Vascular frontiers without borders: Multifaceted roles of platelet-derived growth factor (PDGF) in supporting postnatal angiogenesis and lymphangiogenesis

The platelet-derived growth factor (PDGF) family of growth factors, which primarily serves the function of stabilizing vascular networks, has now been shown to play a role in promoting tumor lymphangiogenesis. PDGF-BB, independent of VEGFR-3 signaling, induces tumor growth and metastasis in part through supporting lymphangiogenesis. These data suggest that targeting the PDGF/PDGF-receptor signaling pathway will provide a novel strategy to block tumor neoangiogenesis and lymphangiogenesis, thereby inhibiting tumor growth and metastasis.

Genetic models have shown that distinct signaling pathways modulate specification, patterning, and remodeling of lymphangiogenesis and angiogenesis during embryonic development. However, the precise identity of cellular and molecular pathways that maintain the integrity and remodeling of the lymphatic and blood vessels postnatally are complex, and these processes are most likely driven by the collaboration of various organ-specific angiogenic and lymphangiogenic factors. VEGF-A, through interactions with its cognate receptors VEGFR-1 and VEGFR-2, supports vasculogenesis and angiogenesis (Carmeliet and Jain, 2000), while VEGF-C and VEGF-D through interaction with VEGFR-3 primarily support lymphangiogenesis (Alitalo and Carmeliet, 2002). However, angiogenic factors, including fibroblast growth factor-2 (FGF-2), and angiopoietins, which were previously thought to exclusively regulate angiogenesis, have also been shown to contribute to the remodeling of lymphatic vessels. In line with these new discoveries, in this issue of *Cancer Cell*, Cao et al. (2004) provide provocative evidence suggesting that the PDGF/PDGF-receptor signaling pathway may not only be important for supporting angiogenesis, but also for promoting tumor lymphangiogenesis independent of VEGFR-3 signaling. This finding sets forth the concept that under certain conditions, vascular growth factors, such as PDGF, may have overlapping functions not only supporting postnatally driven neoangiogenic but also organ-specific lymphangiogenic processes.

It is now well established that VEGF-C and VEGF-D, through interaction with their tyrosine kinase receptor, VEGFR-3, support differentiation and patterning of Prox-1+ endothelial cells into lymphatic vasculature (Alitalo and Carmeliet, 2002; Karkkainen et al., 2004). Targeted inactivation of VEGFR-3 results in embryonic lethality as a result of failure to remodel the primary capillary plexus into a more mature lymphatic vascular network. Later, however, expression of VEGFR-3 is downregulated in blood vessels and becomes restricted to the lymphatic endothelial cells, which sprout from embryonic veins and start to form primitive lymph sacs. In adult tissues, VEGFR-3 is expressed primarily in the lymphatic vasculature and in some fenestrated blood vessel endothelia. VEGF-C/VEGFR-3 pathway is activated in the blood vessels of certain tumors and plays a role in mediating tumor lymphangiogenesis and lymph node metastasis (Mandriota et al., 2001; Stacker et al., 2002).

In addition to VEGFR-3 signaling, other signaling pathways, including angiopoietin/Tie2 and FGF-2, have been shown to play a role in remodeling

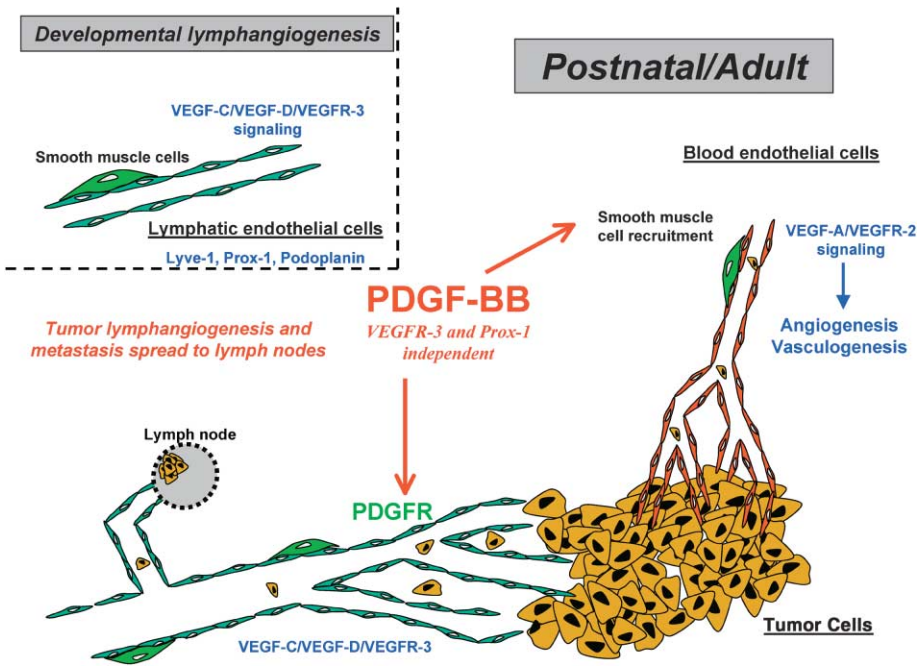

Figure 1. The crosstalk between vascular network stabilization and induction of lymphangiogenesis is modulated by the PDGF/PDGFR signaling pathway

VEGF-C and VEGF-D, through interaction with their tyrosine kinase receptor VEGFR-3, support the development, specification, and maintenance of lymphatic vessel postnatally. VEGF-C/VEGF-D/VEGFR-3 signaling induces Prox-1 expression in endothelial cells, which become committed to lymphatic endothelial cells. PDGF recruits mural cells to coat nascent vessels essential for the stabilization and further establishment of the vascular network. PDGF, by recruiting PDGFR+ lymphatic endothelial cells, induces lymphangiogenesis and lymphatic tumor growth and metastasis through a VEGFR-3- and Prox-1-independent pathway. Lymphatic vessels would then provide the channels for tumor spread to the lymph nodes, and blood vessels would provide a conduit for tumor metastasis to distant organs.

of lymphatic vessels. Angiopoietin-2-deficient mice form lymphatic vessels but have profound defects in lymphatic vessel remodeling, resulting in chylous ascites and defects in pattering and function of lymphatic vasculature (Gale et al., 2002). FGF-2 at low doses induces VEGF-C and VEGF-D expression and promotes lymphangiogenesis through activation of VEGFR-3 signaling (Chang et al., 2004; Kubo et al., 2002). In addition, expression of SLP-76 and Syk signaling proteins is important for the separation of the lymphatic and blood vessels (Abtahian et al., 2003). These data suggest that, although the VEGF-C/VEGF-D/VEGFR-3 signaling pathway is absolutely essential for the initial embryonic development of lymphatic vessels, other signaling pathways may modulate patterning and remodeling of the lymph-angiogenesis in a context-dependent manner. In this regard, the data presented by Cao et al. (2004) suggest that PDGF/PDGFR signaling may also play a critical role in modulating lymphangiogenesis in specific pathophysiological conditions, such as organ-specific tumor growth.

Cao et al. (2004) demonstrate that PDGF-BB can induce intratumoral lymphangiogenesis and lymphatic metastasis by a VEGFR-3-independent mechanism (Figure 1). They report that expression of PDGF-BB in murine fibrosarcoma cells induced tumor lymphangiogenesis, leading to enhanced metastasis in lymph nodes. In addition, they demonstrate that PDGFRs are expressed on newly lymphatic cells and that PDGF-BB is a potent activator of lymphatic endothelial cell motility. Inhibition of VEGFR-3 did not block PDGF-BB-induced lymphangiogenesis. These data suggest that PDGFs may modulate the postnatal remodeling of lymphatic vessels, but not development of rudimentary lymphatic vessels.

Expression of the Prox-1 is essential for VEGF-C/VEGFR-3-mediated initiation and specification of lymphangiogenesis during embryonic development (Oliver and Detmar, 2002). However, PDGFs did not require Prox-1 activation to induce lymphangiogenesis. The finding by Cao et al. that PDGFs support lymphangiogenesis independent of Prox-1 has several implications. It is possible that PDGF/PDGFR signaling is not critical for the Prox-1 dependent developmental generation of the lymphatic vessels. Alternatively, PDGFs, through activation of an as yet unrecognized signaling pathway(s), support lymphangiogenesis during specific pathophysiological conditions.

This unexpected function of PDGF in the regulation of lymphangiogenesis comes as a surprise, since most of transgenic models and in vitro studies had suggested that PDGFs primarily modulate vascular development and stabilization (Betsholtz, 2004). PDGF was purified as a factor from platelets that promote the proliferation of mesenchymal cells. Five members constitute the complete PDGF family: AA, BB, AB, CC, and DD (Betsholtz, 2004). Processes driven by the PDGFRs include angiogenic sprouting and branching of vascular endothelium. PDGF released by endothelial or other cell types triggers pericyte recruitment to coat nascent vessels, which is essential for the stabilization and further establishment of the vascular network (Lindblom et al., 2003). Although no studies so far have demonstrated antitumor effects of targeting PDGF alone, the well-documented effect of PDGF on pericyte and tumor stroma recruitment (Dong et al., 2004) points to the possibility that PDGF may collaborate with other angiogenic factors to support tumor angiogenesis as well as lymphangiogenesis.

Cao et al. (2004) demonstrate that PDGFR-β is expressed on lymphatic endothelial cells conveying signals that support migration and survival of these cells. Therefore, PDGF-BB may induce lymphangiogenesis directly by recruiting and remodeling of PDGFR-β+ lymphatic endothelial cells, thereby supporting tumor growth and metastasis. One other mechanism by which PDGFs, including the newly discovered PDGF-D, may regulate both neoangiogenesis and lymphangiogenesis, is through recruitment of proangiogenic myeloid cells (Uutela et al., 2004). Subsets of hematopoietic cells are recruited to the ischemic niche where, by releasing paracrine factors, they support neoangiogenesis or lymphangiogenesis (Schoppmann et al., 2002). Similarly, PDGF-BB, through recruitment of myeloid cells, may support the assembly of stable blood neovessels and contributes to the remodeling of the lymphatic vessels.

The study by Cao et al. has also raised several major unresolved issues. It remains to be determined whether PDGFs exert their effect on lymphangiogenesis exclusively through PDGFR signaling. Although soluble VEGFR-3 or neutralizing antibodies to VEGFR-3 failed to block PDGF-BB-mediated lymphangiogenesis, it is still possible that PDGFR autophosphorylation through inside-inside intracellular activation of VEGFR-3 tyrosine kinase may induce neoangiogenesis. The effect of PDGF-BB in modulating lymphangiogenesis in VEGFR-3 deficient mice will also determine whether PDGFs may promote lymphangiogenesis independent of VEGFR-3 signaling or through overlapping signaling cascades. In addition, it is important to examine PDGFR-β and PDGFR-α knockout mice for potential lymphangiogenic defects. Although the edema observed in PDGFR-β knockout mice is primarily due to defective pericytic investment of the newly formed vessels, it is possible that subtle lymphangiogenic defects may also contribute to the generation of edema in these mice. Finally, preliminary clinical evidence that certain antibodies to PDGFRs given to patients with advanced solid tumors may induce edema suggests that PDGFs may not only be important for vessel stability, but that they may also support lymphatic vessel integrity.

Although the VEGFR-3 signaling pathway controls many important aspects of lymphatic vasculature growth, accumulating evidence suggests that other players, such as angiopoietins, FGFs, and now PDGF/PDGFR, are involved in generating and remodeling of lymphatic vasculature. Whether organ-specific expression of PDGF or angiopoietins collaborates with VEGF-C/VEGFR-3 pathways to induce lymphangiogenesis remains to be determined. Nonetheless, the data presented by Cao et al. highlight the significance of the PDGF family in regulating angiogenesis and lymphangiogenesis. Monotherapy with PDGFR inhibitors seems to be ineffective in blocking tumor growth. On the other hand, PDGF antagonists combined with chemotherapy have been more effective in blocking tumor growth (Pietras et al., 2002). As such, targeting PDGF in conjunction with chemotherapeutic agents will provide for an effective means to block tumor growth and metastasis.

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Antagonism of Myc functions by Arf

The Arf-Mdm2-p53 tumor suppressor pathway is activated by sustained hyperproliferative signals emanating from oncoproteins such as Myc. A recent study reveals a novel level of feedback control, whereby induced p19^{Arf} binds to Myc and blocks cell proliferation by selectively impairing its transactivation functions.

When faced with an increased threshold of oncogenic signals that provoke cells to divide at an abnormally accelerated pace, the *Arf* tumor suppressor gene is activated. Its encoded product (p19^{Arf} in mouse or p14^{ARF} in humans) antagonizes the ubiquitin E3 protein ligase activity of the p53 negative regulator Mdm2 (Hdm2 in humans) to trigger a p53-dependent transcriptional response that leads to either cell cycle arrest or apoptosis. These protective responses can be disabled through deletion or silencing of *Arf*, by generation of dominant-negative mutants of p53, or through mechanisms leading to Mdm2 overexpression, so enabling incipient cancer cells to thrive.

Myc was the first oncogene recognized to activate *Arf* gene expression (Zindy et al., 1998), although the mechanism by which it does so remains unclear. When Myc expression is enforced in mouse B-lymphocytes in vivo, its enhancing effects on cell proliferation are inhibited by the Arf-Mdm2-p53 axis, but disabling the pathway cancels Myc-induced apoptosis and allows formation of B cell lymphomas (Eischen et al., 1999). Observations that Myc could trigger a p53 response through the agency of Arf were conceptually satisfying, but additional complexities soon became apparent. First, high and sustained Myc activity is required for *Arf* induction, but the promoter is normally insulated from responding to physiologic Myc signals. Second, cells lacking *p53* or harboring mutant forms of the protein display dramatic upregulation of p19^{Arf}, and reintroduction of p53 represses *Arf* transcription. This feedback control by p53 also extends to c-Myc, again through an unknown mechanism (Figure 1). Most importantly, however, mice engineered to lack both *Arf* and *p53*, or all three genes in the pathway, develop (usually multiple) cancers at a faster rate, and the spectrum of tumor types arising in these mice is much broader than that of animals lacking either *Arf* or *p53*, or both *Mdm2* and *p53*, providing genetic evidence that p19^{Arf} must have p53-independent functions (Weber et al., 2000). Indeed, cells lacking *Arf* and *p53* proliferate faster than those lacking either gene alone, and enforced overexpression of *Arf* can arrest the proliferation of *p53* null mouse embryo fibroblasts (MEFs), albeit inefficiently (Eischen et al., 1999; Weber et al., 2000).

Recent studies now suggest that p19^{Arf} can negatively regulate Myc's transcriptional activity through a direct physical interaction that is seemingly independent of Mdm2 and p53 (Qi et al., 2004). These investigators report some surprising findings that include Myc's ability to bind directly to Arf and to relocalize Arf from its usual storehouse in the nucleolus into the nucleoplasm in both wild-type and p53-deficient MEFs. Most striking, however, are their observations that p19^{Arf} associates with Myc on its tar-