Platelet activating factor leads to initiation and promotion of breast cancer

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Bioactive molecules present in the tumor milieu are known to contribute substantially to tumor progression. Phospholipid mediators are a group of molecules that have roles in normal physiology as well as in pathological conditions. Platelet activating factor (PAF), a phospholipid mediator, secreted by cells present in tumor microenvironment has been implicated to have a possible role in cancer progression. Here, we highlight our study of the potential role of PAF in inducing transformation of breast epithelial cells grown as three dimensional cultures. We have also attempted to dissect the motility related molecular pathway activated upon PAF stimulation in MDA-MB 231 cells. This study further calls for detailed analysis of pathways downstream of PAF signalling which would aid in identification of targets and designing of treatment strategies.

Keywords: platelet activating factor; collective cell migration; single cell motility; breast cancer; transformation

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Breast cancer is one of the most common cancers and a leading cause of death in women worldwide. It is a multi-factorial disease caused by a complex combination of genetic and environmental factors. Apart from these, tumor microenvironment has recently been identified as a key factor playing a pivotal role in cancer progression \[1\]. The microenvironment is believed to evolve into an ‘activated state’ thus resulting in a ‘dynamic signaling circuitry’ capable of initiating cancer \[2\]. Immune cells have been found to be one of the major components of this microenvironment. Nevertheless, inflammatory response associated with the tumor has been demonstrated to have tumor promoting capabilities. The inflammatory cells can release various bio-molecules, which may have tumor inducing as well as promoting capabilities. One such group of molecules is the phospholipid mediators, namely LPA (lysophosphatidic acid) PGs (Prostaglandins) and PAF (Platelet activating factor) including PAF - like lipids \[3-5\]. These molecules are known to be secreted by these cells and have been implicated in various physiological and pathological conditions. PAF has been hypothesized to act as an intracellular mediator or messenger and is known to act through the PAF receptor (PAF-R), which is a G - protein coupled receptor \[6\]. Furthermore, given that PAF is a phospholipid mediator, most of the associated pathological effects occur due to excessive accumulation of PAF which maybe a result of either impaired degradation and/or enhanced synthesis \[4\]. Biologically active PAF is synthesized by the cells by hydrolysis of membrane phospholipids catalyzed by phospholipase A2 (PLA2) followed by trans-acetylation by
lysoPAF-acetyltransferase \(^1\) while degradation is carried out by PAF-acetyl hydrolase (PAF-AH), enzyme which hydrolyses and thus inactivates PAF \(^8, 9\). The synthesis generally, may occur in response to physiological cues or due to unregulated oxidative reactions stimulated by various cellular or environmental factors \(^5\). For instance, UV-B irradiations have been demonstrated to induce PAF production by epidermal keratinocytes \(^10\). Apart from this, macrophages, neutrophils and endothelial cells are known to secrete PAF. Bussolati et al have also demonstrated the ability of breast cancer cells to secrete PAF upon growth factor stimulation \(^11\). This correlates with the observation by Camussi’s group that significant amount of PAF is present in breast cancer tissues \(^12\). Impaired degradation is another reason for PAF accumulation, which may result from the inhibition or absence of PAF-AH. Cigarette smoke is considered as one of the factors which result in the accumulation of PAF by inhibiting PAF-AH \(^13, 14\). This accumulated PAF enhances adherence of metastatic breast cancer cells to lung endothelium \(^15\).

Apart from the well-established role of PAF in various immunological processes, PAF has been demonstrated to play a role in neo-angiogenesis \(^16\) and the inhibition of this process has been shown to inhibit growth of tumor in Kaposi’s sarcoma \(^17\), prostate cancer and breast cancer \(^18\). Hepatocyte growth factor (HGF), TNF-α (Tumor Necrosis Factor-α) and thrombopoietin-induced angiogenesis is mediated through PAF\(^19\). In addition to this, PAF has been shown to induce transformation in rat embryonic cells \(^20\) as well as BRCA-1 mutant ovarian cancer cells \(^21\). Axelrad et al reported that PAF induced migration and invasion of HUVECs; these effects could be reversed using a PAF -R antagonist \(^22\). PAF is capable of inducing invasive phenotypes in melanoma cells stimulated by cytokines \(^23\). Apart from this in liver metastasis of colorectal cancer PAF has been shown to promote bFGF (basic fibroblast growth factor) and VEGF (Vascular endothelial growth factor) -induced neoangiogenesis \(^24\). PAF secretion by keratinocytes following UV-B radiation exposure has been reported to be an important mediator of UV-B induced immunosuppression \(^10\). In addition to this, presence of PAF has been shown to reduce the ability of cells to repair DNA damage induced by UV radiations \(^25\). The two phenomena have been hypothesized to be responsible for skin cancer induction \(^26\).

PAF receptor shows differential expression across various cell types. Investigation of the PAF-R status revealed that MDA MB-231 and MCF7 cells showed higher expression as compared to MCF10A cells, which showed a very low expression. A recent study from our lab demonstrated that PAF may play a role in cancer initiation as well as promote cancer progression \(^27\). Three dimensional (3D) cultures of
normal breast epithelial cells as well as cancerous cells have been exploited to study the process of morphogenesis and tumorigenesis [28-30]. We used 3D ‘on top’ cultures of breast epithelial cells to investigate the cancer initiation potential of PAF. In this culture system, breast epithelial cells when grown on laminin-rich extracellular matrix form growth-arrested polarized spheroids that structurally and functionally closely resemble the ‘acini’ of the mammary gland [28-30]. Maintenance of such an architecture mainly depends on the balance between proliferation and apoptosis which translate in 3D cultures into either increase in the number of cells per acini or a luminal filling phenotype [28, 29], as depicted in Figure 1. We observed that when MCF10A cells, considered as ‘near normal’ breast epithelial cells were grown in presence of PAF, resulted in the formation of acinar structures which had a larger volume. Consistent with this finding, we also observed significant increase in the number of cells per acini. Luminal phenotype was also disrupted with a significant number of acini having multiple layers of cells enclosing the luminal space. All these phenotypes demonstrate the ability of PAF to induce proliferation of breast epithelial cells, implying possible attainment of hyperproliferative state, referred to as “escape from proliferative state”, one of the “hallmarks of cancer” [31]. PAF has been shown to induce proliferation of differentiated keratinocytes [32], rat vascular smooth muscle cells [33], epidermal cells [34] as well as breast adenocarcinoma cells. In contrast, PAF is known to induce apoptosis in neurons [35] as well as inhibit proliferation of colon carcinoma cells [36]. Another striking feature of the acini grown in presence of PAF was the formation of “protrusion-like” or “bulb-like” structures. Such structures are known to be characteristic features of invasiveness or migratory cells. Cells grown on 3D substrata that have undergone epithelial-mesenchymal transition (EMT) give rise to protrusion-like structures. When a few cells migrate into such protrusions, these structures may appear as “bulb-like” structures [37, 38]. With the known role of PAF in inducing motility of different kinds of cells including breast cancer cells, these abnormal structures imply possible induction of EMT or motility resulting in escape of cells out of the well structured and regulated acini. Taken together these preliminary results imply that presence of PAF in the microenvironment is capable of inducing transformation. However, studies are being performed to further investigate this process and delineate the mechanism thereof.

To study the role of PAF in breast cancer progression, effect of PAF on migration was studied [27]. Metastasis of cancer cells is one of the many aspects which remain unexplored in cancer pathogenesis and effectively curtailing this phenomenon is the need of the hour [30]. Acquiring migratory potential is one of the early processes in metastasis. Cancer cells are known to invade the surrounding tissue and disseminate to the secondary sites. Research has revealed presence of cluster of cells, which move as sheets during metastasis. This is also supported by the observation of clusters of cells infiltrating the secondary tumor sites. On the other hand, cells with invasive and metastatic characteristic can travel as single entities and lodge themselves into the secondary site. Stimulation of cells with PAF induces motility in a variety of cells including peripheral blood lymphocytes [40] human endothelial cells [41] as well as eosinophils [42] and breast cancer cells. However the mechanism(s) for PAF-induced motility of breast cancer cells remains unknown. Thus, an attempt was made to identify the key pathway(s) involved in PAF-induced increased migration of breast cancer cells. Firstly, we investigated the effect of PAF, under the conditions of our study, on both collective cell migration as well as single cell migration of MDA-MB 231 cells. Previous reports have shown growth factor stimulation to induce PAF secretion and PAF stimulation induced chemotaxis as well as chemokinesis in breast cancer cells such as MDA-MB231 [11]. In addition, a recent report showed the ability of cigarette smoke to enhance motility of MDA-MB 231 cells by inducing PAF accumulation via inhibiting PAF-AH. In agreement with these reports we also observed increased collective cell migration of MDA-MB 231 cells upon PAF treatment in the wound healing assay. At the single cell level, PAF enhanced the velocity as well as distance traversed by the cells without change in directionality [27]. Further, to unravel the mechanistic aspect of PAF induced motility we used small molecule inhibitors of probable pathways predicted from available literature. PAF through PAF-R is known to activate various signal transduction pathways in different cell types. In ovarian cancer cells, Bin Ye’s group have reported PAF induced MMP9 and MMP2 secretion through activation of EGFR/Src/FAK/paxillin and this activation was mediated through PAF-R [43]. Further, PAF-R activates c-Jun N-terminal kinase in hippocampal cells, regulates cell growth, survival and proliferation of macrophage cell line through GBγ- activatable PI3K kinase and while in various cells activates p38 MAP kinases [44]. Apart from this, G-proteins like Ras, Rap as well as other signaling molecules like PLCγ-PLD are regulated by PAF-R [45, 46]. In ovarian cancer cells PAF promotes cancer progression through EGFR/ERK transactivation pathway as well as activates PKC pathway, which couples with activated ERK [47]. FAK and Paxillin are activated following PAF induction in human endothelial cells [48] while in non-cancerous cell lines such as neutrophils [49] and eosinophils [50] it activates various protein kinases such as G-protein kinase, PKC as well as tyrosine protein kinase [48]. Since MAPK pathway and PI3K pathway were the most common downstream targets of PAF in various cell types as well as these pathways are well
known motility pathways, we investigated whether PAF induced motility in invasive breast cancer cells through the MAPK pathway. Our data indicated that the ERK pathway did not play a role in PAF induced motility. Inhibition of the PI3K pathway as well as JNK pathway resulted in the inhibition of motility as compared to PAF stimulated cells. However, PI3K pathway inhibition appeared to partially reduce the motility of PAF stimulated cells. This coupled with the observation that significant reduction of motility was seen in control untreated cells treated with wortmannin (a PI3K inhibitor) alludes to the possible role of PI3K in either PAF induced motility or the inherent motility of MDA-MB 231 cells. On the other hand, JNK pathway inhibition resulted in inhibition of PAF stimulated enhanced motility as well as motility of unstimulated cells, confirming its role in PAF increased motility as well as inherent motility. Taken together, these results raise the possibility that PAF induced increased motility may be occurring through PI3K as well as JNK pathways. Ongoing studies are being performed to dissect out the exact mechanistic pathway involved in PAF stimulated motility in cells.

Role of PAF in various cancers have been studied to some extent. The exact role of PAF and the mechanism(s) thereof have not been reported till date. The results discussed above and supported by few reports available in literature suggests the possible role of PAF in breast cancer initiation as well as promotion of breast cancer by enhancing migratory ability of cells. However, this work warrants further investigation to delineate the pathway(s) involved, which would further help in designing novel therapeutic strategies to combat this heterogeneous condition.

Conflicting interests

The authors have declared that no conflict of interest exists.

Author contributions

L.A.V. performed 3D experiments and prepared the figure. L.A.V. and M.L. wrote the manuscript.

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Abbreviations

PAF: Platelet activating factor; LPA: lysophosphatidic acid; PGs: Prostaglandins; PAF-R: PAF receptor; PLA2: phospholipase A2; PAF-AH: PAF-acetyl hydrolase; HGF: Hepatocyte growth factor; TNF-α: Tumor Necrosis Factor-α; Bfγf: basic fibroblast growth factor; VEGF: Vascular endothelial growth factor; 3D: Three dimensional; EMT, epithelial-mesenchymal transition; MAPK: Mitogen-activated protein kinase; EGFR: Epidermal growth factor receptor; ERK, extracellular signal–regulated kinases; PI3K: phosphatidylinositide 3-kinases; JNK: c-Jun N-terminal kinases; HUVEC: Human Umbilical Vein Endothelial Cells; FAK: Focal adhesion kinase; PLCγ: Phospholipase C; PLD: Phospholipase D; PKC: Protein kinase C.

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