic factors through the activation of NF- κ B [44].

On the other hand, in several cancer models, the presence of PAF-receptor enhances cancer cell apoptosis by chemo-preventing factors through the activation of NF- κ B [62, 63]. NF- κ B activation by oncogenes, viral proteins, inflammatory mediators of cancer such as PAF, etc, controls the expression of genes that take part in processes that on the one hand result in metastatic angiogenesis, while on the other hand result in apoptosis of cancer cells, during the immune response and haematopoiesis [64].

PAF, like NF- κ B, seems to promote distinct biological processes. Dual actions of PAF may relate to the point of action in the cell cycle [65, 66]. Through its binding on its receptor PAF induces mitogenic responses on G₀-rested cells, while it inhibits the transition from G₁ to S phase of the cell cycle. PAF-receptor can on the one hand activate Gi-proteins that are implicated in cellular growth, development and tumor genesis, while on the other hand it can activate Gq-proteins that are implicated in the inhibition of cellular growth and development, exhibiting two distinct and opposite effects on the cell cycle. These mechanisms have not been yet clarified; cyclins (cyclin D1, PRAD1) and their genes / onco-genes may be implicated in [66].

In addition, PAF can also signal cellular damage and potentiate the activity of Natural Killer cells (NK) [67]. NK cells usually attack cancer cells via secretion of the perforin lytic system that requires the co-presence of phosphocholine-lipids in order to destroy cancer cells. Naïve NK when activated also releases PAF that enables the perforin lytic system, since binding of PAF on perforin directs the system to target-cells that express PAF-receptor [68]. Glycoprotein-170 (gp-170) that is expressed on the membrane of cancer cells from MDR1 gene (multidrug resistance, MDR) induce the expression of PAF-receptor on the membrane of these cells that modifies them as more susceptible to NK cell-induced perforin system activity [69].

Furthermore, NK cells secrete IFN- γ as a mediator of this process. IFN- γ induces the expression of PAF-receptor on the membrane of target cells, enhancing their susceptibility to the perforin lytic system. This mechanism occurs in target cells that are susceptible to NK cells, while there are other cells that are not susceptible to NK cells. The variety of PAF-receptor expression of cancer cells may explain in part

the difference in susceptibility of these cells in the perforin lytic system of NK cells.

It seems that PAF is a unique growth regulator with apparently diverse functions. The timing, space and quantity of its production play a significant role in the malignant or beneficial direction of its effects. The clarification of conditions and factors that control timing, location of activity and the quantity of PAF-levels by effecting PAF metabolic enzymes is of great importance.

3. AN ANTI-CANCER APPROACH THROUGH INHIBITION OF PAF AND/OR ITS BIOSYNTHESIS

3.1. Anti-Cancer Properties of PAF-Inhibitors

Recent studies have been focused on the interaction of PAF and several factors with beneficial effects against cancer. These factors are specific PAF-antagonists of PAF-receptor that have been tested *in vitro* and *in vivo* in several cancer models (Table 1) [15, 16, 20, 43, 45, 56, 58, 70-75], or other molecules that exhibit beneficial effects against

tumor-induced angiogenesis through not only their general anti-inflammatory and/or anti-atherogenic activities but also through their ability to improve the condition of the endothelium.

Since atherosclerosis shares common features with those of cancer, several anti-thrombotic and anti-atheromatic molecules such as heparin, varfarin, acenokoumarol and statins, as well as non steroid anti-inflammatory molecules such as indomethacin or COX-2 inhibitors have shown beneficial effects in cancer [76]. Several of these molecules not only exhibit inhibitory effects against PAF-induced biological activities but also against its biosynthetic enzymes [37]. These effects come in accordance with their general anti-cancer activities [76-78].

Several studies have been focused on the correlation between diet and cancer. It has been found that the traditional Mediterranean Diet rich in olive oil, cereals, vegetables, fruits, fish, wine and low in saturated fats and animal foods is correlated with very low risk of several types of cancer [79-82]. This dietary profile is rich in antioxidants

Table1. Anti-Cancer Activities of PAF-Receptor Specific Antagonists

PAF-receptor antagonists	Action	Reference
BN-50730	<ul style="list-style-type: none"> • Inhibition of vascular permeability mediated by VEGF • Inhibitory effect on the proliferation of HUVEC • Reduction of the size of human prostatic carcinoma xenografts in athymic nude mice (without detectable side effects on the mice). • Inhibition of PAF- induction of MMPs, collagenase and urokinase-type plasminogen activator genes 	[15, 76]
BN-52021	Inhibition of vascular permeability mediated by VEGF	[76]
WEB-2170	<ul style="list-style-type: none"> • Inhibition of endothelial cell motility and angiogenesis induced by TNF-α, Hepatocyte Growth Factor (HGF), Vascular Endothelial Growth Factor (VEGF) • Inhibition of angiogenesis in breast cancer cells • Reduction of breast cancer cells proliferation • Inhibition of CD-40/CD-154 induced angiogenesis in Matrigel mouse models • Inhibition of estrogen induced angiogenesis in Matrigel mouse models • Inhibition of proliferation, motility and PAF-production of Renal Cancer Cells • Inhibition of PAF-induced (through NF-kB) expression of angiogenic factors (TNF-a, VEGF, FGF, etc) 	[16, 45, 56, 58, 71-73]
CV-3988	<ul style="list-style-type: none"> • Reduction of breast cancer cells proliferation • Inhibition of angiogenesis in breast cancer cells • Inhibition of CD-40/CD-154 induced angiogenesis in Matrigel mouse models • Inhibition of Photocarcinogenesis 	[16, 73, 74]
WEB-2086	Inhibition of adhesion of cancer cells on endothelial cells	[43]
BN-50739	Inhibition of PAF-induced metastatic process of malignant cancer cells infused in lung of animals	[43]
Y-24180	Cytotoxicity of human prostate cancer cells	[75]
PCA4248	Inhibition of experimental human melanoma lung metastasis	[20]

of natural origin. Several of these antioxidants have exhibited anti-cancer potential through their general anti-PAF activities [37, 83, 84].

3.2. Effect on PAF-Metabolism; A New Approach?

PAF-levels mirror the balance between its production and its degradation. PAF-levels in blood of colorectal cancer patients have been found to be similar to those of healthy subjects [85]. In these patients the activities of the PAF-biosynthetic enzyme PLA₂ and its basic degrading enzyme PAF-AH were significantly higher than those of healthy individuals. Increased levels of PLA₂ have also been observed in patients with advanced cancer [86].

It seems that these pathological implications tend to induce or are correlated with an increase in PAF-levels through induction of its biosynthetic enzymes activities, while a response of increased PAF-AH activity tends to balance PAF-levels via reduction of pathologically increased PAF-levels.

In recent studies it has been shown that when cancer cell lines have been transformed to be able to express PAF-AH directly in the tumor microenvironment, reduction in tumour growth and inhibition of malignant activities such as proliferation, motility and metastatic angiogenesis was achieved. The reduction in PAF-induced signaling pathways due to its direct degradation from regionally produced PAF-AH has been the cause of these beneficial effects [87].

In addition, in patients treated with INF α (a cytokine with anti-cancer activities) PAF-CPT activity of human renal cell carcinoma was significantly reduced in comparison to those who were not treated with this cytokine [34]. The inhibitory effect of INF α against cancer development and growth was achieved also by the reduction in PAF-biosynthesis.

Furthermore, several anti-oxidants exhibit anti-cancer activities not only through their ability to directly inhibit PAF-activity but also through their ability to down regulate PAF-levels.

Inhibition of VEGF-mediated angiogenic activities of several cancer cells by red grape skin polyphenolic extract (SGE) is associated with down-regulation of ERK and p38/MAPK phosphorylation and a decrease in acute PAF synthesis. SGE both prevents and inhibits angiogenesis in the Matrigel model, decreases the basal motility of endothelial and cancer cells, and reverses the chemotactic effect of angiogenic factors [83].

Yuccaols (A, B, C) are phenolic constituents isolated from *Yucca schidigera* bark characterized by antioxidant and anti-inflammatory properties similar to those of resveratrol. The anti-inflammatory properties attributed to *Yucca schidigera* may be due to both resveratrol and Yuccaols with anti-tumor and anti-invasive properties of these phenolic compounds. Resveratrol is a molecule that exhibits a potent inhibitory effect against the biological activities and biosynthesis of PAF [37]. However, Yuccaols were more effective than resveratrol in inhibiting the VEGF-induced Kaposi Sarcoma cell proliferation, as Yuccaols completely inhibited the VEGF-stimulated PAF biosynthesis and enhanced its

degradation. Yuccaol C also abolished PAF-induced cell motility whereas Yuccaol A and Yuccaol B reduced cell migratory activities [84].

Tea-polyphenols also exhibit similar anti-cancer effects [88, 89]. These molecules have also exhibited beneficial effects against atherosclerotic models [90], while they have the ability to inhibit PAF-activities and PAF-biosynthetic enzymes [91].

We have isolated several molecules from Mediterranean Diet foods (such as olive oil, olive pomace, fish, wine, etc.) with anti-PAF activities that exhibit beneficial effects against several inflammatory manifestations such as atherosclerosis [38, 40, 92]. These molecules also have the ability to inhibit/down-regulate PAF-biosynthetic enzymes [37]. Since cancer and atherosclerosis share common features and PAF is implicated in these pathological conditions, one may propose that these molecules may provide beneficial anti-metastatic preventive effects, also through inhibition of PAF and its biosynthesis. This point of view needs to be further studied *in vitro* and *in vivo*.

4. CONCLUDING REMARKS

It is widely accepted that the inflammatory mediator PAF and its receptor are implicated in malignant processes such as tumor development, growth and metastatic angiogenesis. PAF seems to be a significant junction point of several pathways induced by angiogenic factors (such as cytokines and growth factors) in both endothelial and cancer cells. Furthermore PAF has the potential to trigger and enhance such processes by inducing its production, enhancing receptor expression and via angiogenic factors.

PAF-receptor specific antagonists have *in vitro* and *in vivo* exhibited promising results in several cancer models. Several antioxidants with anti-PAF activity exhibit similar effects. In addition, reduced PAF-biosynthesis and induced degradation by such factors may provide an alternative nontoxic approach to anti-cancer therapy.

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