MINI REVIEW

Cellular and molecular mechanisms of PIK3CA-related vascular anomalies

Timothy D Le Cras[1] and **Elisa Boscolo**[2]

[1]Division of Pulmonary Biology, Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati Children's Hospital, Cincinnati, Ohio, USA
[2]Experimental Hematology and Cancer Biology, Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati Children's Hospital, Cincinnati, Ohio, USA

Correspondence should be addressed to E Boscolo: elisa.boscolo@cchmc.org

Abstract

The phosphoinositide 3-kinase (PI3K) pathway is a major mediator of growth factor signaling, cell proliferation and metabolism. Somatic gain-of-function mutations in *PIK3CA*, the catalytic subunit of PI3K, have recently been discovered in a number of vascular anomalies. The timing and origin of these mutations remain unclear although they are believed to occur during embryogenesis. The cellular origin of these lesions likely involves endothelial cells or an early endothelial cell lineage. This review will cover the diseases and syndromes associated with *PIK3CA* mutations and discuss the cellular origin, pathways and mechanisms. Activating *PIK3CA* 'hot spot' mutations have long been associated with a multitude of cancers allowing the development of targeted pharmacological inhibitors that are FDA-approved or in clinical trials. Current and future therapeutic approaches for *PIK3CA*-related vascular anomalies are discussed.

Key Words

- vascular malformation
- vascular anomalies

The phosphoinositide 3-kinase pathway

The phosphoinositide 3-kinase (PI3K) signaling pathway is a critical regulator of the angiogenic process by controlling endothelial cell (EC) proliferation, migration and survival (1). Growth factor stimulation of receptor tyrosine kinases activates PI3K that in turn catalyzes the conversion of phosphatidylinositol (3,4)-bisphosphate (PIP2) to phosphatidylinositol (3,4,5)-triphosphate (PIP3). PIP3 triggers phosphorylation of Pyruvate Dehydrogenase Kinase 1 (PDK1), which phosphorylates the serine and threonine kinase AKT (also known as Protein Kinase B, PKB) (2, 3). This activation of AKT leads to increased cellular proliferation through the mTOR pathway (4, 5). The catalytic subunit of PI3K (p110α) is encoded by the PIK3CA gene. *PIK3CA* 'hot spot' mutations p.E542K (E, glutamic acid replaced by K, lysine at amino acid position 542) and p.E545K (amino acid position 545) in exon 9 (α-helical domain), and p.H1047R/L (H, histidine replaced by R, arginine or L, leucine at amino acid position 1047) in exon 20 (kinase domain) are common in cancer (6), and more recently, have also been identified in pediatric vascular anomalies and overgrowth syndromes. All of the *PIK3CA* mutations associated with vascular anomalies and cancer are non-inherited and lead to sequestration of the p110α subunit to the plasma membrane thereby generating constitutive hyper-activation of the PI3K-AKT pathway (7). Of interest, sustained AKT activation induces pathological angiogenesis and increased blood vessel diameter (8). Germline transmission of the *PIK3CA* oncogenic 'hotspot' mutations is not compatible with life. In mouse, ubiquitous or Tie2-driven heterozygous expression of Pik3ca ^{H1047R} resulted in disrupted vascular remodeling in the embryonic and extraembryonic tissues

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and lethality prior to E11.5 (9). Conversely, ubiquitous expression of Pik3ca ^{H1047R} in 6–8-week-old mice caused increased body weight, organomegaly and metabolic defects (10).

PIK3CA mutations in vascular anomalies

While vascular malformations are primarily present at birth, they can also become apparent during childhood, and they persist throughout adulthood. The International Society for the Study of Vascular Anomalies (ISSVA) created a classification system to aid the diagnosis of these anomalies (11). A number of genes and gene mutations have been implicated with vascular anomalies, in this review we will focus on the disease categories associated with *PIK3CA* mutations (Table 1).

Simple vascular anomalies

Lymphatic malformations (LM)

Lymphatic malformations (LM) are characterized by massively dilated and dysmorphic low-flow lymphatic channels (12, 13, 14, 15, 16) lined with flattened EC. Often, vessels are filled with blood or thrombi as result of intralesional trauma and/or improper communication with the venous system. Expansion of LM is caused by vascular distension generated by fluid accumulation and cellular hyperplasia. LM expands during adolescence, and lesions can cause severe disfigurement, affect vital organs and contribute to infections. Histological markers of LM are Prox-1 and VEGFR3, while Podoplanin levels can be variable (17). Somatic activating *PIK3CA* mutations have been identified in affected tissue resected from the majority (94%) of LM patients (18) and in cells isolated from LM tissue (19, 20, 21). Patient-derived lymphatic endothelial cells (LM-LEC) exhibit pro-angiogenic properties such as increased proliferation, sprout formation, and VEGF-C and VEGFR-3 overexpression. AKT that is downstream of PI3K is constitutively active in the mutant LM-LEC, while ERK1/2 phosphorylation levels are variable (17, 19, 20, 22). A few animal models of LM have been reported and consist of xenografts generated by injection of LM-LEC (17) or LEC progenitor cells (23). In these studies, it is unclear if implanted cells expressed *PIK3CA* mutations. Another model of thoracic LM was generated by overexpression of VEGF-C in mouse airway epithelial cells resulting in lymphangectasia and mTOR pathway activation (24), although the specific activation status of AKT was not assessed. In the future, improved *PIK3CA*-based models of LM would be very beneficial to investigate therapeutic targets.

Generalized lymphatic anomaly (GLA)

Generalized lymphatic anomaly (GLA) is a severe form of LM that is diffuse or multifocal. Patients have increased risk of pulmonary involvement and the dysmorphic lymphatics can infiltrate and compromise the medullary bone causing pain and compromised mobility (25, 26). A high-throughput sequencing effort led to the discovery of *PIK3CA* mutations in affected tissue and LEC in 55% of GLA patients (27). Postnatal transgenic expression of *PIK3CA* p.H1047R in the LEC (*Prox1-CreER^{T2}*) caused lymphatic hyperplasia and dysfunction, modeling important aspects of GLA (27).

Table 1 Genetic mutations in vascular anomalies.

Vascular anomalies	Gene mutations	References
Vascular tumors	*KRAS*, *NRAS*, *HRAS*, *BRAF*, *GNAQ*, *GNA11*, *GNA14*	(72, 73, 74, 75)
Vascular malformations		
Simple		
Capillary malformation	*GNAQ*, *GNA11*, *KRAS*	(76, 77, 78)
Lymphatic malformation	*PIK3CA*	(18, 19, 20, 21)
Venous malformation	*TEK*, *PIK3CA*	(29, 31, 32, 33, 34, 35)
Arteriovenous malformation	*KRAS*, *MAP2K1*, *BRAF*	(76, 79, 80)
Combined		
Lymphatic-Venous malformation	*PIK3CA*	(81)
Capillary-Lymphatic-Venous malformation/KTS, CLOVES	*PIK3CA*	(18, 46, 47, 48, 49)
Capillary malformation-ArterioVenous malformation	*RASA1*, *EPHB4*	(82, 83)
Others		
Fibro-adipose vascular anomaly	*PIK3CA*	(18)
Megalencephaly-capillary malformation	*PIK3CA*	(53)

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Venous malformation (VM)

Venous malformation (VM) manifests as unifocal or multifocal bluish compressible lesions and may affect any tissue or organ. Patients with VM can suffer severe disfigurement, painful swelling, chronic recurrent thrombi and increased risk for pulmonary thromboembolism (13, 28). The histological hallmarks of VM are slow-flow thin-walled ectatic veins with scant smooth muscle cells. Pioneering work by Miikka Vikkula's group identified germline or sporadic somatic activating *TIE2 (TEK)* mutations in about 60% of VM cases (29, 30, 31, 32). More recently, three independent studies described *PIK3CA* mutations in about 25% of VM patients (33, 34, 35). *PIK3CA* mutations are more frequent in intramuscular VM that do not involve the skin (35). *TIE2* and *PIK3CA* mutations are typically mutually exclusive but in few patients both occurred (33, 34, 35, 36). The result of *PIK3CA* or *TIE2* mutation is constitutively active PI3K signaling and downstream AKT activation. While ERK1/2 and STAT1 are activated in *TIE2*-mutant EC they are not in *PIK3CA*-mutant EC. Furthermore, AKT activation is higher in *PIK3CA*-mutated EC compared to *TIE2*-mutant EC. Our studies suggest this stronger AKT phosphorylation is linked to increased vessel density in a *PIK3CA*-mutated patient-derived xenograft model (35, 36, 37, 38). *PIK3CA* mutation-based models include a patient-derived EC xenograft and murine models with transgenic expression of *PIK3CA* p.H1047R in the embryonic mesoderm or in VE-Cadherin+ cells (33, 34, 39).

Combined vascular anomalies (two or more vascular malformations found in one lesion)

Capillary lymphatic venous malformation (CLVM)

Capillary lymphatic venous malformation (CLVM) includes complex lesions that involve overgrowth of multiple vascular components such as capillaries, lymphatics and veins. CLVM appears at birth and enlarge with time (12, 40). Two syndromes having these complex mixed vascular overgrowth lesions are Klippel-Trenaunay syndrome (KTS) (41) and Congenital Lipomatous Overgrowth, Vascular malformations, Epidermal nevi, Scoliosis/skeletal and spinal syndrome (CLOVES) (42). KTS diagnosis depends on three key features, cutaneous capillary malformations, venous and lymphatic malformations, and asymmetric hypertrophy of bones and overlying soft tissue (43, 44). The presentation of KTS is heterogeneous and there is substantial crossover in presentation of KTS with PIK3CA-Related Overgrowth Syndromes (PROS) (40). CLOVES syndrome is characterized by a combination of vascular, skin, lipomatous overgrowth and musculoskeletal abnormalities (45). *PIK3CA* somatic mutations have been identified in a high percentage (~90%) of KTS and CLOVES patients (18, 46, 47, 48). An interesting distinctive feature of KTS is that the *PIK3CA* variant p.C420R, relatively frequent in CLOVES, has not been reported in KTS patients (18, 46, 48, 49). A postnatal murine model of CLOVES was recently generated through ubiquitous expression of a dominant active *PIK3CA* transgene (*P110**) (50, 51, 52).

Other vascular anomalies

A range of overgrowth syndromes combined with vascular anomalies have been associated with *PI3KCA* mutations. Fibro-adipose vascular anomaly (FAVA) (18) is a disease characterized by fibrous and fatty tissue infiltrating the skeletal muscle and abnormalities in the veins or lymphatic vessels. In a recent study, affected tissue from around 60% of FAVA patients had *PIK3CA* mutations. Mosaic post-zygotic or rare germline *PIK3CA* mutations have also been detected in the affected tissue in patients with brain overgrowth or megalencephaly-capillary malformation syndrome (MCAP) (53).

Cellular origin of mutations

In all of the vascular anomalies discussed here, monoallelic expression of gain-of-function *PIK3CA* mutations was initially identified in the affected tissue from patients while not present in blood samples. The allelic frequency of *PIK3CA* mutations in LM tissue ranged from 0.8 to 10% in digital droplet (dd) PCR sequencing analysis (18) suggesting only a distinct cell population within the lesion express the genetic variant. Work from our group and others demonstrated that *PIK3CA* mutations are present in the LM-LEC population, while the non-EC isolated from the affected tissue are mutation-negative (19, 20, 21). In GLA patient tissue, *PIK3CA* variant allelic frequency ranged from 28–33% in EC (27). Similarly, we recently reported that EC isolated from 9/9 patient-derived VM tissue or lesional blood expressed TIE2 or/and *PIK3CA* mutations while non-EC were negative (36). In this study, the allelic frequency of 46.9% (TIE2 p.L914F) and 59.6% (PIK3CA p.C420R) was observed in VM-EC populations from two patients. One VM-EC population contained both a *TIE2* and a *PIK3CA* mutation and single-

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cell-derived clonal expansion revealed that every clone expressed both mutations. Conversely, few studies suggest that in CLVM and in overgrowth syndromes the *PIK3CA* mutations are expressed in the fibroblast population (49, 54, 55), although in these investigations purified EC were not analyzed. Our group have distinct preliminary data that in 7/7 CLVM patients (CLOVES and KTS) *PIK3CA* mutations are present solely in the EC purified from the tissue by CD31 expression (Le Cras T., unpublished) while the remaining cell populations (no CD31 expression detected) were negative. Correlations between any particular *PIK3CA* mutation and the clinical phenotype have not been demonstrated to date. The existence of the same PIK3CA mutations in simple (LM, VM) and combined (CLVM) vascular malformations suggest that it is not the type of mutation that induces different types of vascular anomalies. The type, location and/or severity of the vascular anomaly are likely dependent on when and/or what EC lineage or progenitor cell the mutational event occurs in during embryonic development. *PIK3CA* mutations that affect an early progenitor cell(s) may affect multiple EC lineages descended from those progenitor cell(s) and therefore affect more types of vessels than mutations in a committed cell lineage, although this has yet to be proven. More studies are needed to determine the exact cellular origin of CLVM and pinpoint the effect of the mutation distribution on the disease phenotype and severity.

Therapeutic targets and future treatments

Rapamycin

Rapamycin (Sirolimus) was introduced in 2011 as the first pharmacologic treatment for complicated vascular anomalies and has completely revolutionized the field (56). Prior to its introduction, targeted molecular therapies were not available for most of the anomalies discussed in this review and medical care consisted primarily of symptomatic treatment through surgery and sclerotherapy. Rapamycin is an immunosuppressant agent with efficacy in treating patients with Lymphangioleiomyomatosis (LAM), a lung disease with lymphatic hyperplasia (57). Rapamycin is an mTOR inhibitor that efficiently suppresses mTORC2-dependent AKT signaling in *PIK3CA*-mutant EC (Fig. 1). Rapamycin prevented VM lesion growth in different murine models (33, 37, 58) and restored lymphatic function in a GLA pre-clinical model (27). Rapamycin treatment significantly improved the quality of life of patients with complicated vascular anomalies with tolerable side-effects, although not always associated with lesion size reduction (37, 59, 60). Topical rapamycin was efficacious in patients with superficial microcystic LM lesions (61, 62). Mouse studies demonstrated that the mechanism of action of topical rapamycin is diminished phospho-AKT and phospho-S6 levels (63). Future studies should address the efficacy of rapamycin treatment in relation to the type of *PIK3CA* mutation and other genetic variants (60).

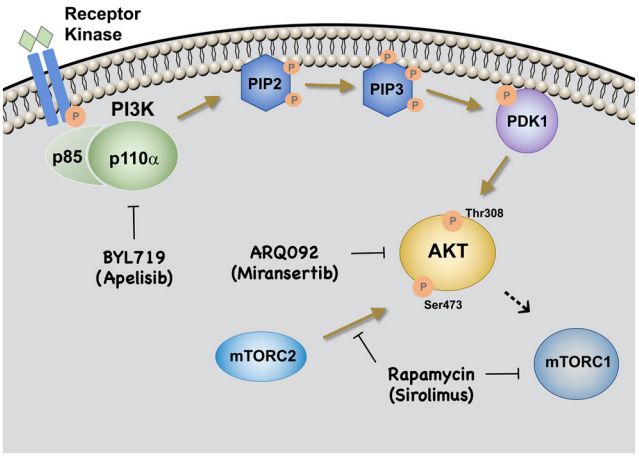

Figure 1
Targeted molecular therapies affecting PI3K pathway activation.

ARQ092

ARQ092 (Miransertib) is an allosteric pan-AKT inhibitor. Several clinical trials for the use of ARQ092 in adult cancer are ongoing (https://clinicaltrials.gov:NCT02476955, NCT01473095). A low dose of ARQ092 (5 mg/m²/day) reduced the levels of phospho-AKT by about half in 83% of biopsies from Proteus patients. Proteus is an overgrowth syndrome caused by somatic activating AKT mutations (p.E17K) (64). Studies using fibroblasts from patients with PIK3CA-related overgrowth syndromes showed that ARQ092 has potent antiproliferative activity (54, 55, 65, 66). Together, these findings lead to a phase I/II clinical trial for the use of ARQ092 for treatment of PIK3CA-related overgrowth syndromes and vascular anomalies (NCT03094832). Expanded access to this drug was recently granted by the FDA (NCT03317366).

BYL719

BYL719 (Apelisib) is a PI3K p110α specific inhibitor. It has a half-maximum inhibitory concentration of 4.6nM for the

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α isoform, while 55–251-fold higher doses are needed to inhibit the β, γ and δ isoforms (67). BYL719 has a tolerable safety profile and encouraging preliminary activity in patients with *PIK3CA*-altered solid tumors (68). Promising results were also obtained in eight CLOVES patients in a study conducted in France where treatment with BYL719 was granted under compassionate use (52). In pre-clinical studies, BYL719 effectively prevented organ dysfunction and death in transgenic dominant active *PIK3CA* mice. In this study and in an independent *in vitro* investigation (35), BYL719 was more effective than rapamycin as it more efficiently blocked AKT phosphorylation at both Serine 473 and Threonine 308. Interestingly, BYL719 treatment also decreased VM lesion size and promoted apoptosis in a transgenic *PIK3CA*^{H1047R} VM murine model (33). A topical formulation of BYL719 was also efficacious in VM superficial lesions (33).

Whether the benefits of long-term treatment with these inhibitors will be limited by side-effects is still unclear although early results seem promising. In cancer, it has been shown that inhibition of the PI3K pathway leads to proliferative arrest rather than cell death, and combination of BYL719 with mTOR inhibitor enhanced the antitumoral activity (69, 70, 71). The combination of PI3K/AKT inhibitors with rapamycin for the treatment of PIK3CA-related vascular anomaly patients could enhance clinical response. Much work still needs to be done in pre-clinical models to explore the efficacy of these therapeutic strategies.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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