Review Article **The Role of PTEN in Tumor Angiogenesis**

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During the past 20 years, the phosphatase and tensin homolog PTEN has been shown to be involved in major physiological processes, and its mutation or loss is often associated with tumor formation. In addition PTEN regulates angiogenesis not only through its antagonizing effect on the PI3 kinase pathway mainly, but also through some phosphatase-independent functions. In this paper we delineate the role of this powerful tumor suppressor in tumor angiogenesis and dissect the underlying molecular mechanisms. Furthermore, it appears that, in a number of cancers, the PTEN status determines the response to chemotherapy, highlighting the need to monitor PTEN expression and to develop PTEN-targeted therapies.

1. Physiological Angiogenesis

Vasculogenesis and angiogenesis are two distinct processes, whereby the first represents vessel formation from differentiated precursors while the second originates from the preexisting vasculature. Capillaries formed during these processes are mainly constituted by endothelial cells which face the lumen of the vessel and sometimes, depending on their size, are surrounded by mural cells comprising pericytes and smooth muscle cells. Angiogenesis is essential during development while in adulthood the vasculature is usually quiescent, except during wound healing and the female reproductive cycle [1]. It is governed by several factors secreted by the targeted tissues and consists of four steps. The first step originates from the existing vessel from which the sprout arises. The combination of nitric oxide and Vascular Endothelial Growth Factor (VEGF) enhances vessel permeability by increasing capillary dilatation and leakiness, respectively. This allows extravasation of various plasma proteins which facilitate endothelial cell migration. Second, endothelial cells, to invade the hypoxic tissue, have to detach from the basement membrane they are laying on. This is mediated by the secretion of proteases such as proteins from the matrix metalloproteinase family (MMP-2, -3, -9) or through the inhibition of protease inhibitors such as TIMP (tissue inhibitor of MMP) proteins family. Third, after

endothelial cells have detached, they proliferate and migrate to invade the hypoxic area, the source of proangiogenic factors, until they find contact to another capillary. During this process, as during axonal guidance, some cells lead the elongation of the sprouting vessel towards the angiogenic chemoattractant source. The endothelial cells forming the sprouting blood vessels exhibit distinct phenotypes. We can distinguish the tip cells at the leading edge of the sprout, stalk cells which follow the tip and phalanx cells which are quiescent cells from the mature vessel. Fate determination of the tip cells is dynamically regulated by VEGF-A/VEGFR1 and 2 and Notch/Dll-4 signaling pathways. Briefly, endothelial cells having the greater VEGFR2 to 1 ratio and higher Dll4 expression are more likely to adopt and keep the tip cell phenotype. VEGF-A-stimulated candidate cells will, therefore, signal through VEGFR2 which has a lower affinity for this ligand but a better protein kinase activity. VEGFR2 signaling leads to Dll-4 expression, activating Notch on the neighbour cell. Notch signaling in these endothelial cells leads to VEGFR1 upregulation while VEGFR2 is downregulated reducing the chance to become a tip cell. This system results in fate determination of the tip cells while keeping their neighbouring cells under a stalk cell phenotype; however, these phenotypes are not fixed over time [2]. First, the neighbourhood of tip cells is constantly changing due to endothelial cell migration, and this impacts on cells' VEGFR expression. Second, VEGFR2 to 1 ratio determines the time length of tip cell turnover, and, altogether, this result in an oscillatory Dll4 expression. Third, the main source of VEGF-A may differs during vessel elongation. After formation of tip cells, the sprout elongates through proliferation of stalk cells and reaches its target under the drive of tip cells. During the last step, cells start to differentiate and form a tube which will be stabilized through the recruitment of mural cells and secretion of extracellular matrix (ECM) [3]. An intact, functional vasculature requires a right balance between pro- and antiangiogenic factors; therefore, physiological angiogenesis is the result of a tightly controlled excess of proangiogenic factors. By contrast, tumor angiogenesis (Figure 1) originates from a disturbed balance between pro- and anti-angiogenic factors rendering endothelial cells unable to become quiescent and consequently support a constant growth of new blood tumor vessels [4]. In this work, we will focus on the following factors involved in tumor angiogenesis: VEGF/VEGFR, angiopoietins, and their receptors: Tie-1 and -2, Eph receptors, and their ephrins ligands, HIF1 α (hypoxia-inducible factor 1 alpha), MMPs, and delineate their relationship with the important tumorsuppressor PTEN (phosphatase and tensin homologue).

2. Key Players in Angiogenesis

VEGF is the most important regulator of endothelial cell fate and acts as ligand of the VEGF receptor which belongs to the receptor tyrosine kinase (RTK) superfamily. VEGF is phylogenetically ancient: in Drosophila it is thought to be involved in blood cell positioning and comprises only one member; however, in mammals, 5 members of the VEGF family: VEGF-A, -B, -C, -D, and placenta growth factor: PLGF have been described. VEGF-A plays a prominent role in angiogenesis as shown by genetic studies of VEGF-A knock-out mice which die embryonically [5]. VEGF-A interacts preferentially with two of the three human members of VEGF receptors: VEGFR1 (Flt-1) and VEGFR2 (Flk1), while VEGF-B and PLGF bind to VEGFR1. Here we will not discuss in details VEGF-C and -D, which bind to VEGFR3 (Flt-4) and are involved in lymphangiogenesis. VEGF signaling through VEGFR1 and -2 results in an upregulation of proteases expression, recruitment of mural cells due to PDGF β secretion, increased vessel permeability, endothelial cell proliferation, migration, survival, and specialization [6].

Angiopoietin (Ang) ligands bind to *Tie* receptors, which are single transmembrane RTK. These proteins possess three immunoglobulin-like (Ig-like) domains, three epithelial growth factor domains (EGF) and three fibronectin type III domains giving the name Tie: Tyr-kinase with Ig and EGF homology domains. The two isoforms of Tie: Tie-1 and -2 are involved in vascular development as demonstrated by loss-of-function studies [7, 8]; however, Tie-2 is much more studied than its isoform. Tie-2 is required early in embryonic development for plexus remodeling and maturation. Tie-2 is predominantly expressed on endothelial cells and principally binds to Ang-1 and -2 which are agonist and antagonist ligands, respectively. Ang-1 stimulation of Tie-2 leads to endothelial cell survival and migration, limits vessel permeability, and stimulates smooth muscle cells recruitment. Tie-2 signaling triggers a negative feedback loop involving the forkhead transcription factor FOXO1 and Ang-2 expression [9].

Another system of receptor/ligand involved in angiogenesis regulation is the Eph/ephrin couple. Eph receptors (first cloned from an erythropoietin producing hepatocellular carcinoma cell line) belong to the RTK superfamily and are involved in embryogenesis, axonal guidance, and angiogenesis [10–12]. Eph receptors are divided into two subfamilies: A and B, which generally bind to ephrin A and B ligands, respectively. In contrast to the other RTK whose ligands are secreted molecules, ephrin ligands are membrane bound. This renders this couple quite unique as (a) it requires two neighbouring cells to signal, and (b) numerous studies have demonstrated that this signal is transmitted by both receptor and ligand. This bidirectional signaling is involved in the regulation of the cytoskeleton leading to modulation of cell adhesion, repulsion, and motility and contributing to cell positioning [13]. Wang et al. first showed that EphB4 and ephrinB2 are key players in the development of the vascular system by the use of transgenic mice. They demonstrated that EphB4 is restricted to veins while ephrinB2 is expressed only by arteries, and a lack of one of these actors is lethal due to perturbed arteriovenous differentiation [14]. Indeed ephrinB2 reverse signaling was demonstrated to participate to tip cell guidance through control of VEGFR2 signaling guarantying a successful vasculature formation [15]. Moreover, ephrinB2 was shown to contribute to vessel maturation as it is expressed on mesenchymal cells such as smooth muscle cells, and its binding to EphB leads to mural cells recruitment [16].

As angiogenesis responds to an increased need in oxygen, hypoxia is a crucial part of the process. The main effector of the adaptive response to hypoxia is the transcription factor HIF1. HIF1 is a member of the basic helix-loop-helix/PAS protein transcription factor family and comprises HIF1 α and HIF2 α , which to be active require heterodimerization with HIF1 β [17]. The α subunit acts as an oxygen sensor as it is degraded under normoxia while the β subunit is constitutively expressed. In normoxic condition, the prolyl hydroxylase enzymes (PHD1-3) are in an active state and hydroxylate HIF1 α on two proline residues (402 and 564) within its oxygen-dependent degradation domain (ODD) [18]. Hydoxy-HIF1 α is recognized by the complex formed by the ubiquitin ligase VHL (von Hippel-Lindau) and the elongin proteins, and this binding triggers the rapid HIF1 α degradation through the proteasomal pathway. In contrast, under hypoxic conditions, HIF1 α forms a heterodimer with HIF1 β , and their translocation to the nucleus acts on gene expression through their binding to the hypoxia response element (HRE). Numerous genes are regulated by HIF1, some related to red blood cell production (erythropoietin), some to the vascular architecture and tone (VEGF, VEGFR1, or Nitric Oxide Synthase 2), some to the metabolism (Glut-1 and Glut-3), and some to cellular proliferation and differentiation (TGF β , Cyclin G2, or p21) [19]. In 2005 our group demonstrated an upregulation of EphB4, ephrinB2,

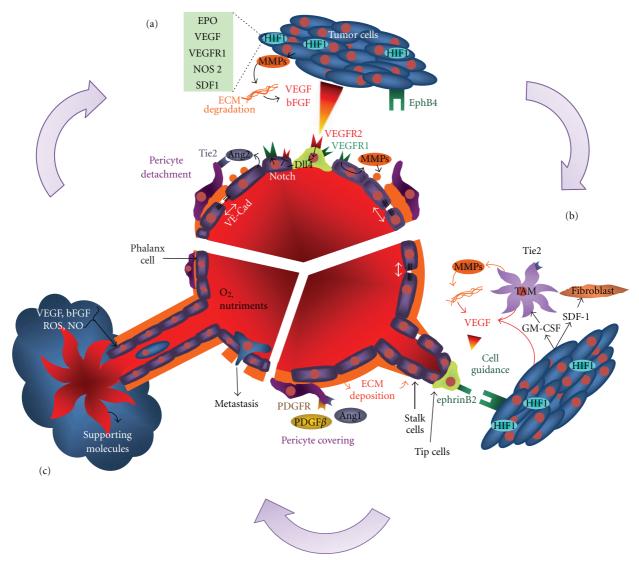


FIGURE 1: Molecular basics of tumor angiogenesis. Key players of tumor angiogenesis and their main functions are depicted here. (a) Once a tumor has reached a volume of $1-2 \text{ mm}^3$, tumor cells start to be hypoxic, and HIF1 α is stabilized. Activated HIF pathway leads to the expression of several genes (within green square) which greatly contribute to VEGF production. VEGF amplify this system through VEGFdependent MMPs expression involved in ECM degradation and growth factors release. VEGF acts as a chemoattractant on endothelial cells from the nearest vessels and triggers vessel sprouting. Stimulated VEGFR2 leads to the expression of Dll4, a Notch ligand which inhibits the tip cell transformation through VEGFR1 upregulation. VEGFR activation mediates proteases expression, VE-Cad complex disruption leading to cell/cell and cell/matrix detachment. In parallel, Tie2 stimulation by Ang2 induces pericytes detachment. This step is required for endothelial cell migration and proliferation. (b) While tip cells drive vessel elongation towards the source of VEGF and through EphB4/ephrinB2 signaling, tumor cells attract stromal cells. These stromal cells, comprising tumor-associated macrophages (TAM) and fibroblasts contribute to tumor angiogenesis through secretion of proangiogenic factors. During vessel elongation, new ECM is synthesized, and few pericytes will cover the neovessel. This—in conjunction with NO production and disruption of adherens junctions—results in a leaky vessel. (c) The resulting vasculature is tortuous with many dead ends and is prone to cell extravasation. Moreover, endothelial cells contribute to tumor growth by secreting supporting molecules in addition to carrying nutrients and oxygen.

EphA2, ephrinA1 mRNA and protein expression in hypoxic tissues [20].

Finally, one of the major sources of angiogenic factors is the ECM, first because it can store growth factors such as VEGF, second because degradation of some of its component leads to the formation of pro- or anti-angiogenic factors. Among the proteases involved in the release of such factors, MMP proteins which are members of a zinc-dependent family of endopeptidases and more precisely MMP2 and 9 are key players. MMP9 was shown to be activated in numerous cancers and to contribute to tumor angiogenesis by increasing the bioavailability of VEGF, basic fibroblast growth factor (bFGF) by degrading collagen types IV, XVIII and perlecan [21, 22]. Moreover, MMPs are involved in cell migration by interfering with the cell-ECM and cell-cell interactions.

3. Tumor Angiogenesis

All the factors mentioned above are not only involved in physiological but also in tumor angiogenesis. Tumor development is first limited to 1-2 mm³ due to the lack of oxygen, nutrients, and growth factors. This restriction may be the cause of tumor latency until the so-called angiogenic switch occurs. Angiogenic switch is defined as the acquisition by tumor cells of properties required for their unopposed growth, where the balance is skewed towards a proangiogenic phenotype [28]. As tumor cells are in a hypoxic state prior to the angiogenic switch, the HIF1 pathway is constitutively active and leads to the expression of angiogenesis-related genes (VEGF/VEGFR, MMPs, Eph/ephrins). Noteworthy, even after the tumor vasculature is fully developed and functional, most of the tumor cells still maintain an activated HIF1 pathway [29]. As mentioned earlier, HIF1 target genes will lead to endothelial cell recruitment to the tumor site mainly through VEGF/VEGFR activity. This effect is amplified by tumor cells, which also have the capability to attract stromal cells as cancer-associated fibroblasts (CAF) [30] and tumor-associated macrophages (TAM) [31]. TAMs seem to play a pivotal role as they secrete VEGF, MMP9, and even immunosuppressive molecules. The resulting vessels are often dilated due to VEGF and leaky because of nitric oxide overproduction and deficient mural cell recruitment [32]. Tumor vessels are characterized by their irregular and tortuous shape consecutive to aberrant endothelial cell proliferation and often possess dead ends leading to a higher risk of hemorrhage. Therefore, a high tumor-associated microvascular density does not reflect high oxygen level in the tumor as blood flows only irregularly through tumor vessels, leading to hypoxic areas within the tumor. Beside the angiogenic factors directly depending on HIF1 pathway, Ang-2/Tie and Eph/ephrins receptor/ligand pairs have also been shown to play a role during the angiogenic switch. Cancer-dependent Ang-2 upregulation occurs principally in tumor-associated endothelial cells and can be used as a biomarker of tumor progression [33]. High levels of Ang-2 trigger endothelial cells apoptosis mediating vessel pruning. This leads to the formation of hypoxic area which in turn upregulates VEGF expression and results in higher vascular density [34]. The roles of EphB4 and ephrinB2 expressed on blood vessels are more controversial. Although several reports showed that EphB4 stimulation impairs tumor growth due to a defect in tumor angiogenesis [35, 36], Kumar et al. demonstrated the opposite [37–39]. Concerning ephrin reverse signaling, Martiny-Baron et al. demonstrated that blocking ephrinB2 stimulation by the use of monomeric soluble EphB4 impaired tumor growth in nude mice and correlates with a decreased microvessel density [40].

4. PTEN and Tumor Angiogenesis

A remarkable property of tumor angiogenesis is that all involved factors described earlier signal through the PI3 Kinase (PI3K) pathway [41–44]. The PI3K pathway is a signaling route involved in many cellular processes such as cell survival, proliferation, or migration. The PI3K protein class IA is activated in response to RTK stimulation, while the class IB is activated by G-proteincoupled receptors [45]. PI3K activation leads to the transformation of phosphatidylinositol-4,5-bisphosphate (PIP2) into phosphatidylinositol-3,4,5-trisphosphate (PIP3). This in turn will activate Akt, a serine-threonine protein kinase which has numerous targets comprising the mammalian target of rapamycin (mTOR), a protein complex stimulated in hypoxic and nutrient-poor environment. The major regulator of this signaling pathway is PTEN, also known as MMAC1 (Mutated in Multiple Advanced Cancer1) or TEP1 (TGF β -regulated and epithelial cell-enriched phosphatase). PTEN gene in human locates on chromosome 10q23.3 and encodes protein of 40-50 kilodaltons as in many organisms, with the exception of PTEN from Caenorhabditis elegans. This protein comprise 4 domains: at the N terminal a PIP2 binding site, then a phosphatase domain, a C2 domain containing phosphorylation sites, followed by a PDZ binding motif at the C-terminal end [46]. PTEN acts as a phosphatase on both lipids and proteins; it antagonizes PI3K pathway by transforming PIP3 into PIP2 (Figure 2) [47] and dephosphorylates proteins such as SHC or FAK [48, 49]. Several studies also report a role for PTEN in cell migration, independent of its phosphatase activity as the expression of a truncated form of PTEN possessing only the PTEN C2 domain inhibits cell migration [50, 51]. PTEN is regulated posttranscriptionally by miRNA, such as miR-21 [52] and posttranslationally through phosphorylation, acetylation, ubiquitylation, or by regulation of its localization [53]. As the PI3K pathway is activated during angiogenesis, PTEN can be considered as a major intracellular regulator of this process.

Before discussing the role of PTEN in tumor endothelial cells, we will first summarize its functions in cancer cells. PTEN is frequently found deleted, mutated, or downregulated in human malignancies. PTEN mutation primarily affects the PTEN phosphatase domain and leads to several diseases including Cowden's and the Bannayan-Riley-Ruvalcaba syndrome, the Lhermitte-Duclos disease, as well as an increased risk of breast, thyroid, and endometrial cancers (Table 1).

Regarding HIF1, PTEN was found to inhibit its stabilization and its transcription factor activity in glioblastoma cell lines [54]. Moreover, several studies report a concomitant loss of VHL and PTEN, two important regulators of the HIF1 pathway, in clear cell renal cell carcinoma [55, 56]. Since HIF1 signals through PI3K pathway, leading to an increased VEGF expression, PTEN loss in cancer cells leads to an increased VEGF expression due to upregulation of HIF1. This was shown by several investigators using different models: Fang with PC-3 cells [57], Tian with HepG2 cells [58]. Takei et al. [59] demonstrated that PTEN reintroduction led to a decreased HIF1- α , VEGF, and PCNA expression in ovarian cancer cells. As to MMPs, a study using a multiple myeloma cell line and cells originating from patients showed that PTEN transfection results in a decreased mRNA and protein expression of MMP2, MMP9 and FAK (Focal Adhesion Kinase), leading to decreased cell migration [60]. Furthermore, microarray analysis of gastric

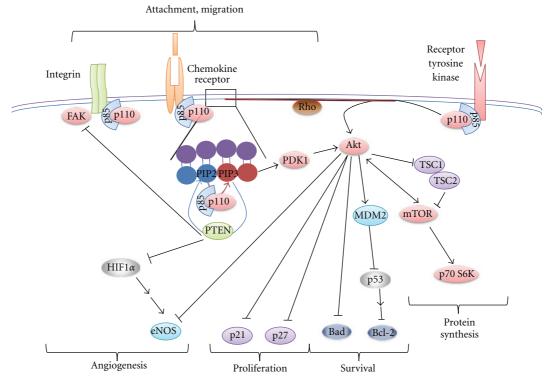


FIGURE 2: The PI3 kinase pathway and PTEN.

TABLE 1: PTEN-related diseases and associated cancer susceptibility. Percentages are indicated when available.

Disease	PTEN defect	Clinical symptoms	Cancer susceptibility
Cowden's syndrome [23, 24]	Splice variants	Trichilemmonas, macrocephaly, papillomatous papules	Breast (65%), thyroid (75%), endometrium (5–10%)
	Deletion: coding sequence		
	promoter (10%)		
	Nonsense mutation		
	Missense mutation (85%):		
	C124: no phosphatase activities, G129: no lipid phosphatase activity, K289: no nuclear translocation		
The Bannayan-Riley- Ruvalcaba syndrome [23, 24]	Deletion (11%)	Macrocephaly, intestinal polyposis, developmental delay, lipomas, speckled penis in male	Breast, thyroid, endometrium, rare colorectal carcinoma
	Nonsense mutation		
	Missense mutation (60%)		
The Lhermitte-Duclos disease [25]	Splice variants	Ataxia, increased	
	Deletion	intracranial pressure, seizures	
	Nonsense mutation	seizures	
	Missense mutation (80%)		
Proteus/Proteus-like syndrome [26]	Missense mutation (20 and 50%, resp.)	Epidermal nevus, disproportionate overgrowth of the skull, limbs, vertebrate, Lipomas, vascular malformation	rare events: cystadenoma of the ovary, testicular tumors, central nervous system tumors, parotid monomorphic adenomas
Autism [27]	Missense mutation (around 10%)	Sometimes associated with macrocephaly	Not demonstrated

carcinomas highlighted a negative correlation between PTEN expression and VEGF, MMP2, and MMP9 expression, and the authors concluded that PTEN has an inhibitory effect on microvascular density [61]. PTEN loss at posttranscriptional level is also involved in tumor angiogenesis. Giovannetti et al. [62] reported that in pancreatic ductal adenocarcinoma, miR-21 is responsible for elevated expression of MMP2, MMP9, and VEGF and this could be abrogated by treatment of the cells with a PI3K inhibitor. As miR-21 is overexpressed in many tumors, this strategy may represent a valuable tool to control tumor angiogenesis [63]. Concerning the Ang/Tie system, it was shown that Ang-1 stimulation of Tie-2 triggers PI3K pathway activation [64]. Moreover, Findley et al. described a Tie2 receptor shedding which is able to bind Ang-1 and -2 and consequently inhibits Tie2 phosphorylation and activation [65]. This process is regulated by VEGF-dependent activation of PI3K pathway, and PTEN overexpression increases Tie-2 shedding.

PTEN localization within the cell is also part of PTEN regulation, since defective PTEN translocation to the cell membrane impairs its control of PI3K signaling [87]. This was shown by Molina et al., who found that the adaptor protein NHERF1 is involved in PTEN translocation to the cell membrane [88]. They proved that impaired PTEN positioning leads to sustained Akt activation. This can be of primarily importance as NHERF1 has been shown to interact with several RTK such as PDGFR β and EGFR [89, 90], which all play a role in tumor angiogenesis. Moreover, PTEN translocation to the cell membrane is not solely involved in regulation of RTK's downstream signaling, but is also emerging as a modulator of their expression [91]. Recent work from our lab has shown that PTEN also interacts with EphB1 signaling (manuscript submitted). As mentioned above, Eph receptors and ephrins are frequently overexpressed by cancer and tumor endothelial cells, and this may lead to an enhanced tumor angiogenesis. Among the various pathways activated by Eph receptors, PI3K has been reported in a number of studies [13, 92]. Moreover, Brisbin showed a direct linkage between VAB-1 and DAF-18, the C. elegans form of Eph and PTEN, respectively [93], an interesting observation in line with our findings. As during tumor angiogenesis both cancer and endothelial cells are involved, PTEN status in endothelial cells is also crucial. Many studies have shown that PTEN expression can be disturbed in both stromal cells and endothelial cells [94, 95]. This can be primordial as reduced PTEN signaling in endothelial cells as well as PI3K activation leads to an enhanced cell proliferation, survival, and migrationall important features for angiogenesis [96–98]. Moreover, tumor vessels are characterized by their inability to become quiescent, and it was recently shown that PTEN is involved in endothelial cell aging [99].

Interestingly, PTEN is susceptible to oxidation. As highly proliferative cells overproduce reactive oxygen species (ROS), this mechanism may account for the reduction of PTEN levels in cancer and cancer-related cells [100]. A vicious circle, therefore, begins as cancer cells produce more ROS, affecting PTEN level and enhancing VEGF secretion [101, 102]. VEGF in turn acts on endothelial cells and stimulates ROS production mainly by NOX1 (NADPH Oxydase 1) [103], potentially affecting PTEN level as well as cell proliferation. VEGF will also amplify endothelial cell migration and contribute to the angiogenic switch enhancing tumor growth [104]. Another derivative of oxygen is NO, which also plays a role in angiogenesis as it contributes to vessel dilatation. Church et al. demonstrated that PTEN is involved in endothelial Nitric Oxide Synthase regulation in both cancer and endothelial cells [105] and should, therefore, affect tumor angiogenesis.

Cancer cells have a supportive effect on endothelial cells; however, the reverse is also true, with the formation of the so-called vascular niche. Koistinen et al. demonstrated that VEGF-stimulated endothelial NO production maintains Acute Myeloid Leukemia (AML) cell growth, and this involved the PI3K pathway [106]. Several examples of endothelial cells stimulating solid tumor growth have also been described. VEGF promotes endothelial cell survival through PI3K activation and increased expression of the antiapoptotic protein Bcl-2, and this can be antagonized by an increased PTEN expression [107]. Recently, it was shown that Bcl-2-expressing endothelial cells also produce IL-8, which acts on tumor cells and leads to an increased invasiveness and metastatic ability [108]. Finally Park et al. demonstrated that Nerve Growth factor (NGF) stimulates endothelial cells to produce MMP2, a process inhibited by PTEN transfection [109].

5. Anti-tumor Therapies and PTEN

It now clearly appears that PTEN is involved in all the different steps leading to tumor angiogenesis. As a consequence, although the PI3K pathway is considered as the major signaling node in physiological and tumor angiogenesis, PTEN can be seen as the main intracellular antagonist to this process. Many efforts have been made to control tumor angiogenesis and related tumor growth, but here again PTEN status appears to be critical [110, 111]. Initial strategies to restrict tumor-associated angiogenesis used anti-VEGF compounds such as monoclonal antibodies against VEGF-A (Bevacizumab) or VEGFR tyrosine kinase inhibitors (Sorafenib, Sunitinib). However, after promising results in clinical trials, tumor resistance to these treatments has emerged as a major problem. These resistances are often related to a switch from VEGF- to bFGF- or EGF-dependent angiogenesis and require the HIF-1 α and PI3K pathways signaling [112–114]. Many studies have now confirmed that resistance to anti-EGFR therapies is often linked to PTEN status in cancer cells, and PTEN loss negatively correlates with clinical response to these therapies [115, 116] or others (Table 2). This problem can be overcome by the use of combined therapies which show synergistic effects and are now tested in clinical trials [117, 118]. MMPs overexpression is another resistance mechanism. We previously discussed the negative correlation between PTEN and MMPs expression; therefore, cancer cells with reduced or lost PTEN activity are more prone to develop this kind of resistance. To avoid the emergence of such resistance, different strategies aiming Journal of Oncology

Type of cancer	Metastatic form	Treatments	References
Colorectal		Cetuximab, panitumab	[66–69]
	×	Cetuximab (+irinotecan)	[70-72]
Breast		Trastuzumab, lapatinib	[73–76]
		Trastuzumab	[76–78]
	×	Endocrine therapy	
Glioblastoma		Gefitinib, erlotinib	[79–81]
		Erlotinib + temozolomid	[79-61]
Gastric		Streptozotocin, doxorubicin, 5-fluorouracil, etoposide/cisplatinum	[82, 83]
	×	Streptozotocin, doxorubicin	[82]
Lung		Gefitinib, erlotinib	[84, 85]
	×	Gefitinib, erlotinib	[85]
Pancreas		Gemcitabine	[62]
Esophageal		5-fluoropyrimidine, taxane, platinum, PI3K pathway inhibitor	[86]

TABLE 2: Clinical trials having shown an impact of the PTEN status on the response to cancer treatment.

at antagonizing the PI3K pathway or reintroducing the expression and/or activity of PTEN have been tried. Many PI3K inhibitors, as well as Akt and mTOR inhibitors, are now being tested in clinical trials [119, 120]. Although there are numerous inhibitors of the PI3K pathway, to date no PTEN inducer has been found, explaining why only PTEN transfection or gene therapy has been tried. Transfection of wild-type PTEN into human prostate cancer cells sensitizes cells to radiation and leads to a decreased tumor-induced angiogenesis [121]. In this work, both cancer- and tumorassociated endothelial cells were affected by this treatment. As previously mentioned, PTEN reintroduction decreases HIF-1 α and VEGF levels [57–59], and Lee et al. showed promising results using this strategy in mice [122]. As PI3K inhibitors monotherapy has often shown mitigated results and gene therapy experiments are just emerging, PTENstimulating molecules are urgently needed. The greatest challenge, however, remains the difficulty to set up a robust PTEN activation model, where molecular stimulators of PTEN could be tested.

6. Concluding Remarks

A myriad of studies have now demonstrated the central role of the PI3K pathway in tumorigenesis and angiogenesis. Overactivation of this pathway, a common event in cancer cells, leads to enhanced and uncontrolled tumor angiogenesis and can be antagonized by the phosphatase and tumorsuppressor PTEN. Moreover, PTEN is involved in the control of cell proliferation, migration, and survival of both cancer and tumor-associated endothelial cells. Ideally, combining PTEN-targeted therapy to classical chemotherapy should be a promising strategy, leading to (a) normalization of the tumor vasculature, (b) sensitization of cancer cells to further chemo- and radiotherapy, and (c) avoidance of resistance to such treatments. Further progress and development of PTEN-targeted therapy are, therefore, required and may lead to improvement of the current anticancerogenic strategies.

Acknowledgments

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References

- P. Carmeliet, "Mechanisms of angiogenesis and arteriogenesis," *Nature Medicine*, vol. 6, no. 4, pp. 389–395, 2000.
- [2] L. Jakobsson, C. A. Franco, K. Bentley et al., "Endothelial cells dynamically compete for the tip cell position during angiogenic sprouting," *Nature Cell Biology*, vol. 12, no. 10, pp. 943–953, 2010.
- [3] R. K. Jain, "Molecular regulation of vessel maturation," *Nature Medicine*, vol. 9, no. 6, pp. 685–693, 2003.
- [4] H. F. Dvorak, "Tumors: wounds that do not heal: similarities between tumor stroma generation and wound healing," *The New England Journal of Medicine*, vol. 315, no. 26, pp. 1650– 1659, 1986.
- [5] P. Carmeliet, V. Ferreira, G. Breier et al., "Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele," *Nature*, vol. 380, no. 6573, pp. 435–439, 1996.
- [6] A. K. Olsson, A. Dimberg, J. Kreuger, and L. Claesson-Welsh, "VEGF receptor signalling—in control of vascular function," *Nature Reviews Molecular Cell Biology*, vol. 7, no. 5, pp. 359– 371, 2006.
- [7] D. J. Dumont, G. Gradwohl, G. H. Fong et al., "Dominantnegative and targeted null mutations in the endothelial receptor tyrosine kinase, tek, reveal a critical role in vasculogenesis of the embryo," *Genes and Development*, vol. 8, no. 16, pp. 1897–1909, 1994.
- [8] T. N. Sato, Y. Tozawa, U. Deutsch et al., "Distinct roles of the receptor tyrosine kinases Tie-1 and Tie-2 in blood vessel formation," *Nature*, vol. 376, no. 6535, pp. 70–74, 1995.
- [9] C. Daly, V. Wong, E. Burova et al., "Angiopoietin-1 modulates endothelial cell function and gene expression via the transcription factor FKHR (FOXO1)," *Genes and Development*, vol. 18, no. 9, pp. 1060–1071, 2004.
- [10] S. Kuijper, C. J. Turner, and R. H. Adams, "Regulation of angiogenesis by Eph-ephrin interactions," *Trends in Cardio*vascular Medicine, vol. 17, no. 5, pp. 145–151, 2007.

- [11] L. J. Stephen, A. L. Fawkes, A. Verhoeve, G. Lemke, and A. Brown, "A critical role for the EphA3 receptor tyrosine kinase in heart development," *Developmental Biology*, vol. 302, no. 1, pp. 66–79, 2007.
- [12] D. G. Wilkinson, "Multiple roles of Eph receptors and ephrins in neural development," *Nature Reviews Neuroscience*, vol. 2, no. 3, pp. 155–164, 2001.
- [13] J. J. Steinle, C. J. Meininger, R. Forough, G. Wu, M. H. Wu, and H. J. Granger, "Eph B4 receptor signaling mediates endothelial cell migration and proliferation via the phosphatidylinositol 3-kinase pathway," *Journal of Biological Chemistry*, vol. 277, no. 46, pp. 43830–43835, 2002.
- [14] H. U. Wang, Z. F. Chen, and D. J. Anderson, "Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-B2 and its receptor Eph-B4," *Cell*, vol. 93, no. 5, pp. 741–753, 1998.
- [15] S. Sawamiphak, S. Seidel, C. L. Essmann et al., "Ephrin-B2 regulates VEGFR2 function in developmental and tumour angiogenesis," *Nature*, vol. 465, no. 7297, pp. 487–491, 2010.
- [16] Y. Oike, Y. Ito, K. Hamada et al., "Regulation of vasculogenesis and angiogenesis by EphB/ephrin-B2 signaling between endothelial cells and surrounding mesenchymal cells," *Blood*, vol. 100, no. 4, pp. 1326–1333, 2002.
- [17] G. L. Wang, B. H. Jiang, E. A. Rue, and G. L. Semenza, "Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension," *Proceedings* of the National Academy of Sciences of the United States of America, vol. 92, no. 12, pp. 5510–5514, 1995.
- [18] M. Ivan, K. Kondo, H. Yang et al., "HIF α targeted for VHLmediated destruction by proline hydroxylation: implications for O2 sensing," *Science*, vol. 292, no. 5516, pp. 464–468, 2001.
- [19] M. M. Baldewijns, I. J. H. van Vlodrop, P. B. Vermeulen, P. M. M. B. Soetekouw, M. van Engeland, and A. P. de Bruïne, "VHL and HIF signalling in renal cell carcinogenesis," *Journal* of *Pathology*, vol. 221, no. 2, pp. 125–138, 2010.
- [20] M. M. Vihanto, J. Plock, D. Erni, B. M. Frey, F. J. Frey, and U. Huynh-Do, "Hypoxia up-regulates expression of Eph receptors and ephrins in mouse skin," *FASEB Journal*, vol. 19, no. 12, pp. 1689–1691, 2005.
- [21] J. Xu, D. Rodriguez, E. Petitclerc et al., "Proteolytic exposure of a cryptic site within collagen type IV is required for angiogenesis and tumor growth in vivo," *Journal of Cell Biology*, vol. 154, no. 5, pp. 1069–1079, 2001.
- [22] J. M. Whitelock, A. D. Murdoch, R. V. Iozzo, and P. A. Underwood, "The degradation of human endothelial cellderived perlecan and release of bound basic fibroblast growth factor by stromelysin, collagenase, plasmin, and heparanases," *Journal of Biological Chemistry*, vol. 271, no. 17, pp. 10079–10086, 1996.
- [23] D. J. Marsh, V. Coulon, K. L. Lunetta et al., "Mutation spectrum and genotype-phenotype analyses in Cowden disease and Bannayan-Zonana syndrome, two hamartoma syndromes with germline PTEN mutation," *Human Molecular Genetics*, vol. 7, no. 3, pp. 507–515, 1998.
- [24] L. C. Trotman, X. Wang, A. Alimonti et al., "Ubiquitination regulates PTEN nuclear import and tumor suppression," *Cell*, vol. 128, no. 1, pp. 141–156, 2007.
- [25] X. P. Zhou, D. J. Marsh, C. D. Morrison et al., "Germline inactivation of PTEN and dysregulation of the phosphoinositol-3- kinase/Akt pathway cause human Lhermitte-Duclos disease in adults," *American Journal of Human Genetics*, vol. 73, no. 5, pp. 1191–1198, 2003.

- [26] X. P. Zhou, H. Hampel, H. Thiele et al., "Association of germline mutation in the PTEN tumour suppressor gene and Proteus and Proteus-like syndromes," *The Lancet*, vol. 358, no. 9277, pp. 210–211, 2001.
- [27] E. A. Varga, M. Pastore, T. Prior, G. E. Herman, and K. L. McBride, "The prevalence of PTEN mutations in a clinical pediatric cohort with autism spectrum disorders, developmental delay, and macrocephaly," *Genetics in Medicine*, vol. 11, no. 2, pp. 111–117, 2009.
- [28] D. Hanahan and J. Folkman, "Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis," *Cell*, vol. 86, no. 3, pp. 353–364, 1996.
- [29] Y. S. Chun, M. S. Kim, and J. W. Park, "Oxygen-dependent and -independent regulation of HIF-1alpha," *Journal of Korean Medical Science*, vol. 17, no. 5, pp. 581–588, 2002.
- [30] R. Kalluri and M. Zeisberg, "Fibroblasts in cancer," *Nature Reviews Cancer*, vol. 6, no. 5, pp. 392–401, 2006.
- [31] M. Murakami, Y. Zheng, M. Hirashima et al., "VEGFR1 tyrosine kinase signaling promotes lymphangiogenesis as well as angiogenesis indirectly via macrophage recruitment," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 28, no. 4, pp. 658–664, 2008.
- [32] S. Morikawa, P. Baluk, T. Kaidoh, A. Haskell, R. K. Jain, and D. M. McDonald, "Abnormalities in pericytes on blood vessels and endothelial sprouts in tumors," *American Journal* of *Pathology*, vol. 160, no. 3, pp. 985–1000, 2002.
- [33] I. Helfrich, L. Edler, A. Sucker et al., "Angiopoietin-2 levels are associated with disease progression in metastatic malignant melanoma," *Clinical Cancer Research*, vol. 15, no. 4, pp. 1384–1392, 2009.
- [34] J. Holash, P. C. Maisonpierre, D. Compton et al., "Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF," *Science*, vol. 284, no. 5422, pp. 1994–1998, 1999.
- [35] X. Huang, Y. Yamada, H. Kidoya et al., "EphB4 overexpression in B16 melanoma cells affects arterial-venous patterning in tumor angiogenesis," *Cancer Research*, vol. 67, no. 20, pp. 9800–9808, 2007.
- [36] M. Kimura, Y. Kato, D. Sano et al., "Soluble form of ephrinB2 inhibits xenograft growth of squamous cell carcinoma of the head and neck," *International Journal of Oncology*, vol. 34, no. 2, pp. 321–327, 2009.
- [37] V. Krasnoperov, S. R. Kumar, E. Ley et al., "Novel EphB4 monoclonal antibodies modulate angiogenesis and inhibit tumor growth," *American Journal of Pathology*, vol. 176, no. 4, pp. 2029–2038, 2010.
- [38] S. R. Kumar, J. Singh, G. Xia et al., "Receptor tyrosine kinase EphB4 is a survival factor in breast cancer," *American Journal* of *Pathology*, vol. 169, no. 1, pp. 279–293, 2006.
- [39] R. Masood, S. R. Kumar, U. K. Sinha et al., "EphB4 provides survival advantage to squamous cell carcinoma of the head and neck," *International Journal of Cancer*, vol. 119, no. 6, pp. 1236–1248, 2006.
- [40] G. Martiny-Baron, T. Korff, F. Schaffner et al., "Inhibition of tumor growth and angiogenesis by soluble EphB4," *Neoplasia*, vol. 6, no. 3, pp. 248–257, 2004.
- [41] S. A. Cunningham, M. N. Waxham, P. M. Arrate, and T. A. Brock, "Interaction of the Flt-1 tyrosine kinase receptor with the p85 subunit of phosphatidylinositol 3-kinase. Mapping of a novel site involved in binding," *Journal of Biological Chemistry*, vol. 270, no. 35, pp. 20254–20257, 1995.
- [42] H. P. Gerber, A. McMurtrey, J. Kowalski et al., "Vascular endothelial growth factor regulates endothelial cell survival

through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway: requirement for Flk-1/KDR activation," *Journal of Biological Chemistry*, vol. 273, no. 46, pp. 30336– 30343, 1998.

- [43] L. Wang, G. Z. Zheng, L. Z. Rui et al., "Matrix metalloproteinase 2 (MMP2) and MMP9 secreted by erythropoietinactivated endothelial cells promote neural progenitor cell migration," *Journal of Neuroscience*, vol. 26, no. 22, pp. 5996– 6003, 2006.
- [44] X. H. Guan, X. F. Lu, H. X. Zhang et al., "Phosphatidylinositol 3-kinase mediates pain behaviors induced by activation of peripheral ephrinBs/EphBs signaling in mice," *Pharmacology Biochemistry and Behavior*, vol. 95, no. 3, pp. 315–324, 2010.
- [45] B. Vanhaesebroeck, S. J. Leevers, G. Panayotou, M. D. Waterfield, and M. D. Waterfield, "Phosphoinositide 3kinases: a conserved family of signal transducers," *Trends in Biochemical Sciences*, vol. 22, no. 7, pp. 267–272, 1997.
- [46] T. Maehama, G. S. Taylor, and J. E. Dixon, "PTEN and myotubularin: novel phosphoinositide phosphatases," *Annual Review of Biochemistry*, vol. 70, pp. 247–279, 2001.
- [47] L. C. Cantley and B. G. Neel, "New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway," *Proceedings* of the National Academy of Sciences of the United States of America, vol. 96, no. 8, pp. 4240–4245, 1999.
- [48] J. Gu, M. Tamura, R. Pankov et al., "Shc and FAK differentially regulate cell motility and directionality modulated by PTEN," *Journal of Cell Biology*, vol. 146, no. 2, pp. 389–403, 1999.
- [49] M. Tamura, J. Gu, K. Matsumoto, S. I. Aota, R. Parsons, and K. M. Yamada, "Inhibition of cell migration, spreading, and focal adhesions by tumor suppressor PTEN," *Science*, vol. 280, no. 5369, pp. 1614–1617, 1998.
- [50] M. Raftopoulou, S. Etienne-Manneville, A. Self, S. Nicholls, and A. Hall, "Regulation of cell migration by the C2 domain of the tumor suppressor PTEN," *Science*, vol. 303, no. 5661, pp. 1179–1181, 2004.
- [51] N. R. Leslie, X. Yang, C. P. Downes, and C. J. Weijer, "PtdIns(3,4,5)P₃-dependent and -independent roles for PTEN in the control of cell migration," *Current Biology*, vol. 17, no. 2, pp. 115–125, 2007.
- [52] F. Meng, R. Henson, H. Wehbe-Janek, K. Ghoshal, S. T. Jacob, and T. Patel, "MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer," *Gastroenterology*, vol. 133, no. 2, pp. 647–658, 2007.
- [53] X. Wang and X. Jiang, "Post-translational regulation of PTEN," *Oncogene*, vol. 27, no. 41, pp. 5454–5463, 2008.
- [54] W. Zundel, C. Schindler, D. Haas-Kogan et al., "Loss of PTEN facilitates HIF-1-mediated gene expression," *Genes* and Development, vol. 14, no. 4, pp. 391–396, 2000.
- [55] A. V. Teichman, E. Compérat, S. Behnke, M. Storz, H. Moch, and P. Schraml, "VHL mutations and dysregulation of pVHL- and PTEN-controlled pathways in multilocular cystic renal cell carcinoma," *Modern Pathology*, 2010.
- [56] I. J. Frew, C. R. Thoma, S. Georgiev et al., "pVHL and PTEN tumour suppressor proteins cooperatively suppress kidney cyst formation," *EMBO Journal*, vol. 27, no. 12, pp. 1747– 1757, 2008.
- [57] J. Fang, M. Ding, L. Yang, L. Z. Liu, and B. H. Jiang, "PI3K/PTEN/AKT signaling regulates prostate tumor angiogenesis," *Cellular Signalling*, vol. 19, no. 12, pp. 2487–2497, 2007.

- [58] T. Tian, K. J. Nan, S. H. Wang et al., "PTEN regulates angiogenesis and VEGF expression through phosphatasedependent and -independent mechanisms in HepG2 cells," *Carcinogenesis*, vol. 31, no. 7, pp. 1211–1219, 2010.
- [59] Y. Takei, Y. Saga, H. Mizukami et al., "Overexpression of PTEN in ovarian cancer cells suppresses i.p. dissemination and extends survival in mice," *Molecular Cancer Therapeutics*, vol. 7, no. 3, pp. 704–711, 2008.
- [60] S. Wang, Z. Cheng, X. Yang et al., "Effect of wild type PTEN gene on proliferation and invasion of multiple myeloma," *International Journal of Hematology*, vol. 92, no. 1, pp. 83–94, 2010.
- [61] H. Zheng, H. Takahashi, Y. Murai et al., "Expressions of MMP-2, MMP-9 and VEGF are closely linked to growth, invasion, metastasis and angiogenesis of gastric carcinoma," *Anticancer Research*, vol. 26, no. 5 A, pp. 3579–3583, 2006.
- [62] E. Giovannetti, N. Funel, G. J. Peters et al., "MicroRNA-21 in pancreatic cancer: correlation with clinical outcome and pharmacologic aspects underlying its role in the modulation of gemcitabine activity," *Cancer Research*, vol. 70, no. 11, pp. 4528–4538, 2010.
- [63] S. D. Sekuklu, M. T. A. Donoghue, and C. Spillane, "miR-21 as a key regulator of oncogenic processes," *Biochemical Society Transactions*, vol. 37, part 4, pp. 918–925, 2009.
- [64] L. M. DeBusk, D. E. Hallahan, and P. C. Lin, "Akt is a major angiogenic mediator downstream of the Ang1/Tie2 signaling pathway," *Experimental Cell Research*, vol. 298, no. 1, pp. 167– 177, 2004.
- [65] C. M. Findley, M. J. Cudmore, A. Ahmed, and C. D. Kontos, "VEGF induces Tie2 shedding via a phosphoinositide 3kinase/Akt-dependent pathway to modulate Tie2 signaling," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 27, no. 12, pp. 2619–2626, 2007.
- [66] M. Frattini, P. Saletti, E. Romagnani et al., "PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients," *British Journal of Cancer*, vol. 97, no. 8, pp. 1139–1145, 2007.
- [67] E. Razis, E. Briasoulis, E. Vrettou et al., "Potential value of PTEN in predicting cetuximab response in colorectal cancer: an exploratory study," *BMC Cancer*, vol. 8, p. 234, 2008.
- [68] A. Sartore-Bianchi, F. Di Nicolantonio, M. Nichelatti et al., "Multi-determinants analysis of molecular alterations for predicting clinical benefit to EGFR-targeted monoclonal antibodies in colorectal cancer," *PLoS ONE*, vol. 4, no. 10, Article ID e7287, 2009.
- [69] A. Sartore-Bianchi, M. Martini, F. Molinari et al., "PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies," *Cancer Research*, vol. 69, no. 5, pp. 1851–1857, 2009.
- [70] P. Laurent-Puig, A. Cayre, G. Manceau et al., "Analysis of PTEN, BRAF, and EGFR status in determining benefit from cetuximab therapy in wild-type KRAS metastatic colon cancer," *Journal of Clinical Oncology*, vol. 27, no. 35, pp. 5924– 5930, 2009.
- [71] F. Loupakis, L. Pollina, I. Stasi et al., "PTEN expression and KRAS mutations on primary tumors and metastases in the prediction of benefit from cetuximab plus irinotecan for patients with metastatic colorectal cancer," *Journal of Clinical Oncology*, vol. 27, no. 16, pp. 2622–2629, 2009.
- [72] F. Perrone, A. Lampis, M. Orsenigo et al., "PI3KCA/PTEN deregulation contributes to impaired responses to cetuximab in metastatic colorectal cancer patients," *Annals of Oncology*, vol. 20, no. 1, pp. 84–90, 2009.

- [73] K. Berns, H. M. Horlings, B. T. Hennessy et al., "A Functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer," *Cancer Cell*, vol. 12, no. 4, pp. 395–402, 2007.
- [74] B. Dave, I. Migliaccio, M. C. Gutierrez et al., "Loss of phosphatase and tensin homolog or phosphoinositol-3 kinase activation and response to trastuzumab or lapatinib in human epidermal growth factor receptor 2—overexpressing locally advanced breast cancers," *Journal of Clinical Oncology*, vol. 29, no. 2, pp. 166–173, 2011.
- [75] Y. Nagata, K. H. Lan, X. Zhou et al., "PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients," *Cancer Cell*, vol. 6, no. 2, pp. 117–127, 2004.
- [76] E. Tokunaga, Y. Kimura, K. Mashino et al., "Activation of PI3K/Akt signaling and hormone resistance in breast cancer," *Breast Cancer*, vol. 13, no. 2, pp. 137–144, 2006.
- [77] C. Christodoulou, I. Kostopoulos, H. P. Kalofonos et al., "Trastuzumab combined with pegylated liposomal doxorubicin in patients with metastatic breast cancer: phase II study of the Hellenic Cooperative Oncology Group (HeCOG) with biomarker evaluation," *Oncology*, vol. 76, no. 4, pp. 275–285, 2009.
- [78] F. J. Esteva, H. Guo, S. Zhang et al., "PTEN, PIK3CA, p-AKT, and p-p70S6K status: association with trastuzumab response and survival in patients with HER2-positive metastatic breast cancer," *American Journal of Pathology*, vol. 177, no. 4, pp. 1647–1656, 2010.
- [79] D. A. Haas-Kogan, M. D. Prados, T. Tihan et al., "Epidermal growth factor receptor, protein kinase B/Akt, and glioma response to erlotinib," *Journal of the National Cancer Institute*, vol. 97, no. 12, pp. 880–887, 2005.
- [80] I. K. Mellinghoff, M. Y. Wang, I. Vivanco et al., "Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors," *The New England Journal of Medicine*, vol. 353, no. 19, pp. 2012–2024, 2005.
- [81] M. D. Prados, S. M. Chang, N. Butowski et al., "Phase II study of erlotinib plus temozolomide during and after radiation therapy in patients with newly diagnosed glioblastoma multiforme or gliosarcoma," *Journal of Clinical Oncology*, vol. 27, no. 4, pp. 579–584, 2009.
- [82] C. L. Nigro, M. Monteverde, M. Riba et al., "Expression profiling and long lasting responses to chemotherapy in metastatic gastric cancer," *International Journal of Oncology*, vol. 37, no. 5, pp. 1219–1228, 2010.
- [83] D. O'Toole, A. Couvelard, V. Rebours et al., "Molecular markers associated with response to chemotherapy in gastroentero-pancreatic neuroendocrine tumors," *Endocrine-Related Cancer*, vol. 17, no. 4, pp. 847–856, 2010.
- [84] M. L. Sos, M. Koker, B. A. Weir et al., "PTEN loss contributes to erlotinib resistance in EGFR-mutant lung cancer by activation of Akt and EGFR," *Cancer Research*, vol. 69, no. 8, pp. 3256–3261, 2009.
- [85] H. Uramoto, H. Shimokawa, T. Hanagiri, M. Kuwano, and M. Ono, "Expression of selected gene for acquired drug resistance to EGFR-TKI in lung adenocarcinoma," *Lung Cancer.* In press.
- [86] M. A. T. Hildebrandt, H. Yang, M. C. Hung et al., "Genetic variations in the PI3K/PTEN/AKT/mTOR pathway are associated with clinical outcomes in esophageal cancer patients treated with chemoradiotherapy," *Journal of Clinical Oncology*, vol. 27, no. 6, pp. 857–871, 2009.

- [87] F. Vazquez and P. Devreotes, "Regulation of PTEN function as a PIP3 gatekeeper through membrane interaction," *Cell Cycle*, vol. 5, no. 14, pp. 1523–1527, 2006.
- [88] J. R. Molina, F. C. Morales, Y. Hayashi, K. D. Aldape, and M. M. Georgescu, "Loss of PTEN binding adapter protein NHERF1 from plasma membrane in glioblastoma contributes to PTEN inactivation," *Cancer Research*, vol. 70, no. 17, pp. 6697–6703, 2010.
- [89] Y. Takahashi, F. C. Morales, E. L. Kreimann, and M. M. Georgescu, "PTEN tumor suppressor associates with NHERF proteins to attenuate PDGF receptor signaling," *EMBO Journal*, vol. 25, no. 4, pp. 910–920, 2006.
- [90] C. S. Lazar, C. M. Cresson, D. A. Lauffenburger, and G. N. Gill, "The Na+/H+ exchanger regulatory factor stabilizes epidermal growth factor receptors at the cell surface," *Molecular Biology of the Cell*, vol. 15, no. 12, pp. 5470–5480, 2004.
- [91] I. Vivanco, D. Rohle, M. Versele et al., "The phosphatase and tensin homolog regulates epidermal growth factor receptor (EGFR) inhibitor response by targeting EGFR for degradation," *Proceedings of the National Academy of Sciences* of the United States of America, vol. 107, no. 14, pp. 6459– 6464, 2010.
- [92] E. V. Wong, J. A. Kerner, and D. G. Jay, "Convergent and divergent signaling mechanisms of growth cone collapse by ephrinA5 and slit2," *Journal of Neurobiology*, vol. 59, no. 1, pp. 66–81, 2004.
- [93] S. Brisbin, J. Liu, J. Boudreau, J. Peng, M. Evangelista, and I. Chin-Sang, "A role for C. elegans Eph RTK signaling in PTEN regulation," *Developmental Cell*, vol. 17, no. 4, pp. 459–469, 2009.
- [94] B. Bussolati, I. Deambrosis, S. Russo, M. C. Deregibus, and G. Camussi, "Altered angiogenesis and survival in human tumor-derived endothelial cells," *FASEB Journal*, vol. 17, no. 9, pp. 1159–1161, 2003.
- [95] A. J. Trimboli, C. Z. Cantemir-Stone, F. Li et al., "Pten in stromal fibroblasts suppresses mammary epithelial tumours," *Nature*, vol. 461, no. 7267, pp. 1084–1091, 2009.
- [96] M. Fosbrink, F. Niculescu, V. Rus, M. L. Shin, and H. Rus, "C5b-9-induced endothelial cell proliferation and migration are dependent on Akt inactivation of forkhead transcription factor FOXO1," *Journal of Biological Chemistry*, vol. 281, no. 28, pp. 19009–19018, 2006.
- [97] T. Nakao, M. Shiota, Y. Tatemoto, Y. Izumi, and H. Iwao, "Pravastatin induces rat aortic endothelial cell proliferation and migration via activation of PI3K/Akt/mTOR/p70 S6 kinase signaling," *Journal of Pharmacological Sciences*, vol. 105, no. 4, pp. 334–341, 2007.
- [98] H. Zheng, T. Dai, B. Zhou et al., "SDF-1α/CXCR4 decreases endothelial progenitor cells apoptosis under serum deprivation by PI3K/Akt/eNOS pathway," *Atherosclerosis*, vol. 201, no. 1, pp. 36–42, 2008.
- [99] A. S. Tarnawski, R. Pai, T. Tanigawa, T. Matysiak-Budnik, and A. Ahluwalia, "PTEN silencing reverses aging-related impairment of angiogenesis in microvascular endothelial cells," *Biochemical and Biophysical Research Communications*, vol. 394, no. 2, pp. 291–296, 2010.
- [100] J. Cao, J. Schulte, A. Knight et al., "Prdx1 inhibits tumorigenesis via regulating PTEN/AKT activity," *EMBO Journal*, vol. 28, no. 10, pp. 1505–1517, 2009.
- [101] K. M. Connor, S. Subbaram, K. J. Regan et al., "Mitochondrial H2O2 regulates the angiogenic phenotype via PTEN oxidation," *Journal of Biological Chemistry*, vol. 280, no. 17, pp. 16916–16924, 2005.

- [102] A. de Luca, F. Sanna, M. Sallese et al., "Methionine sulfoxide reductase A down-regulation in human breast cancer cells results in a more aggressive phenotype," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 43, pp. 18628–18633, 2010.
- [103] K. Bedard and K. H. Krause, "The NOX family of ROSgenerating NADPH oxidases: physiology and pathophysiology," *Physiological Reviews*, vol. 87, no. 1, pp. 245–313, 2007.
- [104] S. Garrido-Urbani, S. Jemelin, C. Deffert et al., "Targeting vascular NADPH oxidase 1 blocks tumor angiogenesis through a PPAR α mediated mechanism," *PLoS ONE*, vol. 6, no. 2, article e14665, 2011.
- [105] J. E. Church, J. Qian, S. Kumar et al., "Inhibition of endothelial nitric oxide synthase by the lipid phosphatase PTEN," *Vascular Pharmacology*, vol. 52, no. 5-6, pp. 191–198, 2010.
- [106] P. Koistinen, T. Siitonen, P. Mäntymaa et al., "Regulation of the acute myeloid leukemia cell line OCI/AML-2 by endothelial nitric oxide synthase under the control of a vascular endothelial growth factor signaling system," *Leukemia*, vol. 15, no. 9, pp. 1433–1441, 2001.
- [107] J. Cai, S. Ahmad, W. G. Jiang et al., "Activation of vascular endothelial growth factor receptor-1 sustains angiogenesis and Bcl-2 expression via the phosphatidylinositol 3-kinase pathway in endothelial cells," *Diabetes*, vol. 52, no. 12, pp. 2959–2968, 2003.
- [108] P. Kumar, Y. Ning, and P. J. Polverini, "Endothelial cells expressing Bcl-2 promotes tumor metastasis by enhancing tumor angiogenesis, blood vessel leakiness and tumor invasion," *Laboratory Investigation*, vol. 88, no. 7, pp. 740–749, 2008.
- [109] M. J. Park, H. J. Kwak, H. C. Lee et al., "Nerve growth factor induces endothelial cell invasion and cord formation by promoting matrix metalloproteinase-2 expression through the phosphatidylinositol 3-kinase/Akt signaling pathway and AP-2 transcription factor," *Journal of Biological Chemistry*, vol. 282, no. 42, pp. 30485–30496, 2007.
- [110] B. Bussolati, M. C. Deregibus, and G. Camussi, "Characterization of molecular and functional alterations of tumor endothelial cells to design anti-angiogenic strategies," *Current Vascular Pharmacology*, vol. 8, no. 2, pp. 220–232, 2010.
- [111] W. C. M. Dempke and V. Heinemann, "Resistance to EGF-R (erbB-1) and VEGF-R modulating agents," *European Journal* of Cancer, vol. 45, no. 7, pp. 1117–1128, 2009.
- [112] P. Alessi, D. Leali, M. Camozzi, A. Cantelmo, A. Albini, and M. Presta, "Anti-FGF2 approaches as a strategy to compensate resistance to anti-VEGF therapy: long-pentraxin 3 as a novel antiangiogenic FGF2-antagonist," *European Cytokine Network*, vol. 20, no. 4, pp. 225–234, 2009.
- [113] S. I. Ueda, Y. Basaki, M. Yoshie et al., "PTEN/Akt signaling through epidermal growth factor receptor is prerequisite for angiogenesis by hepatocellular carcinoma cells that is susceptible to inhibition by gefitinib," *Cancer Research*, vol. 66, no. 10, pp. 5346–5353, 2006.
- [114] Y. H. Shi, L. Bingle, L. H. Gong, Y. X. Wang, K. P. Corke, and W. G. Fang, "Basic FGF augments hypoxia induced HIF-1-alpha expression and VEGF release in T47D breast cancer cells," *Pathology*, vol. 39, no. 4, pp. 396–400, 2007.
- [115] D. Faratian, A. Goltsov, G. Lebedeva et al., "Systems biology reveals new strategies for personalizing cancer medicine and confirms the role of PTEN in resistance to trastuzumab," *Cancer Research*, vol. 69, no. 16, pp. 6713–6720, 2009.

- [116] F. V. Negri, C. Bozzetti, C. A. Lagrasta et al., "PTEN status in advanced colorectal cancer treated with cetuximab," *British Journal of Cancer*, vol. 102, no. 1, pp. 162–164, 2010.
- [117] E. Martinelli, T. Troiani, F. Morgillo et al., "Synergistic antitumor activity of sorafenib in combination with epidermal growth factor receptor inhibitors in colorectal and lung cancer cells," *Clinical Cancer Research*, vol. 16, no. 20, pp. 4990–5001, 2010.
- [118] E. E. Cohen, D. W. Davis, T. G. Karrison et al., "Erlotinib and bevacizumab in patients with recurrent or metastatic squamous-cell carcinoma of the head and neck: a phase I/II study," *The Lancet Oncology*, vol. 10, no. 3, pp. 247–257, 2009.
- [119] J. A. Engelman, "Targeting PI3K signalling in cancer: opportunities, challenges and limitations," *Nature Reviews Cancer*, vol. 9, no. 8, pp. 550–562, 2009.
- [120] R. Marone, V. Cmiljanovic, B. Giese, and M. P. Wymann, "Targeting phosphoinositide 3-kinase—moving towards therapy," *Biochimica et Biophysica Acta*, vol. 1784, no. 1, pp. 159–185, 2008.
- [121] S. Anai, S. Goodison, K. Shiverick, K. Iczkowski, M. Tanaka, and C. J. Rosser, "Combination of PTEN gene therapy and radiation inhibits the growth of human prostate cancer xenografts," *Human Gene Therapy*, vol. 17, no. 10, pp. 975– 984, 2006.
- [122] J. S. Lee, J. H. Lee, H. Poo et al., "Growth inhibitory effect of triple anti-tumor gene transfer using Semliki Forest virus vector in glioblastoma cells," *International Journal of Oncology*, vol. 28, no. 3, pp. 649–654, 2006.



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