

## Review Article

# Lymphangiogenesis and Prostanoids as Regulators in Disease States

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## Introduction

Blood vessel system is a closed circulatory one with microcirculation in every organ. By contrast, lymphatic vessel system is a one-way conduit for tissue fluids and leukocytes including lymphocytes [1]. The critical function of lymphatic vessels is to drain the protein-rich fluid extravasated from the blood vessels and to drive it back into the systemic circulation [1,41]. Lymphatics exhibit significant roles in immune surveillance, and deliver various antigens and antigen-presenting cells to the regional lymph nodes and export immune effector cells and humoral response factors into the circulation. Dysfunction of lymphatics induce lymph accumulation in interstitial spaces, and tissue swellings, lymphedema. Lymphatic functions exhibit major determinant of pathogenesis of various disease states such as inflammatory responses (Schwager et al, 2019), lymphedema (Zheng et al, 2014), endometriosis (Hattori et al, 2020), liver dysfunctions (Nakamoto et al, 2020), and metastasis of tumor cells (Dieterich et al, 2022).

Biologically active lipids including prostanoids exhibit various activities on the cardiovascular systems. Recent evidence indicates that biologically active lipids regulate lymphangiogenesis, similar to angiogenesis, under certain pathological conditions [21,43]. During chronic inflammation, angiogenesis is induced by a variety of inflammatory mediators, such as prostaglandins [40]. However, the accumulated knowledge relevant to bioactive lipid-associated lymphangiogenesis is limited. It was recently clarified the roles of cyclooxygenase-2 and prostaglandinE<sub>2</sub> [21,42] receptor signaling in enhancement of lymphangiogenesis during chronic and proliferative inflammation [21,42]. The same machinery was also active in the

## Abstract

The lymphatic vessels have crucial roles in the regulation of interstitial fluids, immune surveillance and the absorption of dietary fat in the intestine. Lymphatic functions are also closely related to the pathogenesis of various disease states such as inflammatory responses, lymphedema, and metastasis of tumors. Lymphangiogenesis, the formation of new lymphatic vessels from pre-existing lymphatics is critical determinant of the above pathological conditions. Prostanoids including prostaglandins and thromboxanes are the metabolites of arachidonic acid, C-20 unsaturated fatty acid, and they exhibit a variety of actions via specific receptors. Although some kinds of growth factors are well-characterized in the lymphangiogenesis, there is accumulating evidence that prostaglandins and thromboxanes are important regulators of lymphangiogenesis. Previously prostanoids are reported to have immediate actions on the smooth muscles and vasculatures, however, they work as inducers of growth factors or cytokines that regulate lymphangiogenesis, and modulate the prolymphangiogenic microenvironment. This review discusses our current understanding of prostanoids as regulators of lymphangiogenesis, and the emerging importance of the lymphangiogenesis as a therapeutic target.

**Keywords:** Lymphangiogenesis; Inflammation; Lymphedema; Metastasis; Prostaglandin; Thromboxane

secondary lymphedema model, in which lymphedema was induced by a circumferential incision made in the tail of anesthetized mice to sever the dermal lymphatic vessels [29]. This review article summarizes current knowledge on prostanoids relevant to pathological lymphangiogenesis. The receptor signaling and/or biosynthesis of prostanoids will be a promising target to treat the various pathological states related to lymphangiogenesis.

## Biosynthesis and Receptors of Prostanoids

Prostanoids including prostaglandins and thromboxanes are the metabolites of arachidonic acid, C-20 unsaturated fatty acid [31]. Prostaglandins contain a cyclopentane ring with two attached side chains named  $\alpha$  and  $\omega$ ;  $\alpha$  side chain has terminal carboxylic acid. According to the modification of the cyclopentane ring, physiologically important prostaglandins are classified into 4 types: PGD<sub>2</sub>, PGE<sub>2</sub>, PGF<sub>2</sub> $\alpha$  and PGI<sub>2</sub>. ThromboxaneA<sub>2</sub> (TXA<sub>2</sub>), another prostanoid, has an oxane ring instead of the cyclopentane ring. Arachidonic acids are released from membrane phospholipids with various physiological and pathological stimuli by the action of phospholipase A<sub>2</sub>. Arachidonic acids are converted to various prostanoids by sequential actions of cyclooxygenases and the respective prostanoid synthases. There are 2 isoforms of cyclooxygenases, cyclooxygenases-1 and cyclooxygenases-2 [31]. Cyclooxygenases-1 is a constitutive isoform and has housekeeping functions, whereas cyclooxygenases-2 is an inducible isoform and has a proinflammatory nature. PGH<sub>2</sub>, a common precursor of prostanoids that were produced by cyclooxygenases, is converted to each prostanoid via the action of respective prostaglandin synthase or thromboxane synthase [31]. The expression profile of these synthases vary depending on cell types or

tissues.

Prostanoids exhibit their activities acting on the receptors specific for PGD, PGE, PGF, PGI and TX, namely DP, EP, FP, IP and TP receptors. EP receptors are classified into four subtypes, EP1, EP2, EP3 and EP4. Thromboxane A<sub>2</sub> receptor (TP) was first purified from human blood platelets. Homology screening in mouse cDNA libraries subsequently identified the structures of all of the eight types and subtypes of the prostanoid receptors, which belong to a G-protein-coupled receptor super family [39,48]. The most typical actions of prostanoids are relaxation and contraction of various types of smooth muscles. According to these actions of prostanoids on smooth muscles, the classification of the prostanoid receptors has been proposed (Coleman et al., 1994). The contractile receptors, EP1, FP and TP, couple to G<sub>q</sub> and raise intracellular Ca<sup>2+</sup> concentrations. These receptors couple to G<sub>12/13</sub> and activate Rho, a low molecular weight GTP-binding protein that organizes cytoskeleton. By contrast, the relaxant receptors, DP, EP2, EP4 and IP, couple to G<sub>s</sub> and raise intracellular cyclic adenosine monophosphate (cAMP) concentrations. Interestingly EP4 has another signaling pathway related to phosphatidylinositol 3-kinase, activating protein kinase B and extracellular signal-regulated kinases [12]. In contrast, the inhibitory receptor EP3 couples mainly to Gi and decreases intracellular cAMP concentrations, while several EP3 isoforms derived from alternative splicing have other signaling pathways [47].

Highly selective agonists and antagonists had not been developed, thus it had been very difficult to evaluate the functions of each prostanoid receptor to the pathogenesis. However, mouse lacking each prostanoid receptor individually or enzyme mediating prostanoid biosynthesis have been generated by gene targeting, and significances of the prostanoid actions in various pathophysiological processes are being tested [3,21,43]. This review summarizes the current knowledge on roles of prostanoids in the pathogenesis of lymphangiogenesis-associated disease conditions. The field of prostanoids is rich with the candidates of therapeutic tools.

## Lymphangiogenesis and Prostanoids Relevant to Disease States

### Prostanoids regulate Lymphangiogenesis in Inflammation

Under physiological conditions, the plasma is filtered through the semipermeable vascular endothelial layer into the extracellular space. The majority of the extravasated interstitial fluid and macromolecules are absorbed back by the lymphatic vessels (Levick and Michel, 2010). A primary function of the lymphatic system is to provide an accessory return route for the interstitial fluids to the blood (Levick, 2004). When inflammation was sustained, drainage of the interstitial fluid into the circulation was increased together with antigens and immune cells from the periphery to lymph nodes with which adaptive immune responses are operated [33]. Lymphangiogenesis appeared to be critical since applications of prolymphangiogenic factor, vascular endothelial growth factor (VEGF)-C reduced the process of skin inflammation [22,25].

In LPS-induced inflammation, it was demonstrated that cyclooxygenase-2-derived prostaglandins especially PGE<sub>2</sub> enhanced inflammation-induced lymphangiogenesis (Infl) [42]. Infl may modulate the pathological environment by influencing fluid

movement and immunological functions. Inflammation is an intrinsically beneficial event that leads to the removal of harmful factors and restoration of tissue structure and physiological function. Once the noxious stimulus is removed, the inflammatory reaction decreases and resolves. It had been demonstrated previously that in proliferative inflammation, prostaglandin receptor signaling elicited adenylate cyclase-induced angiogenesis [2,3]. Prostaglandins induced vascular endothelial growth factor-A (VEGF-A) in the granulation tissues formed during inflammatory responses. The same pathway was shown to be active in the tumor microenvironment [30]. Among the cyclooxygenase-2-derived metabolites of arachidonic acid, PGE<sub>2</sub> is the most abundantly generated metabolite, and mediates an array of proinflammatory activities [31,39].

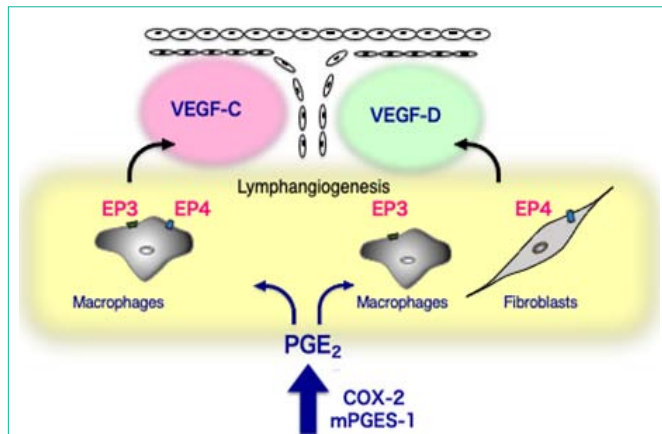
In lipopolysaccharide (LPS)-induced peritonitis, the number of LYVE-1-positive ladder-structured lymphatics in the diaphragm increased temporally [42]. This lymphangiogenic response was accompanied by increased expression of VEGF-C/D in the inflamed tissues. In mice treated with celecoxib, a cyclooxygenase-2 inhibitor, Infl was suppressed with reduced expression of VEGF-C/D. This was also observed in microsomal PGE<sub>2</sub> synthase-1 (mPGES-1) knockout mice. mPGES-1 is an inducible perinuclear enzyme that is functionally linked with cyclooxygenase-2. Cyclooxygenase-2 and mPGES-1 are essential components for PGE<sub>2</sub> synthesis, which may be linked to inflammation, fever, osteogenesis, and cancer. Immunoreactive cyclooxygenase-2 and mPGES-1 were detected in both CD11b-positive and CD3ε-positive cells in the diaphragm. When FITC-dextran was injected into the peritoneal cavities, the amount of residual FITC-dextran was reduced significantly in WT mice injected with LPS, and this reduction was significantly decreased in mPGES-1 KO mice [42].

In a separate experiment, it was clarified that when cultured lymphatic endothelial cells were incubated with pathophysiological concentration of PGE<sub>2</sub> (1 nM and 10 nM), they did not proliferate markedly with no induction of VEGF-A and VEGF-C. By contrast, PGE<sub>2</sub> (1 nM and 10 nM) markedly induced VEGF-A and VEGF-C in harvested peritoneal macrophages (Kashiwagi et al., 2014). These results suggested that cyclooxygenase-2/mPGES-1-derived PGE<sub>2</sub> played a significant role in lymphangiogenesis during inflammation, and represents a novel target for controlling Infl. PGE<sub>2</sub> may act as an inducer for VEGF isoforms acting not on lymphatic endothelial cells, but on the inflammatory cells accumulated in the prolymphangiogenic microenvironment (Kashiwagi et al., 2014).

Lymphangiogenesis is also related to the progression of Inflammatory Bowel Disease (IBD). Blockade of VEGF receptor-3 (VEGFR-3) aggravates inflammatory bowel disease and lymphatic vessel enlargement [23]. Recently it is demonstrated [20] that lymphangiogenesis contributes to mucosal repair in acute colitis elicited by Dextran Sulphate Sodium (DSS) [7,32]. EP4 signaling suppresses the development of this experimental colitis contributing to lymphangiogenesis, in turn promoting mucosal tissue repair. Compared with vehicle, treatment with EP4 antagonist increased signs of colitis, colonic tissue destruction, and CD11b+ cell infiltration, with reduced area of lymphatic vessels. By contrast, an EP4 agonist treatment suppressed the severity colitis with suppressed CD11b+ infiltration and decreased expression levels of inflammatory

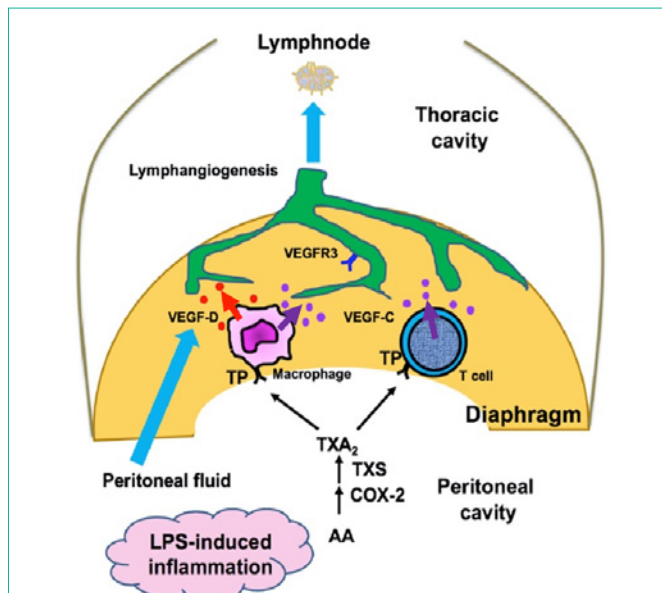
cytokines. These changes were associated with upregulation of prolymphangiogenic growth factors and lymphangiogenesis. Importantly, inhibition of VEGFR-3 signaling delayed mucosal repair, accompanied with impaired lymphangiogenesis. These results suggest that EP4 stimulation enhances mucosal repair in DSS-induced acute colitis by promoting lymphangiogenesis [32].

Inflammation often persists and becomes chronic. Chronic



**Figure 1:** EP receptor signaling relevant to the enhancement of lymphangiogenesis in chronic inflammation.

COX-2/mPGES-1-derived PGE<sub>2</sub> stimulated EP3/EP4 receptors and upregulated VEGF-C and VEGF-D in fibroblasts and macrophages. As discussed, prostanoids act as an inducer of growth factors in the inflammatory sites. COX; cyclooxygenase, mPGES-1; microsomal PGE<sub>2</sub> synthase-1, VEGF; vascular endothelial growth factor. (Cited from *Arterioscler Thromb Vasc Biol.* 2011; 31: 1049-58. with permission).



**Figure 2:** TP signaling in macrophages and T cells enhances lymphangiogenesis with upregulation of VEGF-C and VEGF-D expression in LPS-induced inflammation.

Results from the systemic knockout (KO) of TP, together with macrophage-specific/T cell-specific KO, suggest that TP on macrophages and T cells facilitates the generation of functionally active lymphatic vessels in the diaphragm. TP; Thromboxane A<sub>2</sub> receptor. (Cited from *Arterioscler Thromb Vasc Biol.* 2021; 41: 1390-1407. with permission).

inflammation is characterized by the persistent infiltration of mononuclear cells including macrophages and lymphocytes, and fibrocytes [37]. Lymphangiogenesis observed in the process of chronic inflammation. Growing evidence suggests involvement of chronic inflammatory processes in pathogenesis of a variety of diseases including cancer [62]. In such a disorder, abundant infiltration of inflammatory cells and expression of various pro-inflammatory molecules are found in affected tissues. Substantial evidence has now accumulated indicating that PGs contribute to lymphangiogenesis during the process of chronic inflammation [21].

In a granulation tissue formed around the matrigels injected subcutaneously, which exhibited proliferative inflammation profiles and mimicked tumor microenvironment, lymphangiogenesis was enhanced by PGE<sub>2</sub> [21]. During chronic inflammation in the surgical sponge implants, angiogenesis is induced by a variety of inflammatory mediators including prostaglandins [2,3,40,49,61,66] Majima et al., 1997. It had been demonstrated that cyclooxygenase-2 and PGE<sub>2</sub> receptor signaling enhanced lymphangiogenesis during proliferative inflammation formed around matrigels [21]. Lymphangiogenesis estimated by podoplanin/VEGFR-3/LYVE-1 expression was upregulated during proliferative inflammation seen around Matrigel plugs. A cyclooxygenase-2 inhibitor (celecoxib) reduced lymphangiogenesis, whereas topical PGE<sub>2</sub> application enhanced lymphangiogenesis. Lymphatic flow from the Matrigels was cyclooxygenase-2 dependent. Lymphangiogenesis was suppressed in the granulation tissues of mice lacking either EP3 or EP4, suggesting that these molecules are receptors in response to endogenous PGE<sub>2</sub>. An EP3-selective agonist increased the expression of VEGF-C and VEGF-D in cultured macrophages, whereas an EP4-selective agonist increased VEGF-C expression in cultured macrophages and increased VEGF-D expression in cultured fibroblasts [21]. This study suggested that cyclooxygenase-2 and EP3/EP4 signaling contributes to lymphangiogenesis via VEGF-C and VEGF-D, and may become a therapeutic target for controlling lymphangiogenesis in chronic inflammation (Figure 1).

Recently, an unexpected metabolite of arachidonic acid was reported to induce lymphangiogenesis in the pathological conditions [43]. TXA<sub>2</sub> is an unstable metabolite of arachidonic acid, produced by cyclooxygenase and thromboxane synthase catalysis in various cell types, and exerts its activity through a TP [48]. The roles of TXA<sub>2</sub> in cardiovascular system responses related to ischemic heart disease and brain hemorrhage, as well as platelet aggregation and bronchial smooth muscle contraction [48], are well-understood; however, it was found that TXA<sub>2</sub>-TP signaling also modulates acquired immunity by regulating dendritic cell T-cell interactions [24]. These reports provide a new avenue for exploration of the pathophysiological roles of TXA<sub>2</sub> in immunity, particularly in the lymph system. TP signaling on the platelets was reported to enhance the angiogenesis via platelet adhesion to angiogenic vasculatures [2,5,44]. Platelets supplied proangiogenic factors during the adhesion process. Further, TP-dependent platelet adhesion in the liver microvasculatures restored liver damages [45,53].

The peritoneum provides the lining of the peritoneal cavity and is the largest serous membrane in the body (Gandawidjaja and Hau, 1997). A single layer of mesothelial cells covers the peritoneal cavities. A thin and discontinuous layer of connective tissues and a layer of

fenestrated lymphatic vessels are present under the mesothelial cells [60]. These 3 layers function as an absorptive site for peritoneal fluids [15,18]. Once inflammation was induced in the peritoneal cavities, the fluid reabsorption was highly dependent to the lymphatic function. To analyze the functional relevance of lymphatics to the fluid reabsorption, the diaphragm is a suitable organ [34].

In the peritonitis model, a novel function of thromboxane, the facilitation of lymphangiogenesis via TP signaling was reported using TP knockout mice [43]. Compared with wild-type mice, LPS-induced lymphangiogenesis in systemic TP-knockout mouse diaphragm tissues was suppressed with reduced drainage function from the peritoneal cavity. TP-positive macrophages and T cells accumulated in the diaphragm produced VEGF-C and VEGF-D in a TP-dependent manner. Removal of macrophages and T cells resulted in reduced lymphangiogenesis and lowered expressions of VEGF-C and VEGF-D. Furthermore, TP knockout bone marrow chimeric mice exhibited reduced lymphangiogenesis. When cell-specific TP knockout using TP flox mice (*Tp* F/F) were tested [43], TP knockout specific to macrophages and T cells led to reduced lymphangiogenesis and drainage function in mice with LPS injections. This study suggested that TP signaling exerts prolymphangiogenic activity by acting on macrophages and T cells and that TP signaling represents a novel target for controlling lymphangiogenesis (Figure 2).

### Prostanoids regulate Lymphangiogenesis in Lymphedema

The lymphatic vasculature forms a network of vessels that drain interstitial fluid from tissues and return it to the blood [1]. Surgical treatment of malignant tumors, such as lymphadenectomy in patients with breast cancer, sometimes induces lymphedema that can impact quality of life [11]. The present therapeutic approaches for secondary lymphedema patients are the physical therapies such as a combination of compression, exercise, and massage. When physical therapy is not effective, super microsurgical techniques to make the anastomosis of blood vessels and lymphatic vessels is applied. Although the induction of lymphangiogenesis may be a possible therapeutic approach, it is not clear to what extent therapies aimed at lymphatic regrowth will be effective for the treatment of various types of secondary lymphedema. If lymphedema is attributable to blockade of lymph flow such as surgical removal of lymph nodes, therapies that stimulate lymphangiogenesis to restore lymphatic flow may be a good option for the treatment of secondary lymphedema.

Key regulators in lymphatic development have been demonstrated by transgenic models [52]. VEGFR-3 signaling may be responsible for primary lymphedema [27]. The recent findings suggest that targeting of molecular mechanisms of lymphangiogenesis to improve the lymphatic function may lead to a successful treatment of lymphedema by enhancing the recurrence of lymphatic flow in the secondary lymphedema situation. To clarify the roles of cyclooxygenase-2 in enhancement of lymphangiogenesis during secondary lymphedema, a mouse tail model had been tested [29]. The diameters of the tails around the wounds were markedly increased after surgery, and reached maximum size 2 weeks after wounding in mice without celecoxib. Expression of cyclooxygenase-2 in wound granulation tissues was markedly increased 1 week after surgery compared with unwounded naive control mice. In mice without celecoxib, recurrence of lymphatic flow in the wound granulation

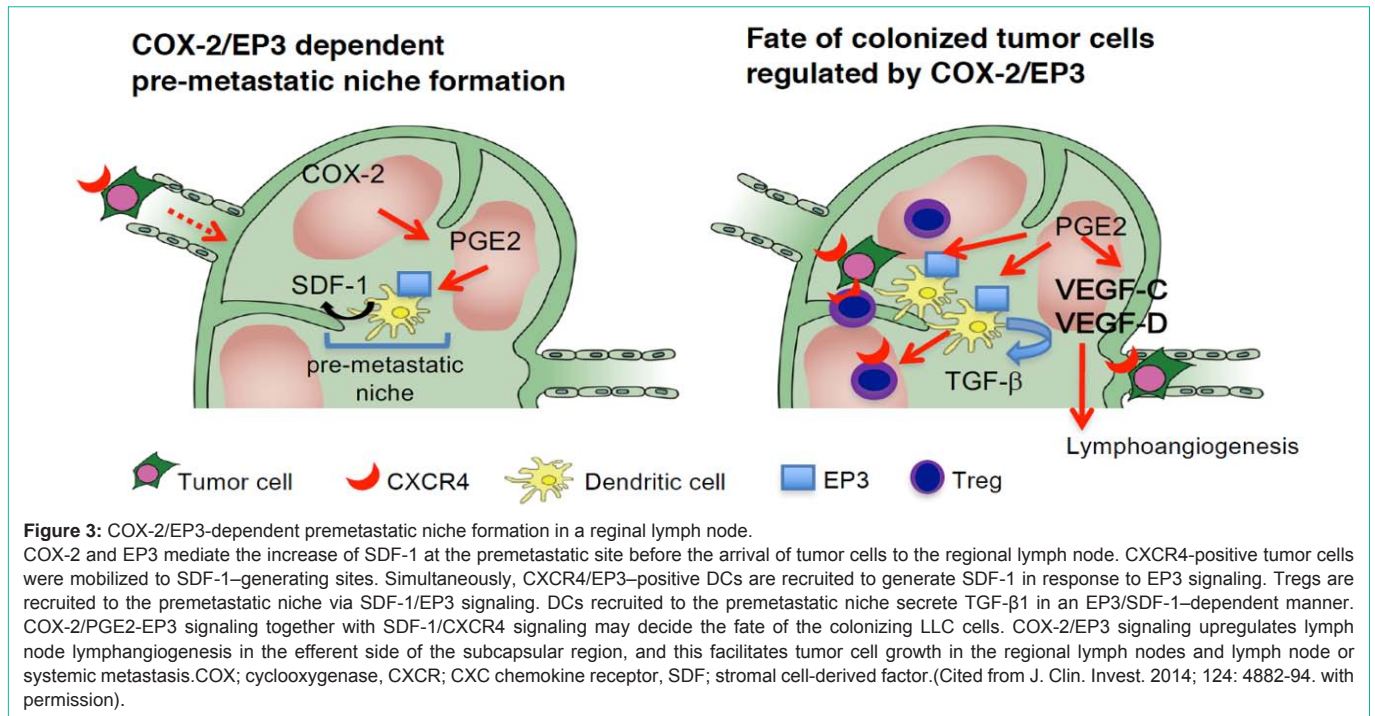
tissues was detected 3 weeks after surgical treatment. In contrast, lymphatic flow was markedly suppressed in mice treated with celecoxib. Newly formed lymphatic structures were identified in the granulation tissues formed at wounded lesions, whereas those were markedly suppressed with celecoxib. Interstitial tissue pressures in the distal areas of the tail wounds were markedly increased in mouse with celecoxib with reduced expression of VEGF-C. This study suggested that lymphangiogenesis, together with recurrence of lymph flow after surgical induction of lymphedema, was upregulated by cyclooxygenase-2 via generation of prostanoids [29].

### Prostanoids regulate tumor - Associated Lymphangiogenesis and Lymph Node Metastasis

It had been repeatedly reported that prostanoids facilitated tumor-associated angiogenesis especially acting on the stromal tissues [63,66]. It was revealed that prostanoids enhanced lymphangiogenesis in tumor microenvironment [36]. Prostanoids are involved in a range of cellular functions, however, it had been emphasized that prostanoids act as an inducer of VEGF isoforms with prolymphangiogenic potentials [21]. The importance of prostanoid-mediated dilation during metastasis was demonstrated in an animal model of lymphogenous spread, in which it was shown that the lymphangiogenic factors VEGF-C and VEGF-D were able to induce dilation of collecting lymphatic vessels draining the primary tumor mass, leading to increased metastatic load in the sentinel lymph node [28]. Tumor studies conducted in EP1-4 receptor knockout mice or using pharmacological inhibition revealed that these receptors have a role in promoting tumorigenesis, angiogenesis and lymphangiogenesis in various tumor settings and therefore provide attractive targets for therapeutic intervention [3,36].

In tumor microenvironment, PGE<sub>2</sub> and E Receptor signaling pathways have been implicated in the promotion of tumor growth, however, little is known about their roles in lymphangiogenesis during tumor development. A previous study clarified that endogenous PGE<sub>2</sub> exhibited a critical role in tumor-associated lymphangiogenesis [36]. Treatment of mice with celecoxib, down-regulated the expression of VEGFR-3 in stromal tissues markedly, and attenuated expression of podoplanin, a marker for lymphatic endothelial cells. EP3 receptor knockout mice exhibited a significant reduction in tumor weight associated with a reduction in VEGFR-3 mRNA expression in tumor stromal tissues [36]. This result suggested that host EP3 receptor signaling regulates tumor-associated lymphangiogenesis by up-regulating expression of VEGF-C and its receptor, VEGFR-3, in tumor stromal tissues. Host EP3 blockade together with cyclooxygenase-2 inhibition may be a novel therapeutic strategy to suppress tumor-associated lymphangiogenesis.

Patients with advanced cancers have a significant risk of local recurrence and distant metastasis after surgical resections of the primary tumors. It is widely accepted that many tumors tend to metastasize to specific organs [55]. The mechanisms that guide tumor cells to specific tissues are largely unknown, although recent evidence suggests that it may involve molecular differences inherent in the tumor cells themselves, modulated by the activities of immune cells, hematopoietic cells, and other tissue components. Lymph Node Metastasis (LNM) is a critical prognostic factor in cancer patients, and lymphatic vessels serve as an important route for the spread of cancer cells [55]. Paget reported that tumor cells



might prepare the lymph nodes for their future arrival, giving a new interpretation to the seed-and-soil hypothesis [54]. The formation of a premetastatic niche, suitable for the arrival of the first tumor cells, facilitates metastasis via the bloodstream [16,17,26]. However, data regarding the factors involved in lymph node premetastatic niche formation are limited [35]. Tumor-associated lymphangiogenesis may enhance metastasis to the regional lymph nodes; however, the involvement of lymph node premetastatic niche formation in the metastatic process is not fully clarified. Among the many cellular components within the tumor microenvironment, Dendritic Cells (DCs) exert profound effects on T cells [64]. The mediators that regulate DC function may also modulate niche formation. PGE<sub>2</sub> and the tryptophan-catabolizing enzyme Indoleamine 2,3-Dioxygenase (IDO) exert strong effects on the maturation and function of DCs [8]. PGE<sub>2</sub> has been identified as a major immunosuppressive soluble factor present in the tumor microenvironment [58]. An important mechanism by which these DCs modulate T cell responses seems to be via PGE<sub>2</sub>-induced expression of IDO. Furthermore, a recent study reported that PGE<sub>2</sub> increased the immunosuppressive potential of regulatory T cells (Tregs) [59]. It had been previously reported that cyclooxygenase-2-derived endogenous PGE<sub>2</sub> enhanced angiogenesis and lymphangiogenesis during tumor development and chronic inflammation [3,21,36]. Furthermore, PGE<sub>2</sub> enhances stromal tissue formation and tumor-associated angiogenesis mediated by tumor stromal chemokines [30]. The function of a broad range of immune cells can be regulated by PGE<sub>2</sub>; however, the reports on the precise contributions of PGE<sub>2</sub> to LNM are limited.

In a study using EP knockout mice, it had been shown that endogenous cyclooxygenase-2-derived PGE<sub>2</sub> stimulated the EP3 receptor on DCs and upregulated the expression of stromal cell-derived factor-1 (SDF-1) in the subcapsular regions of regional lymph nodes following Lewis Lung Carcinoma (LLC) cell injection [50]. SDF-

1 upregulation increased the accumulation of CXCR4<sup>+</sup> LLC cells and facilitated the formation of regional lymph node premetastatic niches. The accumulation of Tregs and lymph node lymphangiogenesis, both of which may influence the fate of metastasized tumor cells, were also cyclooxygenase-2/EP3-dependent. Thus, inhibitors of PGE<sub>2</sub>, together with SDF-1 receptor antagonists and EP3 antagonists, may be effective at suppressing premetastatic niche formation and LNM. These findings strongly suggested a critical function of PGE<sub>2</sub> in the formation of the lymph node premetastatic niche [50] (Figure 3). In a separate experiment, Aspirin had been shown to reduce the metastasis of lung carcinoma cells to the regional lymph nodes, with a significant reduction in mortality [51]. In rodent models of breast cancer, cyclooxygenase-2 inhibition decreased mammary tumor-associated lymphangiogenesis, tumor cell invasion into lymphatics, and metastasis [38].

## Key Findings and Conclusions

Prostanoids exhibit various activities via G-coupled receptors. PGE<sub>2</sub> and TXA<sub>2</sub> are representative molecules that induce the constrictions of various smooth muscles, hypotension, and platelet activation/inhibition. These actions are quite immediate ones, however accumulated results indicate that prostanoids also induce angiogenesis/lymphangiogenesis via up-regulations of growth factors such as VEGF-A, -C, and -D, and cytokines such as SDF-1 [2,6,21]. As discussed in each section of this review, EP3 and EP4 have polylmphangiogenic activities. Although EP3 couples mainly to Gi and decreases intracellular cAMP concentrations, several EP3 isoforms derived from alternative splicing have other signaling pathways [47]. It is reported previously that a variant of EP3 couples to adenylate cyclase/protein kinase A to induce VEGF-A [2]. EP3-mediated increase in cAMP may facilitate lymphangiogenesis in inflammation and tumor microenvironment [21,36]. Further, EP4 couples to Gs and raises intracellular cAMP concentrations. A rise in

the second messenger, cAMP in the cytosol via activation of adenylate cyclase activates Protein Kinase A (PKA), and PKA in turn activates a transcription factor CREB (cAMP response element-binding protein). Although the promoter region of VEGF-C gene contains binding sites for Sp1, AP-2 and NF- $\kappa$ B [9], CREB that belongs to the basic leucine-zipper family and binds to a specific regulatory element in the promoters of the target genes enhances VEGF-C expression [57]. Accumulated results suggest that the prostaglandin receptors that increase cAMP concentrations may activate gene expressions of VEGF-C/D.

Further, it is well-established that TP communicates mainly with Gq and G13, resulting in phospholipase C activation and RhoGEF activation, respectively [65], however TP couples with G11, G12, G13, G14, G15, G16, Gi, Gs and Gh too [46]. Gsa subunit family which stimulates adenylate cyclase [14,56] may stimulate lymphangiogenesis as observed in the LPS-induced peritonitis [43]. This TP activity may be promising to treat pathological conditions including inflammation.

As discussed, gate keepers of the lymphangiogenic process have emerged as a novel class of therapeutic targets. Several antiangiogenic antibodies were applied clinically, however, clinical trials targeted to lymphangiogenesis are very limited. We had summarized present knowledge on prostanoids to induce lymphangiogenesis. PGE<sub>2</sub> has a prolymphangiogenic activities. Interestingly, it does not act on LECs rather on the inflammatory cells derived from the bone marrow in some models. Thus, it exhibits a landscaping effect in the microenvironments during inflammation. Agonists for EP3 and EP4 may become a stimulator of lymphangiogenesis *in vivo* [19,21]. Application of the agonists of these receptors to lymphedema patients will be promising. Biosynthesis of PGE<sub>2</sub> in pathological settings is dependent on cyclooxygenase-2 and mPGES-1. Further, together with inhibitors for cyclooxygenase-2 and mPGES-1, antagonists for EP3 and EP4 will be effective tool to block lymphangiogenesis in some kinds of models [36,42]. Interplay between the immune cells and prostanoids appears important in other pathological conditions related to the lymphatic plasticity [43,50]. Interestingly, inflammation-induced lymphangiogenesis may be blocked by TP inhibition on T cells and macrophages [43], and lymph node metastasis may be blocked via cyclooxygenase-2/mPGES-1/EP3 inhibitions on DCs [50,51]. These suggest that the field of the arachidonic acid metabolites is quite rich with therapeutic candidates. As discussed, prostanoids act as a transcriptional factor to induce VEGF isoforms and other cytokines. Prostanoids act beyond the concepts of the classical activities as smooth muscle contracting substances and vasoactive substances.

## Author Contributions

All authors have been equally involved in writing and editing the manuscript. Figures have been done by KH and MM. All authors contributed equally to the article and approved the submitted version.

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## Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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