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Decoding class II PI3K signaling: From membrane identity to human disease☆

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ABSTRACT

This review provides an integrated overview of the current understanding of class II PI3Ks, with particular attention to their structural and enzymatic properties, lipid substrate specificity, and emerging roles in membrane trafficking, cellular signaling, and disease. Class II phosphoinositide 3-kinases (PI3Ks) are lipid kinases that regulate membrane identity and intracellular signaling by generating phosphatidylinositol 3-phosphate [PI(3)P] and phosphatidylinositol (3,4)-bisphosphate [PI(3,4)P_2] at distinct subcellular compartments. Advances over the past decade have clarified the structural organization, regulatory principles, and lipid output of all the three mammalian isoforms (PI3K-C2α, PI3K-C2β, and PI3K-C2γ). These studies have revealed that class II PI3K function is highly context-dependent, governed by compartment-specific cues and the spatial restriction of lipid products. Dysregulation of class II PI3Ks has been implicated in diverse pathological conditions, including cancer, metabolic disorders, epilepsy, congenital myopathies, vascular dysfunction, and premature aging. These findings establish a framework for understanding how localized phosphoinositide synthesis contributes to cellular homeostasis and disease, and underscore the therapeutic potential of selectively targeting class II PI3K isoforms.

1. Introduction

Phosphoinositide 3-kinases (PI3Ks) are a conserved family of lipid kinases that regulate a broad range of cellular processes by phosphorylating the 3′-hydroxyl group of the inositol ring of phosphoinositides (PtdIns). Through the generation of 3-phosphorylated phosphoinositide species, PI3Ks modulate intracellular signaling and membrane trafficking pathways, regulating cell growth, survival, migration, metabolism, and development. Based on structural organization, substrate specificity, and regulatory mechanisms, the PI3K family is divided into three classes: I, II, and III. Class I PI3Ks have been extensively investigated due to their involvement in growth factor signaling and cancer, while the class III enzyme Vps34 is well established as a central regulator of autophagy [1]. In contrast, class II PI3Ks have remained relatively understudied. First identified in *Drosophila melanogaster*, class II enzymes are conserved in vertebrates and are represented in mammals by three homologous isoforms: PI3K-C2α, PI3K-C2β, and PI3K-C2γ, encoded by the genes *PIK3C2A*, *PIK3C2B*, and *PIK3C2G*, respectively (Fig. 1a). PI3K-C2α and PI3K-C2β are broadly expressed across tissues, whereas PI3K-C2γ displays a more restricted pattern, predominantly in metabolic organs such as the liver and pancreas [2].

Class II PI3Ks are distinguished from their class I and III counterparts by their dual substrate specificity: while they preferentially phosphorylate phosphatidylinositol (PI), they are also capable of using phosphatidylinositol 4-phosphate [PI(4)P] as a substrate, producing PI(3)P and phosphatidylinositol (3,4)-bisphosphate [PI(3,4)P_2], respectively. This biochemical versatility supports their involvement in diverse physiological processes including clathrin-mediated endocytosis, receptor recycling, nutrient stress response, angiogenesis, ciliogenesis, and cytokinesis. In addition, recent studies have implicated class II PI3Ks in pathological contexts, highlighting their potential as therapeutic targets. Notably, the resolution of the PI3K-C2α crystal structure has yielded important insights into the activity and regulation of this enzyme class and may support the development of isoform-selective

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inhibitors.

This review provides an integrated overview of the current understanding of class II PI3Ks, with particular attention to their structural and functional properties and their roles in health and disease. Dedicated sections will focus on each of the three mammalian isoforms, summarizing recent findings that have expanded our understanding of their distinct structural and regulatory principles as well as their emerging biological functions.

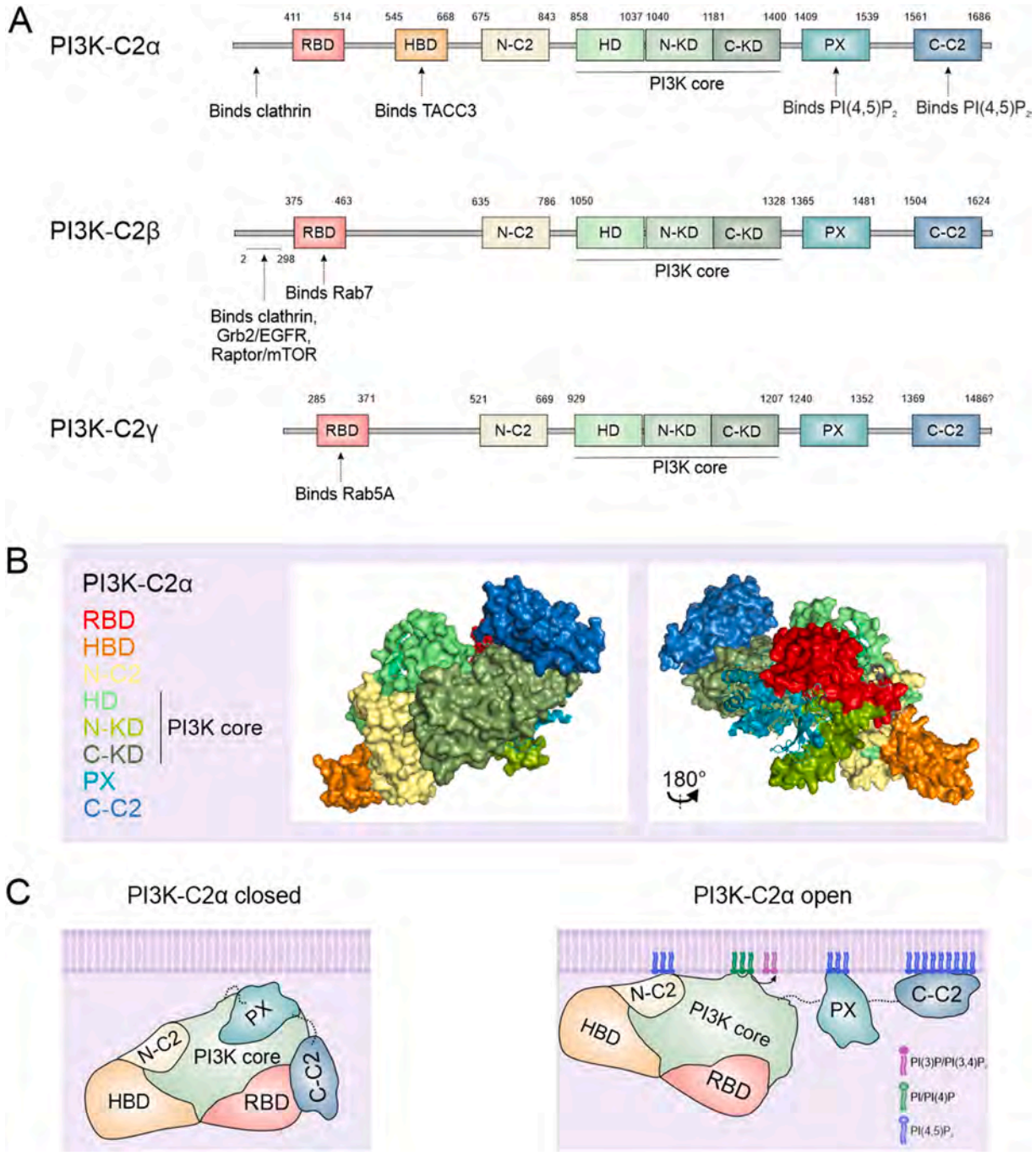

Fig. 1. A. Schematic representation of the domains architecture of the Class II Phosphoinositide 3-Kinase (PI3K) family. PI3K-C2α, -C2β, and -C2γ share both a conserved core (PI3K core), consisting of a helical domain (HD) and a bilobed catalytic domain (N-KD and C-KD), and a C-terminal PX-C2 module. Additionally, PI3K-C2α and -C2β contain an N-terminal clathrin-binding region followed by a Ras-binding domain (RBD). PI3K-C2α possesses a helical bundle domain (HBD), which mediates interaction with TACC3. Predictions by AlphaFold suggest the presence of this domain in the other two paralogues but a formal demonstration is missing. B. Structural model of PI3K-C2α. The model was generated by integrating available cryo-EM and X-ray crystallographic data (PDB entries 7BI2 and 6BTY). Individual domains are coloured according to the schematic representation. C. Model of PI3K-C2α activation. In its closed, inactive conformation, the PX and C—C2 domains form inhibitory contacts with the catalytic core and the RBD. Upon coincident membrane binding of both domains, the enzyme adopts an open and active conformation, which exposes the RBD and the PI3K core, allowing interaction with its lipid substrates. Given the high degree of structural conservation among class II PI3Ks, it is likely that PI3K-C2β and PI3K-C2γ adopt a similar auto-inhibited conformation, in which their PX and C-terminal C2 domains interact with the catalytic core and RBD to maintain the enzyme in an inactive state until membrane binding triggers activation. Additional protein-binding partners that regulate PI3K-C2α activation are omitted here for clarity.

2. Class II PI3K alpha

PI3K-C2α is the most extensively characterized member of the class II PI3K family. It is distinguished by its unique biochemical versatility, which supports its involvement in a broad range of cellular and physiological processes. These include endocytosis, vesicle recycling, ciliogenesis, and mitosis, as well as both physiological and pathological mechanisms such as tissue development during embryogenesis. In particular, it plays roles in angiogenesis, primary cilium dynamics and eye lens transparency and is also implicated in pathological conditions such as premature aging and cancer.

Recent structural studies have uncovered a distinct autoregulatory mechanism governing PI3K-C2α activity, which sets it apart from class I and class III PI3Ks that require regulatory subunits for modulation. In the case of PI3K-C2α, activation and membrane recruitment are orchestrated through its intrinsic domain architecture, enabling localized control of lipid kinase function without auxiliary proteins [3,4]. These insights have significantly refined the understanding of how this enzyme regulates PtdIns synthesis. Although initial studies identified PI(3)P as its primary lipid product [5], accumulating evidence now suggests that PI(3,4)P_2 is likely the dominant output in many cellular contexts [6], underscoring the dynamic and context-dependent nature of PI3K-C2α lipid signaling.

An additional layer of complexity in the study of PI3K-C2α arises from the observation that its complete genetic deletion leads to embryonic lethality in mice, whereas humans lacking functional *PIK3C2A* survive. In mice, *Pik3c2a* knockout results in death during mid-gestation, while in humans, loss-of-function mutations in *PIK3C2A* have been identified in a small number of individuals who develop oculoskeletodental syndrome, a rare syndromic condition with signs related to ciliopathies as well as premature aging. This pronounced phenotypic divergence suggests that PI3K-C2α fulfills essential roles through mechanisms that are functionally complex and only partially conserved across species.

The following sections first examine recent advances in the structural characterization of PI3K-C2α, highlighting insights into domain organization, regulatory mechanisms, and lipid substrate recognition. Subsequent sections review the latest discoveries concerning the cellular functions and physiological roles of PI3K-C2α, with particular attention to how its dysregulation contributes to human disease.

2.1. Structure and regulation of the PI3K-C2α

A recent high-resolution crystal and 4.4-Å cryo-EM structures of PI3K-C2α have revealed a conserved catalytic core composed of the C2 domain, Ras-binding domain (RBD), helical domain, and kinase domain, which resembles that of class I PI3Ks [4] (Fig. 1a,b). In this context, PI3K-C2α enzymatic activity resides in a bilobal kinase domain, comprising N- and C-lobes, that forms a catalytic cleft responsible for ATP and lipid substrate binding. Key mobile elements within this region, including the activation loop, catalytic loop, and P-loop, undergo conformational changes during substrate binding and phosphotransfer [7,8]. Unlike class I PI3Ks, which possess two polybasic motifs for substrate recognition—an N-terminal basic box and a C-terminal KRER sequence—PI3K-C2α retains only a single polybasic motif at the C-terminus, which corresponds to a variant sequence (KRDR) [9]. Structural data show that lysine and arginine residues within the activation loop (K1283, R1284) generate a positively charged pocket, facilitating binding to the 4-phosphate group of PI(4)P, an interaction absent in the PI-selective class III PI3K [4]. This enables PI3K-C2α to produce both PI(3)P [2,5,10,11] and PI(3,4)P2 [6,12], highlighting its unique catalytic capability to generate PI(3,4)P2 directly from PI(4)P [4].

In addition, PI3K-C2α shows a four-helix bundle (HBD), formed by a ~ 100-residue insertion, which is conserved in all mammalian class II PI3Ks and supports the kinase domain, likely acting as a structural scaffold [4]. The HBD is connected to the N-terminal C2 (N—C2) domain, characterized by a β-sandwich fold and a basic loop possibly involved in phospholipid binding, via a flexible linker [4]. Membrane association is a critical determinant of PI3K-C2α activity, as catalytic output is tightly coupled to conformational state (Fig. 1c). Unlike class I and class III PI3Ks, which are recruited and activated through stable interactions with regulatory subunits, class II PI3Ks do not require accessory subunits for allosteric activation and PI3K-C2α is regulated through dynamic, domain-driven interactions with membranes. Structural and biochemical evidence shows that PI3K-C2α, when in solution, adopts a closed conformation in which internal autoinhibitory contacts between domains restrict access to the lipid substrate [4]. Membrane binding is thought to induce a conformational rearrangement that disrupts these autoinhibitory interactions, thereby exposing the catalytic site and enabling lipid phosphorylation (Fig. 1c).

Three different domains contribute to this membrane-triggered activation mechanism, thereby licensing enzymatic activity in specific membrane contexts [13]: a) protein interactions involving the intrinsically disordered N-terminal region, including the clathrin-binding domain, b) association to small GTPases through the Ras-binding domain (RBD) and c) lipid sensing mediated by the PX and C2 domains [13] (Fig. 1c). The unstructured N-terminal region plays a central role in the recruitment of PI3K-C2α by mediating interaction with clathrin. Clathrin enhances the activity of both PI3K-C2α and PI3K-C2β [14,15], and its depletion disrupts PI3K-C2α localization to clathrin-coated pits, highlighting its essential role in clathrin-mediated endocytosis [6,16]. However, the precise mechanism through which clathrin enhances kinase activity is still not fully understood. Direct evidence for small GTPase binding to the RBD of PI3K-C2α is limited. Nonetheless, other class II isoforms such as PI3K-C2β and PI3K-C2γ are known to be recruited to endosomal membranes via Rab GTPases in response to specific stimuli [17,18]. Structural and biochemical data suggest that a similar mechanism may apply to PI3K-C2α. Endocytic Rab proteins may interact with the RBD and displace the inhibitory distal C2 domain, especially in the presence of clathrin and PI(4,5)P_2. This interaction facilitates a conformational transition of the enzyme into an open and active state [4, 5, 11, Franco, 2014 #57]. The PX and C2 domains are critical for both membrane association and regulation of catalytic activity. These domains exhibit high affinity for PI(4,5)P_2 [19], although some studies suggest that the C2 domain may also interact with PI(3,4)P_2 or PI(3,4,5)P_3 [20]. Their spatial arrangement allows for cooperative lipid binding, which increases PI3K-C2α affinity for membranes enriched in PI(4,5)P_2 [4,19]. Calcium ions may influence the interaction between the C2 domain and lipids, although they are not strictly required for binding [19,20]. The PX and C2 domains also regulate a conserved structural element known as the regulatory arch, which consists of helices kα10 to kα12 and controls access to the catalytic site [21]. In the inactive conformation, helix kα12 forms two autoinhibitory contacts. The first, referred to as closed contact I, is formed with the loop between helices kα7 and kα8. The second, known as closed contact II, is formed between the C2 domain and the RBD. These interactions stabilize the inactive state by locking the activation and catalytic loops [4]. Mutations that disrupt either contact result in increased PI3K-C2α activity, while combined mutations produce a synergistic activation effect [4,22]. Upon activation, the PX and C2 module detaches from the RBD, allowing helix kα12 to reposition and form a stabilizing hydrogen bond via residue H1391 with the activation loop. Mutation of H1391 abolishes kinase activity, underscoring its critical role in regulation [4]. Structural studies have also proposed that the C-terminal C2 domain of PI3K-C2α may mediate dimerization [13,23]. Although the functional significance of this dimerization remains uncertain, it may contribute to the regulation or subcellular targeting of the enzyme. Altogether, these regulatory mechanisms confine catalytic activity to specific subcellular locations enriched in PI(4,5)P_2, such as late-stage endocytic pits [6,16], the base of primary cilia [5], and the midbody region during cytokinesis [12].

2.2. PI3K-C2α function in the endocytic and exocytic trafficking

Since its initial characterization, PI3K-C2α has been closely associated with clathrin function. Early studies using the first available antibodies and tagged protein expression systems revealed an expression pattern for PI3K-C2α that closely matched that of clathrin [14]. In this context, PI3K-C2α controls clathrin mediated endocytosis (CME), where it regulates PtdIns metabolism and membrane remodeling at clathrin coated pits (CCP). Coincidental recognition of clathrin together with membranes enriched in PI(4,5)P_2 promotes the localized production of PI(3,4)P_2, which is essential for the recruitment of effectors such as SNX9, SNX18, and FCHSD2 that support the maturation of clathrin coated pits and the scission of vesicles by dynamin [24,25] (Fig. 2a). Loss of either PI3K-C2α or PI(3,4)P_2 impairs the late stages of CCP maturation, leading to the accumulation of incomplete endocytic intermediates [25].

Although the role of PI3K-C2α in CME is well recognized, the exact mechanism underlying effector recruitment remains a topic of discussion. One proposed model suggests that PI(3,4)P_2 is quickly converted into PI(3)P by the phosphatase INPP4A, which in turn promotes SNX9 recruitment and F-actin polymerization [26]. However, recent findings indicate that the synthesis and hydrolysis of PI(3,4)P_2 occur in separate cellular compartments. PI3K-C2α synthesizes PI(3,4)P_2 at CCPs, while INPP4A is localized and active at endosomes, where it mediates PI(3,4)P_2 hydrolysis [25]. Consistently, loss of INPP4A does not affect CCP dynamics, suggesting it is not essential for CME. Instead, INPP4A regulates endosomal PI(3,4)P_2 levels and links endocytic trafficking to Akt and mTORC1 signaling, particularly in cancer cells. In the absence of INPP4A or INPP4B, other phosphatases such as PTEN may compensate for PI(3,4)P_2 hydrolysis at endosmes [25].

Alongside its established role in CME, PI3K-C2α also contributes to clathrin-mediated pinocytosis, functioning in parallel with PI3K-C2β [24]. Although both enzymes are required for fluid-phase cargo uptake, they operate through distinct mechanisms: PI3K-C2α is predominantly localized at clathrin-coated structures, where it supports vesicle formation and internalization, while PI3K-C2β associates with actin-rich membrane regions such as ruffles, promoting F-actin polymerization during vesicle maturation [24]. In addition to its involvement in endocytic pathways, PI3K-C2α has also been implicated in exocytic trafficking processes. Early studies demonstrated its role in neurosecretory granule exocytosis [27], mast cell degranulation [28], and insulin granule release in pancreatic beta cells [29]. More recently, PI3K-C2α has been shown to regulate the delivery of the delta opioid receptor (δOR) from the Golgi to the plasma membrane, further supporting its broader involvement in vesicle trafficking beyond the endocytic axis [30].

Whether these diverse cellular functions can explain the phenotypic traits observed in individuals with PI3K-C2α deficiency [31–33] remains uncertain. However, it is reasonable to speculate that impaired endocytic or exocytic trafficking in neuronal cells may disrupt synaptic signaling, potentially contributing to the intellectual disability observed in some of loss of function patients [32].

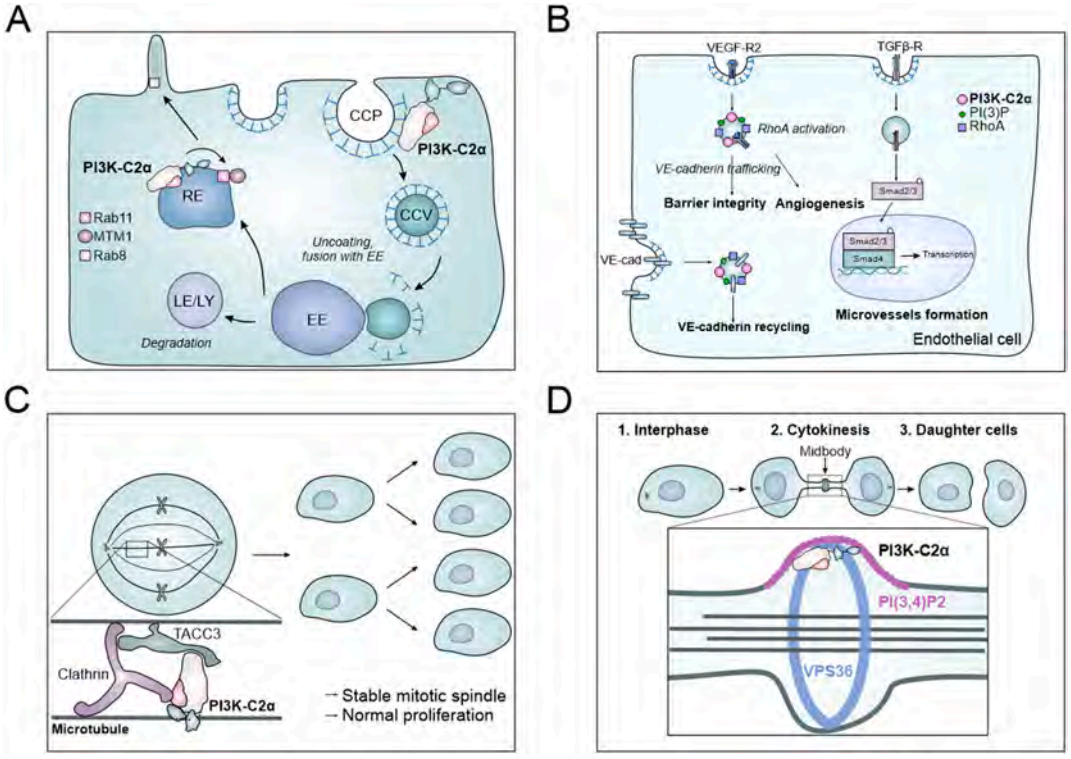

Fig. 2. A. PI3K-C2α function in clathrin-mediated endocytosis (CME) and ciliogenesis. During CME, PI3K-C2α interacts with clathrin via its N-terminal clathrin-binding domain. At clathrin-coated pits (CCPs), it generates PI(3,4)P_2, which is essential for CCPs fission. In ciliated cells, PI3K-C2α localizes to recycling endosomes (RE), where it produces PI(3)P, promoting the activation of the small GTPase Rab11. This in turn triggers Rab8 accumulation at the primary cilium, facilitating its elongation and proper protein trafficking. B. PI3K-C2α in endothelial cells. PI3K-C2α regulates CME of VEGFR2 and TGFβ receptors in endothelial cells, promoting vascular barrier integrity, angiogenesis, and microvessel formation. Additionally, PI3K-C2α-mediated CME is essential for the recycling of VE-cadherin. C. PI3K-C2α function in mitotic spindle stability. During metaphase, PI3K-C2α concomitantly binds clathrin and the motor spindle protein TACC3 through its N-terminal and HBD domain, respectively. This scaffolding function allows the crosslinking of kinetochore fibres, promoting proper spindle organization and chromosomes alignment. D. Schematic representation of PI3K-C2α function during abscission. In late cytokinesis, PI3K-C2α localizes to the midbody, where it generates a pool of PI(3,4)P_2, which anchors the ESCRT-II component VPS36 to the plasma membrane ensuring successful completion of cytokinesis.

2.3. PI3K-C2α role in angiogenesis and platelets with implication for thrombosis

These functional roles in endocytosis are consistent, at least in part, with the phenotype observed in the first *Pik3c2a* knockout mouse model, which exhibited embryonic lethality [34]. This developmental arrest pointed to a requirement for PI3K-C2α during embryogenesis, particularly in the regulation of endothelial cell function, the support of angiogenic signaling, and the maintenance of vascular barrier integrity (Fig. 2b).

Endothelial-specific knockout of PI3K-C2α in mice results in embryonic lethality due to defective angiogenesis and increased vascular permeability [34]. Mechanistically, PI3K-C2α regulates the endocytic trafficking and signaling of key receptors such as VEGFR2 and S1P1 [34,35]. Its loss impairs downstream activation of RhoA and Rac1, thereby disrupting VE-cadherin dynamics, endothelial cell migration, proliferation, and junction formation [34]. Additionally, both PI3K-C2α and PI3K-C2β contribute to endothelial cells morphogenesis in response to sphingosine-1-phosphate (S1P) and its carrier high-density lipoproteins (HDL). In this context, PI3K-C2α primarily supports endothelial cells survival, whereas PI3K-C2β, together with the class I isoform p110γ, regulates migration and branching. [36].

Beyond this, PI3K-C2α also facilitates TGFβ signaling by generating PI(3,4)P2 at endosomes [37], which is essential for TGFβ receptor internalization and Smad2/3 activation. Upon TGFβ binding, ALK5 activation initiates a phosphoinositide signaling cascade involving depletion of PI(4,5)P2 via synaptojanin-1, accumulation of PI(4)P, and PI3K-C2α -mediated production of PI(3,4)P2. This lipid is subsequently converted to PI(3)P by INPP4B, supporting Smad signaling and microvessel formation [37,38]. These molecular and cellular functions of PI3K-C2α in maintaining endothelial integrity and regulating vascular signaling pathways may help explain the cerebrovascular manifestations, including brain strokes and early-onset glaucoma, observed in patients with *PI3KC2A* deficiency [39].

In line with its broader role in vascular biology, PI3K-C2α is also essential for platelet function in mice, particularly in maintaining the open canalicular system (OCS), an internal membrane network that is disrupted in both PI3K-C2α-deficient and kinase-dead models [40–43]. This platelet phenotype further supports a vascular contribution to the cerebrovascular symptoms observed in PI3KC2A-deficient patients. Reduced PI3K-C2α levels provide protection against arterial thrombosis without affecting normal hemostasis [40,41]. Subsequent studies using PI3K-C2α inhibitors showed that pharmacological inhibition reproduces the antithrombotic effects seen in genetic models, reinforcing the enzyme's role in preserving OCS integrity [44]. Although the precise molecular mechanisms remain incompletely defined, PI3K-C2α appears to be crucial for maintaining OCS structure by modulating localized PI(3)P dynamics, thereby impairing prothrombotic platelet functions without interfering with canonical platelet activation pathways [44]. Importantly, unlike standard antiplatelet therapies, PI3K-C2α inhibition remains effective under high shear stress conditions without increasing bleeding risk, and eventually supporting its potential as a complementary strategy to current antithrombotic treatments [44].

2.4. PI3K-C2α in endosomal trafficking and primary cilium dynamics

A second *Pik3c2a* knockout mouse model, published a few years after the initial study, confirmed early embryonic lethality but revealed additional developmental defects suggestive of a ciliopathy phenotype [5]. This later study identified defects in axial rotation, cardiac looping, and left–right asymmetry, all hallmarks of disrupted ciliary function. These phenotypes were attributed to abnormal cilia morphology in the ventral node. Specifically, reduced cilia length and impaired downstream signaling were linked to defective formation and function of the pericentriolar recycling endocytic compartment at the base of the primary cilium. This defect was associated with impaired PI(3)P production and defective Rab11 activation, both of which are essential for the targeted delivery of functional proteins to the primary cilium [5] (Fig. 2a).

Further studies have shown that the pool of PI(3)P generated by PI3K-C2α at the base of the primary cilium is not only critical for ciliogenesis but also for maintaining ciliary signaling, particularly under physiological conditions such as fluid shear stress that activate autophagy through cilium-dependent pathways [10,45]. In this context, PI3K-C2α is upregulated at both transcript and protein levels and is recruited to the ciliary base, where it catalyzes localized PI(3)P production. This lipid pool supports autophagic signaling specifically linked to mechanical stimulation. While nutrient deprivation–induced autophagy primarily relies on the class III PI3K VPS34, which generates PI(3)P at autophagosomal membranes [3,46–49], VPS34 is dispensable under shear stress conditions, underscoring the distinct and non-redundant role of PI3K-C2α in this cilia-mediated autophagic response.

These mechanistic insights have been supported by studies in primary cells derived from individuals with homozygous *PIK3C2A* mutations resulting in complete loss of the protein [33]. Consistent with findings in *Pik3c2a* knockout mouse embryos, these cells exhibit aberrant ciliary morphology, including shortened cilia and accumulation of transport proteins. A more recent study confirmed and expanded upon these findings by analyzing a patient with compound heterozygous *PIK3C2A* mutations—one nonsense and one missense variant abolishing catalytic activity [31]. Despite normal protein levels, the enzymatic defect led to a significant reduction in PI(3)P, particularly at the ciliary base, resulting in defective cilia elongation and impaired Sonic Hedgehog (SHH) signaling. These cellular phenotypes closely resemble those observed in *Pik3c2a*$^{-/-}$ mouse embryonic fibroblasts [5], and are likely responsible for the ciliopathy-associated features seen in patients, including craniofacial and skeletal abnormalities, deafness, and neurodevelopmental impairment. However, the clinical presentation extends beyond typical ciliopathy manifestations, suggesting that additional PI3K-C2α functions unrelated to SHH signaling or ciliary structure contribute to the disease. Taken together, these findings underscore the multifaceted nature of the *PIK3C2A* -related syndrome and the broad cellular roles of PI3K-C2α.

2.5. PI3K-C2α functions during mitosis

Another key phenotypic observations in patients with *PIK3C2A* loss-of-function mutations is reduced stature, often accompanied by features of premature aging such as early-onset cataracts [33]. These traits are likely rooted in impaired cellular proliferation, possibly reflecting premature exhaustion of tissue-specific stem and progenitor cell populations. In line with this, several studies have demonstrated that PI3K-C2α is required for proper execution of mitosis, where it contributes to distinct phases of cell division through mechanistically separable functions.

During metaphase, PI3K-C2α contributes to spindle stability and proper chromosome alignment through a kinase-independent, scaffold-based mechanism. Loss of PI3K-C2α in mouse embryonic fibroblasts or cancer cells leads to a spectrum of mitotic abnormalities, including shortened spindle length, delayed onset of anaphase, chromosome misalignment, and increased chromosomal instability—defects that reflect impaired kinetochore microtubule attachment and disruption of inter-microtubule crosslinking [50]. These structural defects have been mechanistically linked to the HBD, a domain within PI3K-C2α that protrudes into the cytoplasm and serves as a platform for multiple protein–protein interactions. This domain mediates the non-catalytic engagement of PI3K-C2α with the spindle-associated protein TACC3, anchoring the kinase to the mitotic apparatus [4], thereby stabilizing spindle architecture (Fig. 2c).

In contrast, during cytokinesis PI3K-C2α operates in a lipid kinase–dependent manner that is essential for abscission, the terminal stage of cell division (Fig. 2d). In cells lacking PI3K-C2α, abscission is delayed

or fails to occur, leading to persistent intercellular bridges as well as cell-refusion, and eventual binucleation [12,51]. During late cytokinesis, PI3K-C2α is recruited to the midbody through coordinated interactions with PI(4,5)P_2 and γ-tubulin. Its PX–C2 domain module mediates binding to PI(4,5)P_2 at the midbody membrane, while a distinct region within the helical domain interacts with γ-tubulin, ensuring precise localization of the kinase at the site of abscission. Here, PI3K-C2α generates a localized pool of PI(3,4)P_2 that serves as a spatial cue to stabilize the recruitment of VPS36, a core subunit of the ESCRT-II complex. Binding of VPS36 to PI(3,4)P_2 enables its accumulation at the midbody, thereby facilitating the hierarchical assembly of the ESCRT-III machinery required for secondary ingression and membrane fission. In the absence of PI3K-C2α, VPS36 fails to localize to the midbody, recruitment of the ESCRT-III subunit CHMP4B is impaired, and abscission is either delayed or aborted, ultimately resulting in the formation of binucleated cells [12]. In some cell types, these defects could be compensated by a parallel, ALIX-mediated mechanism, that independently promotes ESCRT-III assembly. However, in tissues with low ALIX expression, such as the lens epithelium, the PI3K-C2α-dependent pathway is essential. In this context, loss of PI3K-C2α results in persistent midbodies, binucleation, and the onset of cellular senescence, characterized by increased $p16^{INK4A}$ expression and secretion of senescence-associated cytokines (also known as senescence-associated secretory phenotype or SASP), ultimately leading to early-onset cataract. This phenotype is consistent across species: lenses of zebrafish embryos carrying *pik3c2a* null alleles display elevated $p16^{INK4A}$ and senescence-associated β-galactosidase levels; conditional deletion of *Pik3c2a* in adult mice induces similar senescence markers and cataract formation. In human patients with *PIK3C2A* null mutations, early-onset cataract is one of the most consistent and penetrant clinical features of the disease [31–33].

While the precise molecular connection between cytokinesis failure and the early onset of cellular senescence remains unclear and requires further investigation, a similar constellation of clinical features is not unique to *PIK3C2A* deficiency but is also observed in Lowe syndrome, a congenital disorder caused by mutations in *OCRL*, which encodes another phosphoinositide-metabolizing enzyme, a phosphatase that removes the phosphate at the 5-hydroxyl position of inositol rings within PtdIns [52]. Notably, Lowe syndrome also presents with early-onset cataract and reduced stature [53], two hallmark traits shared with *PIK3C2A* deficiency. In both conditions, defects in cytokinesis have been reported [12,54], suggesting that impaired resolution of cell division may contribute to diminished organ growth and progressive tissue dysfunction. These parallels raise the possibility that cytokinetic failure, followed by the activation of senescence programs, represents a shared mechanistic axis linking phosphoinositide dysregulation to developmental growth impairment and premature aging phenotypes. Within this framework, the dual role of PI3K-C2α during mitosis, scaffold-based stabilization of the spindle and kinase-dependent regulation of abscission, may represent a central determinant of proliferative capacity and tissue maintenance in both physiological and pathological settings. Nonetheless, despite the progress achieved thus far, the extent to which these mechanisms causally account for the clinical features observed in *PIK3C2A* -deficient individuals remains to be fully elucidated.

2.6. PI3K-C2α role in cancer progression and cell migration

Given its function during mitosis, PI3K-C2α has emerged as a context-dependent regulator of tumor progression and a potential therapeutic target. Reduced expression of PI3K-C2α impairs spindle stability during metaphase, which may initially constrain tumor growth by slowing mitotic progression but can ultimately promote chromosomal instability and aggressive behavior due to defective spindle checkpoint activity. Notably, this vulnerability can be exploited therapeutically: tumors with low PI3K-C2α levels display increased sensitivity to microtubule-targeting agents (MTAs) such as taxanes [50,55].

Recent findings have further elucidated the molecular connection between PI3K-C2α and microtubule homeostasis through a pathway that regulates tubulin autoregulation [55]. Tubulin autoregulation is a quality control mechanism that limits tubulin mRNA levels when free, unpolymerized tubulin accumulates, thereby preventing excessive tubulin synthesis. PI3K-C2α participates in this process by interacting with TTC5, a factor that recognizes nascent tubulin chains and promotes mRNA degradation. This interaction is modulated by CARM1, a methyltransferase that modifies PI3K-C2α at residue R175. Methylation stabilizes PI3K-C2α and enables it to sequester TTC5, temporarily suppressing tubulin mRNA degradation. When PI3K-C2α is unmethylated, it becomes unstable and is rapidly degraded, releasing TTC5 to resume the degradation of tubulin transcripts. Disruption of this axis—either through CARM1 knockdown or loss of PI3K-C2α—leads to lower tubulin mRNA levels, defective microtubule assembly, mitotic arrest, and reduced proliferation in cancer cells. These cells also exhibit heightened sensitivity to MTAs such as paclitaxel and vincristine, suggesting that the CARM1–PI3K-C2α–TTC5 pathway not only governs microtubule dynamics but may also serve as a determinant of MTA responsiveness in cancer therapy.

Beyond its role in mitosis, PI3K-C2α has been implicated in cancer metastasis through its kinase activity [56]. High expression of PI3K-C2α correlates with greater tumor aggressiveness and invasiveness in breast cancer models [50, Neve, 2006 #102]. While overall tumor growth may remain unchanged, metastatic spread significantly increases. Overexpression of PI3K-C2α alters the actin cytoskeleton, promoting membrane ruffling, faster focal adhesion turnover, and enhanced motility [56]. Mechanistically, PI3K-C2α is recruited to focal adhesions via its HBD domain, where it interacts with FAK and produces localized pools of PI(3,4)P_2 [56]. This lipid enhances FAK signaling and promotes focal adhesion disassembly. PI(3,4)P_2 also recruits RASA3, which inactivates R-RAS, a small GTPase involved in cell adhesion and migration [57–60]. In cells overexpressing PI3K-C2α, R-RAS activity is significantly reduced, leading to shorter focal adhesion lifetimes and increased migration. This effect depends on the kinase function of PI3K-C2α and is not observed in cells expressing a kinase-dead mutant [56]. Expression of a constitutively active R-RAS mutant restores adhesion stability, confirming this mechanism. An in vivo zebrafish model supports the role of this pathway in metastasis. Breast cancer cells overexpressing PI3K-C2α formed more metastatic foci, and this effect was diminished by RASA3 knockdown [56]. These findings highlight the importance of PI(3,4)P_2 accumulation in disrupting focal adhesion stability. By promoting RASA3 recruitment and R-RAS inactivation, PI3K-C2α overexpression enhances cell motility and metastatic dissemination. This signaling axis identifies PI3K-C2α and RASA3 as promising targets for therapeutic intervention in metastatic breast cancer.

2.7. PI3K-C2α in vascular stress responses and endothelial cell survival

Interest in PI3K-C2α as a therapeutic target for breast cancer metastasis [56] and arterial thrombosis [44], together with the contrast between mouse knockouts that result in early embryonic lethality [5,34] and human PIK3C2A-null patients who can survive into adulthood [31–33], underscored the importance of generating a model that inactivates the catalytic function during adulthood rather than relying on complete genetic loss. This rationale led to the development of the first inducible Pik3c2a mouse line designed to abolish lipid kinase activity while preserving scaffold functions.

This model was created through a dual-allele approach: one allele in which the catalytic motifs were excised, producing a shortened and unstable protein, and another carrying a point mutation (D1268A) previously shown to block catalytic activity [61]. Combined with a tamoxifen-inducible Cre driver, this strategy ensured efficient postnatal inactivation of kinase function while maintaining residual protein for scaffold-related roles. The model was thus intended to more closely mimic the effects of pharmacological PI3K-C2α inhibition than complete knockout approaches [62].

Under baseline conditions, systemic inactivation in adult mice was well tolerated, consistent with reports of human PIK3C2A-null or loss-of-function carriers [31–33]. Strikingly, however, mice became highly susceptible to lipopolysaccharide (LPS)–induced endotoxic shock, revealing a previously unrecognized role for PI3K-C2α in inflammatory stress. This vulnerability was shown to be endothelium-specific: vascular endothelial deletion reproduced the systemic phenotype, whereas myeloid deletion had no effect. The response was dependent on caspase-8 and RIPK3–mediated extrinsic apoptosis, indicating that PI3K-C2α protects endothelial cells from cytokine-driven death receptor signaling during systemic inflammation [62].

Compared to the earlier constitutive knockout models, which revealed that PI3K-C2α is essential for embryonic development, angiogenesis, vascular integrity [34,35], ciliogenesis [5], and mitosis [12,50], the inducible adult kinase-dead model offers a complementary perspective. By preserving noncatalytic scaffold functions and restricting kinase inactivation to postnatal stages, this model bypasses the early lethality observed in total-body knockouts, more closely reflecting the relative tolerance seen in human PIK3C2A-deficient individuals. While earlier models highlighted developmental and proliferative roles, the inducible model uncovers a previously unappreciated, stress-specific function: PI3K-C2α protects endothelial cells from cytokine-driven apoptosis during systemic inflammation [62]. Thus, this approach not only confirms and extends findings from prior models regarding vascular biology but also expands the functional repertoire of PI3K-C2α to include the regulation of endothelial stress responses in adult organisms, providing mechanistic insights with potential relevance for therapeutic targeting.

3. Class II PI3K beta

Despite its structural similarity to PI3K-C2α, PI3K-C2β has evolved distinct biological functions, primarily centered on nutrient sensing and metabolic regulation. Like its paralogue, it catalyzes the production of PI(3)P and PI(3,4)P_2 at specific subcellular compartments, thereby shaping membrane identity and regulating vesicular trafficking. However, differently from to PI3K-C2α, no human cases with germline homozygous loss-of-function mutations have yet been identified but complete loss of PI3K-C2β is compatible with life in mice [63]. These differences underscore a divergence in physiological roles and suggest that PI3K-C2β operates in conditionally restricted or tissue-specific contexts different from PI3K-C2α. This divergence likely reflects not only subtle sequence variation, but also differences in how conserved structural domains are functionally deployed to direct interactions with specific membranes, binding partners, or regulatory cues that shape isoform-specific specialization.

3.1. Structure and regulation of the PI3K-C2β isoform

Although no high-resolution crystal structure of full human PI3K-C2β has been deposited to date, much of its structural organization can be inferred from homology with PI3K-C2α, for which crystallographic data are available [4]. Like other class II phosphoinositide 3-kinases, PI3K-C2β displays the conserved domain architecture characteristic of this class, including an N-terminal C2 domain (N—C2), a helical domain, a bilobed kinase domain, and a C-terminal extension composed of a PX domain and an additional C2 domain (C—C2) [22] (Fig. 1a). Additionally, like all class II PI3K isoforms, PI3K-C2β possesses a Ras-binding domain (RBD), whose role is gaining increasing relevance in the regulation of PI3K-C2β subcellular localization and activity. PI3K-C2β can form a potentially direct complex with Rab7, particularly with its active GTP-bound form, promoting the localization of PI3K-C2β to late endosomes and lysosomes [17]. Reflecting the functional importance of this domain, a mutation in PI3K-C2β (R458Q) affecting the RBD has been identified in a small cohort of patients with high-grade gliomas, where it correlated with temozolomide resistance, disease relapse, and poorer prognosis. While this suggests a potential role for the RBD in disease, its precise biological implications require further investigation [64,65].

Central to PI3K-C2β function is its bilobed kinase domain, which constitutes the catalytic core and determines substrate specificity. Structural interrogation of this region has shown that subtle sequence elements within the substrate-binding pocket are critical for defining phosphoinositide selectivity. To explore this, a substrate-selectivity mutant—referred to as the “KPLP mutant”—was engineered by replacing the endogenous substrate-binding motif of class II PI3Ks with a corresponding sequence from the class III PI3K VPS34 [6]. This modification preserves the ability to phosphorylate phosphatidylinositol (PI) to generate PI(3)P but abolishes utilization of PI(4)P, thereby eliminating PI(3,4)P_2 synthesis. Originally developed in PI3K-C2α to probe lipid product-specific functions [6], the same substitution was introduced into PI3K-C2β, where it confirmed that the structural determinants governing substrate selectivity are conserved across class II isoforms and are embedded within the catalytic core [66].

Beyond the kinase domain, regulation of PI3K-C2β is fine-tuned by its extended N-terminal region, which contains proline-rich motifs crucial for subcellular localization and enzymatic modulation. Two segments within this region (residues 80–130 and 200–280) mediate interaction with the mTORC1 component Raptor, thereby stabilizing the association of PI3K-C2β to lysosomal membranes [67]. Furthermore, upon EGF stimulation, these motifs engage with the epidermal growth factor receptor (EGFR) and the adaptor protein Grb2; the latter interaction is thought to promote enzymatic activation by relieving autoinhibition. This hypothesis is supported by observations that deletion of residues 1–148 or 1–261 enhances catalytic activity, confirming the negative regulatory role of the N-terminal region [15,68].

At the opposite end of the protein, the C-terminal PX and the C—C2 domains also contribute to regulation, predominantly through autoregulatory mechanisms. In PI3K-C2α, these domains mediate inhibitory control by folding back onto the kinase and Ras-binding domains, thereby stabilizing an autoinhibited conformation [22]. Due to high sequence conservation, a similar mode of autoinhibition is likely preserved in PI3K-C2β (Fig. 1c).

Finally, PI3K-C2β activity can also be regulated by lipid interaction through the C2 domains. Lysophosphatidic acid (LPA) promotes plasma membrane recruitment of the enzyme, while PI(4,5)P_2 serves as an essential cofactor for kinase activation, as demonstrated in PI3K-C2α and inferred to be conserved in PI3K-C2β [22]. Supporting this notion, two mutations identified in heterozygous human patients, a missense substitution (I1554M) and a truncating variant (Q1533*), map specifically to the C—C2 domain. Both mutations cause loss of catalytic activity [66]. Rather than disrupting autoinhibition, these mutations likely impair the ability of the C—C2 domain to bind PI(4,5)P_2 and properly localize PI3K-C2β to the plasma membrane, underscoring that interaction with membrane lipids drives enzymatic activation.

3.2. PI3K-C2β and mTORC1 signaling in cellular function and epilepsy pathogenesis

The structural features and activation mechanisms of PI3K-C2β have laid the foundation for understanding how this enzyme selectively generates PI(3)P and PI(3,4)P_2 in specific subcellular compartments. The role of these lipids in signaling was initially associated with metabolic regulation, particularly through the Akt/mTOR axis. However, the precise mechanisms underlying this connection emerged gradually and, somewhat unexpectedly, revealed an inhibitory function for PI3K-C2β on mTOR signaling in opposition to the well-established activating role of class I PI3Ks.

Initial studies uncovered this paradoxical role for PI3K-C2β in metabolically active tissues such as liver, muscle, and adipose tissue. In these contexts, genetic inactivation of PI3K-C2β with mutation abolishing its catalytic function led to enhanced insulin-stimulated Akt activation, without affecting MAPK signaling [69]. This effect was

linked to reduced PI(3)P levels and expansion of the APPL1-positive early endosomal compartment, which altered insulin receptor trafficking and ultimately amplified class I PI3K–dependent Akt signaling. These findings indicate that PI3K-C2β catalytic activity can act as a brake on the Akt/mTOR pathway.

More recent work has expanded this view by showing that PI3K-C2β also functions as a direct negative regulator of mTORC1, independently of Akt. Under nutrient-deprived conditions, PI3K-C2β localizes to lysosomes and late endosomes, where it synthesizes PI(3,4)P_2, leading to mTORC1 repression. This effect is mediated through association with the Raptor subunit of mTORC1 and recruitment of inhibitory 14–3-3γ, a mechanism dependent on local PI(3,4)P_2 production [67]. This process is further regulated by interaction with the GTP-bound form of Rab7, which mediates endolysosomal recruitment of PI3K-C2β and is essential for its inhibitory function [17]. Under nutrient-rich conditions, however, PI3K-C2β is phosphorylated by protein kinase N2 (PKN2), acting downstream of mTORC2. This phosphorylation promotes the formation of a complex between PI3K-C2β and 14–3-3 proteins, which sequesters the kinase in the cytosol and prevents endolysosomal localization, thereby relieving mTORC1 repression. Disruption of this regulatory circuit through PKN2 loss or mutation of the PI3K-C2β phosphorylation site restores lysosomal localization and mTORC1 inhibition, supporting the existence of a feedback loop in which mTORC2 controls mTORC1 activity via PKN2 and PI3K-C2β (Fig. 3a, b) [17].

Building on this role in nutrient signaling, recent evidence has demonstrated that disruption of PI3K-C2β–mediated mTORC1 control contributes directly to human disease. In particular, a comprehensive genetic and functional study identified ultra-rare heterozygous loss-of-function variants in *PIK3C2B* as causative in a subset of patients with focal epilepsy. These variants, clustered within the catalytic or C-terminal lipid-interacting domains, impair PI(3,4)P_2 synthesis and lead to mTORC1 hyperactivation in vitro (Fig. 3c). Notably, the p.E1294Q, p.Q1533*, and p.I1544M variants abolished kinase activity and significantly increased S6K phosphorylation, consistent with mTORC1 activation. In contrast, a variant retaining catalytic function (p.R1118L) did not alter mTORC1 signaling and was associated with a milder clinical phenotype [66].

Animal models further validated these findings and showed in both *Pik3c2b* heterozygous and knockout mice a dose-dependent increase in mTORC1 activity in hippocampal neurons, which was reversed by acute rapamycin treatment. Electrophysiological recordings from hippocampal slices revealed increased synaptic transmission, impaired GABAergic inhibition, and polyspiking activity. These phenotypes were rescued by mTORC1 inhibition, strongly implicating mTORC1 hyperactivity as the driver of network hyperexcitability. Functionally, this translated into a dramatic increase in seizure susceptibility. Upon pilocarpine challenge, *Pik3c2b* knockout mice rapidly progressed to status epilepticus and experienced high mortality, while heterozygous mice showed delayed but significant seizure onset. Similarly, mice carrying a G1501fs*1 mutation, closely resembling the Q1533* human variant, display a haploinsufficiency and more severe seizures than wild-type controls in response to pilocarpine treatment The phenotype was fully rescued by pretreatment with everolimus, an mTORC1 inhibitor, demonstrating the therapeutic relevance of this pathway.

Taken together, these findings implicate PI3K-C2β as a critical negative regulator of mTORC1 in different cell types, including neurons. In such cells, loss of PI3K-C2β impairs PI(3,4)P_2-dependent lipid signaling, disrupts neuronal excitability, and predisposes to seizures. The data support haploinsufficiency as the underlying mechanism and position PI3K-C2β within the growing list of mTOR pathway components involved in focal epilepsy [66,70].

3.3. *PI3K-C2β in tumor biology*

The link between PI3K-C2β and Akt/mTORC1 signaling, particularly under nutrient stress, raises the possibility of relevance to cancer

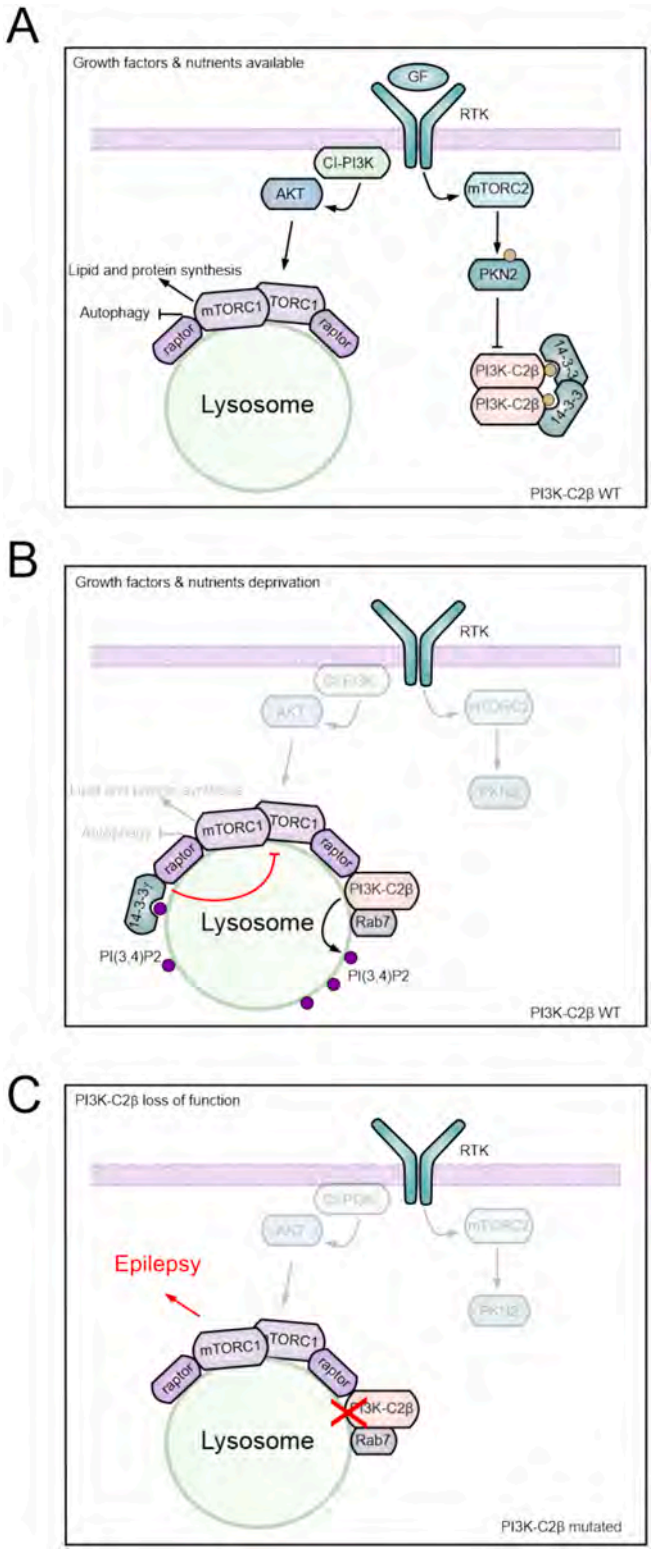

Fig. 3. Schematic representation of PI3K-C2β-mediated mTORC1 regulation. A. Under nutrient and growth factor rich conditions, PI3K-C2β is phosphorylated by PKN2 and, in turn, inactivated in the cytoplasm by 14–3-3 protein binding. B. Under nutrient and growth factors deprivation, PI3K-C2β is not phosphorylated by PKN2 and translocates to the lysosomes via Rab7interaction, where it binds mTORC1 component Raptor. Here, it generates a localized pool of PI(3,4)P2 that recruits 14–3-3γ to Raptor, suppressing mTORC1 activity. C. Loss-of-function mutations in PI3K-C2β leads to defective PI(3,4)P2 production, resulting in mTORC1 hyperactivation. In vivo, this translates to neuronal hyperexcitability and seizure susceptibility, ultimately leading to epilepsy.

biology, where dysregulated growth, metabolic adaptation, and survival pathways converge. Yet, while PI3K-C2β limits anabolic signaling by repressing mTORC1 activity under nutrient-deprived conditions, accumulating evidence suggests that it may also support tumor progression in distinct cellular and pathological contexts. In various malignancies, including acute myeloid leukemia, glioblastoma multiforme, medulloblastoma, neuroblastoma, and small cell lung cancer, elevated *PIK3C2B* expression has been reported. Experimental interference with PI3K-C2β in these settings reduces proliferation, increases apoptosis, and enhances sensitivity to chemotherapy [71]. While the underlying mechanisms remain incompletely defined, these observations suggest that PI3K-C2β may contribute to tumor maintenance and therapeutic resistance in specific contexts.

Consistent with this view, and in further contrast to its role in dampening mTORC1 signaling under nutrient stress, PI3K-C2β has also been implicated in tumor cell proliferation and mitotic control. Downregulation of PI3K-C2β delays the transition from G2/M to G1 phase, reduces colony formation in vitro, and impairs tumor growth in vivo [72]. Its loss sensitizes tumor cells to chemotherapeutic agents such as docetaxel; both transient and stable knockdown synergize with low-dose treatment, nearly abolishing colony formation and markedly suppressing tumor progression in xenograft models. These findings suggest that PI3K-C2β promotes mitotic progression and survival in proliferative tumors, independently of any role in nutrient-responsive signaling. Although some of these effects may resemble the scaffold-based function described for the paralogue PI3K-C2α [50], the molecular basis that controls PI3K-C2β during the cell cycle remains to be elucidated.

The ability of PI3K-C2β to promote therapy resistance may also extend to non-dividing cancer cells that escape chemotherapy by entering senescence. In this post-mitotic state, tumor cells activate a specialized engulfment program, internalizing neighboring healthy cells to acquire nutrients and sustain viability. This cannibalistic behavior, which has been associated with tumor relapse, critically depends on PI3K-C2β activity [73]. In breast cancer models, chemotherapy-induced senescent cells acquire a phagocytic-like phenotype and engage in whole-cell engulfment through mechanisms resembling macropinocytosis, efferocytosis, and endocytosis. Phosphoinositide profiling during this process revealed that PI(3)P is the only phosphoinositide consistently enriched at the interface between predator and prey, particularly during late stages of internalization. Among all PI(3)P-generating kinases tested, only PI3K-C2β was required to produce this localized lipid pool, as *PIK3C2B* knockout significantly impaired engulfment efficiency, while deletion of other class II or class III PI3Ks had no measurable effect. Mechanistically, this function appears to depend on clathrin, a known interactor of both $PI(4,5)P_2$ and PI3K-C2β. Rather than participating in classical coated-pit endocytosis, clathrin in senescent cells organizes actin-rich structures that facilitate the engulfment and mechanical processing of prey cells. These observations broaden the functional landscape of PI3K-C2β, highlighting its role in coordinating membrane remodeling and cytoskeletal dynamics that support cancer cell survival under therapeutic stress.

PI3K-C2β activity in cancer cells can be further regulated by distinct lipid signals. Ceramide has been shown to interact with the PIK catalytic domain of PI3K-C2β, altering its localization and suppressing its activation, ultimately impairing lamellipodia formation and cell motility in ovarian cancer [36]. In contrast, lysophosphatidic acid (LPA) stimulates PI3K-C2β, driving PI(3)P production and promoting cell migration in ovarian and cervical cancer cells [74].

Altogether, PI3K-C2β contributes to cancer persistence through multiple mechanisms, by sustaining mitotic progression in proliferating cells, controlling cell migration and enabling engulfment-based survival in senescent ones. While these findings highlight the potential of PI3K-C2β inhibition as a therapeutic strategy, any such approach will require careful context-specific evaluation, as blocking PI3K-C2β may inadvertently promote tumor growth by relieving mTORC1 repression under nutrient-stressed conditions.

3.4. PI3K-C2β role in cell migration and metastatisation

PI3K-C2β not only supports proliferative and survival signaling, but also critically contributes to the acquisition of migratory and invasive properties in tumor cells. By orchestrating cytoskeletal remodeling, focal adhesion turnover, and membrane trafficking, it serves as a key mediator between intracellular signaling and the physical dynamics required for cancer cell motility. Through localized production of specific phosphoinositide signals, PI3K-C2β enables directional migration and has been implicated in the metastatic dissemination of several tumor types.

In agreement, its downregulation impairs lysophosphatidic acid (LPA)–induced motility in ovarian and cervical cancer cell lines, whereas overexpression enhances cell movement, lamellipodia formation, and adhesion turnover [74,75]. This migratory function is strictly dependent on enzymatic activity, as kinase-inactive mutants fail to support these processes [75,76]. PI3K-C2β–driven motility is further modulated by upstream regulatory RNAs and oncogenic signals. In metastatic neuroblastoma, miR-362-5p targets *PIK3C2B* and suppresses migration and invasion [77]. In prostate cancer, PI3K-C2β promotes motility through regulation of the transcription factor Slug, independently of MEK/ERK signaling [78]. In breast and ovarian cancer, downregulation of *PIK3C2B* reduces metastatic dissemination, and PI3K-C2β-deficient cells become refractory to the anti-metastatic effects of ceramide liposomes [70,76,79]. Together, these findings underscore a role for PI3K-C2β–generated PtdIns in driving focal adhesion dynamics and metastatic behavior.

Mechanistically, PI3K-C2β generates $PI(3,4)P_2$ at focal adhesion sites, where it promotes disassembly in response to environmental changes [80]. This process is regulated by a signaling cascade in which reduced growth factor input is sensed by PKN2 (a downstream effector of mTORC2), which phosphorylates PI3K-C2β and promotes its sequestration by inhibitory 14–3-3 proteins, thereby limiting its activity [17]. When this inhibition is relieved, PI3K-C2β is recruited to adhesion sites by the disassembly factor DEPDC1B and locally synthesizes $PI(3,4)P_2$. This lipid product recruits the RhoA GTPase-activating protein ARAP3, leading to RhoA inactivation, stress fiber disassembly, and focal adhesion turnover. These findings define a $PI(3,4)P_2$-based lipid switch that integrates nutrient sensing with cell-matrix adhesion remodeling, a mechanism that can be exploited by cancer cells to regulate motility and invasion under metabolic or microenvironmental stress.

3.5. PI3K-C2β in endothelial function and stroke

While PI3K-C2β promotes tumor cell survival, migration, and adaptation to stress, its function extends beyond cancer biology into vascular physiology, where it plays an important role in endothelial development and the regulation of vascular architecture. Endothelial cells rely on tightly coordinated membrane trafficking and lipid signaling to maintain junctional stability and control cell size, two processes sensitive to growth factor and nutrient cues and now known to involve class II PI3Ks. In this context, PI3K-C2β has been implicated in angiogenesis and endothelial remodeling, particularly during pathological conditions such as ischemia or inflammation. These roles are increasingly understood as part of a broader function for PI3K-C2β in limiting mTORC1 signaling, which serves to modulate endothelial growth responses and preserve vascular homeostasis under stress or injury.

Although class II PI3Ks are increasingly recognized as essential regulators of endothelial cell function, the roles of PI3K-C2β diverge markedly from those of the closely related isoform PI3K-C2α. PI3K-C2α is indispensable for endothelial survival, adherens junction assembly, and angiogenesis; its loss disrupts VE-cadherin trafficking, compromises junctional integrity, and increases vascular permeability [34]. These defects result from a reduction in PI(3)P-positive endosomal compartments and impaired delivery of junctional cargo. In contrast, inactivation of PI3K-C2β leads to enlarged blood vessels, a phenotype linked not

to defective proliferation or migration but to increased endothelial cell size during sprouting angiogenesis in early postnatal mice carrying a kinase-inactivating mutation of *Pik3c2b* [65]. Mechanistically, PI3K-C2β restrains endothelial cell hypertrophy by suppressing mTORC1 activity through localized production of lysosomal PI(3,4)P_2. These in vivo findings are consistent with previous reports describing PI3K-C2β as a negative regulator of mTORC1 signaling under nutrient-deprived conditions, and support a model in which PI3K-C2β modulates growth factor responses in the endothelium to ensure balanced vascular development. In *Pik3c2b* mutant mice, sustained mTORC1 activity drives endothelial enlargement, which can be reversed by rapamycin treatment, confirming that PI3K-C2β functions to limit mTORC1-dependent cell growth. These observations reveal distinct but complementary functions for class II PI3K isoforms: PI3K-C2α promotes junctional integrity and vessel formation, while PI3K-C2β restricts endothelial cell size and vessel diameter by tempering mTORC1 signaling [34,65].

PI3K-C2β also functions as a context-specific regulator of endothelial barrier remodeling, particularly under injury-induced stress. In a model of ischemic brain injury, genetic inactivation of PI3K-C2β protects against vascular leakage, brain edema, infarct size, and the associated inflammatory response [81]. In cultured human cerebral microvascular endothelial cells, PI3K-C2β loss enhanced Rab11-dependent recycling of VE-cadherin to the plasma membrane, stabilizing intercellular junctions and preserving barrier integrity. These effects are PI(3)P-dependent and suggest that PI3K-C2β contributes to junctional destabilization during ischemic or inflammatory insult.

The barrier-stabilizing phenotype observed upon PI3K-C2β inactivation parallels its role in restraining mTORC1-driven endothelial hypertrophy during vascular development, and contrasts with the junctional defects caused by PI3K-C2α deficiency. Together, these findings support non-redundant, mechanistically distinct functions for the two class II PI3K isoforms: PI3K-C2β governs dynamic remodeling under pathological stress, while PI3K-C2α sustains basal junctional stability and angiogenesis [34,65].

These mechanistic insights also point to potential therapeutic relevance. Since vascular permeability is a major driver of secondary brain injury in stroke, selectively targeting PI3K-C2β could offer a strategy to preserve barrier integrity and limit tissue damage. Importantly, recent advances in the development of isoform-selective PI3K inhibitors have begun to include class II isoforms such as PI3K-C2β [70]. The prospect of inhibiting PI3K-C2β without compromising baseline endothelial function opens new avenues for selectively modulating stress-induced vascular remodeling, particularly in cerebrovascular disorders.

3.6. PI3K-C2β in cell adhesion and implications in muscle pathophysiology

The therapeutic potential of PI3K-C2β inhibition, suggested by its protective role in vascular remodeling during stroke, may extend to genetic disorders affecting skeletal muscle. In particular, PI3K-C2β has emerged as a disease modifier in X-linked myotubular myopathy (XLMTM), a severe congenital myopathy caused by mutations in the PtdIns-phosphatase MTM1 [82]. MTM1 hydrolyzes PI(3)P and PI(3,5)P_2, into PI and PI(5)P, respectively, maintaining endosomal lipid homeostasis and supporting vesicular trafficking. Loss of MTM1 results in aberrant PI(3)P accumulation in skeletal muscle, impaired β1-integrin trafficking, and defective focal adhesion architecture [82].

The first evidence implicating PI3K-C2β as a disease modifier in XLMTM came from genetic studies showing that muscle-specific inactivation of *Pik3c2b* in *Mtm1*-deficient mice fully prevents disease onset and rescues muscle architecture, function, and survival even when applied post-symptomatically, highlighting PI3K-C2β inhibition as a powerful therapeutic strategy [83]. This rescue might be due to normalization of PI(3)P levels, the main substrate of MTM1. Support to this theory comes from recent mechanistic insights into endosomal mapping in a muscle cell line derived from *Mtm1*-KO mice, which recapitulates key features of XLMTM [84]. In this model, MTM1 is shown to regulate a PI(3)P pool localized on EEA1-positive early endosomes. In the absence of MTM1, PI(3)P accumulates abnormally on EEA1-positive endosomes, leading to impaired biogenesis of Rab4-positive recycling vesicles and disrupting cargo trafficking. This compartment-specific dysregulation appears particularly relevant in myotubes, where Rab4-mediated recycling is a critical pathway for maintaining membrane dynamics and protein surface expression. Notably, PI3K-C2β depletion in *Mtm1*-KO cells not only normalizes PI(3)P levels on EEA1-positive endosomes but also restores the formation and density of Rab4-positive vesicles, effectively rescuing trafficking defects and supporting the notion that MTM1 and PI3K-C2β cooperatively regulate endosomal homeostasis in muscle [84].

However, other studies refined this view, demonstrating that loss of PI3K-C2β specifically restores trafficking of vesicles containing β1-integrin adhesion receptors not by globally reducing PI(3)P, but through modulation of integrin-specific endocytic sorting driven by PI(3,4)P_2 synthesis [85]. In *Mtm1*-deficient muscle cells, the absence of PI3K-C2β selectively impairs an endocytic degradative route, favoring retention or recycling of β1-integrins to the plasma membrane and restoring surface adhesion complexes. This mechanism is independent from clathrin-mediated endocytosis and appears to involve PI3K-C2β association with cargo-selective endocytic adaptors such as intersectin-1 and Dab2 [85]. These findings suggest that PI3K-C2β and MTM1 exert antagonistic control over β1-integrin trafficking, operating on distinct pools of PI 3-phosphates to balance integrin endocytosis, retention, and recycling. While MTM1 facilitates β1-integrin delivery to the membrane via Rab11- and exocyst-dependent routes, PI3K-C2β promotes their clearance through lysosomal degradation. Therefore, in XLMTM, MTM1 deficiency disrupts β1-integrin recycling and leads to its pathological intracellular retention/degradation, which is corrected by PI3K-C2β inhibition through rebalancing of endocytic routing rather than direct phosphoinositide normalization.

In line with these observations, selective inactivation of PI3K-C2β catalytic activity in *Mtm1*-deficient mice rescues key features of XLMTM, including muscle atrophy, triad/sarcomere disorganization, and impaired motor function, while normalizing mTORC1 activity and endosomal trafficking pathways [86]. These improvements are not observed in other myopathy models, such as those caused by BIN1 deficiency, highlighting the specificity of the rescue and indicating that PI3K-C2β inhibition selectively benefits conditions driven by PI(3)P dysregulation. Together, these findings support a model in which MTM1 and PI3K-C2β antagonistically regulate distinct pools of PI(3)P and PI(3,4)P_2, with downstream effects on β1-integrin localization, adhesion architecture, and vesicle recycling. In this context, PI3K-C2β emerges as a key node linking phosphoinositide metabolism, adhesion dynamics, and membrane trafficking in skeletal muscle. Its inhibition thus represents a promising and selective therapeutic approach for phosphoinositide-driven myopathies such as XLMTM.

4. Class II PI3K gamma

Among the class II PI3K isoforms, PI3K-C2γ remains the least characterized. Originally identified as a novel PI3K isoform in normal breast tissue [87], PI3K-C2γ exhibits a distinct tissue distribution compared to PI3K-C2α and PI3K-C2β. It is predominantly expressed in the liver and pancreas, with additional presence in the breast, prostate and small intestine [18,87]. Like its counterparts, PI3K-C2γ can generate both PI(3)P and PI(3,4)P_2 in vitro. However, in vivo studies showed that a spatially restricted pool of PI(3,4)P_2 is produced on endosomal membranes following insulin stimulation [18] and on lysosomes under conditions of glutamine deprivation [88].

4.1. Structural features of PI3K-C2γ

PI3K-C2γ retains the core domain architecture characteristic of class

II PI3Ks, including a Ras-binding domain (RBD), a N-terminal C2 domain (N—C2), a helical domain, a bilobed catalytic core, a PX phosphoinositide-binding domain, and a second C2 domain at the C-terminus (C—C2) (Fig. 1a,b). Despite this conservation, PI3K-C2γ diverges from its paralogues PI3K-C2α and PI3K-C2β by lacking a clathrin-binding motif, suggesting exclusion from clathrin-mediated endocytic pathways and implying functional specialization [2]. Structural data on PI3K-C2γ remain sparse: to date, only the PX domain has been resolved at atomic resolution, while the full-length structure is lacking. Alphafold predictions reveal extensive unresolved regions, especially in the N-terminal domain, which shows limited homology to the corresponding regions of the other class II isoforms. Conversely, the C-terminal half appears more conserved and may support an autoinhibitory mechanism involving the PX and C—C2 domains, as described for PI3K-C2α, although this remains experimentally untested. The limited structural insight leaves open the possibility that PI3K-C2γ harbors isoform-specific regulatory features. Structural information would help to clarify the molecular basis for distinct lipid substrate preferences, impacting on the selective recruitment of PI3K-C2γ to membranes, and would shed the light on isoform specific interactors. For example, so far PI3K-C2b have been linked with Rab7 proteins [17], whereas PI3K-C2γ preferentially associates with Rab5 [18]. In addition, both isoforms contribute to inhibition of the mTOR pathway, but via different mechanisms: PI3K-C2β through its interaction with Raptor [67], the regulatory subunit of mTORC1, and PI3K-C2γ primarily through its catalytic activity [88].

Elucidating the complete three-dimensional structure will be essential to define the molecular basis of PI3K-C2γ regulation and its functional divergence from other class II PI3Ks.

4.2. PI3K-C2γ in metabolic signaling and vesicular trafficking

In hepatic tissue, where its expression is particularly enriched, PI3K-C2γ has been implicated in metabolic signaling and vesicular trafficking. PI3K-C2γ was initially proposed to function in hepatocyte maturation [89], but later studies also indicated a role in sustaining long-term insulin signaling in the liver [18]. While class I PI3Ks rapidly activate insulin signaling at the plasma membrane, a delayed sustained phase is orchestrated within intracellular compartments. Upon insulin stimulation, PI3K-C2γ is recruited to early endosomes through its interaction with active Rab5-GTP. At this site, it promotes the accumulation of PI(3,4)P_2 and the consequent prolonged Akt2 activation. In hepatocytes and mouse livers lacking PI3K-C2γ, this specific endosomal PI(3,4)P_2 pool is substantially reduced, highlighting the role of this enzyme in both spatial and temporal regulation of insulin pathways. This compartmentalized signaling cascade selectively affects glycogen synthase, without altering other downstream targets of Akt such as the mTORC1/S6K pathway or FoxO1–3 transcription factors. Consequently, the absence of PI3K-C2γ compromises glycogen storage in hepatocytes and redirects glucose metabolism toward triglyceride synthesis. Although knockout mice for PI3K-C2γ are viable and display normal development, they progressively develop metabolic abnormalities, as hyperlipidemia, triglyceride accumulation, increased adiposity and insulin resistance, with age or upon exposure to a high-fat diet [18]. Consistent with these findings, genome-wide association studies (GWAS) have implicated *PIK3C2G* in human metabolic disease. Variants in the gene have been associated with increased susceptibility to type 2 diabetes in a Japanese population [90] and with elevated body mass index, a key risk factor for diabetes, in Aboriginal Australians [91]. Additional GWAS results have linked polymorphisms in *PIK3C2G* to hyperlipidemia and increased risk of myocardial infarction, further supporting the involvement of this gene in metabolic dysregulation [92].

4.3. PI3K-C2γ in cancer

Unlike class I PI3Ks, which are frequently activated in cancer and drive oncogenic signaling, PI3K-C2γ has recently emerged as a potential tumor suppressor, a function that is both unexpected and distinct from the more nuanced, context-dependent roles reported for the other class II isoforms. The role of PI3K-C2γ in cellular metabolism has been explored in oncogenic contexts, particularly in pancreatic ductal adenocarcinoma (PDAC), a malignancy known for its pronounced metabolic flexibility. Approximately 30 % of PDAC patients display downregulated *PIK3C2G* expression, which correlates with shorter overall survival and a more aggressive disease phenotype [88,93]. These clinical associations are supported by experimental findings in KPC (*Kras*^{G12D}; *Trp53*^{R172H}; *Pdx1*-Cre) genetically engineered mouse models, where PI3K-C2γ deletion accelerates tumor progression and promotes metastatic spread. Mechanistically, loss of PI3K-C2γ leads to aberrant mTORC1 activation under glutamine-starved conditions, through disruption of the Asap1/Arf1 signaling axis that normally restrains mTORC1 activity. The resulting metabolic rewiring enhances glycolytic flux and glutamine-dependent anabolism, promoting biomass accumulation and proliferation during nutrient stress (Fig. 4). This glutamine reliance sensitizes PI3K-C2γ-deficient tumors to glutaminase inhibition, and therapeutic efficacy is further potentiated by concomitant mTORC1 blockade with everolimus.

Beyond PDAC, alterations in *PIK3C2G* have been associated with poor clinical outcomes in additional malignancies, though the functional implications appear to diverge from the tumor-suppressive role described in pancreatic cancer. In intrahepatic cholangiocarcinoma, *PIK3C2G* mutations are linked to reduced survival and unfavorable prognosis [94], while in stage III colorectal cancer, patients with low *PIK3C2G* copy number receiving oxaliplatin show increased risks of recurrence and mortality [95]. In lung adenocarcinoma, *PIK3C2G* emerged from whole-exome sequencing as a prognostic marker associated with worse survival; functional experiments confirmed that its silencing reduces proliferation, migration, and invasion while promoting apoptosis and disrupting cell cycle progression in A549 cells [96]. In hepatocellular carcinoma models, PI3K-C2γ has been implicated in promoting tumor invasiveness. Specifically, PI(3,4)P_2 generated downstream of PI4KIIIα activates Akt2 and supports the formation of invadopodia-like protrusions involved in extracellular matrix degradation and cell motility [97]. Elevated PI4KIIIα expression in clinical samples correlates with poor prognosis, further supporting the involvement of this lipid signaling axis in aggressive disease behavior.

These findings reveal that PI3K-C2γ can function as either a tumor suppressor or an oncogenic facilitator, depending on cellular context and the particular signaling functions exploited by the tumor. In PDAC, PI3K-C2γ constrains mTORC1 activity and limits metabolic plasticity under nutrient stress, acting as a barrier to tumor progression. Conversely, in hepatocellular carcinoma and potentially other settings, its lipid products may amplify PI3K/Akt signaling and promote cytoskeletal remodeling, thereby enhancing invasiveness and dissemination. This functional duality highlights the context-dependent nature of PI3K-C2γ signaling and suggests that its contribution to tumor biology is shaped by tissue-specific roles and the selective pressures of the tumor microenvironment. Further investigation will be required to delineate these mechanisms and assess how PI3K-C2γ might be targeted or modulated in a cancer-type-specific manner.

5. Class II PI3K inhibitors

The development of selective inhibitors for class II PI3Ks has opened new opportunities to investigate isoform-specific functions and assess their therapeutic potential. Among the three isoforms, PI3K-C2α has been the most extensively studied. Its involvement in endocytosis, angiogenesis, thrombosis, metabolic regulation, and cytokinesis has made it a compelling target for pharmacological intervention [2,98]. Early-generation compounds, including PIK-90, MIPS-19416, and MIPS-21335, were derived from class I scaffolds and displayed dual activity toward class I and class II enzymes. MIPS-21335 showed moderate

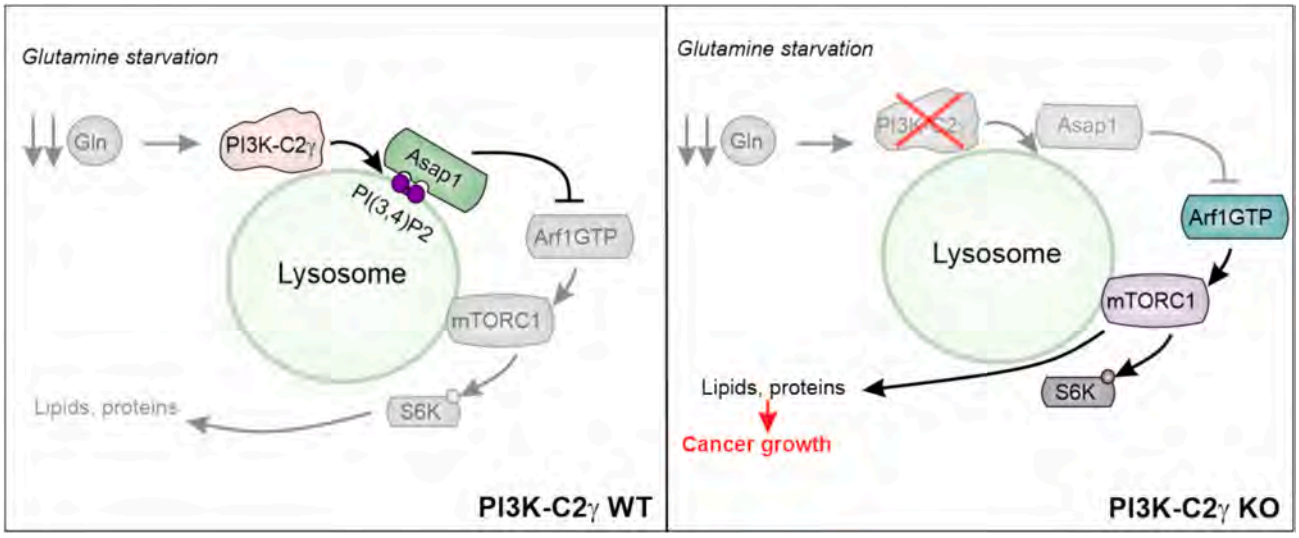

Fig. 4. PI3K-C2γ regulation of mTORC1 in response to glutamine levels. Under conditions of glutamine deprivation, PI3K-C2γ generates $PI(3,4)P_2$ at the lysosomal membrane, facilitating the recruitment of Asap1 and suppressing mTORC1 activation. In pancreatic ductal adenocarcinoma (PDAC), loss of PI3K-C2γ is associated with mTORC1 hyperactivation, leading to a rewiring of glutamine metabolism that supports enhanced tumor cell proliferation.

selectivity for PI3K-C2α over class I PI3Ks but lacked the specificity required for precise biological studies [44].

Recent medicinal chemistry efforts led to the identification of the PITCOIN series (PITCOIN1, PITCOIN2, PITCOIN3 and PITCOIN4), a group of highly selective PI3K-C2α inhibitors with nanomolar potency [99,100]. Among these, PITCOIN3 and 4 are the most selective compounds, binding the ATP-binding pocket of PI3K-C2α through interactions with non-conserved residues (such as M1136, N1134, and S1113 for PITCOIN3). These inhibitors show negligible off-target activity against >100 lipid and protein kinases, including PI3Kα and Vps34 [99]. PITCOIN compounds phenocopy the effects of genetic PI3K-C2α deletion, blocking $PI(3,4)P_2$ synthesis at clathrin-coated pits and at the midbody, as well as impairing PI(3)P production on endosomes [12,99]. In platelets, PI3K-C2α inhibitors reduce basal PI(3)P levels, alter membrane morphology, and significantly diminish thrombus formation under flow [44,99]. These findings support PI3K-C2α as a promising therapeutic target in thrombosis [44], cancer [56], viral infection [101,102], VEGF signaling and endocytosis [6,34], cytokinesis [12], and metabolic disorders including diabetes [61].

Beyond PI3K-C2α, interest is growing in the development of selective inhibitors for PI3K-C2β [70]. This isoform contributes to mTORC1 regulation, endocytosis, focal adhesion dynamics, and platelet function. Preclinical studies suggest that targeting PI3K-C2β could provide therapeutic benefit in ischemia as well as consequent deleterious inflammatory response [81]. However, PI3K-C2β is also expressed in the brain and inhibits neuronal mTORC1 activity that when unchecked can lead to epilepsy. Therefore, systemic inhibition may carry the risk of neurological side effects. To address this, future drug design should prioritize the development of compounds that do not cross the blood–brain barrier, ensuring efficacy in vascular and metabolic tissues while preserving central nervous system function.

PI3K-C2γ remains the least characterized member of the class II PI3K family. It has been implicated in the generation of PI(3,4)P2 on endosomes following insulin stimulation [18], and in the regulation of hepatic glucose and lipid metabolism. Recently, highly potent and selective PI3K-C2γ inhibitors have been reported: the lead compound (compound 23, IC50 = 4 nM) shows >250-fold selectivity over class I PI3Ks, preferential liver distribution, and effectively blocks insulin-stimulated PI(3,4)P2 synthesis and hepatic glycogen accumulation, highlighting its potential as a therapeutic tool for glycogen storage diseases [103]. Notably, the restricted expression of PI3K-C2γ in liver, pancreas, and certain epithelial tissues may offer opportunities for therapeutic targeting with reduced systemic impact. However, any effort to inhibit this isoform must be carefully evaluated in light of recent findings in PDAC, where PI3K-C2γ appears to act as a tumor suppressor. Loss of PI3K-C2γ in PDAC models results in enhanced mTORC1 activation under glutamine-limited conditions, driving metabolic reprogramming and promoting tumor aggressiveness [88]. These data underscore the importance of tissue-specific context when considering PI3K-C2γ as a therapeutic target. In settings such as PDAC, inhibition could unintentionally exacerbate disease progression, highlighting the need for precise molecular stratification and careful therapeutic design.

In summary, the availability of highly selective PI3K-C2α inhibitors has significantly advanced our understanding of class II PI3K biology. Expanding this progress to include PI3K-C2β and PI3K-C2γ will be essential to realize the full therapeutic potential of this enzyme family. The design of isoform-specific, tissue-targeted inhibitors with appropriate pharmacokinetic profiles remains a key priority for the next phase of translational research in this area.

6. Concluding remarks and perspectives

Over the past few years, the field of class II PI3K research has witnessed substantial progress, particularly in our understanding of isoform-specific functions and the structural underpinnings of PI3K-C2α. However, our understanding of PI3K-C2β and PI3K-C2γ remains comparatively limited. Thus, the future development of isoform-specific inhibitors for PI3K-C2β and PI3K-C2γ represents a key step toward translating the unique properties of class II PI3Ks into clinically actionable strategies. In parallel, greater attention must be paid to the biosynthetic pathways and subcellular availability of their lipid substrates, particularly PI and PI(4)P, which critically shape the localization and signaling output of class II PI3Ks. A more comprehensive mapping of phosphoinositide precursor fluxes and their integration with metabolic cues and membrane trafficking networks will be essential to fully understand the spatial and temporal logic of class II PI3K activity. As we continue to uncover the distinct and overlapping functions of the three isoforms, class II PI3Ks are emerging not just as specialized kinases but as central integrators of membrane identity, vesicular dynamics, and cellular signaling, a role that warrants further exploration both for fundamental biology and therapeutic innovation.

CRediT authorship contribution statement

Emilio Hirsch: Supervision, Funding acquisition, Writing – original draft. **Arezou Kahnamouei:** Writing – review & editing. **Maria Chiara De Santis:** Writing – original draft. **Federico Gulluni:** Writing – original draft. **Lorenzo Prever:** Writing – original draft. **Emanuele Fantastico:** Writing – original draft. **Roberta Rubino:** Writing – original draft. **Gabriele Squillero:** Writing – original draft.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used ChatGPT (GPT-5) to enhance the clarity and style of the scientific writing. Following its use, the authors carefully reviewed and edited the text, and they take full responsibility for the final content of the publication.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Emilio Hirsch reports a relationship with Kither Biotech that includes: equity or stocks. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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