An Overview of Class II Phosphoinositide 3-Kinases

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Contents

Abstract Phosphoinositide 3-kinases (PI3Ks) catalyse the synthesis of specific members of the family of lipids collectively known as 'phosphoinositides'. These PI3Ks products can in turn modulate activation of many downstream proteins, ultimately regulating several cellular processes. Mammalian cells possess eight PI3Ks which are grouped into three classes based on their structure and substrate specificity. While class I and III PI3Ks have been extensively investigated, our understanding of the three class II members has only improved in most recent years. This chapter will summarise some of the available information on mammalian class II PI3Ks and their physiological roles.

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List of Abbreviations

DAG	Diacylglycerol
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
HCC	Hepatocellular carcinoma
IL	Interleukin
LPA	Lysophosphatidic acid
LPS	Lipopolysaccharide
MEF	Mouse embryonic fibroblast
NADPH	Nicotinamide adenine dinucleotide phosphate
OA	Osteoarthritis
PI3K	Phosphoinositide 3-kinase
PtdIns	Phosphatidylinositol
PtdIns3*P*	Phosphatidylinositol 3-phosphate
PtdIns4*P*	Phosphatidylinositol 4-phosphate
PtdIns(4,5)P_2	Phosphatidylinositol 4,5-bisphosphate
PtdIns(3,4)P_2	Phosphatidylinositol 3,4-bisphosphate
PtdIns(3,4,5)P_3	Phosphatidylinositol 3,4,5-trisphosphate
PX	Phox homology
RA	Rheumatoid arthritis
TNF	Tumour necrosis factor

1 Introduction

Phosphoinositide 3-kinases (PI3Ks) are a conserved family of lipid kinases that catalyse the phosphorylation of specific phosphoinositides (Cantley 2002; Vanhaesebroeck et al. 2012; Maffucci 2012), a group of lipids consisting of a *myo*-inositol headgroup linked to a diacylglycerol (DAG) through a phosphodiester bond (Fig. 1). Specifically, PI3Ks catalyse phosphorylation on position three within the *myo*-inositol ring of phosphatidylinositol (PtdIns), phosphatidylinositol 4-phosphate (PtdIns4*P*) and phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2], leading to the synthesis of phosphatidylinositol 3-phosphate (PtdIns3*P*), phosphatidylinositol 3,4-bisphosphate [PtdIns(3,4)P_2] and phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)P_3], respectively (Vanhaesebroeck et al. 2001; Domin and Waterfield 1997) (Fig. 1). These lipids in turn regulate the activation of several proteins, ultimately controlling a plethora of intracellular functions (Vanhaesebroeck et al. 2010, 2016; Engelman et al. 2006; Ghigo et al. 2012; Falasca and Maffucci 2007, 2012; Gulluni et al. 2019; Bilanges et al. 2019). Eight mammalian PI3Ks exist and they were grouped into three classes based on their structure and substrate specificity (Vanhaesebroeck et al. 2001; Domin and Waterfield 1997). Class I PI3Ks, by far

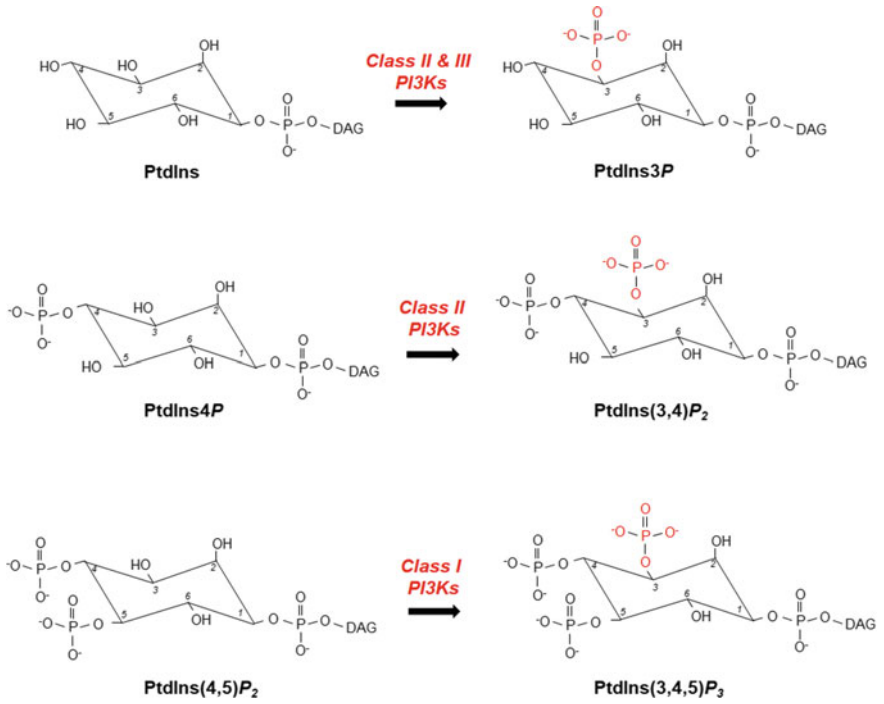

Fig. 1 Schematic representation of the lipid substrates and products of the distinct classes of PI3Ks. Positions within the inositol ring are indicated by numbers

the best characterised amongst the members of this family, are dimers of a regulatory and one of four catalytic subunits, and are mainly responsible for synthesis of PtdIns(3,4,5)P_3 in vivo. Class II PI3Ks have been reported to catalyse synthesis of both PtdIns3*P* and PtdIns(3,4)P_2. Class III PI3K catalyses synthesis of the bulk of intracellular PtdIns3*P* (Fig. 1).

For a long time, class II PI3Ks have been the least investigated amongst all PI3Ks but our understanding of these enzymes has massively improved in most recent years. This chapter will focus on mammalian class II PI3Ks and their physiological roles. It must be noted, however, that accumulating data in literature also points to a role for them in several human diseases (Vanhaesebroeck et al. 2016; Ghigo et al. 2012; Falasca and Maffucci 2012; Gulluni et al. 2019; Bilanges et al. 2019).

2 Identification and Tissue Distribution

A class II PI3K was first identified in *Drosophila melanogaster* (MacDougall et al. 1995). It was later discovered that class II enzymes are conserved from *Caenorhabditis elegans* to humans but they are not expressed in yeast [reviewed in Gulluni

et al. (2019), Bilanges et al. (2019), Margaria et al. (2019)]. Three members of class II PI3Ks exist in mammalian cells, namely PI3K-C2α, PI3K-C2β and PI3K-C2γ, encoded by the genes *PIK3C2A*, *PIK3C2B* and *PIK3C2G*, respectively. Human PI3K-C2α was cloned from U937 cells (Domin et al. 1997) while human PI3K-C2β was isolated from a cDNA library of MCF7 (Brown et al. 1997) and subsequently from U937 cells (Arcaro et al. 1998). Original studies indicated that PI3K-C2α was ubiquitously expressed with highest levels in heart, placenta and ovary (Domin et al. 1997). Expression of PI3K-C2β was shown to be high in thymus and placenta, while expression of PI3K-C2γ was identified in liver and prostate tissue as well as in breast and salivary glands (Rozycka et al. 1998; Ono et al. 1998). It is now well established that PI3K-C2α and PI3K-C2β are ubiquitously expressed while PI3K-C2γ shows a more restricted expression pattern (Consortium GTEx 2013). Interestingly, a recent study reported an increase of PI3K-C2β both at the mRNA and protein levels in fibroblasts obtained from individuals carrying homozygous loss-of-function mutations in *PIK3C2A* as well as upregulation of PI3K-C2β upon downregulation of PI3K-C2α in HeLa cells (Tiosano et al. 2019). Whether this is a compensatory effect or whether this study has unveiled a general mechanism of regulation of the expression levels of these two enzymes remains to be established. PI3K-C2γ levels can also be modulated, as suggested by a study reporting increased expression of this enzyme during liver regeneration following partial hepatectomy (Ono et al. 1998).

3 Structure of Class II PI3Ks

Class II PI3Ks are monomers of high molecular weight, possessing the PI3K core common to all family members (consisting of a C2 domain, a helical domain and the catalytic domain), a Ras binding domain (also found on the catalytic subunits of class I PI3Ks) but characterised by unique N-terminal and C-terminal extensions compared to the other PI3Ks (Fig. 2) (Vanhaesebroeck et al. 2010; Falasca and Maffucci 2012; Gulluni et al. 2019; Margaria et al. 2019). The C-terminal extension is common between the three class II PI3Ks and consists of a Phox homology (PX) domain and a C2 domain (Fig. 2). The N-terminal extensions differ between the three enzymes and allow some of them to bind to distinct proteins. For instance,

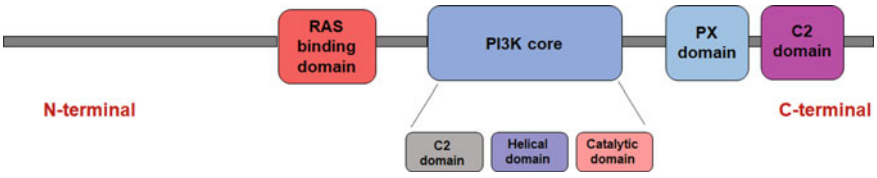

Fig. 2 Schematic representation of the common structure of class II PI3Ks, comprising an N-terminal region, a Ras binding domain, the PI3K core (consisting of a C2, a helical and a catalytic domain) and a common C-terminal, comprising a PX and a second C2 domain

both PI3K-C2α (Gaidarov et al. 2001) and PI3K-C2β (Wheeler and Domin 2006) can bind to clathrin via their N-terminal region, but PI3K-C2β can also interact with Raptor (Marat et al. 2017). In addition, PI3K-C2β possesses proline-rich motifs which have been investigated for their potential role in activation of the enzyme (Wheeler and Domin 2006), while PI3K-C2α can bind to transforming acidic coiled-coil-containing protein 3 (TACC3) via a region localised between the Ras binding domain and the PI3K core (Gulluni et al. 2017). Less is known about the structure of PI3K-C2γ which, in general, is the least characterised of the three enzymes, but it does not appear to possess a clathrin binding domain (Margaria et al. 2019).

4 Lipid Products

The differences between class II and class I PI3K enzymes were immediately clear, as indicated by an original study reporting that only 1% of the in vitro activity of PI3K-C2β was directed towards PtdIns(4,5)P_2 (Arcaro et al. 1998), the main substrate of class I PI3Ks. On the other hand, class II PI3Ks were found to catalyse phosphorylation of PtdIns and PtdIns4*P* (MacDougall et al. 1995; Arcaro et al. 1998, 2000; Gaidarov et al. 2001; Virbasius et al. 1996; Misawa et al. 1998), with PtdIns being the main substrate in vitro (Falasca and Maffucci 2012). While it was soon clear that PtdIns3*P* and, to a lesser extent, PtdIns(3,4)P_2, were the main products of class II PI3Ks in vitro, identification of their in vivo products was complicated by the absence of selective inhibitors for these enzymes. Indeed, data only started appearing following the advent of antisense/siRNAs/shRNAs-based techniques and, later on, with the generation of transgenic mice (Falasca et al. 2007; Maffucci et al. 2005; Wen et al. 2008; Boukhalfa et al. 2020a, b; Valet et al. 2015; Alliouachene et al. 2015; Franco et al. 2014; Yoshioka et al. 2012).

4.1 PtdIns3P

Over fifteen years ago, we reported that PI3K-C2α and PI3K-C2β catalyse the synthesis of PtdIns3*P* in vivo in response to insulin (Falasca et al. 2007) and lysophosphatidic acid (LPA) (Maffucci et al. 2005). These studies were amongst the first lines of evidence supporting a role for class II PI3Ks in the regulation of pools of PtdIns3*P* specifically synthesised in response to cellular stimulation. Consistent with this, PI3K-C2α-dependent pools of PtdIns3*P* were also detected in PC12 cells upon stimulation of exocytosis (Wen et al. 2008) and have been observed, more recently, in kidney epithelial cells in response to shear stress (Boukhalfa et al. 2020a, b). The possibility of a cell cycle-dependent, class II PI3Ks-mediated synthesis of this phosphoinositide was also supported by in vitro assays demonstrating that PtdIns3*P*, but not PtdIns4*P*, PtdIns(4,5)P_2 or PtdIns(3,4,5)P_3, increased in the nuclei and nuclear

envelopes of HL-60 cells during transition into the G2/M-phase, likely due to activation of PI3K-C2β (Visnjić et al. 2003). Transgenic mouse models revealed that class II PI3Ks can also regulate pools of PtdIns3*P* in basal, unstimulated conditions, as detected in platelets from mice heterozygous for a catalytically inactive (kinase-dead) PI3K-C2α (Valet et al. 2015) or in unstimulated hepatocytes from a mouse model expressing a kinase-dead PI3K-C2β (Alliouachene et al. 2015). This latter study further indicated that PI3K-C2β modulation of PtdIns3*P* was cell type-specific, as levels of this phosphoinositide were reduced in hepatocytes but not in mouse embryo fibroblasts (MEFs) or splenocytes (Alliouachene et al. 2015).

Generally, class II PI3Ks have been implicated in localised synthesis of PtdIns3*P* within specific cellular compartments. For instance, the previously mentioned insulin- and LPA-dependent PtdIns3*P* was detected at the plasma membrane (Falasca et al. 2007; Maffucci et al. 2005). Similarly, PtdIns3*P* was specifically generated in large dense-core vesicles (Wen et al. 2008) or in the primary cilium area (Boukhalfa et al. 2020b) during exocytosis and in response to shear stress, respectively. Furthermore, MEFs lacking PI3K-C2α displayed reduced levels of a basal pool of PtdIns3*P* specifically localised around the base of the cilium but showed no alteration on this phosphoinositide within the rest of the cell (Franco et al. 2014). Similarly, selective ablation of PI3K-C2α in endothelial cells was shown to decrease the levels of PtdIns3*P* in endosomes (Yoshioka et al. 2012). Taken together these studies indicate that PI3K-C2α and PI3K-C2β can control synthesis of very localised pools of PtdIns3*P*, sometimes in response to specific cellular stimulation. To the best of our knowledge, there is no indication so far that PI3K-C2γ can also catalyse synthesis of PtdIns3*P* in vivo.

4.2 PtdIns(3,4)P_2

PtdIns(3,4)P_2 is the only in vivo product of PI3K-C2γ identified so far, and it appears to be localised specifically on Rab5-positive early endosomes (Braccini et al. 2015). Evidence that the other class II PI3Ks can also catalyse the synthesis of PtdIns(3,4)P_2 in vivo also exists, including the observation that transient downregulation of PI3K-C2α inhibited the insulin-induced synthesis of PtdIns(3,4)P_2, but not PtdIns(3,4,5)P_3 or levels of PtdIns3*P* in MIN6 pancreatic β cells (Leibiger et al. 2010) and more recent data indicating that PI3K-C2α and PI3K-C2β catalyse the synthesis of PtdIns(3,4)P_2 at late-stage endocytic compartments (Posor et al. 2013) and in lysosomes and late endosomes (Marat et al. 2017). Consistent with this, fibroblasts derived from patients displaying homozygous loss-of-function mutations in *PIK3C2A* revealed reduced PtdIns(3,4)P_2 overall and reduced PtdIns3*P* specifically at the ciliary base (Tiosano et al. 2019).

5 Mechanisms of Activation

Although our understanding of the physiological roles of class II PI3Ks has improved massively in the past decade, still little is known about the mechanisms of their activation (Falasca and Maffucci 2012; Bilanges et al. 2019). Association of class II PI3Ks to some growth factor receptors was reported (Falasca and Maffucci 2012), such as interaction of PI3K-C2α and PI3K-C2β with the epidermal growth factor receptor (EGFR) (Arcaro et al. 2000), of PI3K-C2α with the insulin receptor B (Leibiger et al. 2010), and of PI3K-C2β with the platelet-derived growth factor receptor (Arcaro et al. 2000) and c-Kit (Arcaro et al. 2002). This, however, has not led to a clear indication of whether such associations are part of a generic mechanism of activation of these enzymes, as well established for class I PI3Ks. In this respect, it is worth mentioning that binding of PI3K-C2α and PI3K-C2β to EGFR and ErbB-2 was already detectable in quiescent A431 cells although it increased upon EGF stimulation (Arcaro et al. 2000) and association of PI3K-C2β with c-Kit in small cell lung carcinoma cell lines did not appear to increase upon stem cell factor stimulation (Arcaro et al. 2002).

Original studies reported that the in vitro activity of PI3K-C2α increased upon removal of the region encompassing the clathrin-binding sites (Gaidarov et al. 2001) and that deletion of the proline-rich motifs of PI3KC2β affected its activity (Wheeler and Domin 2006), suggesting that the N-terminal extensions could be involved in regulation of their enzymatic activity. On the other hand, the involvement of the C-terminal PX and C2 domains in regulation of PI3K-C2α activation has been demonstrated by a recent study showing that these two domains fold onto the catalytic domain preventing its activity when the enzyme is in solution (Wang et al. 2018). Once the enzyme is recruited to the membrane, interactions of the N-terminal region with clathrin and of both PX and C2 domains with PtdIns(4,5)P_2 remove such an inhibition over the catalytic domain (Wang et al. 2018). Interestingly, Ca^{2+} has been reported to affect the interaction of PI3K-C2α PX-C2 domains with the membrane (Chen et al. 2018) and previous studies demonstrated that the activity of this enzyme could be increased by increasing concentration of Ca^{2+} (Wen et al. 2008) and by stimuli able to increase intracellular Ca^{2+} concentration (Wang et al. 2006). Currently, it is not known whether such an autoregulatory mechanism exists in all class II enzymes although it is worth mentioning that increased enzymatic activity of PI3K-C2β in vitro was observed upon deletion of the C-terminal C2 domain (Arcaro et al. 1998). Being the PX/C2 domains a characteristic feature of class II PI3Ks, it is tempting to speculate that this or similar mechanisms might regulate the activation of all class II PI3Ks.

Association with other proteins has been also proposed as a potential mechanism of activation (Falasca and Maffucci 2012), as in the case of PI3K-C2β and intersectin (Das et al. 2007). More recently, a study demonstrated that protein kinase N induces PI3K-C2β inactivation upon mitogen stimulation by promoting its association to 14-3-3 proteins (Wallroth et al. 2019). Evidence of post-translational modifications of PI3K-C2β is also present in the literature, from growth factor-induced tyrosine phosphorylation (Arcaro et al. 2002) to possible proteolysis (Visnjić et al. 2003) and

nitrotyrosylation (Chiang and Postlethwaite 2006). The specific role of these post-translational modifications in the modulation of class II PI3Ks enzymatic activity, however, remains to be clarified.

As discussed previously, an interesting aspect of class II PI3Ks biology is their ability to generate localised pools of PtdIns3*P* and PtdIns(3,4)P_2. Consistent with this, studies have reported intracellular relocation of class II enzymes as the way by which such localised synthesis of their products can be achieved. Examples include the insulin-induced translocation of a GFP-tagged PI3K-C2α to the plasma membrane of L6 cells (Falasca et al. 2007), the LPA-dependent translocation of a Myc-tagged PI3KC2β to the plasma membrane of HeLa and SKOV-3 cells (Maffucci et al. 2005) and the shear stress-induced relocation of PI3K-C2α to the primary cilium (Boukhalfa et al. 2020b).

Much work is still required to come to a full understanding of how the enzymatic activity of class II PI3Ks can be modulated in different cellular contexts.

6 Physiological Roles of Class II PI3Ks—Insights from Animal Models

6.1 PI3K-C2α

Our understanding of the physiological roles of class II PI3Ks has improved in the past ten years due to the generation of several mouse models, as recently summarised (Gulluni et al. 2019). Briefly, a critical role for PI3K-C2α during development was demonstrated by two studies reporting embryonic lethality upon full knockout of the enzyme, due to defects at the level of the primary cilium structure and function (Franco et al. 2014) and in vascular development and angiogenesis due to its key role in endothelial cells specifically (Yoshioka et al. 2012). Embryonic lethality was also reported in homozygous mouse models for either a kinase-dead (Alliouachene et al. 2016) or truncated versions (Mountford et al. 2015) of PI3K-C2α. Heterozygous mice from the kinase-dead model were viable and fertile although male mice presented early onset leptin resistance, mild age-dependent obesity, insulin resistance and glucose intolerance (Alliouachene et al. 2016). Heterozygous mice expressing the truncated versions of PI3K-C2α showed alteration of the internal membrane structure of platelets and impaired thrombosis (Mountford et al. 2015). These defects were not detected in platelets from PI3K-C2β deficient mice and were not enhanced in platelets from mice deficient in both enzymes (Petitjean et al. 2016), confirming a non-redundant role for PI3K-C2α in platelets.

6.2 PI3K-C2β

In contrast to PI3K-C2α, a PI3K-C2β full knockout mouse model was reported to be viable and fertile (Harada et al. 2005). This study focused in particular on the impact on the epidermis and reported no alteration in epidermal growth, differentiation or in the barrier function in knockout mice (Harada et al. 2005). Knockout mice were also shown to be resistant to passive cutaneous and passive systemic anaphylaxis (Srivastava et al. 2017). A mouse model expressing a kinase-dead PI3K-C2β revealed increased insulin sensitivity selectively in metabolic tissues as well as increased glucose tolerance and protection against liver steatosis induced by high-fat diet (Alliouachene et al. 2015).

6.3 PI3K-C2γ

A PI3K-C2γ global knockout mouse model was also reported to be viable and to develop normally. These mice showed reduced accumulation of glycogen in the liver and altered lipid metabolism and developed insulin resistance with age or upon high-fat diet (Braccini et al. 2015).

7 Physiological Roles of Class II PI3Ks—First Insight from Human Studies

A recent study reported the identification of the first monogenic disorder linked to mutations of a class II PI3K (Tiosano et al. 2019). The authors described five individuals from three unrelated consanguineous families showing similar clinical features, including short stature, cataracts, dysmorphic facial features, skeletal and teeth abnormalities. Most individuals also presented neurological abnormalities and secondary glaucoma. Hearing loss was also observed in some patients. Next-generation sequencing analyses showed that all affected family members, but none of the unaffected members, were homozygous for loss-of-function variants in *PIK3C2A* and indeed PI3K-C2α was not detected in patient-derived fibroblasts (Tiosano et al. 2019). This study indicated a critical role for PI3K-C2α in human development but it also highlighted a stark difference with the transgenic mouse models where lack of PI3K-C2α results in embryonic lethality. Whether this difference is due to different physiological roles in humans or potential compensatory effects of other PI3Ks (possibly PI3K-C2β, whose expression levels increase in fibroblasts from affected individuals (Tiosano et al. 2019)) remains to be established.

8 Cellular Functions Regulated by Class II PI3Ks

8.1 PI3K-C2α

Investigation into the molecular mechanisms responsible for the phenotypes detected in the different animal models revealed that PI3K-C2α is involved in several intracellular trafficking processes, including transport of cargos and signalling proteins to the primary cilium (Franco et al. 2014, 2016) as well as trafficking of vascular endothelial growth factor receptor-2 and sphingosine-1-phosphate receptor 1 (Yoshioka et al. 2012), vascular endothelial-cadherin (Yoshioka et al. 2012) and leptin receptor (Alliouachene et al. 2016). Studies using cell lines also indicated a role in clathrin-dependent (Gaidarov et al. 2001; Posor et al. 2013) and in dynamin-independent endocytosis (Krag et al. 2010) as well as in neurosecretory (Wen et al. 2008; Meunier et al. 2005) and insulin (Leibiger et al. 2010; Dominguez et al. 2011) granule exocytosis.

Data also indicate the involvement of PI3K-C2α in several different cellular processes, from regulation of vascular smooth muscle cell contraction (Wang et al. 2006; Yoshioka et al. 2007) to human cytomegalovirus replication (Polachek et al. 2016) and Kaposi's sarcoma-associated herpesvirus reactivation from latency (Abere et al. 2018). PI3K-C2α has been also implicated in regulation of autophagy (Merrill et al. 2017) through its ability to generate a localised pool of PtdIns3*P* which is critical during initiation of this cellular process (Nascimbeni et al. 2017a; Roberts and Ktistakis 2013). Recent data, in particular, have revealed a specific role for the PI3K-C2α-dependent PtdIns3*P* pool in shear stress-induced autophagy in kidney epithelial cells, as opposed to the PtdIns3*P* pool generated by class III PI3K which is required for activation of autophagy upon cellular starvation (Boukhalfa et al. 2020b).

A role for PI3K-C2α in insulin signalling was also suggested by data demonstrating that its downregulation reduced translocation of the glucose transporter GLUT4 to the plasma membrane and glucose uptake in muscle cells (Falasca et al. 2007) and it induced a switch toward mitogenic rather than metabolic signalling in pancreatic β cells (Leibiger et al. 2015). On the other hand, heterozygous mice for a kinase-dead PI3K-C2α did not display alteration of insulin signalling in insulin target tissues (Alliouachene et al. 2016). Although it cannot be excluded that the different results are due to the partial inactivation of the enzyme as opposed to its downregulation, the potential contribution of PI3K-C2α to insulin signalling requires further investigation.

8.2 PI3K-C2β

Compared to PI3K-C2α, the cellular functions ascribed to PI3K-C2β are somehow more limited, although they seem to be more common between different cell types. For example, PI3K-C2β is involved in regulation of migration of several cell types,

from HEK293 (Domin et al. 2005) and human umbilical vein endothelial cells (Tibolla et al. 2013) to different types of cancer cells (Maffucci et al. 2005; Mavrommati et al. 2016; Chikh et al. 2016; Katso et al. 2006). Investigation of the kinase-dead mouse model revealed a role for PI3K-C2β in the regulation of insulin signalling pathways through regulation of endosomal trafficking, in particular of the insulin receptor (Alliouachene et al. 2015). PI3K-C2β has been also involved in negative regulation of mTORC1 during growth factor deprivation (Marat et al. 2017) and it has been proposed as a host factor for influenza virus entry (O'Hanlon et al. 2019).

8.3 PI3K-C2γ

Investigation of the mechanisms responsible for the defective metabolism detected in PI3K-C2γ knockout mice revealed a role for this enzyme in the regulation of glycogen synthase activity in the liver upon insulin stimulation (Braccini et al. 2015). Overall, very little is still known about the physiological roles of PI3K-C2γ, which remains the most obscure of the three class II PI3Ks.

8.4 Co-operative Roles?

While most studies indicate distinct functions for class II PI3Ks, evidence of the involvement of both PI3K-C2α and PI3K-C2β in the regulation of some cellular processes has also appeared in the literature. Examples include studies indicating a role for both enzymes in vascular smooth muscle cell contraction (Sarker et al. 2019; Islam et al. 2020), in clathrin-dependent pinocytosis (Aung et al. 2019) and in the regulation of cell mitosis (Gulluni et al. 2017; Cisse et al. 2019). Understanding the relative contribution of each enzyme in these processes will be very interesting. It is known, for example, that they play distinct roles in the regulation of pinocytosis (Aung et al. 2019). On the other hand, the exact role of PI3K-C2β during mitosis has not been described yet (Cisse et al. 2019), therefore its involvement in this process is still not clear, in particular, compared to PI3K-C2α, which is critical for mitotic spindle assembly and anaphase onset (Gulluni et al. 2017). Interestingly, it has been demonstrated that the enzymatic activity of PI3K-C2α is not required during mitosis as it contributes to the process by acting as a scaffold protein (Gulluni et al. 2017). As the involvement of PI3K-C2β was identified using siRNA/shRNA-mediated downregulation (Cisse et al. 2019), it remains to be established whether the enzymatic activity of PI3K-C2β is required instead. In general, it will be interesting to define the exact mechanisms of action of each enzyme in cellular processes that involve both of them and to understand why two members of the same class of PI3Ks are required.

9 Class II PI3Ks, Immune Cells and Inflammatory Responses

Very few data suggesting a possible role for class II PI3Ks in immune cells are available and some of them are summarised here. Evidence includes the observation that downregulation of PI3K-C2α reduced the FcεRI-mediated release of the enzyme β-hexosaminidase and a tagged neuropeptide-Y in RBL-2H3 cells (Nigorikawa et al. 2014). These data, together with the detected localisation of PI3K-C2α on large vesicles generated upon FcεRI stimulation, suggested that this enzyme is involved in FcεRI-mediated degranulation (Nigorikawa et al. 2014).

A role for PI3K-C2β in the activation of the K^+ channel KCa3.1 was first suggested by the observation that siRNA-mediated downregulation of the enzyme inhibited the channel activity in Jurkat cells overexpressing KCa3.1 and in naïve human $CD4^+$ T cells (Srivastava et al. 2009). A specific role for PtdIns3*P* was also reported (Srivastava et al. 2009), consistent with previous studies (Srivastava et al. 2005, 2006a, b). In addition, reduced FcεRI-mediated KCa3.1 activation was detected in bone marrow-derived mast cells from PI3K-C2β knockout mice and this was rescued by dialysing a PtdIns3*P* able to insert into the plasma membrane (Srivastava et al. 2017). Interestingly, stimulation with anti-CD3 antibodies did not appear to increase PI3K-C2β enzymatic activity in Jurkat cells overexpressing both KCa3.1 and a GFP-tagged PI3K-C2β, but it appeared to recruit the enzyme to the immunological synapse, suggesting that relocation of the enzyme was crucial to impact on KCa3.1 (Srivastava et al. 2009). Additional analysis confirmed an important role for this PI3K-C2β/PtdIns3*P*/KCa3.1 pathway in mast cell activation, as bone marrow-derived mast cells from PI3K-C2β knockout mice displayed reduced Ca^{2+} influx, cytokine production and degranulation upon FcεRI stimulation (Srivastava et al. 2017). The additional observation that PI3K-C2β knockout mice were resistant to IgE-mediated passive cutaneous and systemic anaphylaxis led the authors to propose this enzyme as a potential therapeutic target for IgE-mediated diseases (Srivastava et al. 2017).

Involvement of class II PI3Ks in inflammatory responses in pathological conditions are also suggested by some data in the literature, although the specific contribution of these enzymes is not always defined. For instance, a recent study reported increased expression of PI3K-C2γ in the synovial fluid of rheumatoid arthritis (RA) compared to osteoarthritis (OA) patients, in cells of the synovial lining layers and inflammatory infiltrates in the synovial tissue of RA compared to OA patients and in peripheral blood mononuclear cells from RA patients compared to healthy individuals (Kim et al. 2020). These authors further demonstrated that the chemical compound PBT-6, which they showed inhibits PI3K-C2γ, was able to increase cell death in tumour necrosis factor (TNF)-α-induced synovial fibroblasts (MH7A cells) and in lipopolysaccharide (LPS)-activated Raw 264.7 macrophages. Furthermore, PBT-6 decreased levels of interleukin (IL)-6 in TNF-α-treated MH7A cells and secretion of TNF-α and IL-6 by LPS-activated Raw 264.7. Migration of activated macrophages towards the conditioned medium of TNF-α-induced fibroblasts was

also inhibited by addition of PBT-6. Importantly, the authors showed that PBT-6 inhibited osteoclastogenesis in vitro and reduced severity of collagen-induced arthritis in mice (Kim et al. 2020). On the other hand, a potential role for PI3K-C2α in OA has been suggested by a recent study reporting regulation of this enzyme by the potassium voltage-gated channel subfamily Q member 1 overlapping transcript 1 in chondrocytes (Liu et al. 2021). Specifically, the authors detected down-regulation of the channel in osteoarthritic compared to normal chondrocytes and showed that overexpression of PI3K-C2α reduced the secretion of inflammatory factors, reduced apoptosis and increased viability of osteoarthritic chondrocytes. These observations suggest that deregulation of this pathway might be involved in development or progression of OA (Liu et al. 2021).

A few observations suggesting a possible involvement of PI3K-C2β in the immune response in some pathological conditions also exist. Examples include a recent study evaluating the levels of nine tumour-infiltrating T cell types in hepatocellular carcinoma (HCC) and non-tumour tissues (Li et al. 2020). These authors suggested that driver genes including *PIK3C2B* might be associated with the reduced T cell infiltration detected in HCC (Li et al. 2020). Similarly, *PIK3C2B* has been found to be one of only two genes whose expression levels seem to be able to discriminate chronic lymphocytic leukaemia and monoclonal B cell lymphocytosis cases from normal polyclonal and mono/oligoclonal B lymphocytes (McCarthy et al. 2015).

Additional evidence in the literature still requires further investigation in terms of their significance. An example includes the observation that the antioxidant delphinidin, which was shown to ameliorate psoriasis in vitro and in vivo, was also reported to bind to some kinases, including PI3K-C2β (Chamcheu et al. 2017). The importance of such an interaction and whether it is associated with the detected beneficial effects was not clarified.

Whether future studies will reveal additional roles for class II PI3Ks in immune response in physiological and/or pathological conditions remains to be established. In this respect, it is worth mentioning that a pool of PtdIns3*P*, whose synthesis is mediated by class III PI3K, is produced in phagosomes and is important for phagocytosis (Vieira et al. 2001; Birkeland and Stenmark 2004; Ellson et al. 2001a), in particular for modulation of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex and superoxide production (Suh et al. 2006; Ellson et al. 2001b). In fact, the existence of cyclical waves of PtdIns3*P* synthesis during this process has been also reported (Chua and Deretic 2004). Although the potential involvement of class II PI3Ks in modulating PtdIns3*P* levels in this process was excluded by a study investigating the regulation of the NADPH oxidase during phagocytosis of *Staphylococcus aureus* and *Escherichia coli* (Anderson et al. 2008), whether additional studies will provide further information on this topic remains to be seen.

Similarly, it has been reported that autophagy is important for the regulation of the innate immune system (Germic et al. 2019a, b). Indeed, autophagy, a process generally used to degrade cellular components, such as damaged organelles or unnecessary and/or potentially harmful molecules, or to provide metabolic intermediates, can also provide materials for presentation by innate immune cells (Germic et al. 2019a). The process requires generation of a double membrane structure named

autophagosome and its fusion to lysosomes (Levine and Klionsky 2004; Zhao et al. 2021) and several studies have established that synthesis of PtdIns3*P* by class III PI3K is critical for autophagosome formation (Nascimbeni et al. 2017b; Backer 2008). Emerging data in the literature, however, also point to the contribution of PI3K-C2α to the process (Merrill et al. 2017), including recent evidence of a specific type of autophagy involving this enzyme (Bischoff et al. 2021). It will be interesting to investigate whether class II PI3K-mediated autophagy plays any role in immune cells.

10 Conclusion

Interest towards the identification of the physiological roles of class II PI3Ks and their potential involvement in human diseases has massively increased in the past fifteen years. This has led to a better understanding of the cellular functions regulated by these enzymes. On the other hand, some of the limitations that had previously hindered the investigation of class II PI3Ks still exist, namely the limited availability or lack of selectively inhibitors (Falasca et al. 2017), which has also strongly limited the impact and translational relevance of the accumulating lines of evidence pointing to their involvement in human diseases (Falasca et al. 2017). Much work is still required to shed more light into these PI3Ks and to fully understand their contribution to physiological and pathological processes.

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